# The role of C1q in (auto) immunity

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# Chapter 1

## Introduction

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# Introduction



### The immune system

The immune system plays an important role in protecting the body against infectious agents. The immune response is delicately regulated and should only be initiated when necessary. This also requires timely termination, since inappropriate immune activation can result in persistent inflammatory disease or autoimmunity [1]. The immune system can be divided into the innate system and the adaptive system. The innate immune system is the first line of defence that provides a quick non-specific response compared to the adaptive immune system. The adaptive immune response is a specific response that is mainly established by two cell types, the B and T cells. The B cells are able to recognize their antigen in their native form. The antigen recognition together with activation signals will activate the B cells and differentiate them in antibody secreting plasma cells [2]. T cells are responsible for producing these activation signals, but are also able to produce inflammatory cytokines and kill infected cells. In contrast with B cells, T cells are only able to recognize processed antigens presented by antigen presenting molecules also known as the human leukocyte antigens (HLA) [3]

### The innate immune system

The innate part of the immune system is known to respond quickly and non-specifically. Behind the mechanical and biological barriers, including the skin and mucus in the lungs and intestine, innate immune cells can be activated in case of infection with a pathogen. The innate immune cells can be triggered via pattern recognition molecules (PAMPS) or via signals of damaged and stressed cells called danger-associated molecular pattern molecules (DAMPS). Characteristics of the innate immune system are activation of the complement system, the recruitment and activation of immune cells by cytokines, recognition and clearance of pathogens in the tissue, and induction of the adaptive immune response via antigen presentation. Innate immune cells are, amongst others, macrophages, dendritic cells, neutrophils, mast cells, basophils and natural killer cells. The complement system is seen as a part of the innate immune system because the activation of the complement system can be quick and non-specific. Macrophages, dendritic cells and mast cells are important cells in the production of complement components and thereby also important in facilitating proper functioning of the complement system [4-7].

**Macrophages**

Macrophages are large mononuclear phagocytic cells that are able to present antigens, but are also very important to engulf and degrade cellular debris and pathogens. Myeloid progenitor cells are derived from the bone marrow that differentiate into monocytes. The monocytes will migrate into tissues were they can maturate towards macrophages. One of the important functions of the macrophage is to engulf and to kill microorganisms. Macrophages were identified in the 70's as major producers of C1q [8]. Next to macrophages, it is now known that immature dendritic cells are also a major source of C1q production [9, 10]. C1q produced by macrophages and dendritic cells play an important role in the clearance of dead cells and cellular debris [11, 12].



**Dendritic cells**

Dendritic cells (DCs) are derived from the same myeloid progenitor cells as macrophages and are the most potent antigen-presenting cells (APCs). Derived from the bone marrow, monocytic cells travel through the circulation into the tissue where they become immature DCs. In the tissue, the immature DCs are able to interact with microbial-associated molecules that can activate the DCs. Subsequently, DCs can migrate towards secondary lymph nodes under influence of cytokines and chemokines. During the migration the DCs will undergo a process of maturation. After entering the lymph nodes, the DCs are capable to present antigens in combination with co-stimulatory molecules resulting in T-cell activation. Immature DCs are an important source of C1q production and after reaching a mature stage, the C1q production is diminished [9, 10].

**Mast cells**

Mast cells (MCs) are best known for their role in anaphylactic allergy responses and to protect the body against parasitic worms. They are predominantly present in mucosal and skin surfaces and thereby close to the host/environment interface [13-15]. As effector cells they can also play an important role in other diseases like atherosclerosis, contact dermatitis, cancer and arthritis [16-19]. One of the markers of MCs is the high affinity IgE receptor (FcεRI). Via this receptor MCs can become activated followed by degranulation. As IgE is already bound to the high-affinity FcεRI, crosslinking is of FcεRI is a quick process leading to a swift activation of MCs. Degranulation can also occur after triggering by the complement components C3a and C5a. Interaction of C3a or C5a with mast cells will activate the mast cells resulting in degranulation where cytokines, chemokines and bioactive lipids will be released[20-23].

## The complement system

The complement system is an essential part of the innate immune system of the human body. It consists of a set of soluble proteins, mainly present in blood and other body fluids, as well as an intricate system of membrane bound complement receptors and regulators. Complement activation can take place via three distinct pathways; the classical pathway, the lectin pathway and the alternative pathway. Each pathway is activated in a different manner. Together, the complement system consists around 30 proteins and via a cascade of enzymatic reactions it results in the production of biologically active fragments.



