Molecular Immunology 116 (2019) 117–130

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Complement activation by IgG containing immune complexes regulates the interaction of C1q with its ligands

C. Garren Hester*, Michael M. Frank

Department of Pediatrics, Duke University Medical Center, Durham, NC 27710, USA

ARTICLE INFO

Keywords:
Complement regulation
C1q-C3c complex
C1q receptors
C1q/C1 transfer
Low C1q
C1q dissociation

ABSTRACT

Classical pathway activation of the compl ement system is initiated by the binding of the globular head domains of glycoprotein C1q to its corresponding ligand leading to both C1 activation and C3 convertase formation. However, the whereabouts and function of C1q after complement activation have only been marginally investigated. This report presents two mechanisms of action that remove bound C1q from a complement activating IgG immune complex in concentrated serum. The first mechanism details that sequential activation of the classical and alternative pathways releases bound C1q from an immune complex and that the dissociated C1q is subsequently found in complex with complement fragment C3c. The second mechanism is the displacement of C1q from an immune complex by the addition of near physiologic concentrations of purified or serum C1q. This activity can also be demonstrated using serum depleted of C3, normal serum chelated in EDTA, or purified C1. Fresh C1q in C3-depleted serum was found to replace dissociated C1q on the immune complex. C1q dissociated from immune complexes by the mechanism of C1q displacement is able to bind B and T lymphoblastoid cells that express receptors and ligands for both the collagen like region and the globular head domains of C1q. C1q dissociated from immune complexes by the mechanism of C3 activation do not bind these cells. This result suggests that C3 bound to C1q during complement activation and dissociation interferes with the ability of released C1q to access C1q receptors and ligands, particularly receptors for the globular head domains. These underlying mechanisms that regulate the interaction of C1q with its ligands reveal a novel function for complement activation during the immune response.

1. Introduction

Complement protein C1q is a large, highly positively charged, multifunctional glycoprotein with a molecular weight of 410 kD and a plasma concentration of approximately 70 μg/ml (Ziccardi and Cooper, 1977). Unlike most complement proteins that are primarily produced in the liver, C1q is synthesized extrahepatically by macrophages, dendritic cells, endothelial cells, fibroblasts, mast cells, microglial cells, trophoblasts, and mesenchymal cells with broad tissue expression in the spleen, lymph nodes and lung (Ghebrehiwet et al., 2012, 2017). C1q is comprised of 18 polypeptide chains, each consisting of an 81 amino acid collagen like region called the CLR or cC1q domain and an 136 amino acid globular head region called the gC1q domain (Kishore et al., 2004; Kouser et al., 2015; Thielens et al., 2017). The polypeptide chains originate from three similar but nonidentical chains (C1qA, C1qB, C1qC) that are products from three different genes on chromosome locus 1p36. These chains will form six triple helical strands in which the N-terminal collagen like region of each strand forms the triple helical stalk, the C-terminal region becomes a cluster of three multivalent globular heads (ghA, ghB, ghC), and inter-chain disulfide and non-covalent bonds stabilize the six stalks together forming a “bouquet of flowers” configuration. Similar collagen like domains are found in the N-terminal regions of the collectins (mannose-binding lectin, surfactant protein A, surfactant protein D), the ficolins, and some C1q/TNF-related proteins (CTRPs), but these proteins are derived from one polypeptide chain and a single gene (Lu and Kishore, 2017). The collagenous area of C1q is capable of binding fibrinogen, C-reactive protein, DNA, heparin, complement receptor 1 (CR1), calreticulin (CT or cC1qR) and various extracellular matrix proteins such as decorin, biglycan, lumican, fibronectin, laminin and chondroitin 4-sulfate proteoglycan (Kishore et al., 2004; Nayak et al., 2011). However, these ligands may have restricted access since the collagen like region also serves as the scaffold for the zymogens C1r and C1s that form the calcium dependent tetrameric $C1r_2C1s_2$ complex with C1q termed C1. This pentameric complex is the complement C1 activation site that can be triggered by the binding of the gC1q head domains to Fc regions of IgG

* Corresponding author.
E-mail address: cgarrenh@gmail.com (C.G. Hester).

https://doi.org/10.1016/j.molimm.2019.10.004
Received 19 March 2019; Received in revised form 1 October 2019; Accepted 3 October 2019
Available online 18 October 2019

or IgM antibodies or one of the numerous self, non-self or altered-self ligands it recognizes (Ghaia et al., 2007; Kishore et al., 2004). The binding versatility of C1q is attributed to the charge pattern recognition ability of its heterotrimeric globular domains, and such globular domain modules are found in many noncomplement proteins existing as either homotrimers (precerebellum, adiponectin, multimerins, EMILINS, saccular collagen, type VIII and type X collagen) or heterotrimers (C1q, chipmunk hibernation protein) that individually exhibit diverse functions despite a common homology (Kishore et al., 2004). The globular domains ghA, ghB and ghC of C1q can interact with the same ligands with varying affinities and different ligands independently (Lu and Kishore, 2017). Ligand binding to the globular head domains of C1q can induce a change in the configuration of the collagen like stalk, and this coupled together with the loss of calcium is thought to cause the activation of the C1 complex and the initiation of the classical pathway of the complement system (Kojouharova et al., 2010).

Most plasma complement C1q circulates as a part of a C1 complex, and a normal plasma concentration of C1 inhibitor, a multifunctional serine protease inhibitor, restrains the C1 complex from auto-activation and dissociation (Ziccardi and Tschopp, 1982; Ziccardi, 1982). Immune complex formation that occurs during an immune response however can overcome the regulatory role of C1 inhibitor and activate C1 bound to the Fc region of antibody (Ziccardi, 1984). During complement activation by IgG containing immune complexes, C1q binds the C_H2 domain of the Fc fragment of IgG, and the C_H3 domain of the Fc fragment serves to stabilize the C1 complex and prevent inactivation by C1 inhibitor (Okada and Utsumi, 1989). C1 is now a potent activator of the complement system generating C3 convertases from both the classical pathway and alternative pathway (Takahashi et al., 1976). Immune complexes consisting of soluble antigens and IgG antibodies are considered opsonized or solubilized after C3 activation and C3 fragment decoration of the antigen and antibody, and this process occurs without the aid of C5 and the terminal lytic complex C5b-C9 (Takata et al., 1984). Such C1q activating immune complexes have the potential to be natural adjuvants that can direct the course of an immune response to a soluble antigen through the binding of immune complexes to receptors for immunoglobulins and complement fragments on lymphocytes and antigen presenting cells (Boackle et al., 1998; Sörman et al, 2014; Thorton et al., 1994).

Complement C3 is typically the primary marker for complement activation as it is the focal point of the three described pathways of complement activation. During the immune response, a cooperative role for C3 activation and C3 fragments during B cell activation and T cell dependent antibody responses has been well documented (Dempsey et al., 1996; Pepys, 1976). C3 activation also leads to the generation of the potent anaphylatoxins C3a and C5a, important mediators and now therapeutic targets of the inflammatory response, reinforcing the importance of C3 (Hawksworth et al., 2017; Verschoor et al., 2016). Currently, there has been an important shift from extracellular to intracellular C3 activation and C3 fragmentation in cell apoptosis, cell danger signaling, T cell homeostasis and T cell effector mechanisms once again demonstrating a central role of C3 in both innate and adaptive functions (Hess et al., 2016; Liszewski et al., 2017). Over the last few decades there have also been numerous studies that have investigated C1q separate from its role as an initiator of complement activation, and there is now a conglomeration of innate and adaptive immune functions attributed to C1q and/or cellular receptors for C1q (Ghebrehiwet et al., 2017; Lu et al., 2008; Nayak et al., 2011; Thielens et al., 2017). Receptors for C1q can be specific for the globular head domains (gC1qR/p32/p33/C1QBP/HAPB1, DC-SIGN, cC1qR or CT, CD33) or the collagen like region (cC1qR or CT, CD93, LAIR-1) and are found ubiquitously expressed extracellularly and intracellularly on nucleated cells. A receptor for C1q also exists on human erythrocytes (CR1 or CD35). Despite the interdependent roles of C1q and C3 during the course of complement activation, many investigators study C1q and C3 independently and inconsequentially. Reviewing an era when the complement system was first dissected into the classical and alternative pathways of activation, a relationship between C1q and C3 had emerged that presented more than the activation of one protein by the activation of the other or whether one protein is deficient or consumed. One aim of this report is to reintroduce this obscure but important research in order to reinvigorate the relationship between C1q and C3 during and after complement activation.

