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REVIEW

# Autoantibodies against complement component C1q in systemic lupus erythematosus

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**Abstract**

Systemic lupus erythematosus (SLE) is the archetype of a systemic autoimmune disease, but the multifaceted pathogenic mechanisms leading to inflammation and organ damage are not fully understood. Homozygous deficiency of complement C1q, the first component of the classical pathway of complement, is strongly associated with the development of SLE, thus pointing at a primarily protective role of C1q. However, while most SLE patients do not have hereditary C1q deficiency, there is indirect evidence for the importance of C1q in the inflammatory processes of the disease, including hypocomplementemia as a result of activation via the classical pathway, deposition of C1q in affected tissues and the occurrence of autoantibodies against C1q (anti-C1q). The growing body of knowledge on anti-C1q led to the establishment of a biomarker that is used in the routine clinical care of SLE patients. Exploring the binding characteristics of anti-C1q allows to understand the mechanisms, that lead to the expression of relevant autoantigenic structures and the role of genetic as well as environmental factors. Lastly, the analysis of the pathophysiological consequences of anti-C1q is of importance because C1q, the target of anti-C1q, is a highly functional molecule whose downstream effects are altered by the binding of the autoantibody. This review summarises current study data on anti-C1q and their implications for the understanding of SLE.

**Keywords:** anti-C1q antibodies, C1q, complement, SLE, systemic lupus erythematosus

## INTRODUCTION

Systemic lupus erythematosus (SLE) is the archetype of a systemic autoimmune disease, but the multifaceted pathogenic mechanisms leading to inflammation and organ damage are not fully understood. SLE is considered to be the consequence of intrinsic (genetic) as well as extrinsic (environmental) factors.[1] Variations in different genes, that are mostly involved in the regulation and function of the immune system, can affect the risk of developing SLE, and often multiple genetic factors are thought to play a role. Homozygous deficiency of complement C1q was found to be the most potent genetic disease susceptibility factor for human SLE, thus pointing at

a critical role for complement C1q in the pathogenesis of SLE.[2,3] C1q is the recognition and starter molecule of the classical pathway of the complement system. It is a 460 kDa glycoprotein consisting of 18 polypeptide chains that have an N-terminal collagen-like domain. These chains form six triple helices assembling to a structure that resembles a bouquet of tulips with the stalks being formed by the collagen-like regions while the C-terminal parts form the flower-like globular head regions of the molecule, which primarily mediate the binding of C1q. A comprehensive (but not exclusive) explanation for the role of C1q in SLE was provided by the so-called 'waste disposal hypothesis'.[2,4] This hypothesis assumes that systemic autoimmunity in SLE is driven by the defective clearance of apoptotic cells, that could become antigenic and, as a consequence, induce an autoimmune response. Typical autoantigens (e.g. nuclear antigens and phospholipid-associated antigens), that are targeted in SLE, are found on the surface of apoptotic cells.[5] The non-inflammatory phagocytosis of apoptotic cells is decreased in SLE patients,[6,7] and C1q has been shown to bind to apoptotic cells and to accelerate the clearance of self-antigens generated during apoptosis.[8–11] As suggested by data from Ogden *et al*.,[12] C1q binds to apoptotic cells via the globular heads and directly initiates their uptake by interacting with phagocyte receptors for its collagenous tail region. In addition to this function as a bridging molecule for phagocytes, the phagocytosing capacity of professional phagocytes is further increased by a priming effect of C1q, leading to an indirect enhancement of phagocytosis after exposure of phagocytes to bound C1q.[13,14] Moreover, macrophages from C1q-deficient mice and C1q-deficient patients show a delayed clearance of apoptotic cells *in vitro*.[15] Thus, C1q may be involved in preventing autoimmunity through a role in the disposal of dying and dead cells. Although C1q is not essential for the clearance of apoptotic cells,[16] but has an accelerating effect on phagocytosis, one needs to keep in mind that even small delays in clearance might have detrimental effects over an extended period of time, considering the estimation that approximately one million cells die per second in the course of physiological human tissue turnover.[17] In addition, the way how apoptotic cells are recognised and eliminated by the presence/absence of C1q is important, as C1q was shown to modulate the phenotype of professional phagocytes.[13,14,18]

