Complement biology in health and disease

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CHAPTER 9

SUMMARY AND GENERAL DISCUSSION

SUMMARY

The complement system is an integral part of the immune system and tissue homeostasis. The complement system has long been viewed as a straightforward proteolytic cascade to combat invading pathogens, however, the complement system has been shown to be far more complex and our knowledge and understanding of the complement system is still expanding. In this thesis we report on local complement production and possible significance of complement proteins outside of the traditional complement system activity. We focus in this thesis on C1q in autoimmunity and tuberculosis (TB), in addition, we studied the relatively new concept of intracellular C3.

Complement component C1q is locally produced by isolated articular chondrocytes
Inflammation and innate immune responses may contribute to development and progression of Osteoarthritis (OA). Chondrocytes are the sole cell type in articular cartilage and produce extracellular-matrix molecules. How inflammatory mediators reach chondrocytes is incompletely understood. Previous studies have shown that chondrocytes express mRNA encoding complement proteins such as C1q, suggesting local protein production, which has not been demonstrated conclusively. **Chapter 2** describes the analyses of the chondrocytes from osteoarthritic cartilage, which were isolated from patients that underwent total knee replacement. The chondrocytes isolated were able to produce and secrete C1q *ex vivo*, as well as able to bind C1q *ex vivo*. Since chondrocytes were able to secrete and bind C1q we explored whether incubation *in vitro* with C1q could affect RNA expression of various complement and collagen genes. In a pilot experiment we demonstrated that incubation with 100 µg/ml C1q for 24 hours, negatively affected the expression of *MMP13* and the *COLL2*:*COLL10* ratio. We concluded therefore, that C1q protein can be expressed and secreted by human articular chondrocytes and that C1q is able to bind to chondrocytes influencing the relative collagen expression.

Complement C3 cleavage by Cathepsin-L leads to formation of C3a-desArg and is not essential for survival in a HAP-1 model cell line
A novel intracellular role has been described for complement protein C3 expressed in CD4+ T-cells, by indicating that intracellular C3 can be cleaved by Cathepsin-L (CTSL), resulting in signalling by C3a via the intracellular C3aR. This intracellular C3 pathway was hypothesized to be pivotal for the survival of CD4+ T-cells. The studies described in **Chapter 3** explored the presence of protein C3 in cell lysates, the CTSL cleavage site in C3 and survival of C3 deficient cells. We were unable to detect C3 in cell lysates without incubation with NHS prior to lysis. The analysis of C3 cleavage by CTSL *in vitro* by mass spectrometry indicated that the C3 cleavage by CTSL results in the formation of C3ades-Arg, since the arginine was still attached to the N-terminus of the C3b molecule. This is an interesting finding, since prior research indicated that intracellular

C3 was downstream modulated by the C3aR. However, C3ades-Arg is unable to bind the C3aR, indicating that the hypothesized intracellular pathway could not occur. In addition, we generated a C3 deficient HAP-1 cell line by the use of CRISPR/Cas9, and no differences were observed regarding proliferation, morphology and metabolism between the wildtype and C3 deficient HAP-1 cell lines. In conclusion, we demonstrate that processing of C3 by CTSL likely generates C3a-desArg and not C3a, and further that C3 or its derived products are not essential for cell survival in a HAP-1 cell line model.

Complement component C1q as serum biomarker to detect active tuberculosis

Tuberculosis (TB) is a major global health threat, which is caused by infection by Mycobacterium tuberculosis (*Mtb*). Diagnosis of active TB is hampered by the lack of specific biomarkers that discriminate active TB disease from other (lung) diseases or latent TB infection (LTBI). Early diagnosis and treatment of TB disease is important to reduce transmission of infection and prevent disease associated mortality. Recently, complement has been highlighted as candidate biomarker for active TB disease. Based on integrated human gene expression results from literature, genes encoding for the different C1q chains and *SERPING1* (encoding for the C1-inhibitor (C1-INH)) were increased in TB disease. Therefore, we analysed the protein levels for C1q (**Chapter 4**) and C1-INH (**Chapter 5**). In the studies described in **Chapter 4** we measured C1q protein levels in four different geographical TB cohorts from Italy, The Gambia, Korea and South Africa. C1q levels were increased in patients with active TB compared to all relevant control populations. Importantly, longitudinal follow up of TB patients during treatment revealed that serum C1q levels normalized to those of endemic controls, indicating that the upregulation of C1q was associated with disease and not intrinsic to the individuals. To further evaluate the potential of C1q as a biomarker for active TB, C1q levels of TB disease patients were compared to those of patients with conditions for which differential diagnoses are difficult to make in clinical practice. Patients with TB had significantly higher circulating C1q levels as compared to patients with sarcoidosis or patients with pneumonia. Likewise, higher C1q levels were found in patients with TB compared to patients with leprosy, that were analysed as additional control. Thus, the upregulation of C1q likely is does not reflect a general response to inflammation. Moreover, C1q protein was locally detected at an increased level in the lungs of TB patients (n=3) compared to controls. In analogy with human TB, C1q also validated as a biomarker of TB disease in rhesus macaques, in both serum and bronchoalveolar lavage (BAL). Increased C1q levels were only observed in animals that progressed to active disease and not in those that controlled the infection. In summary, C1q levels were elevated in patients with active TB compared to LTBI in four independent cohorts. Therefore, we propose that the addition of C1q measurements to current biomarker panels may provide added value in the diagnosis of active TB.