In 1966 Peter Lachmann described a homeostatic feedback mechanism for C3 on the activation of C1. Using antibody sensitized sheep erythrocytes (EA) as a complement activator, he observed that C3 activation by EA with bound C1, C4 and C2 inhibited the ability of bound C1 to activate more C2 that in turn would activate more C3 (Lachmann, 1966). He was able to exclude a role for C1 inhibitor and questioned whether C1 had eluted from the activating immune complex. In 1986 Robert Ziccardi detailed a mechanism in whole serum reminiscent of Lachmann's work and demonstrated that C1 activation and consumption by IgG containing immune complexes is controlled by the sequential activation of C4, C2 and C3 (Ziccarid, 1986). One year later Fishelson and Müller-Eberhard showed that purified C1q is able to agglutinate sheep erythrocytes with bound C3b (EC3b), and this binding is abrogated if EC3b is converted to EC3bi or EC3d (Fishelson and Müller-Eberhard, 1987). They also showed that purified intact C1 was able agglutinate EC3b and that collagen was unable to inhibit this reaction therefore implicating the globular head domains over the collagenous stalk of the C1q molecule as the active region. Additionally, the binding of C1q to EC3b prevented the binding of factor B of the alternative pathway of complement as well as the complement regulatory protein, factor H. A negative feedback relationship between C1q and C3 was evident, but Lachmann's question about the whereabouts of C1q remained elusive and even extraneous through the years.

A similar phenomenon was found in the laboratories of Michael Loos and Ulf Nilsson as well as this laboratory (Kaul and Loos, 1997; Nilsson, 2001; Hester and Frank, 2002). It was observed that the signal for C1q bound to solid-phase antigen-antibody immune complexes or immobilized IgG was lost after incubation in high concentrations of normal serum under physiologic ionic strength buffer conditions. Nilsson demonstrated this phenomenon to be C3 dependent but C5 independent and most effective if both the classical and alternative pathways were functional. Nilsson reasoned that C1q, like IgG in the immune complex or immobilized on a plate, is antigenically quenched during complement activation by the binding of C3, and that labeled antibodies to C1q, like labeled antibodies to IgG, cannot bind its respective antigen. To test this, Nilsson monitored the amount of C1q released from immune complexes during the reaction and found that there were significant amounts of C1q in the supernatants as opposed to IgG. However, C1q was released in both normal and complement inactivated serum, and no direct relationship could be determined between the binding of C3 and the amount of C1q released from the immune complex.

This report seeks to consolidate the findings of the aforementioned complement investigators and presents two mechanisms that remove bound C1q from an immune complex. These mechanisms of action serve to regulate the ability of C1q to remain on an immune complex and perpetuate complement activation. Furthermore, these mechanisms may also determine the complement functions as well as the noncomplement functions of C1q after it is released.

2. Materials & methods

2.1. Complement buffers

The following veronal buffered saline solutions were used: 1) veronal buffered saline containing 1 mM $MgCl_2$ and 0.15 mM $CaCl_2$ (VBS^{++}) 2) 12.5 mM EDTA in veronal buffered saline without calcium and magnesium (EDTA-VBS) 3) low ionic strength veronal buffered saline containing 1 mM $MgCl_2$, 0.15 mM $CaCl_2$ and dextrose ($DVBS^{++}$)

4) low ionic strength 8 mM EGTA in DVBS containing 5 mM $MgCl_2$ (Mg-EGTA-DVBS) and 5) low ionic strength 10 and 20 mM EDTA in veronal buffered saline without calcium and magnesium (EDTA-DVBS). All complement buffers have a pH of approximately 7.35. VBS buffers contain physiologic amounts of NaCl (0.14 M), and DVBS buffers contain subphysiologic amounts of NaCl (0.056 M).

2.2. Human sera

Normal human serum was obtained from healthy volunteers. Complement inactivation of serum was performed at 56 °C for one hour. Cobra venom factor (Complement Technology, Tyler, TX) treated serum was prepared by incubating 50 μg of CVF per 1 ml of serum at 37 °C for 30 min. Complement-depleted sera (C1q-, C2-, C3-, factor B-, factor P- and C5-depleted) were obtained commercially (Complement Technology, Tyler, TX). These reagents were confirmed have normal titers of the major complement proteins that were not depleted and an intact classical or alternative pathway when the depleted complement protein was restored. C4-depleted serum was prepared in this laboratory by passing one milliliter of normal serum over a mini-column of affinity purified IgG anti-C4 bound to cyanogen bromide activated Sepharose CL-4B (Sigma Chemical, St. Louis, MO). The serum was collected and analyzed by C4 hemolytic titration and showed greater than 95% reduction in C4 activity. From a previous study, two serum samples from two different patients with acquired angioedema type II with C1 inhibitor deficiency due to an autoantibody to C1 inhibitor were also utilized. Both patient samples were classical pathway deficient and contained low antigenic levels of C1q. All sera were stored at −70 °C before use.

2.3. Proteins and antibodies

Purified BSA (Sigma Chemical, St. Louis, MO), greater or equal than 98% pure, was prepared in PBS and frozen at −35 °C until use. Purified complement proteins human C1q, C1 and C4 were purchased commercially (Complement Technology, Inc., Tyler, TX) and stored at −70 °C until use. Heat inactivation of C1q was performed at 56 °C for one hour after dilution to a physiologic serum concentration.

IgG anti-BSA antibody was produced in rabbits under the care and supervision of the Division of Laboratory Animal Services at Duke University Medical Center in Durham, NC. Animals were first immunized with BSA emulsified in complete Freund's adjuvant and subsequently boosted with BSA emulsified in incomplete Freund's adjuvant. The resulting antiserum was collected every two weeks and antibody production was monitored by Ouchterlony double immunodiffusion in 1% agarose. High affinity IgG antibodies were purified by caprylic acid (Sigma Chemical, St. Louis, MO) followed by ammonium sulfate precipitation. The IgG anti-BSA was further purified by immunoadsorption on polymerized BSA prepared using glutaraldehyde. The adsorbed antibodies were eluted in 0.1 M Glycine-HCL, 0.5 M NaCL buffer pH 2.8, and the eluate was immediately brought to pH 7.4 with 1 M Tris followed by concentration, filtration and dialysis into PBS. Before each use this material was centrifuged in an airfuge (Beckman Coulter, Indianapolis, IN) for 15 min to remove any aggregates of IgG that may have formed during storage at 4 °C.

The following IgG antibodies to human complement proteins were commercially available: anti-C4c and anti-C3c (Dako Corporation/Agilent Technologies, Santa Clara, CA), anti-C1q and anti-Properdin (The Binding Site, San Diego, CA), anti-C4d and anti-C3d (Quidel Corporation, San Diego, CA), and anti-Rabbit IgG (Sigma Chemical, St. Louis, MO).

2.4. Raji B and Jurkat T lymphoblastoid cells

The Raji B cell line is a germinal center derived cell line from a patient with Burkitt's lymphoma. The Jurkat T cell line is a peripheral blood derived cell line from a patient with T cell leukemia. Both cell lines were grown and maintained in RPMI-1640 with 10% fetal bovine serum and supplemented with sodium pyruvate, HEPES and D-glucose (ThermoFisher, Waltham, MA). Both cell lines are commercially available (ATCC, Manassas, VA). Cells were harvested, centrifuged and suspended in 10 mM EDTA-PBS (Kitamura et al., 1978). Cells were then incubated at 37 °C for 10 min followed by centrifugation and washing in PBS. Cells were counted for concentration and viability using a Bright-Line hemacytometer (Reichert, Buffalo, NY). Cells were centrifuged and suspended in $DVBS^{++}$ or EDTA-DVBS just before use.

2.5. Radioiodination of HuC1q and IgG anti-BSA

Purified human C1q, IgG anti-BSA or IgG anti-Human C1q was radiolabeled with ^{125}Iodine (Perkin Elmer, Waltham, MA) using pre-coated iodination tubes (Pierce Chemical, Dallas, TX), a procedure formerly known as the IODO-GEN method. Briefly, 200 μCi $Na^{125}I$ was activated in washed pre-coated iodination tubes in 0.1 mL 0.1 M sodium phosphate buffer pH 7.0 for 6 min at room temperature. The activated ^{125}I was then added to 200 μg of purified C1q, IgG anti-BSA or IgG anti-Human C1q diluted in 0.1 M sodium phosphate buffer pH 7.0 and incubated at room temperature for 12 min. Free iodine was separated from radiolabeled protein by centrifugation using BioSpin 6 columns (Bio-Rad, Hercules, CA) washed in VBS^{++}. Protein concentrations were determined by the Pierce BCA Protein Assay (ThermoFisher, Waltham, MA). For radiolabeled C1q only, 30% glycerol was added and the protein concentration adjusted accordingly. Radiolabeled C1q was either used immediately after the iodination procedure or stored at −70 °C until use. Radiolabeled IgG anti-BSA and IgG anti-Human C1q were stored at 4 °C until use. Typical radioactivity for these preparations was approximately 1×10^6 cpm/μg. Radioiodination of C1q was the method of choice over biotin or FITC in order to limit the use of secondary reagents and to measure C1q binding activity as quickly as possible.