In the context of the ongoing autoimmune process, inflammation in SLE patients involves many components of the immune system, of which again complement C1q seems to be of importance. While most SLE patients do not have hereditary C1q deficiency, there is indirect evidence for the importance of C1q in the inflammatory processes of the disease:

a. Low levels of the components of the classical pathway of complement, including C1q, are frequently observed in SLE patients and often can be attributed to complement activation, particularly during flares.[19,20] Interestingly, low complement levels were found to be associated with a decreased uptake of dying cells in SLE patients.[21]
b. C1q deposition is a specific histological finding in severe lupus nephritis where it is detected in electron-dense deposits of the renal subendothelial space and/or the glomerular basement membrane.[22,23]
c. In about one third of unselected SLE patients and in more than 90% of patients with proliferative lupus nephritis, autoantibodies targeting C1q (anti-C1q) can be detected, and these anti-C1q strongly correlate with disease activity and hypocomplementemia.[24]

The growing body of knowledge on anti-C1q autoantibodies (anti-C1q) has led to the establishment of a biomarker that is used in the routine clinical care of patients with SLE. Additionally, exploring the binding characteristics of the antibody and its functional consequences also provides insights into the complex pathogenic mechanisms of the disease. This view is based on the observations that (1) the target of anti-C1q is a highly functional molecule,[25] whose functions are likely to be altered by the binding of an autoantibody, (2) C1q itself has been shown to have a strong association with SLE as outlined before and (3) anti-C1q show binding characteristics allowing insights into basic mechanisms of the disease. This review summarises the implications of current study data on anti-C1q for the understanding of SLE.

## ASSOCIATION OF ANTI-C1Q WITH DISEASE

Anti-C1q autoantibodies (anti-C1q) were first suspected by Agnello *et al*. in 1971[26] and eventually clearly described in 1988.[27,28] Anti-C1q were mostly

seen in patients with SLE and the Hypocomplementemic Urticaria Vasculitis Syndrome (HUVS), but they are not very specific for these diseases. Although to a lesser extent, anti-C1q can also be found in patients with different autoimmune and/or renal diseases (e.g. mixed connective tissue disease, rheumatoid vasculitis, cryoglobulinemia, acute poststreptococcal glomerulonephritis, autoimmune thyroid disease and others), in HIV-positive patients and even in healthy individuals.[29–33] In a normal population, positivity for anti-C1q ranged from 4% in middle aged (40–49 years old) up to 18% in the elderly (70–79 years old).[34] Furthermore, positivity for anti-C1q among normal blood donors strongly depends on the composition of the assay (usually, anti-C1q are quantified by ELISA using a high molar salt buffer to avoid binding of immune complexes to plate-bound C1q) and the definition of a positive test result.[35] Consequently, anti-C1q cannot be regarded as a diagnostic marker of SLE, even though the highest titres of anti-C1q were described in SLE patients and patients with the closely related HUVS.[36,37] However, anti-C1q have been found to be a useful marker of disease activity in SLE.[38] In particular, anti-C1q levels and the percentage of SLE patients being positive for anti-C1q strongly dependent on the presence of active lupus nephritis at the time of sampling.[24,35] As a biomarker for the occurrence of proliferative lupus nephritis in patients with SLE, anti-C1q seem to be superior to determining 'classical' parameters such as anti-dsDNA or complement (C3, C4).[39] These observations also showed that anti-C1q, in contrast to other autoantibodies in SLE, tend to disappear in patients with no or low disease activity (i.e. declining to titres being below the lower limit of detection). Most strikingly, in the absence of detectable anti-C1q the development of severe lupus nephritis in the following months is very unlikely with a negative predictive value ranging up to 100%.[24,39–44] This is not only useful information for the clinician but also suggests a pathogenic role of anti-C1q: Anti-C1q seem to be an essential but not sufficient factor for the development of proliferative lupus nephritis.[44]