The three pathways have their own activation and recognition mechanisms that lead to the formation of C3 convertases where the central complement component C3 will be cleaved into anaphylatoxin C3a and opsonin C3b. After formation of the C3 convertases C4b2a or C3bBb, the terminal pathway will be initiated, which starts with the formation of a C5 convertase. This convertase cleaves C5 into C5b and the anaphylatoxin C5a. The fragment C5b will interact with C6 and C7 and form a complex, which is able to bind to cell membrane where it can interact with C8 that will insert into the cell membrane. To form a lytic pore, several C9 fragments are needed and will finally form the terminal complement complex (TCC, C5b9, membrane attack complex (MAC)) (Figure 1).

**Classical pathway**

The initiator molecule C1q, which forms, together with the serine proteases C1r and C1s, the C1 complex, activates the classical pathway. C1q can bind to different ligands like antigen-bound IgG and IgM, apoptotic cells, cellular debris and acute phase proteins. When C1q binds to a ligand it undergoes a conformational change, which allows the serine proteases C1r and C1s to become activated. The serine protease C1s will subsequently cleave C4 into C4a and C4b. The covalent binding of C4b follows this on the target together with C2a, which is a cleavage product of C2 that is cleaved by C1s into C2a and C2b, forming the C4b2a complex. This classical pathway C3-convertase cleaves C3 in to C3a and C3b. C3b can covalent bind to its target and C3b act as a opsonin, C3a functions as an anaphylotoxin. After the formation of the C3 convertase the C5 convertase will be formed, leading to the formation of the terminal pathway [24].

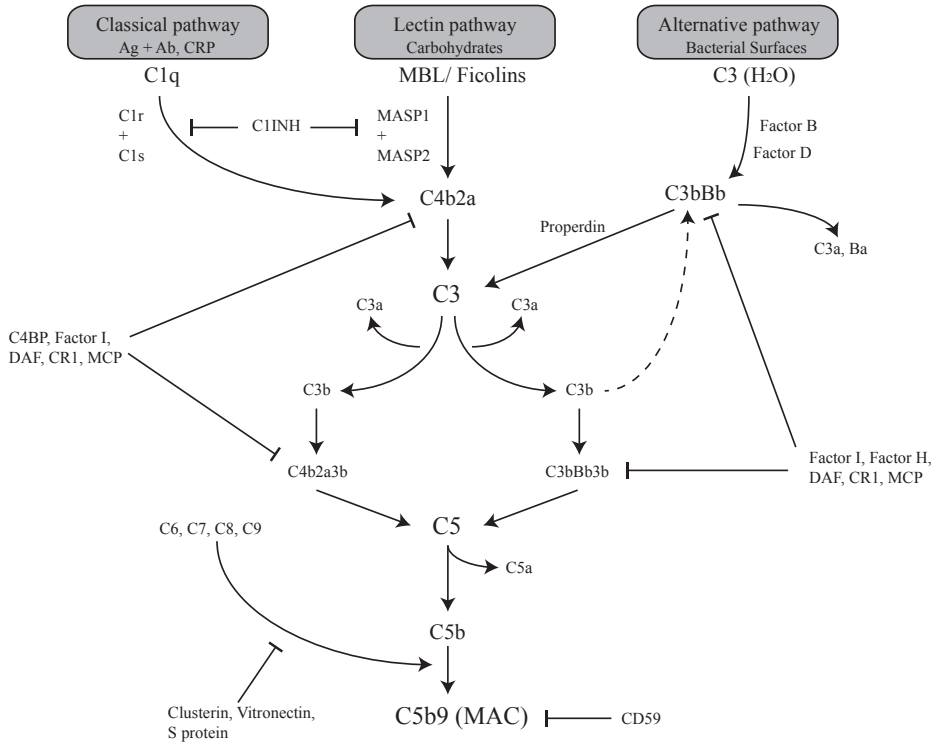


**Figure 1. Activation pathways of the complement system.** The central complement component C3 is activated via three pathways. The classical pathway is activated by binding of immune surveillance molecules on the activating surface different from the lectin pathway that is initiated by carbohydrate residues on the activating surface. Direct binding of C3b to the activating surface triggers the alternative pathway. All the three pathways lead to an enzymatic convertases that cleaves C3 (into C3a and C3b) either C5 (into C5a and C5b). Furthermore, C5b triggers the terminal pathway, which lead to the formation of a multimeric membrane attack complex (C5b-9), resulting in a pore in the target cell membrane.