2.6. Biotinylation of antibodies

IgG antibody was first dialyzed overnight at 4 °C into 0.1 M Na-Borate pH 8.8. 250 μg of N-hydroxysuccinimide biotin ester (Sigma Chemical, St. Louis, MO) was added per 1 mg of antibody for 4 h at room temperature. The reaction was stopped using 1 M NH_4Cl for 15 min at room temperature followed by extensive dialysis into PBS pH 7.4. IgG antibodies to C1q, C4c, C4d, C3c, C3d and Properdin were biotinylated with this method. Biotinylated IgG anti-Rabbit IgG was commercially available (Sigma Chemical, St. Louis, MO).

2.7. Preparation of solid-phase immune complexes with bound C1q

50 μg/ml of BSA in 0.1 M carbonate buffer pH 9.5 was added to Nunc Maxisorp Tubes (ThermoFisher, Waltham, MA) for 1 h at 37 °C followed by washing in PBS-Tween. This was followed by the addition of rabbit IgG anti-BSA antibody at varying concentrations in PBS for 1 h at 37 °C followed by further washing in PBS-Tween. Radiolabeled purified human C1q was then added at varying concentrations in VBS^{++} and incubated for 30 min at 37 °C followed by washing two times in VBS^{++} and one wash in $DVBS^{++}$.

2.8. Dissociation of radiolabeled C1q from immune complexes

20–25% normal serum, complement protein depleted or deficient serum, or patient serum was added to washed solid-phase BSA-IgG anti-BSA immune complexes with bound radiolabeled C1q in $DVBS^{++}$, EDTA-DVBS or Mg-EGTA-DVBS and incubated for 1 h at 37 °C. Maxisorb tubes were washed extensively in respective buffers, and the amount of radioactivity in each well was determined in a Packard Cobra II gamma counter.

2.9. Human C1q capture ELISA

20 μg/ml of IgG anti-Human C1q 0.1 M carbonate buffer pH 9.5 was added to Nunc 96 Immuno Module Maxi Breakapart ELISA plates (ThermoFisher, Waltham, MA) and incubated for 1 h at 37 °C followed by washing in PBS-Tween. Serum supernatants containing radiolabeled C1q released from solid-phase immune complexes were diluted 1:5 in 12.5 mM EDTA-VBS and applied to IgG anti-human C1q coated microwells. The samples were incubated for one hour at 37 °C followed by extensive washing in PBS-Tween. Biotinylated antibodies to C1q, C4c, C4d, C3c, C3d, Properdin or Rabbit IgG were diluted in PBS and added to microwells for one hour at 37 °C followed by washing. Streptavidin peroxidase diluted in PBS was then added for 30 min at 37 °C followed by extensive washing. The OPD substrate (Sigma Chemical, St. Louis, MO) was prepared fresh and added to wells for up to 60 min at room temperature, and the absorbance was measured using a Molecular Devices microplate reader and Softmax software.

2.10. Incubation of dissociated radiolabeled C1q with Raji B or Jurkat T lymphoblastoid cells

Supernatants of solid-phase immune complexes with bound radiolabeled C1q incubated in C1q-depleted or C3-depleted serum or purified C1 or C1q were collected and chilled to 4 °C in glass tubes as well as counted for total radioactivity. Raji B and Jurkat T cells at concentrations up to 5×10^6 cells were added to the supernatants in a total volume of 0.5 ml and incubated for one hour at 4 °C in $DVBS^{++}$ or EDTA-DVBS. The cells were washed twice in its respective buffer and counted for radioactivity. Because purified C1q can potentially bind to glass tubes and cause background problems, especially in low ionic strength buffer, the counted cells were suspended in 0.5 ml $DVBS^{++}$, mixed gently and aspirated without centrifugation to remove the cells (Borsos and Rapp, 1965). The glass tubes were then washed two more times with 2 ml of buffer and counted again for residual C1q radioactivity. This radioactivity was subtracted from the radioactivity recorded containing the cells. The background results were very similar in activity to adding supernatants containing radiolabeled C1q to glass tubes without cells, and the amount of radiolabeled C1q that bound to the glass tubes remained bound throughout the washing procedure (results not shown).

3. Results

3.1. C1q is removed from solid-phase pre-formed immune complexes in C1q-depleted serum

A solid phase immune complex assay consisting of BSA and rabbit IgG anti-BSA antibody was developed to study the binding of purified human C1q to immune complexes and the subsequent loss of the C1q signal as a result of the complement activation after the addition of concentrated serum. First, both the antibody to BSA and radiolabeled C1q were titrated to achieve suboptimal doses of C1q binding to the preformed immune complexes. The amount of radiolabeled C1q binding in Fig. 1A first indicates that background binding to BSA and the reaction tube is increased at C1q concentrations more than 2.5 μg/ml. Second, suboptimal binding of all concentrations of radiolabeled C1q tested occurred between 20 and 40 μg/ml of IgG anti-BSA. The experiments in this report therefore utilized 2.0–2.5 μg/mL of radiolabeled C1q to limit the amount of background C1q binding to BSA and 20–25 μg/mL of rabbit IgG anti-BSA as the antibody concentration.

The next experiment examines the effect of the restoration of complement system proteins on C1q binding and stability and utilized concentrated C1q-depleted serum. Low ionic strength buffer $DVBS^{++}$ was employed to aid with the reassembly of C1r and C1s with radiolabeled C1q bound to the immune complex (Golan et al., 1981). Heat inactivated C1q-depleted serum was used as a control in addition to $DVBS^{++}$ buffer alone. Fig. 1B shows that radiolabeled C1q binding progressively diminishes over time after exposure to C1q-depleted serum and that the amount of C1q that dissociates from the immune complex approaches 80% after one hour of incubation. C1q does not dissociate from immune complexes incubated in heat inactivated C1q-depleted serum or $DVBS^{++}$ buffer alone.

3.2. Radiolabeled C1q released from immune complexes in C1q-depleted serum is captured by IgG anti-C1q and in complex with complement C3c

The supernatants from immune complexes treated with C1q followed by C1q-depleted serum or heat inactivated C1q-depleted serum were removed, diluted in EDTA-VBS and added to microwell plates coated with IgG anti-C1q. Biotinylated antibodies to Rabbit IgG, C4c, C4d, C3c, C3d or Properdin were used to detect complement protein binding to captured C1q. Fig. 2A shows that the C1q released from immune complexes is bound almost exclusively to C3c. Minor amounts of C4c, C4d and C3d binding were detected but the C3c signal had an absorbance value approximately seven times higher. There is no signal using an antibody to rabbit IgG indicating that rabbit IgG anti-BSA does not dissociate with C1q from solid-phase BSA. After the development of the assay, the microwells were also counted for radioactivity. Only the wells that contained C1q-depleted serum recorded radioactivity demonstrating that the radiolabeled C1q was transferred from the solid-phase immune complex assay to the C1q-C3c capture ELISA (results not shown).

An additional experiment was performed to ensure that rabbit IgG anti-BSA does not dissociate from solid-phase BSA. Nonlabeled rabbit IgG anti-BSA was replaced with radiolabeled rabbit IgG anti-BSA during immune complex preparation, and these immune complexes were then exposed to C1q-depleted and heat inactivated C1q-depleted serum, normal and heat inactivated normal serum, and C3-depleted and heat inactivated C3-depleted serum. Fig. 2B demonstrates that complement dependent dissociation of rabbit IgG anti-BSA from solid-phase BSA did not occur. The supernatants were tested in the C1q capture ELISA, and strong positive signals were found associated with complement C3c and not C4d (Fig. 2C).

3.3. Radiolabeled C1q is released from immune complexes in C3-depleted serum and is replaced by fresh serum C1q

If C1q is bound to C3c when it is dissociates from immune complexes in C1q-depleted serum, it was hypothesized that radiolabeled C1q would remain bound to immune complexes in the absence of C3. Hence, C3-depleted serum was examined for the ability to dissociate C1q from immune complexes. Because C3-depleted serum contains physiologic amounts of C1q that may bind to available antibody sites present on immune complexes with suboptimal amounts of bound radiolabeled C1q, two sets of identical samples were utilized to monitor both radiolabeled and fresh serum C1q binding. The first set specifically monitored the amount of radiolabeled C1q binding to immune complexes while the second set utilized a radiolabeled sheep IgG anti-C1q antibody to detect fresh serum C1q binding and measured the radioactivity of both the purified C1q and the IgG anti-C1q. The first set of samples was then subtracted from the second set to determine if fresh serum C1q bound to the immune complexes. Fig. 3 shows that C3-depleted serum, like C1q-depleted serum, is able to equally and effectively dissociate radiolabeled C1q from immune complexes. Furthermore, radiolabeled IgG anti-C1q antibody shows that C3-depleted serum deposited fresh C1q on immune complexes unlike C1q-depleted serum. Heat inactivated C3-depleted serum did not have either activity and is similar to heat inactivated C1q-depleted serum and the buffer control. This result reveals that C1q can be released from immune complexes by a C3 dependent and a C3 independent mechanism.