Determining anti-C1q may also be of clinical help in other diseases, for example acute poststreptococcal glomerulonephritis (APSGN), which shares several characteristics with lupus nephritis, and autoimmune thyroid disease (AITD). In both entities, anti-C1q were found to correlate with disease severity. In children with APSGN anti-C1q were associated with hypocomplementemia, proteinuria, elevated creatinine, occurrence of oliguria, hypertension and delayed resolution of the disease, and in patients with AITD with the thyroid function (hypo- and hyperthyroidism).[30–32] These findings suggest that anti-C1q have pathogenic roles outside of SLE as well, but the role of anti-C1q in those diseases is much less well studied, partially because of the relative rareness and/or the more difficult determination of disease activity, and therefore remains more speculative.

## CHARACTERISTICS OF ANTI-C1Q

Anti-C1q are mostly IgG with a predominance of the IgG1 and IgG2 subclasses.[45–48] In contrast to immune complexes, that bind to the globular heads of C1q, anti-C1q mostly bind to the collagen-like region of the molecule.[49] Their binding is mediated via the antigen-binding fragments (Fab) and of high affinity. Interestingly, anti-C1q do not or only weakly bind to unbound (soluble) C1q or to C1q within the C1 complex which consists of the three subcomponents C1q, C1r and C1s.[27,28] Thus, a possible pathogenic role might be limited to tissues or organs in which C1q is deposited and not associated with the serine proteases C1r and C1s.[50] In contrast to plasma C1q, which mainly assembles with C1r/s to form the C1 complex, free C1q is locally synthesised in tissues, mainly by dendritic cells and macrophages.[51–53] Such a situation would also be given in lupus nephritis by the presence of resident as well as infiltrating macrophages and dendritic cells,[54,55] and C1q might even be produced by resident kidney cells, for example glomerular mesangial and/or epithelial cells.[56] Of note, anti-C1q were found to enhance the C1q production of macrophages *in vitro*.[57] In line with the hypothesis of a local effect, anti-C1q could be isolated from glomerular basement fragments of patients with proliferative lupus nephritis,[58] and the deposition of anti-C1q seems to occur via binding to deposited C1q.[50,59] Furthermore, C1q undergoes changes in conformation upon binding,[60] and Golan *et al*.[61] could show that binding of C1q to immune complexes or other C1q-binding surfaces exposes new antigenic sites. These observations led to the conclusion that anti-C1q bind to one or several cryptic epitopes that are exposed on the collagen-like region of C1q as a result of changes of conformation of the molecule that occurs after binding to immune complexes or other specific surfaces. Exploring

these conformational properties, anti-C1q were found to specifically target C1q bound on early apoptotic cells, whereas anti-C1q do not bind to C1q bound on immunoglobulins or artificial immune complexes.[62] Although C1q bound to immune complexes exposes neoepitopes as well, these neoepitopes were not recognised by patient-derived anti-C1q, suggesting that the conformational changes of C1q are complex and strongly dependent on the nature of the ligand. This observation does not only question the classical view on the role of immune complexes in SLE but also provides a direct link between SLE, apoptosis and C1q, thus supporting the hypothesis that SLE is driven by impaired clearance of apoptotic material.

