Contributions to the study of the complement system in IgA nephropathy and dialysis
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CHAPTER

Summary, Discussion
and Future perspectives
SUMMARY, DISCUSSION AND FUTURE PERSPECTIVES.

Deregulation or excessive activation of the complement system is now considered a fundamental aspect of the pathogenesis of a wide-spectrum of immune-mediated and inflammatory diseases. The approval by the US Food and Drug Administration (FDA) of complement inhibitor eculizumab, a monoclonal antibody directed against C5, first in 2007 for paroxysmal nocturnal hemoglobinuria (PNH), and afterwards for the rare kidney disease atypical hemolytic uremic syndrome (aHUS) in 2011, and the subsequent success that patients with these diseases experienced, proved the efficacy of complement-targeted therapies and therefore increased the interest into its clinical use, especially in kidney diseases. Furthermore, lessons drawn from the clinical experience with eculizumab use over the past years has helped to increase the knowledge on how the complement system works, and to explore the remaining and emerging challenges in complement-associated kidney disease. Notably, various trials testing eculizumab in other conditions associated with complement activation failed. This points to the fact that a more refined approach to modulate the complement cascade is needed, especially since targeting of the system can be done at different levels (i.e. the initiators, convertases, complement receptors, and regulators); and that a more precise identification of subgroups of patients benefiting from complement therapeutics can contribute to its success. For that, a better understanding of the mechanisms involved in complement activation both in kidney pathology driving CKD progression and in the systemic uremic milieu in patients with advanced kidney disease is needed. Also, the identification of appropriate biomarkers representing these processes is essential to increase the contribution of complement targeting treatment in slowing CKD progression and prevention of morbi-mortality in advanced CKD states such as those seen in patients on maintenance dialysis.

The current work presented in this thesis contributes to, and expands the notion of, the involvement of the complement system in various pathological processes taking part in the progression of CKD and in the uremic milieu in dialysis patients. Particularly, in a common glomerular disease such as IgA nephropathy (IgAN), it was shown that C4d, as both a glomerular and potentially vascular biomarker of lectin pathway (LP) activation, identifies a subgroup of patients with a worse prognosis in this very complex and heterogeneous disease, that may benefit from different clinical approaches. Also, in hemodialysis (HD) patients with higher levels of intra-dialytic complement activation indicated by an increased C3d/C3-ratio, a higher risk of a cardiovascular event was
identified. Furthermore, in CKD and HD patients who were administered intravenous iron formulations, particularly iron sucrose, complement activation measured through sC5b-9 levels was documented, expanding the list of potential side effects of this commonly administered drug in HD patients. Finally, in peritoneal dialysis (PD), both local and systemic complement activation was demonstrated to occur through apparently different triggering pathways, and strikingly, systemic sC5b-9 levels were significantly higher than those found in HD patients.

The discovery of the involvement of the LP in a subgroup of IgAN patients has opened new avenues for research in IgAN, and expanded the early role attributed to complement in this disease. An extremely heterogeneous glomerular disease, both at morphological patterns and outcome levels, demands a more refined approach than just the one guided by proteinuria, hypertension and eGFR. Indeed, personalized medicine in glomerular disease clinics has relied mostly on morphological patterns, unlike other clinical areas of medicine, such as Oncology, where the use of immunohistological panels and genomic approaches have become essential for clinical decisions. If well implemented, these new approaches may also establish themselves as part of the standard of care for glomerular disease. Therefore, in Chapter 2, beyond the confirmation of the predictive role of mesangial C4d in our cohort of IgAN patients as previously demonstrated, we propose the use of an immunohistological panel of biomarkers where the combination of glomerular C4d and tubulointerstitial CD3 (a T-cell maker, indicating tubulointerstitial inflammation) could be applied. This could be a way to further document the importance of the glomerular tubular cross-talk in IgAN and the importance that tubulointerstitial inflammation has in renal function deterioration in this disease. Furthermore, the vascular compartment has recently seen a renewed interest in IgAN due to studies showing that the subgroup of patients with microangiopathic lesions have worse prognosis. In Chapter 3, we, therefore, expanded our analysis of C4d to investigate if this could be a marker of arteriolar lesion and found that this marker was associated both with vascular lesions and independently with outcomes in IgAN. To conclude, our studies reinforced the potential role that C4d may have in predicting outcomes in IgAN, and expanded its potential diagnostic applications by focusing on the arterioles. A simple biomarker, already commonly used in the clinics, particularly in transplant pathology, can therefore be applied in pathology analyses of IgAN biopsies, where both the glomerular and the vascular compartments could provide relevant and complementary information.
Although most of the research in complement and kidney disease has focused on the contribution of this system to the progression of CKD in various inflammatory kidney diseases, a growing interest exists in the contribution of the complement system to the systemic inflammatory milieu itself in advanced kidney disease. Accordingly, in Chapter 4 the current knowledge about the complement system in dialysis patients is summarized. In both hemodialysis (HD) and peritoneal dialysis (PD), bioincompatibility seems to be an important factor leading to complement activation. In the case of HD, this relies on repeated exposure of the blood to an extracorporeal circuit, particularly dialysis membranes, whereas in PD, the bioincompatibility may arise from the constant exposure to a dialysis fluid in the peritoneal cavity, which can also be closely linked to the presence or loss of the constitutively expressed complement regulators in the peritoneal membrane. Based on the current literature, the proposed mechanisms for complement activation in HD involve both the lectin and the alternative pathways, which subsequently trigger inflammation and coagulation. Also, the classical pathway and alternative pathway have been implicated in complement activation both at local and systemic levels in PD.

