C1q and anti-C1q autoantibodies in (auto)immunity

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APPENDICES

English summary

The immune system is an important factor in the protection against disease and infection, and it consists of an innate part and an adaptive part. The innate part recognizes several common molecules and patterns associated with infection and damage, and rapidly reacts to such signals. Cells of the adaptive immune system can be very specific towards one molecule, but need more time to develop and mature in a primary immune response. Notably, in the adaptive immune response, memory cells are formed that shorten the reaction time when their particular target is encountered again. Antibodies are produced by a specific cell type in the adaptive immune system, called B cells. These antibodies exist as various types and specifically bind a target with their antigen-binding region. When bound to their target, they can activate other elements of the immune system as an effector mechanism. One of these elements is the complement system, which is a group of proteins in the innate immune system that can activate each other in a highly regulated chain reaction. Complement activation starts with the classical, lectin or alternative pathway depending on the activator. The initiator molecule of the classical pathway is called C1, which consists of recognition protein C1q and enzymes C1r and C1s. C1q is capable of recognizing several different structures, such as bacterial proteins, dying cells, and target-bound antibodies.

The immune system is critical in the defense of the human body against pathogens. However, as introduced in **chapter 1**, the immune system sometimes inadvertently turns against the body it should be protecting; this is called autoimmunity. This situation may arise through genetic disorders causing over-activation of the immune system, or when cells of the adaptive part of the immune system start recognizing structures that belong to the body itself, so-called self-antigens. When this recognition of self-antigens occurs in B cells and is not halted, it leads to the production of antibodies against self-antigens, called autoantibodies. Various types of autoantibody are associated with different autoimmune diseases, highlighting the relevance of these antibodies. In this thesis, discussion of autoantibodies mainly focuses on those targeting the initiator of the classical pathway of complement activation, C1q. An interesting feature of anti-C1q autoantibodies is their specificity to solid-phase C1q, a conformational state which C1q adopts upon binding to its ligands. This type of autoantibody is strongly associated with systemic lupus erythematosus (SLE) and especially lupus nephritis. Due to the crucial role of autoantibodies as well as the complement system in (auto)immunity, this thesis describes C1q and anti-C1q autoantibodies in the context of several diseases.

In **chapter 2**, we reviewed the role the complement system plays in several rheumatic diseases. The role of complement in the pathogenesis of rheumatoid arthritis (RA) is

mainly ascribed to the alternative pathway, while the classical pathway only plays a minor role. In the meantime, the influence of complement on the rheumatic disease SLE can be two-fold. Firstly, although rare, deficiency of C1q almost always leads to development of SLE. Secondly and more commonly, SLE severity is correlated with large-scale complement activation and deposition in tissues. Based on the evidence, one would expect complement to play a large role in the processes that drive RA and SLE. Complement-inhibiting drugs (including the anti-C5 therapeutic antibody eculizumab) were tested, but were deemed to have insufficient impact on both diseases. Conversely, in the case of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) there was initially no suspicion for a large role of complement. This changed when stimulation of neutrophils through their C5a receptor was shown to be critical for the disease process. Subsequent research and development led to the approval and marketing of avacopan, a compound blocking the C5a receptor. While complement activation is observed in many rheumatic diseases, therapeutic inhibition of complement was shown to only be effective in some diseases.

Tuberculosis (TB) can occur as active disease or in a latent form. The diagnosis of active TB disease continues to prove difficult, as current tests either do not distinguish between latent and active infection, or take a long time to reach conclusion. This is a problem for pulmonary TB as well as extrapulmonary manifestations of TB, such as TB-associated uveitis. In **chapter 3**, we confirmed the value of C1q as a biomarker for active pulmonary TB disease and also established the association between high serum C1q concentration and TB uveitis. Furthermore, we observed an inverse correlation between high C1q concentration and expression of a previously published signature set of interferon-stimulated genes. The combination of serum C1q concentration and the interferon signature could almost fully separate healthy individuals from patients with active pulmonary TB disease, therefore this combination may be useful for future clinical diagnostics.