As shown for most autoantibodies being detected in lupus, classical anti-C1q were not yet found to cross-react with other autoantigens.[43,63] More specifically, anti-C1q do not cross-react with collagen type II or the structurally related collectins (lung surfactant protein A, mannan-binding lectin, bovine conglutinin). In addition, anti-C1q usually do not bind to denatured C1q, suggesting that the antibody only recognises intact C1q molecules or fragments of the collagenous part expressing the same conformation-dependent epitopes of bound C1q.[64] Up to now, knowledge on the precise epitope(s) of anti-C1q is limited. In an early attempt to compare anti-C1q derived from patients with different diseases, no striking differences in binding characteristics between anti-C1q from SLE patients and patients with HUVS could be found.[65] In both diseases, anti-C1q were found to bind to neoepitopes expressed on the collagen-like region of C1q upon binding of C1q to a surface. However, Western blot analyses suggested differences in epitope specificity between the two entities.[64] Thus, assuming that more than one neoepitope is expressed on the collagen-like region upon binding of C1q, it is possible that anti-C1q may differ in their epitope specificity between different diseases. In a more recent attempt of an epitope mapping for anti-C1q as occurring in SLE, Vanhecke *et al*. identified a major linear epitope of C1q targeted by anti-C1q, the so-called 'A08'.[66] This epitope is cryptic and confirms the notion that anti-C1q primarily bind to neoantigens that are only exposed after the binding of C1q to a target structure.[66,67] In addition, the identification of 'A08' as an epitope allowed establishing a peptide-specific anti-C1q ELISA that could be used as a diagnostic tool.[68] The 'A08' epitope is also of interest as it includes the so-called arginine-rich region of the collagen-like region of the C1q A chain that had been shown to mediate the binding of non-immunoglobulin molecules to C1q, such as lipopolysaccharide (LPS), C-reactive protein (CRP), DNA, heparin, fibronectin, urate crystals, human serum amyloid P and von Willebrand factor. The binding of at least some of these molecules has even been shown to activate the classical pathway,[69–73] which could also be the case for the binding of anti-C1q. Last, as a result of the position of the positively charged amino acid sequence, the peptide residues 14–26 of the C1q A chain (that are mostly identical with 'A08') could potentially be presented on HLA to be recognised by appropriate T-cell receptors.[74]

## PATHOGENIC MECHANISMS LEADING TO THE GENERATION OF ANTI-C1Q

In the context of an impaired clearance of apoptotic material, it is plausible that C1q, bound to the surface of apoptotic bodies by its role as a bridging molecule, becomes antigenic in analogy to nuclear components that usually are not exposed to the immune system. Prolonged exposure of new epitopes to the immune system eventually could lead to an autoimmune response against C1q. In this respect, processes leading to the development of anti-C1q would not fundamentally be different from those being believed to drive the generation of antinuclear and antiphospholipid antibodies. However, as a result of the specificity of anti-C1q for apoptotic cell surface-bound C1q, anti-C1q provide specific support for the hypothesis that impaired clearance of apoptotic material indeed is a fundamental problem in patients with SLE. In addition, the analysis of human monoclonal anti-C1q Fabs generated from a bone marrow-derived phage display library of an SLE patient demonstrated that the development of anti-C1q is the consequence of an antigen-driven, affinity-matured immune response.[75] Beyond this characteristic, the analysis of monoclonal anti-C1q did not reveal antibody properties that would suggest the use of unusual germline sequences or abnormal maturation processes. Thus, as a starting point of the disease, it might not be the antibody response that is abnormal in SLE but the processes that allow the exposure of autoantigens such that they can be recognised by antibodies.[76] Understanding these processes will be of crucial importance for the understanding of SLE. It is of

interest to note that anti-C1q were more likely to be elevated in unaffected siblings of patients as well if the patient had elevated levels of that antibody.[77] Furthermore, anti-C1q were also detectable in the healthy parents of the SLE probands, and there was a strong association between the presence of anti-C1q in the (healthy) parents and their healthy, unaffected children. Therefore, anti-C1q formation is at least partly genetically determined.

Apart from the expression of the relevant antigenic sites, additional triggers for the generation of anti-C1q are necessary. In this regard, the striking sequence homology of 'A08' with an antigenic site of the Epstein–Barr virus (EBV) led to the hypothesis that anti-C1q are the consequence of an immune response primarily targeting EBV. The observation was of interest since EBV infection seems to be of crucial importance for the development of SLE. This hypothesis is based on observational data demonstrating a 95–99% seropositivity for EBV in adult SLE patients, which exceeds the rate of seropositivity found in matched healthy controls (about 85 to 95%).[78,79] In addition, SLE patients were shown to have a more diverse antibody response against the EBV-derived antigenic structure Epstein–Barr virus nuclear antigen-1 (EBNA-1) than controls, which includes a more pronounced immune response against the C-terminal regions of EBNA-1.[80] In fact, some C-terminal antigenic sites of EBNA-1 are able to trigger autoantibody production *in vivo* as observed in lupus patients (i.e. antibodies against double-stranded DNA, Ro, the Smith antigen (Sm) and the U1 nuclear ribonucleoproteins (nRNP)).[81–83] In line with these findings, we could demonstrate, that anti-C1q can be induced *in vivo* by the Epstein–Barr virus-derived antigenic site 'EBNA348' (also being part of the C-terminal EBNA-1). In addition, while for example there was no sequence homology between Ro and the antigenic site of EBNA-1, that was described to induce anti-Ro,[81] 'EBNA348' has a short sequence homology with 'A08' of C1q that includes the amino acids being essential for the binding of anti-C1q.[79] However, more study data are required to determine the role of EBV infection in the generation of anti-C1q.