Analysis of the endogenous complement regulator C1-inhibitor, the product of *SERPING1*, in active tuberculosis

We already reported in **Chapter 4** that increased levels of C1q can be used a host serum biomarker that can discriminate active TB disease from LTBI and non-TB pulmonary infections or sarcoidosis. In serum, C1q forms a complex with C1r and C1s to generate the full C1 molecule which can activate the classical pathway of the complement system. Importantly, the C1 complex is regulated by a natural inhibitor, namely C1-inhibitor (C1-INH). Transcriptional biomarker signatures have indicated that expression of *SERPING1*, which encodes the inhibitor C1-INH, discriminates between active and latent TB disease. The studies described in **Chapter 5** aimed to determine serum levels of *SERPING1* encoded C1-INH in four geographically diverse cohorts of patients with active TB, LTBI, disease controls and patients that have been successfully treated for TB. The aim of these studies was to investigate a possible application as host biomarker for active TB. C1-INH serum levels were significantly increased in TB patients compared to endemic controls in two out of four cohorts analysed. In addition, in the Gambian cohort with TB patients followed over time the increased C1-INH decreased rapidly upon initiation of TB treatment. It is interesting that SERPING1 expression is consistently increased in patients with TB whereas its protein product C1-INH is found to be increased in only half of the cohorts analysed in this study. It is unclear why C1-INH upregulation was observed in only two out of four cohorts. Overall, upon comparing C1q and C1-INH protein level in the different populations analysed, C1q was uniformly increased in TB cohorts whereas C1-INH in two out of four TB cohorts. The observation that increased C1-INH serum levels were only detected in cohorts of patients with active TB, but not in patients with similar clinical conditions and the notion that C1-INH decreased rapidly during TB treatment, suggests that *Mtb* is actively regulating these complement proteins. An increase in C1q and C1-INH could therefore represent an immune-escape mechanism of *Mtb* enabling immunosuppressive actions of C1q, without enhancing the classical pathway activity.

Systemic and pulmonary C1q as biomarker of progressive disease in experimental non-human primate tuberculosis

Non-human primate (NHP) *Mtb* infection models are widely used to study pathogen-host interactions and pre-clinical evaluation of vaccine candidates. NHP and macaque species (*Macaca spp*) in particular, are considered highly relevant models for TB, due to their close phylogenetic relationship to man, outbred nature and large similarity in TB pathogenesis. Macaques are applied across the whole spectrum of TB research, both in preclinical evaluation of TB vaccines and therapeutics as well as basic research on TB disease development. Modelling in these species has the advantage of having controlled and accurate time-response-conditions relative to infection. Depending on macaque (sub)species, *Mtb* strain and challenge dose, TB disease manifestation in macaques mimics the diversity seen in humans. After the demonstration presented in **Chapter 4** that C1q was increased after high dose TB challenge in NHP, we exploited the diversity

in TB disease manifestation in NHPs to examine the dynamics of C1q as a biomarker of TB disease in more detail. The results of these studies are presented **Chapter 6**. We assessed systemic and pulmonary C1q levels after experimental infection using a high or low single dose as well as repeated limiting doses *Mtb* challenge of macaques. We show that increasing C1q levels, either peripherally or locally, correlate with increased TB pathology and with decreased survival following challenge with high or low dose *Mtb* in different macaque species. Upregulation of C1q did not precede detection of *Mtb* infection by a conventional interferon-gamma release assay, confirming its association with disease progression but not TB infection per se. Lastly, we show that pulmonary vaccination with Bacillus Calmette Guérin (BCG), also results in a temporal increase in pulmonary C1q. However, whether this local C1q production plays a role in protection against *Mtb* infection and disease remains to be investigated. Our observations confirm and further support C1q as a marker of progressive TB disease. As C1q can be readily measured over the course of *Mtb* infection, it could therefore be applied to monitor TB disease progression in a resource-limited setting.