## Lectin pathway

The lectin pathway acts in a similar way in activation as the classical pathway because the recognition molecule of the lectin pathway, mannose-binding lectin (MBL) is structurally highly related to C1q and interacts also with serine proteases. However, it recognizes other targets. The lectin pathway will be activated by the recognition of certain carbohydrates ligands by mannose binding lectin (MBL) as well as H-ficolin or L-ficolin. When MBL is bound to mannose residues on pathogens, serine proteases (MASPs) will become activated in the same manner as C1r and C1s. The MASPs associated with MBL are MASP-1, MASP-2 and MASP-3. MASP-2 is responsible for activating C4 and C2 will generate the C3 convertase C4b2a, which is similar to the C3 convertase in the classical pathway.

**Alternative pathway**

The alternative pathway is spontaneously activated by hydrolysis of C3 in the circulation. This activation process is also known as “tick-over”. Here, C3 is spontaneously hydrolysed into C3(H20) or C3i. This hydrolysis can be amplified when C3 is interacting with biological and artificial interfaces like gas bubbles, biomaterial surfaces, and lipid surfaces and complexes [25-28]. Upon hydrolysis the C3 conformation changes allowing factor B (FB) to bind to C3(H20). This will be cleaved by the serine proteases Factor D (FD) resulting in the cleavage product C3(H20)Bb. This C3-convertase is able to continuously cleave C3 into C3a and C3b fragments. In the presence of an activating surface, C3b will covalently bind to the surface and initiate a positive feedback loop to the alternative pathway [28].



**Complement regulation**

During activation of the complement system several regulators are involved. In the classical pathway and the lectin pathway the soluble regulator C1 inhibitor (C1-INH) plays a role. The major role of C1INH in the complement system is the inhibition of proteases including as C1s, C1r and MASP2 [29, 30]. Furthermore, C1-INH is also able to interfere with the interaction of C3b with FB via a reversible interaction and inhibit the alternative pathway [31]. Further down the classical pathway, complement regulators like C4b-binding protein (C4BP), Complement Receptor type 1 (CR1) and Decay-Accelerating Factor (DAF, CD55), which all can bind to C4b and to C3b and can inhibit further activation of the classical and the lectin pathway [32-34].

In the alternative pathway the major fluid phase complement regulators are Factor H (FH), and properdin [35]. Factor H acts as a co-factor of Factor I mediating the cleavage of C3 and decay accelerating activities for the alternative pathway C3 convertases [36]. Properdin regulates the alternative pathway by stabilizing the short lived C3 convertase C3bBb [37]. Furthermore, the alternative pathway can be regulated on the cell surface including, membrane co-factor protein (MCP, CD46), Complement receptor 1 (CR1, CD35) and DAF. MCP acts as a co-factor for factor I and thereby mediates the cleavage of C3b and C4b. CR1 is a receptor for C3b and C4b and enhances the phagocytic activity of neutrophil-mediated phagocytosis [38]. DAF can accelerate the decay of C3 convertases as described above, but is also able to accelerating the decay of C5 convertases [33, 39].

The terminal pathway of the complement system will result in the formation of the MAC. During this formation several regulatory proteins can regulate this process. CD59, vitronectin and clusterin are regulatory proteins that inhibit the formation of the MAC [40-42].

**Systemic Lupus Erythematosus (SLE)**

Systemic Lupus Erythematosus (SLE) is an autoimmune disease, which can affect various organs and thereby can be characterized by clinical features including glomerulonephritis, rashes, serositis, haemolytic anaemia, thrombocytopenia and the central nervous system can also be involved. SLE is characterized by B-cell hyper-activation, autoantibody production and immune complex formation [43, 44]. Patients with SLE have a long list of clinical presentations, which can make it difficult for the clinician to diagnose the patient with SLE. The American College of Rheumatology (ACR) established a set of criteria for the diagnosis of SLE as well as for reasons of scientific research [45]. Overall 20-150 in 100.000 people are affected by SLE and it predominantly affects women (80%) [46].



The exact aetiology of SLE is unknown but it involves different factors like the genetic background and environmental factors [47, 48]. Deficiencies in the classical pathway of the complement system is highly associated with the development of SLE [49]. In C1q deficiency around 80% will develop lupus, in C1r/C1s deficiency the lupus incidence is around 68%, in C4 deficiency 75% and in C2 deficiency 10%[50-52].