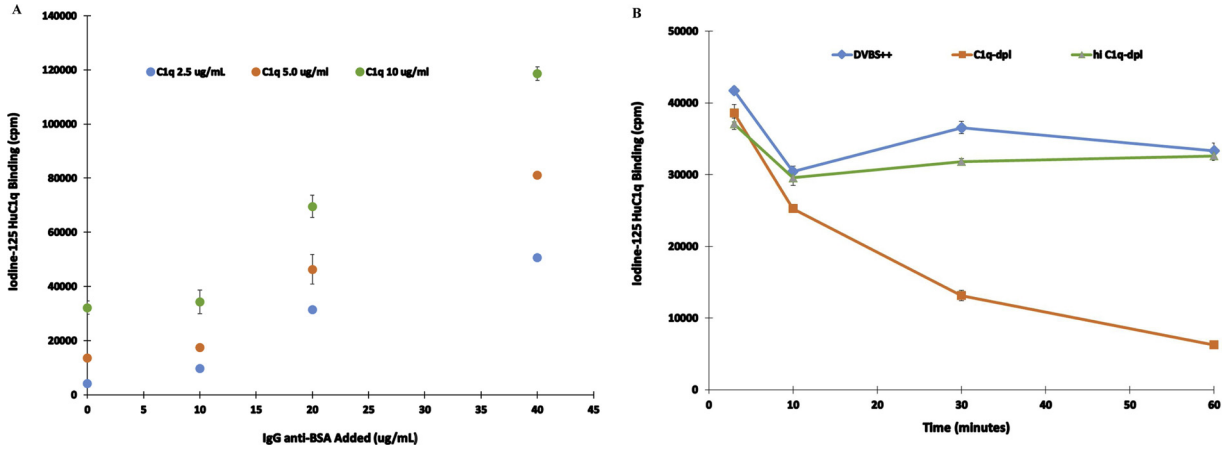

Fig. 1. A. Determination of suboptimal C1q binding to solid-phase IgG immune complexes. 0, 10, 20 and 40 μg/ml of affinity purified rabbit IgG anti-BSA were added to solid-phase BSA and incubated at 37 °C for 60 min. After washing, **2.5**, **5** and **10 μg/ml** of radiolabeled **C1q** in VBS^{++} were added and incubated at 37 °C for 30 min. The amount of radiolabeled C1q bound was measured for background binding to BSA alone and suboptimal binding to solid-phase immune complexes. Duplicate samples were performed, and the mean (SD) was determined for each sample. **B.** The restoration of serum components to immune complexes with bound C1q. C1q-depleted serum diluted in $DVBS^{++}$(**Clq-dpl**), heat-inactivated C1q-depleted serum diluted in $DVBS^{++}$(**hi Clq-dpl**), or $DVBS^{++}$ alone (**DVBS++**) were added to immune complexes with bound radiolabeled C1q and incubated at 37 °C for 3, 10, 30 and 60 min. After extensive washing, the amount of radiolabeled C1q bound to the preformed immune complexes was determined. Duplicate samples were performed, and the mean (SD) was determined for each sample.

3.4. EDTA, EGTA inhibit C1q release activity in C1q-depleted serum but not in C3-depleted serum

To explore the possible role of complement system proteins in the release of C1q from immune complexes beyond the effect of heat inactivation of serum, the chelating agents EDTA or EGTA were added to low ionic strength DVBS buffer to inhibit either both the classical and alternative pathways or the classical pathway only, respectively. Fig. 4 shows that the ability of C1q-depleted serum to dissociate C1q was completely inhibited by both EDTA-DVBS and Mg-EGTA-DVBS buffers. C3-depleted serum however maintained the ability to release radiolabeled C1q from the immune complex. These results indicate that the C3 dependent mechanism of C1q dissociation requires calcium and the C3 independent mechanism of C1q dissociation does not.

3.5. Purified Human C1 and Human C1q displace radiolabeled C1q from immune complexes in DVBS++ and EDTA-DVBS similar to C3-depleted serum

Because C3-depleted serum is able to dissociate C1q from immune complexes in the presence of EDTA or EGTA, purified human C1 or purified human C1q was added to immune complexes with bound C1q instead of C1q containing C3-depleted serum to test the possibility that either C1 or C1q itself can dissociate C1q. Purified C1 or purified C1q was added to radiolabeled C1q bound to immune complexes in $DVBS^{++}$ or EDTA-DVBS. Fig. 5 shows that 50 μg/mL of either purified C1 or C1q alone can dissociate radiolabeled C1q from immune complexes in $DVBS^{++}$ or EDTA-DVBS. It is noteworthy that the presence of C1r and C1s in the C1 complex did not interfere with the ability of C1q to displace radiolabeled C1q from the complex. This suggests that since C1q binds to the Fc fragment of IgG antibody within the immune complex via its globular heads, the displacement of C1q by another C1q also occurs via the same region. Heat inactivation destroys the ability of purified C1q to dissociate radiolabeled C1q from immune complexes similar to C3-depleted serum (results not shown).

3.6. Serum dilution significantly reduces the activity of both C1q-depleted and C3-depleted serum to release radiolabeled C1q from immune complexes

Different concentrations of serum were tested to determine if either C1q-depleted or C3-depleted serum would lose its ability to release C1q from immune complexes upon dilution. Fig. 6 demonstrates that the ability of C3 and C1q dependent mechanisms to dissociate radiolabeled C1q from immune complexes is sensitive to serum dilution. As the concentration of serum decreases, C3-depleted serum remains slightly more effective at releasing radiolabeled C1q than C1q-depleted serum, but serum concentrations less than 25% in either serum show significant decreases in this ability. Purified C1q and purified C1 show similar decreases in activity as the concentration of C1q or C1 fall below physiologic concentrations (results not shown). The amount of radiolabeled C1q that typically binds immune complexes in a microtube was calculated to be approximately 35–70 ng per well. In 25% of serum, there exists approximately 3.5 μg of C1q if the normal serum concentration of C1q is 70 μg/ml. Therefore, 3.5 μg of additional purified C1q, or 50–100 times the amount of C1q that is bound to the immune complexes, is required to dissociate radiolabeled C1q efficiently. This experiment suggests that low levels of serum C1q/C1 may influence the amount of C1q bound and released from an immune complex.

3.7. Radiolabeled C1q incubated and dissociated in C3-depleted serum, purified C1 or purified C1q binds Raji and Jurkat lymphoblastoid cells

Radiolabeled C1q bound to immune complexes was released by either incubation in C1q-depleted serum or incubation in C3-depleted serum. The supernatants containing the dissociated radiolabeled C1q were then incubated with Raji B Lymphoblastoid or Jurkat T Lymphoblastoid cells in order to determine if dissociated C1q will bind cell lines known to express C1q receptors. Fig. 7A and B show that radiolabeled C1q dissociated from immune complexes in C3-depleted serum binds Raji and Jurkat cells. 5×10^6 Raji cells bound more than 20 of the 70 ng (32%) of dissociated radiolabeled C1q added, and 5×10^6 Jurkat cells bound more than 8 of the 50 ng (18%) of dissociated radiolabeled C1q added. 5.9% and 1.8% of released radiolabeled C1q from immune complexes incubated in C1q-depleted serum bound to Raji or Jurkat cells, respectively. These results indicate that in the presence of excess C1q and in the absence of C3, C1q can dissociate from an IgG immune complex and transfer to receptors and ligands for C1q on B and T cell lymphoblastoid cells. These results also suggest that the C3c bound to the C1q during C1q dissociation does not allow for this transfer leaving C1q-C3c complexes in the fluid-phase.

In Fig. 7C and D, the supernatants from radiolabeled C1q dissociated from immune complexes incubated with purified C1 or