Complex medical history of a lupus patient with a compound heterozygous mutation in *C1QC*

Genetic deficiencies, caused by homozygous mutations in one of the C1q genes are rare and are strongly associated with development of Systemic Lupus Erythematosus (SLE). C1q deficiency is a rare condition with just over 70 documented cases from at least 45 different families. These deficiencies are all the result of homozygous mutations in one of the three C1q genes, except in one case with a compound heterozygous mutation in *C1QA*. Patients with C1q deficiency have various clinical presentations and outcome. Most common is the diagnosis of Systemic Lupus Erythematosus (SLE) in early childhood and recurrent infections. In **Chapter 7** we describe a C1q deficient patient with a compound heterozygous mutation in the *C1QC* gene: c.100G>A p.(Gly34Arg); c.205C>T p.(Arg69X). The medical history of this patient was complex and involved various infections, SLE, cerebral involvement, vascular problems and bone lesions. This patient was treated with fresh frozen plasma (FFP) for over a decade. Even though the C1q levels and CP activity effects were relatively short lived, the symptomatic relief and substantial improvement in quality of life following FFP treatment was sustained for various weeks. Over time there were several adverse reactions to the FFP therapy as well. However, despite these adverse reactions, the patient preferred the FFP therapy, because of reduction of fatigue, arthralgia and number of infections. Nonetheless, because of a serious anaphylactic reaction, FFP treatment was eventually discontinued.

Carbamylation reduces capacity of IgG for hexamerisation and complement activation

Post-translational modifications (PTM) of proteins following biosynthesis are common in the human body, and are important in the regulation of activity, stability and folding of proteins. Dysregulation of PTMs has been linked to inflammatory and autoimmune

conditions. Carbamylation is the chemical conversion of a positively charged lysine into an uncharged homocitrulline. Carbamylated proteins are frequently targeted by autoantibodies in patients suffering from Rheumatoid Arthritis (RA). Several proteins have reported to be carbamylated *in vivo*, interestingly, it was reported that carbamylation of IgG impacts on its capacity to activate complement. Moreover, cryo-electron microscopy analyses have demonstrated that binding of C1q requires a hexameric arrangement of monomeric IgG complexes, which assemble via non-covalent Fc:Fc interactions. The studies described in **Chapter 8** were performed to better comprehend a possible role of a prominent post-translational modification associated rheumatic disease that is formed after carbamylation. More specifically, we studied the interaction between carbamylated IgG in relation to the ability to activate the complement system. We identified several peptides of human immunoglobulins (including IgG) to be carbamylated *in vivo* in the synovial fluid of RA patients. We confirmed that carbamylation of IgG results in a decrease in its ability to mediate complement activation, both in ELISA and in complement dependent cytotoxicity (CDC) assays. Nevertheless, when the carbamylated variants were mixed with the non-modified IgG, there was no dominant inhibitory effect on complement activation. CDC activity is highly dependent of IgG1 hexamer formation, and in the CDC assay it was unclear whether ca-IgGs form hexamers in the presence of non-modified IgGs. Therefore, we explored whether the effect of reduced complement activation by ca-IgG is also present when using pre-formed ca-IgG-hexamers. Next, we analysed the effect of carbmaylation on hexamerisation and complement activation by making use of a triple mutant variant of the IgG1-DNP which enhance the ability of the antibody to form hexamers both in solution and on the cell surface (designated IgG1-DNP-RGY). Upon carbamylation, the IgG1-DNP-RGY was no longer able to hexamerise in solution, indicating that carbamylation negatively affects Fc:Fc interaction resulting in the loss of C1q binding by IgG. In conclusion, the inability of ca-IgG to activate the complement system is the combined effect of both decreased binding of C1q to the modified Fc of IgG and the reduced capacity of ca-IgG to form hexamers.