Taken together, besides the abnormal exposure of antigen, additional triggers for the generation of anti-C1q are necessary. One such trigger could be a previous EBV infection with an aberrant antibody response against the virus leading to the development of the autoimmune response in SLE. This hypothesis is outlined in Figure 1b.

## CONSEQUENCES OF THE BINDING OF ANTI-C1Q

With regard to potential secondary pathogenic mechanisms induced by the presence of anti-C1q, the clinical findings mentioned above suggest that the presence of anti-C1q is necessary but not sufficient for the development of severe lupus nephritis. This hypothesis is supported by observations made in more than 1000 individuals of families in which at least one member had SLE showing that anti-C1q were associated with a history of lupus nephritis, but the mere presence of anti-C1q, for example in healthy relatives, was not.[77] Thus, pathogenic effects of anti-C1q appear to be limited to individuals that are susceptible to lupus, and in these individuals, anti-C1q accelerate or exacerbate the disease.

There are no data showing that anti-C1q directly activate the complement cascade in normal blood, but it is at least likely that binding of anti-C1q to C1q leads to secondary amplification of complement activation, for example by increasing the amount of deposited IgG, which enhances complement activation and may eventually result in a self-perpetuating mechanism.[84] This assumption is supported by observations made in an *in vitro* system in which bound anti-C1q from SLE patients were found to secondarily activate the complement system via the classical and lectin pathways.[85] However, the effect of anti-C1q on complement activation is controversial as another *in vitro* study found that affinity-purified anti-C1q, when compared to control IgG, inhibited the deposition of C3c on circulating immune complexes in a dose-dependent manner.[86] Differences in observations can be explained by the different experimental settings that were used and that - among other factors - might lead to different exposure of binding sites of C1q as outlined before.

It is also possible that anti-C1q interfere with the physiological roles of C1q that are not solely dependent on downstream complement activation, for example the uptake of immune complexes and/or apoptotic bodies.[50] As a result of binding characteristics of anti-C1q, this interference is expected to inhibit or to alter the effects of bound C1q. In an *in vivo* model, Trouw *et al*. demonstrated that the injection of a monoclonal anti-C1q antibody that is specific for the collagen-like region of C1q resulted in glomerular deposition of the antibody together