Antibodies against a wide variety of self-antigens are present in SLE patients. Some of these autoantibodies can be already present in serum years before onset of disease [53]. Many of the antibodies are targeting nuclear antigens and are known as anti-nuclear antibodies (ANA's) [53]. ANA's can be divided into several subtypes including anti-dsDNA antibodies, anti-Ro antibodies, anti-histone antibodies, anti-Sm antibodies and anti-hnRNP antibodies. Other autoantibodies, which can be present in SLE patients, are directed against complement components like C1q (anti-C1q) or anti-C1s antibodies [54, 55].

In the circulation autoantibodies can form immune complexes and deposits in tissues like the skin or the kidney or immune complexes can form locally in tissues. Studies show that SLE patients have higher immunoglobulins in serum compared to healthy controls which correlates with disease activity [56].

Due to the unknown cause and complexity of SLE, patients are mainly treated on an individual basis. Most of the patients receive immunosuppressive drugs like prednisone. In case of deficiency in a complement component like C1q, patients benefit from treatment with Intra-venous Immuno-Globulins (IVIG) [57] and fresh frozen plasma [58]. A more radical treatment is Haematopoietic Stem cell transplantation in which has been successfully in two SLE patients with C1q deficiency [59, 60].

**Neuropsychiatric Systemic Lupus Erythematosus (NPSLE)**

One of the manifestations in SLE is the involvement of the nervous system, which includes a diversity of neurological and psychiatric events. Around 39-50% of SLE patients are presenting NPSLE symptoms [61]. There is a difference in NPSLE manifestation attributed to SLE compared to neurological manifestations as a complication of the disease or due the treatment of the SLE. The American College of Rheumatology (ACR) established in 1999 criteria which the clinicians use for the diagnosis of NPSLE [62]. In these criteria clinicians can segregate the NPSLE in diffuse and focal NPSLE.



Patients with NPSLE are treated in many different ways, due to the different types of disease and inflammation, also the type of symptoms in NPSLE play an important role in the type of treatment [63].

Complement factors are known to contribute to the pathology of inflammatory central nervous system (CNS) and neurodegenerative diseases [64]. In cerebrospinal fluid (CSF) an increase of complement components C3, C4 and C5b9 were found [65, 66]. This may impact on disease progression as in mice that were deficient for alternative complement pathway activity, less lupus cerebritis was seen as compared to controls [67].

Recently, research groups demonstrated that activated microglia are important producers of complement components, which can be of importance in inflammatory conditions in the brain like depression, but also in NPSLE [68].

Furthermore, the breakdown of the blood brain barrier (BBB) is prominent in NPSLE as well as the presence of autoantibodies against brain antigens like glutamate receptors. These antibodies against glutamate receptors are known to play an important role in neurodegenerative diseases [69-71]. Also autoantibodies against complement components could play a role in NPSLE, but the exact mechanism is still unknown.

**The role of complement in arthritis**

There is strong evidence that complement plays an important role in the pathogenesis of rheumatoid arthritis (RA) and osteoarthritis (OA) [72]. One study in 2013 showed that single nucleotide polymorphisms (SNPs) in and around the C1q genes were associated with the susceptibility of RA. These results could be explained because the genetic variants would increase the production of C1q leading to an increased capacity to activate the classical pathway [73], and suggest that more complement activation associates with more joint damage. As seen in several studies, a decreased haemolytic activity and complement components was found in the synovial fluid, indicating local complement activation and consumption [74-76]. In

mouse models of inflammatory arthritis it was found that complement activation is involved in joint damage especially complement component C5a [77].
One of the factors activating the classical pathway is binding of C1q to immune complexes and to microparticles from apoptotic granulocytes, where deposition of C1, C3 and C4 was seen in synovial fluid [78]. The alternative pathway has been suggested to be more involved in the complement activation in the joint for there was an increase Bb fragments found in RA synovial fluid [79] and in rodent arthritis models it is demonstrated that factor B or C5 deficient mice were protected from arthritis whereas C4 deficient mice not [80, 81]. Cartilage fragments are recently implicated as complement regulators. Several studies indicate that several components of the extracellular matrix (ECM) are enhancing and others are inhibiting complement activation [82-84].