GENERAL DISCUSSION

Circulating Complement

In this thesis we have explored both low and high C1q serum concentrations in relation to disease and disease manifestations. On one hand we described a patient with C1q deficiency in **Chapter 7** [1], on the other hand in **Chapter 4**, we described the presence of elevated C1q levels in patients with active (pulmonary) TB [2]. C1q deficiency is associated with the development of Systemic Lupus Erythematosus (SLE) [3-5], and we reported on a new identified case with a rare mutation, a compound heterozygous mutation in *C1QC*, as described in **Chapter 7**. This patient has a complex medical history with a wide variety of clinical manifestations, indicative that C1q is, either directly or indirectly, involved in various biological processes. The notion that C1q has functional properties independent of the complement system is also indicated by the hierarchical association of complement deficiencies and the risk to develop SLE. C1q deficiency has the largest risk for SLE development, while in C1r/s deficiency this risk is some-what lower, for C4/C2 deficiency the risk is even less, and C3 deficiency is not a very strong risk factor [6, 7]. If all effects of C1q where via the CP then all deficiencies in that pathway should have the same risk. It is remarkable that genetic deficiencies of the CP predispose to SLE rather than that they protect against SLE development since in most SLE patients complement activation is suggested to contribute to inflammation and organ damage [8]. For C1q it is known that it plays an important role in the clearance of apoptotic cells [9]. Proper clearance of apoptotic cells is important to prevent the release of immunogenic material which could trigger development of an autoimmune diseases such as SLE and glomerulonephritis. Moreover, recently, C1q was reported to act as an inhibitor for T-cell responses, suggesting that a C1q deficiency could give rise to T cells aiding autoreactive B-cells [10]. The complement system is both friend and foe in the pathogenesis of SLE. Because of the complexity of SLE, both in clinical presentation and multifactorial aetiology, it is unlikely that one process is sufficient on its own is causative for disease onset.

On the opposite side of C1q deficiency, is an increased concentration of circulating C1q. In **Chapter 4**, we described the presence of increased C1q levels in patients with active TB. We assume that the increased levels of C1q we observed are rare since the only other clinical condition in which increased levels of C1q have been reported is visceral leishmaniasis, also known as kala azar [11]. Whether the increased C1q levels are protective for the host or the pathogen, more specifically *Mtb* as described in **Chapter 4**, is unknown. Interestingly, the infection itself occurs rather local, in the macrophages of the lungs, whereas the increased C1q expression is found systemically, in circulating cells collected from peripheral blood. Also *SERPING1* expression is elevated, though this increase did not translate to uniformly increased C1-INH protein levels in the measured cohorts as demonstrate in **Chapter 5**. Other components from the complement system do not appear to be increased in various studies in the circulation. These results are

interesting as they suggest an independent role of C1q outside of complement system. TB is caused by an intracellular pathogen and C1q has been shown to be of importance in $CD8^+$ T-cell biology where C1q dampens the $CD8^+$ T-cell responses [10]. Given that *Mtb* is an intracellular pathogen it could be of importance for *Mtb* to stimulate higher C1q production which will subsequently contribute to decreased $CD8^+$ T-cell anti-microbial activity. It is interesting to speculate that the rise in C1q levels serves a functions outside of the complement system cascade since the other component of the CP do not seem to be increased based on the expression profiles. Whether the *Mtb* directly upregulates the C1q expression or indirect is unclear. In the NHP model, increased C1q levels were associated with pathology and was found to be a marker of progressive TB disease and not the infection itself as demonstrated by the studies presented in **Chapter 6**. These observations correspond to the observation made in the human situation. We hypothesize that the increase in C1q is not a direct consequence of infected macrophages, but an indirect consequence of the milieu created by infected macrophages. For example, it is conceivable that infected macrophages produce mediators which stimulate surrounding cells to increase the production of C1q. This concept can be further studied *in vitro* by adding the supernatant of cultured infected macrophages to naïve cultured PBMCs / monocytes. The increased levels of C1q would lead to a potentially more active CP, which would conceivably contribute to enhanced clearance of *Mtb*. For increased levels of C1q to impact on T-cell biology without the concomitant effect of enhanced CP activity, C1-INH also needs to be present in increased levels to prevent further cascade activation.