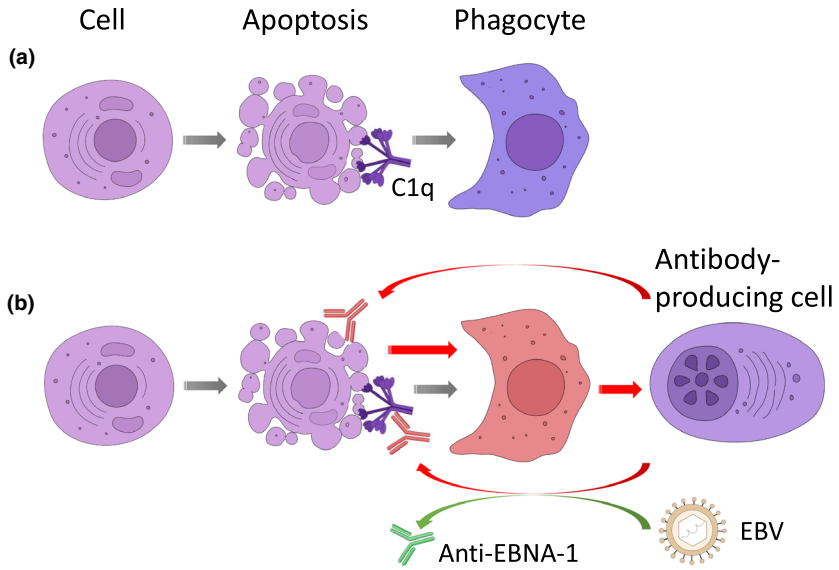


**Figure 1.** Conceptual model of the origin of anti-C1q. **(a)** Physiologically, the clearance of apoptotic cells by professional phagocytes is an anti-inflammatory process partially mediated by bound C1q. **(b)** In the context of an altered clearance of apoptotic cells by phagocytes, those phagocytes could induce an immune response, eventually leading to the production of autoantibodies targeting antigens that are exposed on the surface of apoptotic cells, such as many intracellular antigens as well as surface-bound bridging molecules including C1q. The process of autoantibody generation against C1q seems to be facilitated by the previous infection with the Epstein–Barr virus (EBV) as a result of the presence of preformed antibodies cross-reacting with EBNA-1 of EBV and cryptic antigens being expressed on bound C1q.

with C1q. This deposition was accompanied by mild neutrophil influx but could not induce severe renal damage.[87] However, the situation was different when additional glomerular immune complexes had been induced by a pre-injection of subnephritogenic doses of a C1q-fixing anti-glomerular basement membrane (anti-GBM) antibody. In this setting, the following injection of the anti-C1q antibody could exacerbate the pre-existing subclinical renal disease. Although the model does not precisely reflect the situation in SLE patients for several reasons, the authors could demonstrate that an anti-C1q antibody can lead to disease exacerbation. In addition, concerning the molecular mechanisms, the study could demonstrate that deposition of C1q, complement activation involving C4 as well as C3, and Fcγ receptors mediate inflammation after binding of anti-C1q, pointing to complex downstream effects. These findings were supported by more recent *in vitro* data in which anti-C1q were found to significantly decrease the phagocytosis of early apoptotic cells being opsonised with C1q by macrophages.[86] In addition, Thanei *et al.*[14] could demonstrate that anti-C1q induced a pro-inflammatory phenotype in human monocyte-derived macrophages, by reversing the anti-inflammatory effects of C1q alone. This pro-inflammatory effect was mediated by FcγRII, and macrophages that were exposed to C1q/anti-C1q complexes had a significantly lower phagocytic activity of early and late apoptotic cells that was accompanied by a reduced Mer tyrosine kinase expression. Thus, anti-C1q seem to not only exacerbate complement activation and to alter the function of C1q directly, but even enhance clearance defects of apoptotic material as being observed in SLE patients and, as a consequence, might induce a vicious circle. This concept is summarised in Figure 2.

## OPEN QUESTIONS

Although our understanding of SLE and of anti-C1q has clearly improved over the last decades, a number of unanswered questions remain. A more obvious one is the question of why anti-C1q have a strong association with renal lupus, although many mechanisms outlined above would point to a rather systemic or at least not kidney-specific effect. With regard to the expression of C1q epitopes, C1q deposited in glomeruli might express critical epitopes that are not exposed in other tissues. Expression of neoepitopes on bound C1q does not seem to have a simple yes/no character but to follow a more complex pattern with the appearance of