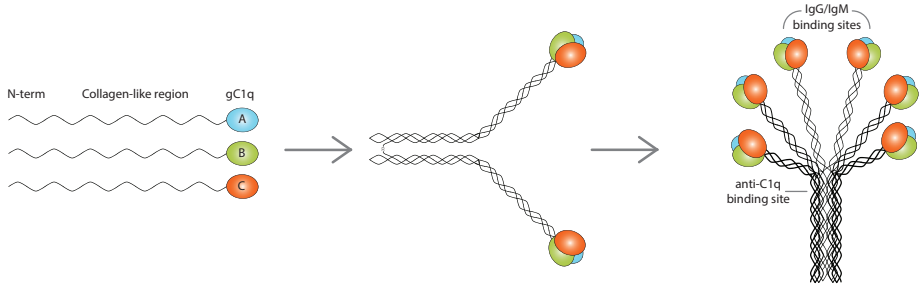


Figure 2. Schematic overview of the complex build-up of the C1q molecule.

## Complement component C1q

### C1q gene and protein

The genes C1QA, C1QB and C1QC are located on the p-arm of chromosome 1 (chromosomal location: 1p34-1p36.3). These genes encode for the constituents of the C1q molecule, the C1q A, B and C peptide chains that each form one helical strand and all contain a short N-terminal region, a collagen-like region and a C-terminal globular region (gC1q domain) (Figure 1). The collagen-like regions assemble into helical conformations. Six of these structural triple helix units form, because of non-covalent interactions via two C-chains, the hexameric C1q molecule that has an overall structure that resembles a bouquet of tulips [85] or an 'Eiffel-tower'-like structure [86]. Structurally the C1q molecule (460 kDa) is composed of 18 polypeptide chains (6xA, 6xB and 6xC).

### C1q production

In contrast to most other complement factors, C1q is not produced by hepatocytes but mainly by macrophages and immature dendritic cells [9, 10]. Following their maturation, dendritic cells completely shut down C1q production [9, 10] suggesting a role for C1q in adaptive immune responses [4, 7]. Indeed, a role for C1q in adaptive immunity can also be concluded from in-vivo studies regarding antigen presentation [87], cellular activation of immune cells [88, 89] and setting a tolerance threshold [90-92].



### C1q binding to immunoglobulins; monomeric versus hexameric

C1 driven complement activation can be triggered via the binding of C1q to the Fc-region of IgM or IgG and activation of C1r-C1s tetramer [93]. For IgM, the most efficient activation is associated with the hexameric form, representing a minor fraction of total IgM (5% of total IgM) [94]. Hexameric IgM (IgMh) differs from pentameric IgM (IgMp) in the absence of J-chain, which is an evolutionary conserved peptide that covalently links pentameric IgM and functions to its transepithelial transport by binding the poly-Ig receptor. The lack of J-chain in IgMh possibly represents a mechanism to prevent polymeric Ig, with high complement activation potential (approximately one-log compared to IgMp, [95], to be transported to the mucosal surfaces where the antigenic load is generally high [96]. The physiological function of human IgMh is still largely unknown. Melis et al. propose that IgMp and IgMh should be seen as two distinct subclasses [97] and not as a side product of evolution.

The C1q binding sites are hidden in IgMp, but are forced outwards by a star-to-staple conformational change upon antigen binding [98-100] and at the same time the complex glycans are turned inside. These conformational changes may decrease the clearance rate of IgMp and initiate complement activation after binding of C1q [101, 102]. The hexameric form, on the other hand, appears to be a planar molecule in which all of the variable regions are available for antigen and Fc-binding [101, 103].

The binding of one of the C1q head domains to an IgG-Fc part is of low affinity [104, 105]. Activation of the classical pathway by IgG requires that at least two IgG molecules bind to C1q (at least for non-nucleated cells). Only in rare cases certain paraproteins have been reported to induce C1 activation while in monomeric form [106]. Attachment of IgG to antigens in close proximity to each other, allows C1q to bind more than one Fc-region, thereby activating complement [98, 107]. Simple proximity binding however appears to be insufficient for maximal complement activation by IgG. Diebolder et al. recently demonstrated that IgG molecules form

ordered hexameric structures after they bind cell surface-expressed antigen. These hexamers form a docking station for C1 binding and activation. Mutations that interrupted hexamer formation led to strongly decreased C1q binding and CDC. All features to potentiate levels of complement activation described above, together with the structural data for C1q, IgG and IgM hexamers therefore fit with the notion that Ig hexamerization is required for potent activation of the classical pathway of complement [86, 108].