Tuberculosis and biomarkers

Common diagnostic tests as the tuberculin skin test and interferon (IFN)-γ release assay are unable to discriminate between active and latent TB infection. It is important to discriminate active TB from LTBI in order to promptly initiate treatment to prevent mortality and further spread of the pathogen, here, it appears that host biomarkers play a significant role. In the search for biomarkers it is important to take along LTBI, since only comparing active TB with healthy controls is not representative or informative for an environment where TB is endemic. Ideally, biomarkers should be able to discriminate between TB and other respiratory infections that present with similar symptoms and abnormalities on chest X-rays. In this thesis, **Chapter 4** and **Chapter 5**, we have focussed on complement proteins as biomarker for TB. From a feasibility perspective, we preferred protein measurements instead of RNA expression profiles for biomarker signatures, since protein measurements are often easier, more stable, more accessible, cheaper and less time consuming compared to RNA procedures. In **Chapter 4** we demonstrated that increased C1q levels were specifically increased in active TB, not in LTBI or other respiratory infections. The ROC analysis of C1q resulted in an AUC of 0.77 for active TB versus LTBI, which may seem unimpressive, in the TB field, for a single protein biomarker, this is considered quite good. C1q is abundantly present, easy to measure and stable, therefore, it would be interesting to further evaluate its

potential in biomarker panels for TB diagnostics. The availability of the NHP model of tuberculosis for C1q research, as we demonstrated in **Chapter 4** and **Chapter 6**, could greatly accelerate and facilitate further research into biomarkers for TB. Further research can also be performed more into the host-pathogen interactions in the NHP for TB, but for a biomarker it is most important that it has a high specificity and sensitivity, this can be explored independent of its role in the pathogenesis.

Local Complement

The bulk of the complement proteins that are present in serum are produced and secreted by the liver, in particular by hepatocytes. However, serum does not reach all sites in the body where complement activation is needed. There are also cells that produce complement proteins locally at serum restricted sites. Local complement production and activation play a role in the initiation phase of the immune response. This activation impacts on the permeability of the local vasculature that subsequently will allow more systemic plasma proteins to leave the vessel and contribute to, or even take-over, the initial local response. We have reviewed the literature and found that there was also a profound ability for immune cells to produce local complement components [12]. Some cells are capable of producing all components needed for a functional pathway (monocytes), whereas others are only able to produce one or two complement components. This could point to other local functions of these complement proteins outside the complement system. We have further explored local production of complement and demonstrated that C1q can be produced by chondrocytes, as described in **Chapter 2.** In diseased joints, as found in rheumatoid arthritis (RA) and osteoarthritis (OA), higher levels of complement components are detected in the synovial fluid. This could be due to leakage from serum in pathogenic conditions. However, we found that C1q was produced by chondrocytes in unstimulated conditions *ex vivo*. This is interesting, especially considering the nutrient poor environment of the articular cartilage. From the cells perspective it could be disadvantageous to use a large amount of energy for the production of a protein without functionality. Therefore, it is tempting to speculate that C1q must have some functionality for the cartilage environment, as otherwise the cell would not produce this molecule. Articular cartilage is avascular and relies on diffusion of molecules. The structure is heterogenous and the ability of proteins to diffuse into the cartilage decreases with increasing molecular size [13, 14]. C1q is a large, 460 kDa, protein and the size limit of proteins that can move freely in cartilage is estimated to be around 65 kDa [15]. Therefore, it is likely that C1q produced by the chondrocytes has a local rather than systemic function. Conceivably, C1q would function in an autocrine setting in stimulating the chondrocytes, or alternatively C1q could be involved in local complement activation and / or cartilage biology. We speculate that C1q and complement activation may be involved in the maintenance of a lacuna for the chondrocyte. In such a scenario the released C1q may trigger the degradation of matrix molecules that are (too) close to the cell body of the chondrocytes. There are local interactions between complement proteins and matrix

molecules [16-20] and given the relation between complement and arthritic symptoms [21], it is interesting to explore the contribution of local production of C1q to cartilage morphology and biology with for example dedicated experiments with C1q deficient mice as a model.