Chapter 4 describes our research into complement and anti-complement autoantibodies during pregnancy and in the pregnancy complication preeclampsia. Here, we observed that serum concentrations of both C1q and factor H were higher in women with a healthy pregnancy than in nonpregnant controls. Possibly, C1q concentration is increased to fulfill its role in the construction of new blood vessels and in tissue remodeling, for which there is great need in the placenta. On the other hand, factor H is a complement regulator and may be useful to limit complement activation in the remodeling placenta. Autoantibodies to C1q and factor H were also investigated in the same chapter, but presence of these antibodies was not significantly different between preeclampsia and control pregnancy

groups. We observed lower serum factor H concentration in women with preeclamptic pregnancies than in women with control pregnancies. This difference was driven by early-onset cases of preeclampsia, as factor H concentration was lower in these cases compared to late-onset cases. These results indicate a role for C1q and factor H during pregnancy, and suggest that low factor H concentration may be involved in the pathogenesis of preeclampsia.

The rheumatic disease systemic sclerosis involves fibrosis of the skin and internal organs. Pulmonary fibrosis and pulmonary arterial hypertension are the most common lung-associated conditions in systemic sclerosis, contributing highly to mortality. In **chapter 5**, we investigated the presence of anti-C1q autoantibodies among systemic sclerosis patients, in order to find a prognostic marker for these conditions. We found that anti-C1q was not present at a higher rate in systemic sclerosis patients than in healthy controls. Moreover, lung fibrosis and pulmonary arterial hypertension were not increased in patients that did have anti-C1q autoantibodies, contrary to an earlier report. Therefore we concluded that anti-C1q autoantibodies would not have a good prognostic value for lung conditions in systemic sclerosis patients. Furthermore, the observed association of anti-C1q with anti-topoisomerase antibodies, which was already reported to associate with lung complications, would detract from any added value of anti-C1q in systemic sclerosis prognostics.

In **chapter 6**, we characterized several human anti-C1q autoantibodies. We isolated B cells that produce anti-C1q antibodies from healthy donors, and subsequently produced these antibodies recombinantly. This allowed for the molecular characterization of 9 monoclonal human anti-C1q antibodies. The anti-C1q autoantibodies were of the immunoglobulin G (IgG) isotype and contained variable domains with a considerable number of mutations from the germline sequence. We confirmed the specificity for solid-phase C1q, and measured binding of anti-C1q to C1q while C1q was bound on a range of its natural ligands such as IgG, IgM, CRP and necrotic cells. We observed that the antibodies bound specifically to the collagen-like region of C1q and competition experiments reveal that at least 2 distinct epitopes are targeted by human anti-C1q. Electron microscopy narrowed down the location of at least one of these epitopes to be in a region close to the globular heads of C1q, and further showed that this epitope is present multiple times on a C1q molecule.

The anti-C1q autoantibodies isolated in **chapter 6** showed competition for binding to C1q with anti-C1q in the serum of SLE patients, indicating that they bind the same or similar epitopes on C1q. Addition of anti-C1q antibodies to C1q-containing immune

complexes did not yield additional complement activation, but did increase binding of Fcγ-receptors. In cellular assays, this translated into the ability of anti-C1q to enhance the phagocytosis of C1q-opsonized particles by human phagocytes, demonstrating the capacity to functionally engage Fc receptors. These data led us to propose a model for the involvement of anti-C1q autoantibodies in lupus nephritis, a disease they are strongly associated with. We know from literature that deposited immune complexes are necessary for anti-C1q to be pathogenic. These immune complexes bind C1q and activate complement. According to our hypothesis, complement activation attracts immune cells through anaphylatoxins, however Fc receptors on these cells are not yet engaged because C1q masks the antibodies in the immune complex. Only when anti-C1q autoantibodies bind to deposited, C1q-containing complexes, do the immune cells have a target for their Fc receptors, leading to cellular activation and damage to the kidney of the SLE patient. In healthy individuals, such as the donors from whom we isolated the anti-C1q antibodies, no immune complexes are deposited in the kidney, therefore they do not develop nephritis even if they have anti-C1q autoantibodies.

Together, the research described in this thesis shows that complement, and mainly C1q, confers pathogenic effects, contributes to physiology, is a therapeutic target and a diagnostic tool, all besides its traditional role in fighting infections. Additionally, studies characterizing autoantibodies against C1q allowed for new understanding of their molecular features and immunological impact. The research presented here therefore urges further investigation into C1q and anti-C1q autoantibodies, and hopefully provides a foundation for future clinical application.