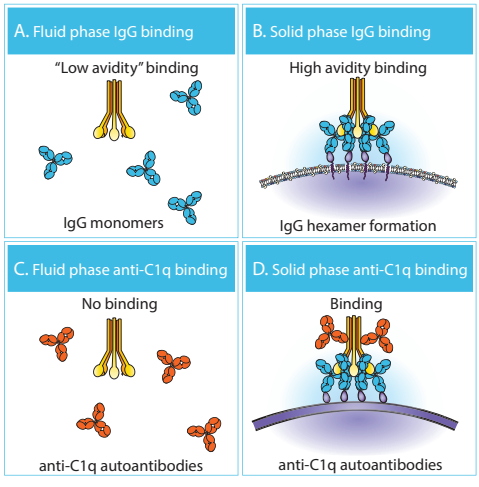


**Figure 3. Schematic overview of the interactions between C1q and immunoglobulins**
**A.** In fluid-phase; C1q avidity too low for binding to monomeric IgG molecules under physiological conditions and therefore no activation. **B.** Antibody binding to antigens allows hexamerisation via Fc-Fc interactions, this generates an optimal platform for C1q binding and activation. **C.** Anti-C1q autoantibodies do not bind to fluid-phase C1q and are therefore non-depleting. **D.** Anti-C1q autoantibodies bind to solid-phase C1q and amplify complement activation [109].

## C1q traditional and non-traditional roles

The traditional view on the role of C1q is restricted to the activation of the classical pathway following its binding to ligand bound IgM or multimeric IgG [110]. The list of ligands for C1q has grown considerably, now also including DNA [111], CRP [112], and matrix molecules such as decorin [83, 113, 114].

In the observation regarding the accumulation of apoptotic cells received a lot of attention. Normally apoptotic cells are hardly detectable in tissues as these are rapidly eliminated by e.g. macrophages. Hence the "waste disposal" theory was introduced suggesting that C1q, by binding to dying and dead cells would stimulate phagocytosis [115]. Indeed dedicated in-vitro experiments revealed that early, and especially late apoptotic cells could bind C1q [116, 117], which resulted in more efficient uptake by phagocytes [118, 119]. The uptake of C1q-opsonized

apoptotic cells by phagocytes not only clears a potential source of autoantigens but also impacts on the immune status of the phagocyte [120]. The complement activation that occurs as a result of C1q binding to dead cells needs to be well controlled to allow for efficient opsonisation, including C3b fragments, without the risk of lysis and release of autoantigens [121, 122]. Next to C1q, apoptotic and necrotic cells were also shown to bind MBL [123] and properdin [124] to enhance complement mediated opsonisation. The process of apoptotic cell clearance is essential in physiology and is characterized by redundancy. Therefore it was postulated that C1q may instead, or additionally, impact on the threshold for auto-reactivity in the adaptive immune response. Indeed both in-vitro studies and studies in C1q-deficient mice indicate that C1q impacts on the adaptive immune response [87, 90-92, 125].



Apart from the effects of C1q as part of the complement activation cascade described above, several reports have implicated C1q in processes that may not per se involve additional complement activation. For example C1q has been reported to play an important role in nerve pruning [126] Wnt/Beta-catenin signalling [127, 128]. Also a role for C1q was reported on neoangiogenesis [129]. C1q could potentially exert such effects in the absence of complement activation by the activation of cellular C1q receptors. Over the years several receptors have been considered to be C1q receptors and several have since then been abandoned. The gC1q-Receptor and the cC1q-Receptor are still considered bonafide C1q receptors [130] and additionally a role for C1q binding to the inhibitory collagen receptor LAIR-1 was reported [131].

**Genetic C1q deficiency**

Genetic deficiency of C1q is strongly associated with development of Systemic Lupus Erythematosus (SLE) [132]. This observation defined the lupus-paradox, where in most SLE patients C1q contributes to tissue damage by inducing complement activation via tissue-deposited immune complexes (ICs), whereas genetic deficiency of C1q is also associated with development of SLE [133]. Clearly C1q is not essential for the clinical presentation of SLE, but how C1q contributes to the protection against the development of SLE remains to be solved. A major breakthrough was achieved when the first C1q knock-out mice were generated [134]. These mice developed a lupus-like disease and importantly the investigators observed increased numbers of apoptotic cells [134]. Later it was shown that the lupus-like disease observed in these first C1q-deficient mice was largely due to the breeding strategy in which a large genetic segment containing autoimmunity-prone genes was co-transferred [135]. Next to a variety of papers describing the effects

of complete genetic deficiency of C1q, several reports that describe associations between SNPs in C1q and clinical phenotypes have appeared. The presence of SNPs in the C1q genes has been associated with differences in response to rituximab treatment for lymphoma treatment [136, 137] as well as with development of SLE [138-143] or development of RA [73].