We also explored the effect of the post translation modification (PTM) homocitrulline, formed by carbamylation, on complement activity. Carbamylation is the chemical conversion of a positively charged lysine into an uncharged homocitrulline and can occur on all proteins. Carbamylated proteins are frequently targeted by autoantibodies in patients suffering from RA [22, 23]. Upon carbamylation of IgG (ca-IgG), the IgGs were incapable of activating the complement system via the CP, which is initiated by C1q binding to a hexameric formation of IgGs. Some therapeutics, such as cell-depleting (monoclonal) antibodies, rely on complement activity for their functionality, so in theory, these could become ineffective by Carbamylation *in vivo*. In **Chapter 8** we demonstrate that IgGs can indeed become carbamylated *in vivo* in the synovial fluid of RA patients. Therapeutic antibodies (-mabs) could therefore also become carbamylated and lose their complement dependent effects. However, with our titrating experiments we demonstrated that for a mixture of antibodies to lose their complement effect, the majority has to become carbamylated. We still expect the majority of IgG to be unmodified, even in an inflammatory environment, and therefore the effect of *in vivo* carbamylation on therapeutics is considered negligible. Next to therapeutics that rely on complement activity for their mode of action, there are also antibodies for which complement activation is an unwanted side effect. For therapeutic antibodies for which complement activation would be considered to be a risk factor [24], the simple carbamylation of the antibodies would render the antibodies completely inert regarding complement activity. However, if therapeutics are administered in a carbamylated version, this could also give rise to anti-carbamylated antibodies, which can consequently form immune complexes, depositing in the glomeruli which then still results in the activation of the complement system. Another possibility is to explore the effectivity and safety of those therapeutics by replacing the lysines in the C1q binding region of the Fc portion of the IgG, thereby preventing the possibility to carbamylate lysines. However, this could influence the effectivity and safety of the therapeutics or enhance their immunogenicity and should therefore be thoroughly evaluated.

Intracellular Complement component C3

Complement component C3 is, together with Factor B, one of the ''oldest'' proteins in the complement system. Intracellular complement, or the ''complosome'' emerged recently as an interesting new perspective in the field of complement immunology [25-28]. However, some precaution put forward in this recent literature may be warranted, since replication of published articles remains necessary and has been challenging, as demonstrated by the studies described in **Chapter 3**. The new notion of intracellular complement has been met with mixed reactions in the field. Challenges lie in, but

are not limited to, the definition of ''intracellular complement'' since all proteins are made intracellular and are at some point fully formed inside the cell before they are secreted. Another terminology that is being challenged is ''C3 deficiency'', since confocal microscopy data from a C3 deficient patient demonstrated presence of intracellular C3, though this data cannot be replicated independently because of the antibodies used in these experiments are not available anymore. In this thesis we have aimed to find a new alternative antibody but were unsuccessful in finding one that gave similar results as published by Liszweski et al.. This also begs for the question that if only one antibody is able to show these results, is it justified to rely on this one polyclonal antibody? More data has been published pointing to an intricate and complex relation between: complement, metabolism and autophagy [29-34]. There are still information gaps in these networks which could be relevant for understanding the possible role for intracellular complement in biology. I do believe we are collecting the pieces to finish this puzzle but it remains challenging to allocate them. For example, Liszweski et al. proposed that intracellular C3a, resulting from intracellular C3 cleavage by CTSL, would signal via the C3aR and have further downstream effect in the mTOR pathway [28]. In **Chapter 3** we have analysed the cleavage fragments of C3 by CTSL and found the terminal arginine attached to the C3b instead of the C3a, indicating production of C3a-desArg. Interestingly, this C3a-desArg is unable to bine C3aR [35, 36] and consequently the pathway proposed earlier involving the intracellular C3aR would not be relevant. Moreover, C3a-desArg, which is also annotated as Acylation Stimulating Protein (ASP) in literature, has been linked to lipid metabolism [37]. In **Chapter 3** we also analysed the necessity of C3 for cell survival in the HAP-1 cell line as a model. We found no differences between C3 sufficient and deficient cells so far regarding survival and growth. This is supported by a recent study where C3 was also knocked out in different cell lines [32], however, they also demonstrated that C3 expression was required for normal autophagy regulation. Ideally, the use of freshly isolated CD4+ T-cells should be pursued for future intracellular C3 research. These are exiting new times which could possibly open up to a new area for complement therapeutics, though we should remain critical how to interpret these novel data and continue to replicate and validate those findings.

Conclusion

In this thesis we have explored the role and presence of complement proteins in both auto-immune and infectious diseases as well as cell biology of C1q production and intracellular C3. The diversity of the findings presented in this thesis further demonstrate the complexity and intrinsic relations that exist in the human body and urges the need to have a broad focus and challenges to look beyond the often conventional compartmentalized research perspectives. New evidence in this thesis has been presented that supports the consensus that complement proteins have conceivably other, local, functions outside of the scope of the complement system.

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