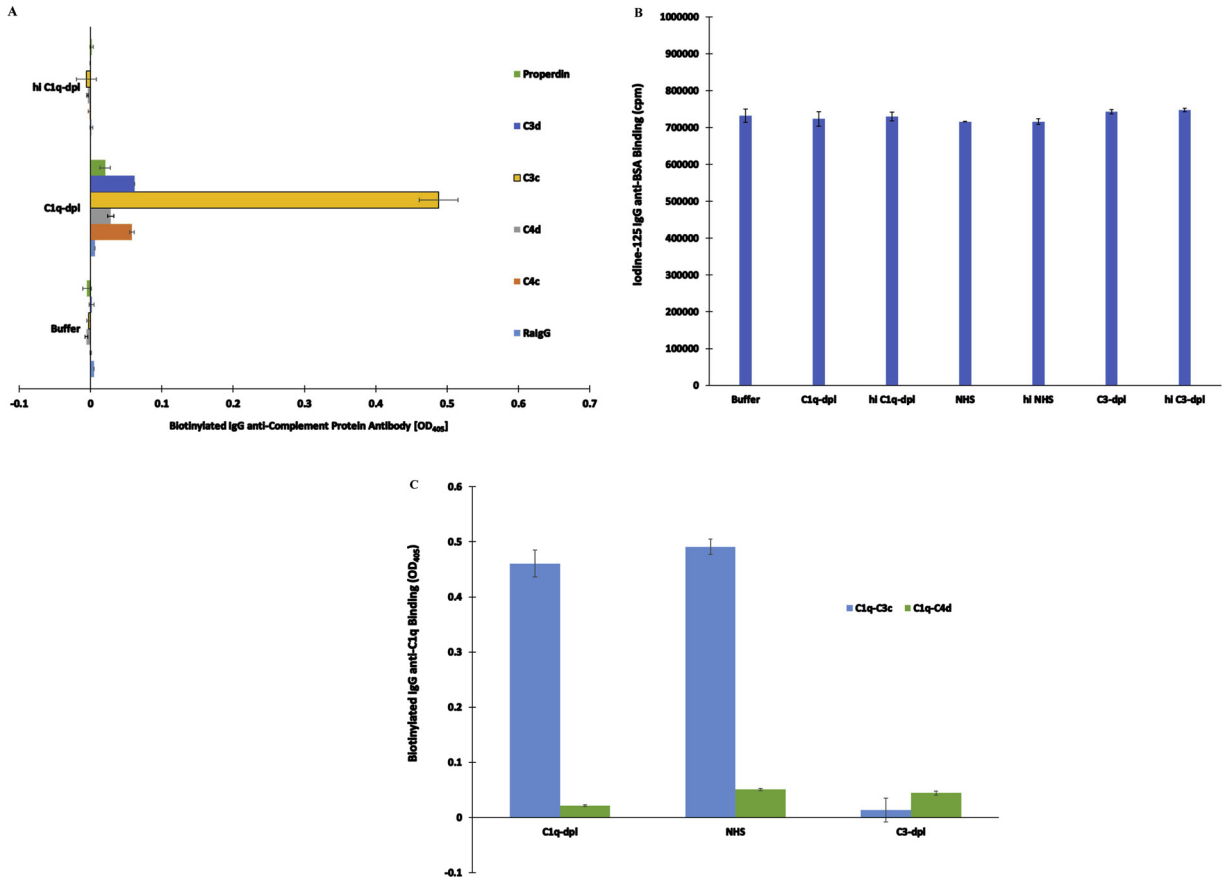

Fig. 2. A. C1q dissociated from immune complexes is captured in a C1q capture ELISA with bound C3c. Supernatants from reactions in Fig. 1B (**Buffer**, **C1q-dpl**, **hi C1q-dpl**) were added to microwells pre-coated with IgG anti-Human C1q. Biotinylated IgG antibodies were used to detect **rabbit IgG** and human complement proteins and fragments **C4c**, **C4d**, **C3c**, **C3d** and **Properdin** bound to C1q. Microwells were developed with an OPD substrate and measured for optical density at a wavelength of 405 nm. Duplicate samples were performed, and the mean (SD) was determined for each sample. **B.** Rabbit IgG anti-BSA does not dissociate from solid-phase BSA during complement activation. Iodine-125 rabbit IgG anti-BSA was added to solid-phase BSA followed by the addition of nonlabeled C1q to generate immune complexes with bound C1q. C1q-depleted (**C1q-dpl**) and heat inactivated C1q-depleted serum (**hi C1q-dpl**), normal (**NHS**) and heat inactivated normal serum (**hi NHS**), and C3-depleted (**C3-dpl**) and heat inactivated C3-depleted serum (**hi C3-dpl**), were then added and incubated at 37 °C for 60 min. A Buffer control (**Buffer**) was utilized as a positive control for radiolabeled IgG anti-BSA binding. Extensive washing was performed between each step. The amount of radiolabeled IgG anti-BSA that remained bound to BSA was measured. Duplicate samples were performed, and the mean (SD) was determined for each sample. **C.** C1q-C3c complexes are generated in C1q-depleted serum and normal human serum during complement activation by immune complexes. Supernatants from reactions in Fig. 2B (**C1q-dpl**, **NHS**, **C3-dpl**) were added to microwells pre-coated with IgG anti-Human C1q. Biotinylated IgG antibodies were used to detect human complement fragments **C4d** and **C3c** bound to C1q (**C1q-C3c** and **C1q-C4d**). Microwells were developed with an OPD substrate and measured for optical density at a wavelength of 405 nm. Duplicate samples were performed, and the mean (SD) was determined for each sample.

purified C1q were applied to both Raji and Jurkat cells and compared to radiolabeled C1q dissociated in C3-depleted or C1q-depleted serum. As in the previous assay, radiolabeled C1q dissociated in C1q-depleted serum showed minimal binding to either cell line. Radiolabeled C1q dissociated with purified C1q exhibited the most binding to either cell line with binding more than 10 fold the binding that occurs in C1q-depleted serum. Although significantly lower than using purified C1q, substantial amounts of radiolabeled C1q bind both Raji and Jurkat cells when radiolabeled C1q is dissociated using purified C1. Furthermore, the binding of radiolabeled C1q dissociated in C3-depleted serum gave almost identical results to radiolabeled C1q dissociated using purified C1. Both purified C1 and C3-depleted contain and bring potential C1r and C1s to the reaction, and it may be possible that C1r and C1s play a role for this decrease.

To explore the effect of calcium in these experiments, radiolabeled C1q was dissociated from immune complexes but added to Raji B lymphoblastoid cells in the presence of EDTA. In contrast to Fig. 7C, E shows that the presence of EDTA reverses the inhibition seen in $DVBS^{++}$ and allows radiolabeled C1q dissociated in C1q-depleted serum to bind Raji cells. The amount of radioactivity is 7 to 8 fold greater in EDTA-DVBS than $DVBS^{++}$. Radiolabeled C1q dissociated in C3-depleleted or in purified C1 continues to bind Raji cells in EDTA-DVBS, but the binding decreases approximately 30–40%. The binding of radiolabeled C1q dissociated in purified C1q decreases approximately 60% in EDTA-DVBS. This experiment highlights that in the presence of EDTA and irrespective of the mechanism of dissociation, radiolabeled C1q binds equivalently to Raji lymphoblastoid cells.

3.8. Complement-depleted serum missing an early classical pathway (C4, C2) or alternative pathway (factor B, Properdin) protein, or normal serum treated with cobra venom factor (CVF), partially dissociate radiolabeled C1q from preformed immune complexes

Radiolabeled C1q dissociates from immune complexes almost completely if incubated in C1q-depleted or C3-depleted serum. Other complement protein depleted sera as well as CVF-treated normal serum were utilized to determine whether or not radiolabeled C1q can be released from immune complexes using these reagents. Fig. 8A and B indicate that the absence of classical pathway or alternative pathway proteins can have a significant effect on C1q dissociation. More radiolabeled C1q remains on immune complexes that were incubated in C4-depleted serum, C2-depleted serum, factor B depleted serum,

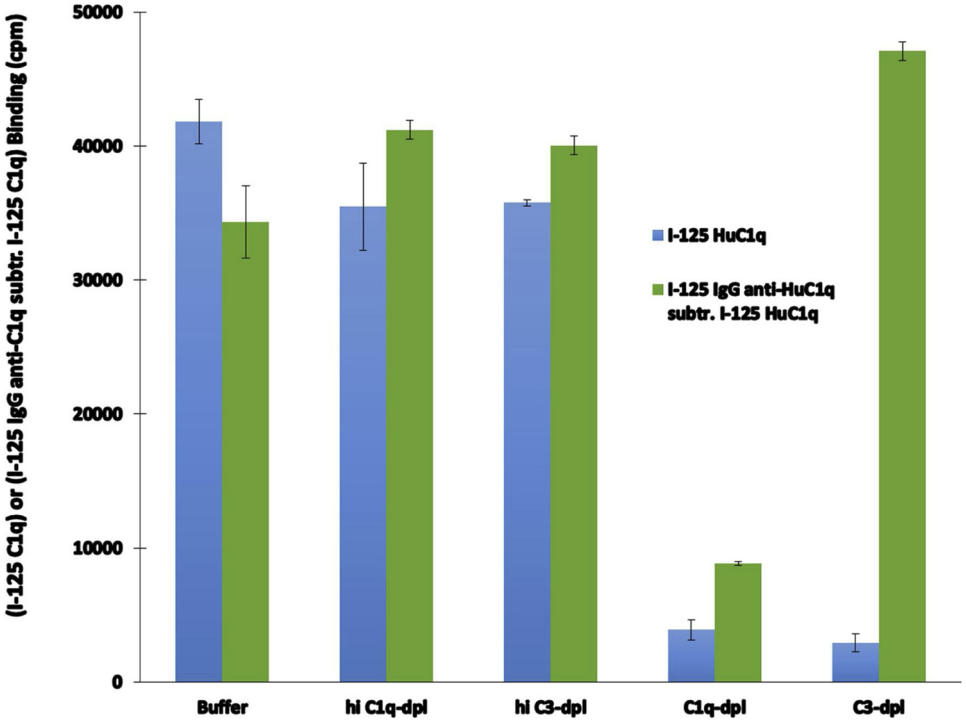

Fig. 3. C3-depleted serum displaces C1q from immune complexes with more C1q. Immune complexes with bound radiolabeled C1q were treated with C1q-depleted (**C1q-dpl**) and heat-inactivated C1q-depleted serum (**hi C1q-dpl**) or C3-depleted (**C3-dpl**) and heat-inactivated C3-depleted serum (**hi C3-dpl**). The amount of radiolabeled HuC1q (**I-125 HuC1q**) that remains bound to immune complexes was measured. Radiolabeled IgG anti-HuC1q (**I-125 IgG anti-HuC1q subtr. I-125 HuC1q**) was also utilized to determine if additional C1q binds to immune complexes. A buffer control with no serum (**Buffer**) was used as a positive control for both radiolabeled C1q and radiolabeled IgG anti-human C1q. Duplicate samples were performed, and the mean (SD) was determined for each sample.