## Antibodies against the C1 complex

### Anti-C1q autoantibodies



The past four decades anti-C1q autoantibodies have been studied in a wide variety of autoimmune and renal conditions as well as in infectious diseases [144]. In the healthy population the prevalence of anti-C1q autoantibodies ranges between 2% and 8% [106, 145-148] and increases with age [149]. Hypocomplementemic Urticarial Vasculitis Syndrome (HUVS) represents the clinical condition with the highest percentage of anti-C1q positivity; 100% [150]. Other conditions characterized by high anti-C1q antibody prevalence are, mixed connective tissue disease (94%), Felty's syndrome (76%), and SLE (30–60%) [144, 146, 148, 151].
The occurrence of anti-C1q autoantibodies was shown to have familial clustering, indicating that there is a genetic risk factor that together with environmental cues may precipitate the production of these antibodies [152]. Anti-C1q autoantibodies have also been described to occur in infectious diseases although at a frequency of around, for example 13% of HIV infected individuals vs. 5% in healthy controls [153] or up to 26% in patients suffering from hepatitis C virus infection as compared to 10% of healthy controls [154].
Anti-C1q autoantibodies cannot be used as a diagnostic tool however, the presence of anti-C1q autoantibodies can provide prognostic information. This has been particularly instrumental in the renal complication of systemic lupus erythematosus (SLE), lupus nephritis (LN). Anti-C1q autoantibodies were reported to occur in 30–60% of the SLE patients and to be especially present in those patients that among the many clinical presentations also suffered from LN [144, 155].
Several studies provide evidence that anti-C1q autoantibodies are superior to other serological markers in identifying a flare of LN [156, 157]. However, other studies indicate that combinations of anti-C1q antibodies with other serological markers are superior to anti-C1q antibodies alone [158-160]. Especially striking is the strong negative predictive value of anti-C1q testing for LN. In the absence of anti-C1q autoantibodies it is very unlikely that a patient with LN will develop a flare [106, 156, 158, 161-163]. As many of these studies report on rather small patient populations from very diverse ethnic backgrounds ranging from Brazil [163], China [164], India

[165], and Egypt [166] it is likely that considerable variation exists in the strength at which anti-C1q antibodies are associated with and is predictive for LN flares. Several of the larger studies from Europe and Hong Kong point in the same direction [156, 159, 167] and also a recent meta-analysis confirmed the diagnostic value of serum anti-C1q antibodies for LN [168]. Whether or not anti-C1q antibodies are also associated with the disease activity of LN remains to be established, as currently there is no consensus on this issue [147, 159, 169]. Also in post-streptococcal nephritis anti-C1q autoantibodies were reported to associate with a worse outcome [170]. Collectively these data suggest that anti-C1q by itself may not be pathogenic to the kidney, as anti-C1q autoantibodies are also found in the healthy population. This would imply that only following a specific insult to the kidney these anti-C1q autoantibodies could enhance the local pathology, as seen in the enhanced LN in SLE and PSGN. Experimental evidence for a pathogenic contribution of anti-C1q autoantibodies came from murine studies [171-174]. Administration of anti-C1q antibodies to healthy mice results in a modest deposition of C1q and anti-C1q antibodies but this does not induce renal impairment [172]. In sharp contrast anti- C1q autoantibodies strongly aggravate immunecomplex-mediated nephritis [173]. The mode of action was depending on the presence of C1q in the immune complexes in the glomeruli, as anti-C1q autoantibodies were not able to aggravate glomerulonephritis induced by non-C1q fixing immune complexes [173]. Anti-C1q autoantibodies may also play pathogenic roles in other conditions such as autoimmune thyroid disease [148].

One mechanism to clear anti-C1q autoantibodies from the circulation is to use immunoabsorption on C1q-columns [175]. This method depleted next to circulating immune complexes also anti-C1q autoantibodies and was shown to be beneficial in SLE patients [176, 177].