Chapter 5 investigates the implications that the complement system can have in the mechanisms leading to HD-associated cardiovascular outcomes. Intradialytic complement activation predominantly occurred in the HD patients that would later develop a cardiovascular event. Moreover, the complement activation seen in these patients was accompanied by an enhanced thrombo-inflammatory response. This suggests that, in HD, the mechanism behind the higher cardiovascular risk could be attributed, at least in part, to complement, inflammation, and coagulation. Besides the long term effects of repeated intra-dialytic complement activation in HD patients, the recent resurgence and increased reporting of dialyzer reactions during HD sessions, and the still poorly defined etiology of the high prevalence of sudden death during the initial dialysis sessions, provide the rationale for increasing research on the complement system and HD therapies.

Exposure to an extracorporeal circuit or bioincompatible fluids is not the only source of potential inflammation in dialysis patients. The repeated administration of drugs, such as intravenous iron formulations, can also contribute to inflammation in these patients. Although a recent trial has diminished the concerns regarding intravenous iron therapies, many questions remain unanswered about the possible role of repeated iron administration contributing to the inflammatory uremic milieu.
that increases the risk of infection and cardiovascular events in the CKD population. Furthermore, evidence for iron overload, anaphylactic reactions, oxidative stress, atherosclerosis, and cognitive impairment have been documented as important side effects of iron therapy.\textsuperscript{22,24,25} Recently, our group has demonstrated for the first time that iron formulations can activate complement \textit{in vitro}, expanding the concept of complement activation related pseudo-allergy (CARPA) to the field of iron formulations therapy.\textsuperscript{26} Particularly the levels of complement activation demonstrated after iron dextran administration could be responsible for the occurrence of clinically evident hypersensitivity reactions.\textsuperscript{26} In Chapter 6 we demonstrated that in both non-CKD, non-dialysis CKD, and most importantly in CKD patients on maintenance hemodialysis, where the exposure to extracorporeal circuit stimuli is already responsible for a significant degree of complement activation, iron therapy itself, was responsible for a significant increase in sC5b-9 levels. Although this increase was significant, it was not sufficient to produce an acute clinically recognizable reaction, as it was significantly lower than the amount proposed to provoke this (~5-10 fold increase).\textsuperscript{27} Another interesting finding was that complement activation occurred after iron sucrose but not ferric carboxymaltose administration.

In PD, evidence regarding the importance of peritoneal membrane complement regulators (Cregs) has been established by several studies, by demonstrating an association with peritoneal solute transport ratio (PSTR) as well as complement activation.\textsuperscript{14,28} It is, however, difficult to document the status of Cregs on the peritoneal membrane due to the lack of access to the membrane itself. Peritoneal membrane biopsies are rarely performed in the clinical setting and remain mostly a technique used at times of PD catheter insertion or PD discontinuation.\textsuperscript{29} As is the case with mesothelial cells in peritoneal dialysis fluid, the availability of measurable soluble forms of Cregs may serve as surrogates for their histological expression in the peritoneal membrane. Therefore, in Chapter 7, we measured the soluble form of CD59 at both a local and systemic level in PD patients and investigated its relationship with complement activation and clinical outcomes. Surprisingly, a strong relationship between the dialysate sCD59 and complement activation indicated by sC5b-9 was not found. In contrast, local and systemic sCD59 independently associated with outcomes concerning both membrane and residual renal function in this cohort of PD patients. The mechanisms beyond complement activation leading to the association of sCD59 with PD outcomes remain to be determined. In Chapter 8, we further explored the complement
profile of patients on PD, through a comprehensive complement analysis both at local and systemic level including components and activation products, and investigated its relationship with clinical data. In line with the landmark study of Lambie et al. that described a dissociation between systemic and local cytokine levels with different clinical implications, different initiation pathways seem to lead to complement activation either at circulation or at intra-peritoneal milieus. Furthermore, systemic complement activation was compared to a cohort of HD patients and, unexpectedly, significantly higher levels of sC5b-9 levels were found in our PD cohort. Further studies should be performed to confirm this intriguing finding. Finally, our study points to the potential value of complement component C1q as a predictor of a significant clinical outcome in PD, such as the case of peritonitis events. Together, both studies contribute to the growing knowledge of complement system involvement in PD and show that its clinical relevance goes beyond the assessment of the traditional activation products. In fact, the measurement of complement components and regulators may have the potential to become important biomarkers aiding clinical decisions.