properdin-depleted serum and CVF-treated normal serum compared to C1q-depleted and C3-depleted serum. It was initially expected that CVF-treated normal serum may completely dissociate radiolabeled C1q from immune complexes like C3-depleted serum. However, the results suggest that C3 inactivated serum and C3 depleted serum may behave differently due to the presence of C3 fragments generated during CVF activation that could potentially bind C1q and interfere with the C1q mechanism of dissociation. Interestingly, there are small but increased levels of C1q-C3c complexes that appear in normal serum after activation with CVF (results not shown). The ability of C4-depleted serum to dissociate radiolabeled C1q from immune complexes was restored with purified C4, but this repletion was not effective if C4-depleted serum was treated with CVF prior. These results suggest that disruptions in either the classical or alternative pathway allow more immune complexes to contain C1q during the complement opsonization process. Normal human serum and C5-depleted serum both released radiolabeled C1q from immune complexes similar to C1q-depleted and C3-depleted serum.

3.9. Serum samples from two patients with acquired angioedema with C1 inhibitor deficiency cannot dissociate C1q by mechanisms of C3 or C1q

Patients diagnosed with acquired angioedema type II with C1 inhibitor deficiency have excessive complement activation due to an autoantibody against C1 inhibitor, the serine protease inhibitor that regulates the activity of intact C1. These patients have extremely low amounts of C1 inhibitor, and as a result, they also have low levels of hemolytic C4 and C2 as well as low antigenic levels of C1q (Brasher

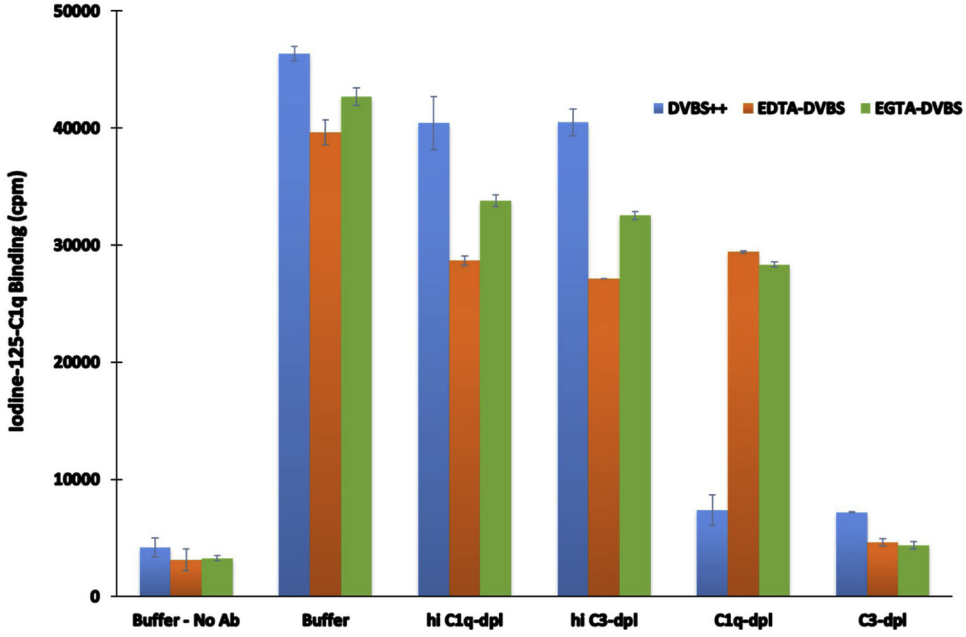

Fig. 4. C3-depleted serum displaces C1q from immune complexes in the presence of calcium chelators. Immune complexes with bound radiolabeled C1q were treated with C1q-depleted (**C1q-dpl**) and heat-inactivated C1q-depleted serum (**hi C1q-dpl**) or C3-depleted (**C3-dpl**) or heat-inactivated C3-depleted serum (**hi C3-dpl**) in either **DVBS++**, **EDTA-DVBS** or **EGTA-DVBS**. The amount of radiolabeled C1q that remains bound to immune complexes was measured. A buffer control with no serum (**Buffer**) was used as a positive control for radiolabeled C1q binding. A negative control (**Buffer - No Ab**) was used that contains no IgG anti-BSA. Duplicate samples were performed, and the mean (SD) was determined for each sample.

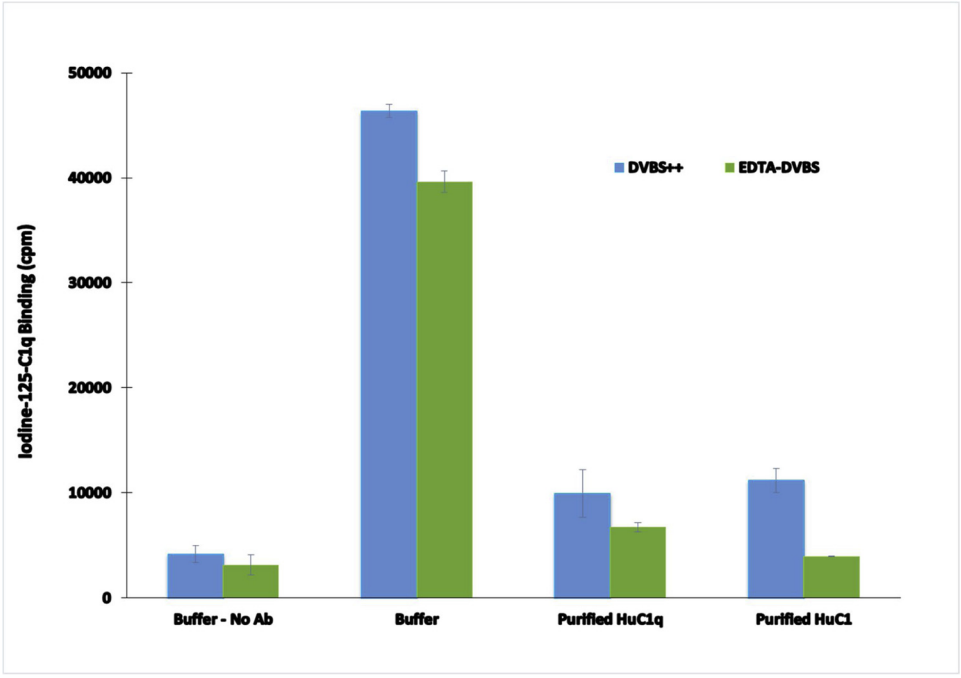

Fig. 5. Purified human C1 and purified human C1q displace C1q from immune complexes in the presence of calcium chelators. Immune complexes with bound radiolabeled C1q were treated with human C1 (**Purified HuC1**) and human C1q (**Purified HuC1q**) in **DVBS++** or **EDTA-DVBS**. The amount of radiolabeled C1q that remains bound to immune complexes was measured. A buffer control with no serum (**Buffer**) was used as a positive control for radiolabeled C1q binding. A negative control (**Buffer - No Ab**) was used that contains no IgG anti-BSA. Duplicate samples were performed, and the mean (SD) was determined for each sample.