**Assays to detect anti-C1q**

Over time several assays have been developed to detect anti-C1q autoantibodies both in humans and in experimental animal models. The first assays employed a direct coating of intact C1q, which necessitated the use of high salt conditions to discriminate between immune complex binding and anti-C1q autoantibody binding [178]. Already early in the history of anti-C1q autoantibodies it was discovered that the majority of these autoantibodies is directed against the collagen-like part of the C1q molecule [179]. From equilibrium studies and from the observation that anti-C1q antibodies can be found in the presence of freely circulating C1q it was argued that anti-C1q antibodies may interact with epitopes that are not exposed in C1q in fluid phase [180]. Later these arguments were supported by elegant studies

using phage display technology generated Fab fragments that only interacted with solid-phase C1q [181]. Next, assays have been developed that utilized only the C1q collagen-like region, generated by enzymatic digestions as antigen [145, 179]. This eliminated the need to use high ionic strength buffer. A recent paper reports on the use of peptides derived from C1q that have interesting properties to detect a major linear epitope in a high percentage of the patients in the absence of high-ionic strength buffer [182]. In contrast to the assays reported before that anti-C1q antibodies only target the collagen-like region of C1q in 2007 it was discovered that there are also antibodies that specifically target the globular head regions of C1q [183].

To study anti-C1q antibodies in experimental animal models, assays were developed that used coating of purified mouse C1q and high salt conditions similar to the human situation [171, 172]. Next, in order to circumvent the purification of mouse C1q, an assay was developed which employed a coating of C1q binding peptides, that captured C1q from Rag-/- serum, as the antigenic entity for the anti-C1q ELISA [173, 174]. (Table 1)

| Year | Milestone | |
|---|---|---|
| 1971/88 | Identification of C1q as the target of autoantibodies | [179, 185] |
| 1982 | C1q in solid phase exposes neo-epitopes | [180] |
| 1984 | Identification of the collagen-like stalk as the main binding site of anti-C1q antibodies | [186, 187] |
| 1987 | Anti-C1q associates with the occurrence of LN | [188]reviewed a.o. [144] |
| 1991 | Identification of anti-C1q in mice | [122] |
| 1996 | Anti-C1q also present in healthy population and increase with age | [171] |
| 1993 | Anti-C1q also present in healthy population and increase with age | [149] |
| 2004 | Experimental evidence on how anti-C1q can be pathogenic to the kidney in LN but not in healthy individuals | [173] |
| 2007 | Identification of anti-C1q antibodies that target the globular heads | [183] |

**Table 1. History of anti-C1q antibodies**

**Anti-C1s autoantibodies**

Autoantibodies targeting C1s have been described to occur in around 50% of the SLE patients [184]. The binding of these autoantibodies to C1s enhances its activity and has been suggested to be partially responsible for the reduced levels of C4 observed in SLE [184]. As these antibodies have only been described in one study, future studies will have to reveal the clinical implications of the presence of these antibodies.



## Scope of the thesis

The scope of this thesis is to describe several aspects of the role of C1q in immunity and autoimmunity in a clinical- and cellular perspective.

**The clinical association of C1q and anti-C1q in autoimmunity**

C1q deficiencies are relatively rare, but have a strong association in the development of SLE. Around 80% of the C1q deficient patients will develop SLE, but the clinical outcome is variable. In **chapter 2**, we describe a Dutch child with C1q deficiency presenting only recurrent infections without any signs of autoimmunity. This made us curious about other C1q deficient patients and their clinical outcome. In **chapter 3**, we investigated the variability in clinical presentation and outcome of C1q deficient patients worldwide using questionnaires. Some C1q deficient patients with SLE are diagnosed with a genetic mutation resulting in the production of low levels of C1q that has a low molecular weight (LMW-C1q). In **chapter 4**, we describe a C1q deficient patient producing low levels of C1q with a low molecular weight who is diagnosed with SLE and NPSLE. The role of complement activation and NPSLE is not well known. The studies presented in **chapter 5**, describes if there is an association between complement activation and anti-C1q antibodies in patients with NPSLE.

**Local Production of C1q by immune and non-immune cells**

As described by several groups the main producers of C1q are immature dendritic cells and macrophages. Because both cells are from myeloid origin we focused on another cells originating from the same progenitor. In **chapter 6**, we describe the production of C1q in mast cells, indicating that mast cells can contribute to complement activation in tissue where mast cells are predominantly present like the skin or synovial tissue. In **chapter 7**, we discuss the role of C1q produced by chondrocytes, which are non-immune cells and are important in the formation of cartilage.

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