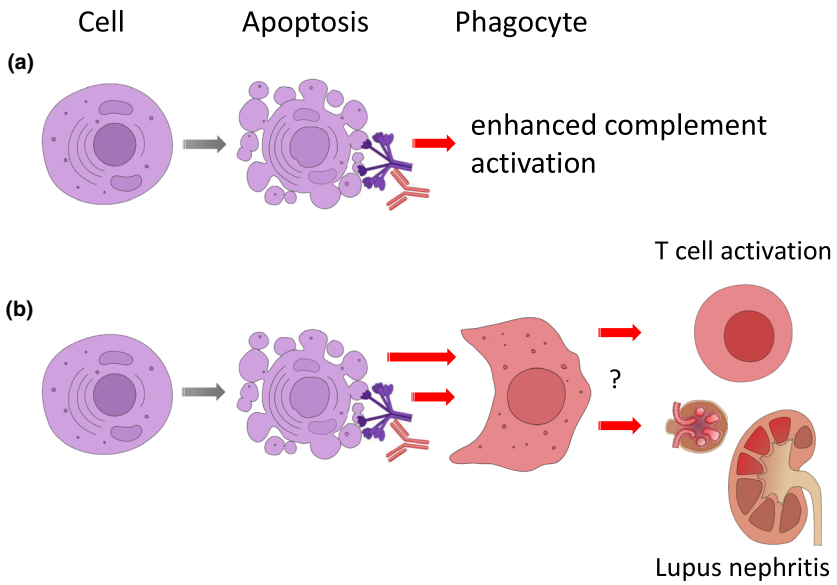


**Figure 2.** **(a)** Secondary effects of anti-C1q. Once being present, anti-C1q can enhance complement activation and **(b)** secondarily enhance the induction of pro-inflammatory phagocytes with reduced phagocytic activity. These inflammatory cells together with complement activation may trigger further downstream inflammation including T-cell activation, and lead to the development of proliferative lupus nephritis.

certain neoepitopes being dependent on the binding partner for C1q.[62] Of note, accumulation of apoptotic cells in glomeruli of SLE patients or secondary deposition of apoptotic material in the glomerulus could provide such a critically important surface as a binding partner for C1q.[88–90] However, of course, additional factors might play an essential role as well.

Among the factors that have been described to be involved in the development of SLE, complement C1q is by far not the only one. Another one, whose important role is supported by increasing evidence, are type I interferons (IFN). The complex role of IFN type I in SLE has been reviewed recently.[91] Regarding C1q, an association between C1q deficiency and defective regulation of IFN-$\alpha$ has been reported,[92,93] and C1q-containing ICs were shown to markedly reduce the expression of the majority of IFN-response genes.[94] However, how the presence of anti-C1q influences the effects of C1q on the interferon signature remains to be examined.

Another question that needs to be studied is the long-term effect of anti-C1q on the morbidity of SLE patients. Thus far, studies on anti-C1q in SLE focused on overall disease activity, particularly at the time of sampling. Little is known about how the presence of anti-C1q might affect the physiological and pathogenic role of C1q in an extended period of time. For example, the chronic presence of anti-C1q might affect the development of atherosclerosis in SLE patients by interfering not only with deposited C1q but also with components of the haemostatic system and macrophages. This topic is of relevance since SLE is associated with considerable cardiovascular morbidity.[95] As demonstrated in a mouse model of atherosclerosis, C1q has protective effects for early atherosclerosis, and human *in vitro* data show C1q deposition on cholesterol crystals that modulate the phenotype of phagocytosing macrophages. The binding of C1q to cholesterol crystals also leads to the exposure of new binding sites that might allow the binding of anti-C1q with potential impact on disease progression.[96,97]

Last, although this review focused on the role of C1q in apoptotic cell clearance, the role of C1q in SLE seems to be complex. Previous work described a direct effect of C1q on T-cell proliferation.[98,99] In a more recent study using a mouse model of autoimmunity, C1q was found to control the response to self-antigens by modifying the mitochondrial metabolism of $CD8^+$ T cells, which can themselves propagate autoimmunity.[100] These data suggest not only a link between C1q and $CD8^+$ T-cell metabolism but provide an alternative (or additional) explanation of how C1q protects against lupus. In addition, the observation might have implications for the role of viral infections in the maintenance of autoimmunity. It will be interesting to study

whether anti-C1q play a role in this concept as well.

## CONCLUSIONS

Anti-C1q are an exciting biomarker of SLE disease activity and for the occurrence of active proliferative lupus nephritis in patients. In addition, the exploration of binding characteristics of anti-C1q and its functional consequences provides important insights into pathogenic mechanisms of the disease involving complement deposition and activation, the clearance mechanisms of apoptotic cell debris, phagocyte function and the role of genetic as well as environmental factors such as EBV infection.

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## CONFLICT OF INTEREST

The author declares no conflict of interest.

## AUTHOR CONTRIBUTION

**Marten Trendelenburg:** Conceptualization; Formal analysis; Supervision; Validation; Visualization; Writing-original draft; Writing-review & editing.

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