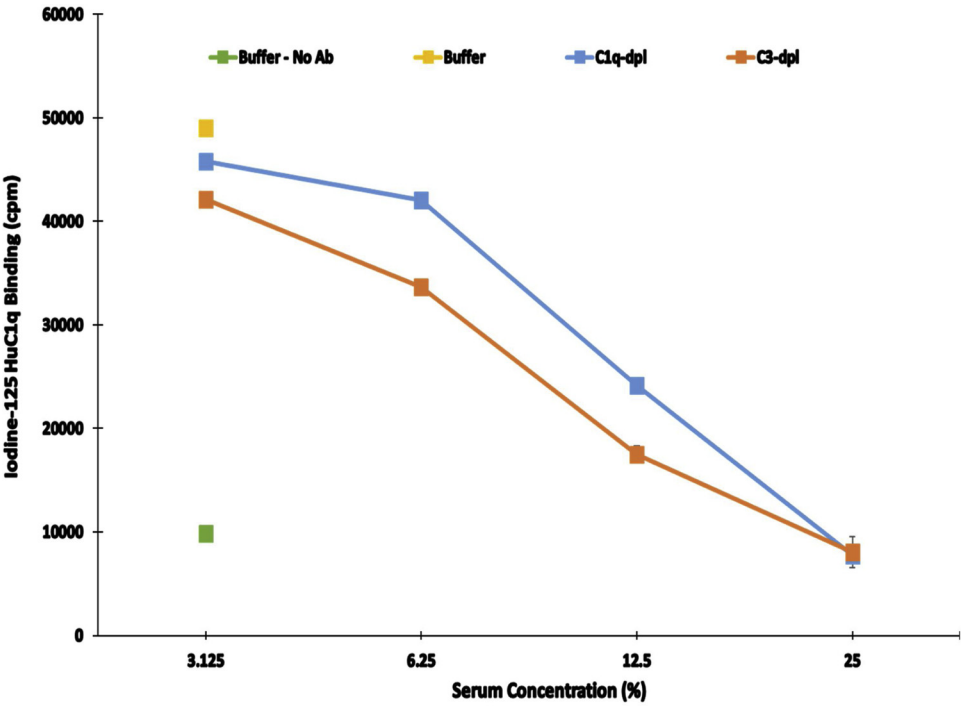

Fig. 6. The ability of C1q-depleted and C3-depleted serum to dissociate C1q from immune complexes is serum concentration dependent. Immune complexes with bound radiolabeled C1q were treated with different concentrations of C1q-depleted (**C1q-dpl**) or C3-depleted (**C3-dpl**) serum in DVBS++. The amount of radiolabeled C1q that remains bound to immune complexes was measured. A buffer control with no serum (**Buffer**) was used as a positive control for radiolabeled C1q binding. A negative control (**Buffer - No Ab**) was used that contains no IgG anti-BSA. Duplicate samples were performed, and the mean (SD) was determined for each sample.

et al., 1975; Brasher, 1976). For this reason, two patient serum samples with this disease were tested for the ability to dissociate radiolabeled C1q from immune complexes. Low ionic strength DVBS^{++} or DVBS-EDTA were utilized to distinguish between C3 and C1q mechanisms of C1q dissociation. Fig. 9 shows that neither patient sample is able to dissociate radiolabeled C1q from immune complexes in either buffer indicating defects in complement and C1q activity. Normal serum however is able to dissociate radiolabeled C1q almost completely from immune complexes in DVBS^{++} or in EDTA-DVBS.

4. Discussion

As predicted by Lachmann, the experiments in this report demonstrate that C1q bound to high affinity IgG containing immune complexes is indeed eluted as the result of C3 activation. This function requires the formation of both the classical and alternative pathway C3 convertases to efficiently remove C1q alluding to the work of Ziccardi, Fishelson and Müller-Eberhard, and Nilsson. C1q that is dissociated from solid phase immune complexes in concentrated C1q-depleted serum can be captured by IgG anti-C1q and IgG anti-C3c antibodies. This work also produced another mechanism of C1q dissociation from immune complexes orchestrated by C1q in concentrated C3-depleted serum. Purified C1 and C1q perform the same function as serum C1q at similar concentrations. The presence of the calcium chelators EDTA or EGTA do not interfere with the ability of C1q to dissociate C1q bound to immune complexes distinguishing this mechanism from the mechanism

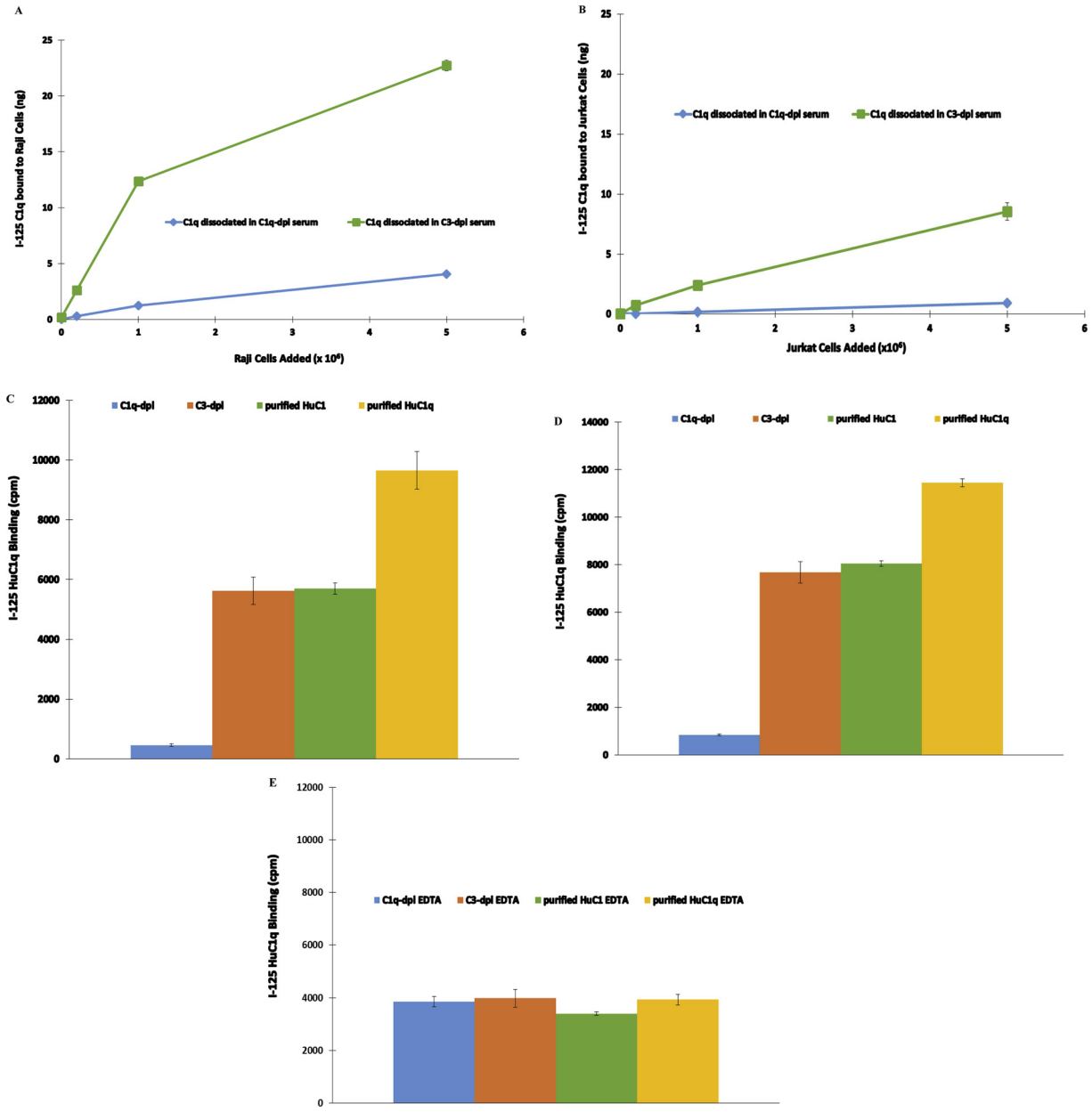

Fig. 7. A. C1q dissociated from immune complexes in C3-depleted serum binds Raji lymphoblastoid cells. Radiolabeled C1q bound to solid-phase immune complexes was dissociated with either C1q-depleted serum (**C1q dissociated in C1q-depleted serum**) or C3-depleted serum (**C1q dissociated in C3-depleted serum**). The supernatants were subsequently incubated with different concentrations of Raji lymphoblastoid cells at 4 °C. The cells were washed and measured for the amount of radiolabeled C1q bound. Duplicate samples were performed, and the mean (SD) was determined for each sample. **B.** C1q dissociated from immune complexes in C3-depleted serum bind Jurkat lymphoblastoid cells. Radiolabeled C1q bound to solid-phase immune complexes was dissociated with either C1q-depleted serum (**C1q dissociated in C1q-depleted serum**) or C3-depleted serum (**C1q dissociated in C3-depleted serum**). The supernatants were subsequently incubated with different concentrations of Jurkat lymphoblastoid cells at 4 °C. The cells were washed and measured for the amount of radiolabeled C1q bound. Duplicate samples were performed, and the mean (SD) was determined for each sample. **C.** C1q dissociated from immune complexes in C3-depleted serum, purified C1 or purified C1q bind Raji lymphoblastoid cells. Radiolabeled C1q bound to solid-phase immune complexes was dissociated with C1q-depleted serum (**C1q-dpl**), C3-depleted serum (**C3-dpl**), purified C1 (**purified HuC1**) or purified C1q (**purified HuC1q**). The supernatants were subsequently incubated with Raji lymphoblastoid cells at 4 °C. The cells were washed and measured for the amount of radiolabeled C1q bound. Duplicate samples were performed, and the mean (SD) was determined for each sample. **D.** C1q dissociated from immune complexes in C3-depleted serum, purified C1 or purified C1q bind Jurkat lymphoblastoid cells. Radiolabeled C1q bound to solid-phase immune complexes was dissociated with C1q-depleted serum (**C1q-dpl**), C3-depleted serum (**C3-dpl**), purified C1 (**purified HuC1**) or purified C1q (**purified HuC1q**). The supernatants were subsequently incubated with Jurkat lymphoblastoid cells at 4 °C. The cells were washed and measured for the amount of radiolabeled C1q bound. Duplicate samples were performed, and the mean (SD) was determined for each sample. **E.** C1q dissociated from immune complexes in C1q-depleted serum, C3-depleted serum, purified C1 or purified C1q bind similarly to Raji lymphoblastoid cells in EDTA-DVBS. Radiolabeled C1q bound to solid-phase immune complexes was dissociated with C1q-depleted serum (**C1q-dpl EDTA**), C3-depleted serum (**C3-dpl EDTA**), purified C1 (**purified HuC1 EDTA**) or purified C1q (**purified HuC1q EDTA**). The supernatants were subsequently incubated with Raji lymphoblastoid cells at 4 °C. The cells were washed and measured for the amount of radiolabeled C1q bound. Duplicate samples were performed, and the mean (SD) was determined for each sample.