The pathophysiological contributions of the complement system, particularly in IgAN and dialysis, have been further elucidated with the work described in this thesis. However, the link between the complement system and the local and systemic inflammatory response observed in these conditions, has shown conflicting results in our studies. Indeed, in Chapter 2 and Chapter 3, the IgAN biopsy studies were not conclusive as to whether complement activation leading to mesangial and arteriolar C4d is a cause of active inflammation, or whether it’s just a mesangial or endothelial stress marker of bystander activation in necrotic or fibrotic areas. Concerning glomerular C4d, Segarra et al. demonstrated that its expression in the mesangium occurs at the early stages of the disease, providing a rationale for its involvement in the early inflammatory processes instead of merely being a marker of late injury. Also, in our IgAN cohort, a subgroup analysis with patients without advanced CKD confirmed the association of arteriolar C4d with outcomes, also favoring the involvement in early processes of the disease, but the presence of C4d was not associated with morphological markers of inflammation such as mesangial, endo or extracapillary proliferation. Furthermore, in Chapter 5, the ex-vivo hemodialysis model showed that targeting complement would abolish the production of inflammatory markers, while in Chapter 6, associations between complement activation and MPO, an oxidative stress marker, were found in our hemodialysis cohort, but not for PTX-3, a pentraxin shown to be a sensitive early marker of inflammation induced by
Finally, in Chapter 7, sCD59 was shown to predict the peritoneal solute transport rate (PSTR) which has been closely linked to inflammation, but in Chapter 8, sC5b-9 and the evaluated complement components showed no local association with IL-6 but did with a tissue remodeling marker, MMP-2. Together, these findings open up questions regarding to how complement activation is linked to the general inflammatory response occurring and influencing outcomes in CKD patients. Furthermore, perhaps these findings provide further clues of potential new functions of complement system in disease, apart from generating an inflammatory response. Moreover, instead of seeing the inflammatory response as a generalized reaction, we should discriminate between the different types of inflammatory responses seen in diseases. Some of which will be complement-mediated, while others are complement-independent.

FUTURE PERSPECTIVES

The content of this thesis further expands the knowledge of the potential implications of complement as a tool for personized medicine, as well as a therapeutic target in CKD to improve patient outcomes. Ideally, assessment of the complement system could be progressively refined and further implemented in clinical practice to guide therapy in CKD and Dialysis clinics. Several trials on various kidneys diseases are already ongoing with complement inhibitors that target at different levels in distinctive pathways. At the time when most of the results of these trials become known, an increased need to understand which patients will be more prone to benefit from these potential complement therapeutics will be essential. For that, an accurate understanding of the complement pathways involved in the different pathological processes in CKD, as well as robust biomarkers representing them, will be needed. Candidate biomarkers include C4d, sC5b-9, C3d/C3 and C1q and could easily be used in clinical practice. The current thesis has confirmed, expanded, and opened a hypothesis for their potential use.

There are, however, open questions that warrant further investigation before implications to the clinics can be made. First, the value of arteriolar C4d should be further investigated in larger IgAN patient cohorts. Furthermore, confirming and understanding the reasons for the found differences between the complement activation patterns in HD versus PD will be an interesting topic of research. We hypothesize that complement activation in HD may occur predominantly at a C3-level, while terminal pathway activation is predominant in PD. This may become even more relevant as dialysis technique comparisons are at the core of nephrologists’
concerns in their counseling practice towards advanced CKD patients. Biopsy studies may eventually give new information that can explain these differences between the dialysis techniques, and particularly in PD, between the local and systemic complement activation. Identifying the initial trigger for systemic and local complement activation in PD could also further help to identify new potential treatments for these patients. Furthermore, in HD, the relationship between complement activation, inflammatory markers and patient outcomes should be further explored, combining both the current work available in this thesis with previously published data by other groups.\textsuperscript{15} Especially, the link between complement exhaustion and infectious risk is an area of research that has received little to no attention thus far. Also, considering complement activation as the major source of bioincompatibility, research and development of “complement-friendly” dialysis membranes seems an appealing solution to improve outcomes in these patients. Finally, our studies have confirmed \emph{in vivo} another potential mechanism from which iron formulations may cause harm; i.e., through complement activation. Interestingly, these effects were shown for iron sucrose but not for ferric carboxymaltose, but the latter formulation was only tested in non-dialysis patients. Therefore, it would be interesting to confirm whether ferric carboxymaltose has the same complement activation effect in HD as the one observed for iron sucrose, and to expand the same complement analysis to other commonly used iron formulations such as iron isomaltoside. Furthermore, expanded HD cohorts with increased follow-up time, different time-points sampling, and an enlarged panel of biomarkers could further allow the investigation of the clinical relevance of the effect of repeated iron administration to these patients. Increasing our understanding about the impact of modern medicine (nanoparticles, liposomes, monoclonal antibodies) on the complement system would help to understand, and potentially prevent/treat, certain side-effects (i.e. CARPA), whereas for other drugs (i.e. anti-CD20) using this mechanism might increase their efficacy.\textsuperscript{36,37}
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Summary, Discussion and Future perspectives.