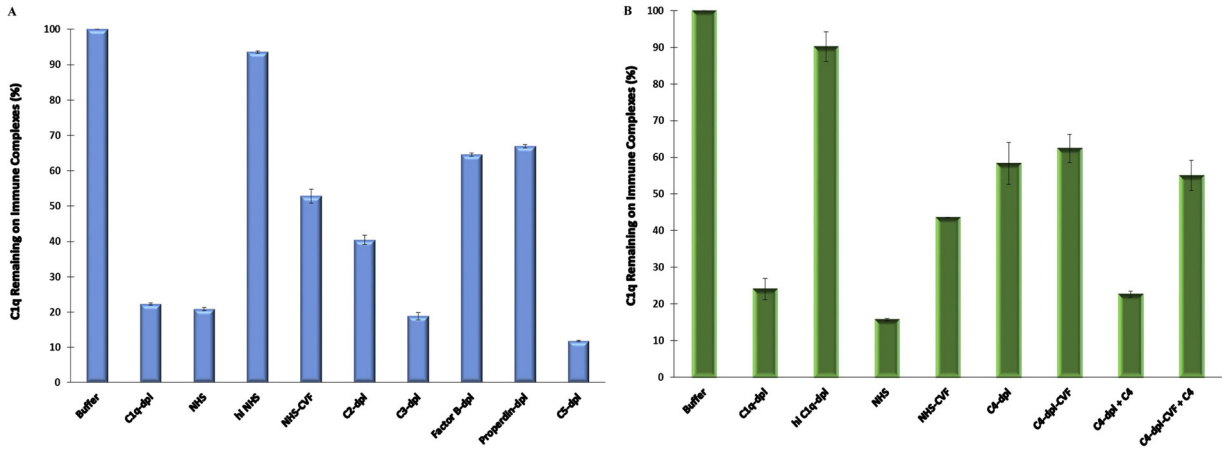

Fig. 8. A. Sera depleted of classical or alternative pathway proteins or cobra venom factor-inactivated normal human serum show reduced ability to dissociate C1q from immune complexes. Immune complexes with bound radiolabeled C1q were treated with C1q-deplted serum (**C1q-dpl**), normal human serum **(NHS**), heat-inactivated normal human serum (**hi NHS**), cobra venom factor-inactivated normal human serum (**NHS-CVF**), C2-depleted serum (**C2-dpl**), C3-depleted serum (**C3-dpl**), factor B-depleted serum (**Factor B-dpl**), properdin-depleted serum (**Properdin-dpl**) or C5-depleted serum (**C5-dpl**). A buffer control with no serum (**Buffer**) was used as a positive control for radiolabeled C1q binding. Duplicate samples were performed, and the mean (SD) was determined for each sample. **B.** Sera depleted of classical or alternative pathway proteins or cobra venom factor-inactivated normal human serum show reduced ability to dissociate C1q from immune complexes. Immune complexes with bound radiolabeled C1q were treated with C1q-deplted serum (**C1q-dpl**), heat-inactivated C1q-depleted serum (**hi C1q-dpl**), normal human serum **(NHS**), cobra venom factor-inactivated normal human serum (**NHS-CVF**), C4-depleted serum (**C4-dpl**), cobra venom factor-inactivated C4-depleted serum (**C4-dpl-CVF**), C4-depleted serum with the addition of purified C4 (**C4-dpl + C4**), or cobra venom factor-inactivated C4-depleted serum with the addition of purified C4 (**C4-dpl-CVF + C4**). A buffer control with no serum (**Buffer**) was used as a positive control for radiolabeled C1q binding. Duplicate samples were performed, and the mean (SD) was determined for each sample.

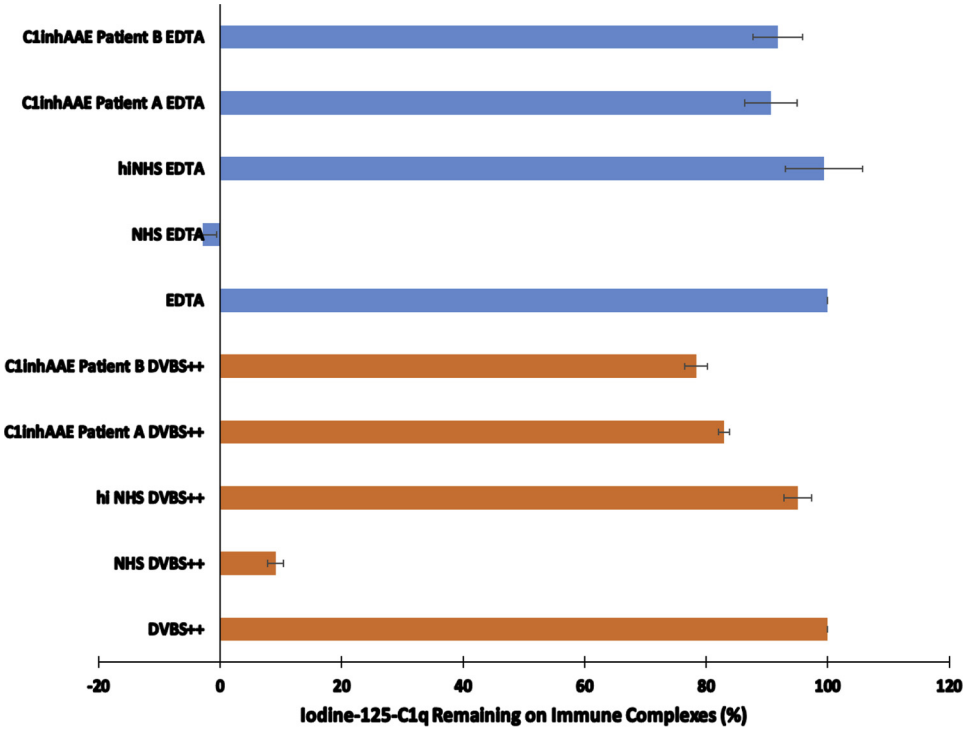

Fig. 9. Sera from two patients with acquired angioedema with C1 inhibitor deficiency do not dissociate C1q from immune complexes in DVBS++ or EDTA-DVBS. Immune complexes with bound radiolabeled C1q were treated with normal human serum (**NHS**), heat-inactivated NHS (**hi NHS**), patient A serum (**C1inhAAE Patient A**) or patient B serum (**C1inhAAE Patient B**) in either DVBS++ or EDTA-DVBS. A buffer control with no serum (**DVBS++ or EDTA**) was used as a positive control for radiolabeled C1q binding. Duplicate samples were performed, and the mean (SD) was determined for each sample.

of C3. Fresh unlabeled C1q is observed to replace radiolabeled C1q on the immune complex after treatment in C3-depleted serum. Because the presence of C1r and C1s in complex with C1q does not influence the release of C1q by the binding of C3 or the binding of subsequent C1q, the globular head domains of C1q are the critical regions both mechanisms of C1q dissociation serve to modulate.

The first observation that the dissociation of C1q from immune complexes by the activation and binding of C3 may have a physiologic explanation. C3 has been shown to bind via ester bonds to both C_H2 and C_H3 domains of the IgG Fc fragment in an immune complex during complement activation, and these domains are also where C1 is bound and stabilized (Vivanco et al., 1999; Okada and Utsumi, 1989). C3 binding to IgG therefore may disturb C1q binding and C1 stability and contribute to the release of C1q from IgG. It may also be possible that C3 can bind both the IgG Fc fragment via its thiolester C3d region and C1q via its C3c region. Subsequently, C3b would be degraded to C3d by serum factors C4 binding protein, factor H, factor I, soluble CR1 or combinations thereof, releasing C1q-C3c complexes from the immune complex and leaving behind C3d fragments that tag the immune complex for C3 receptors (Fig. 10).

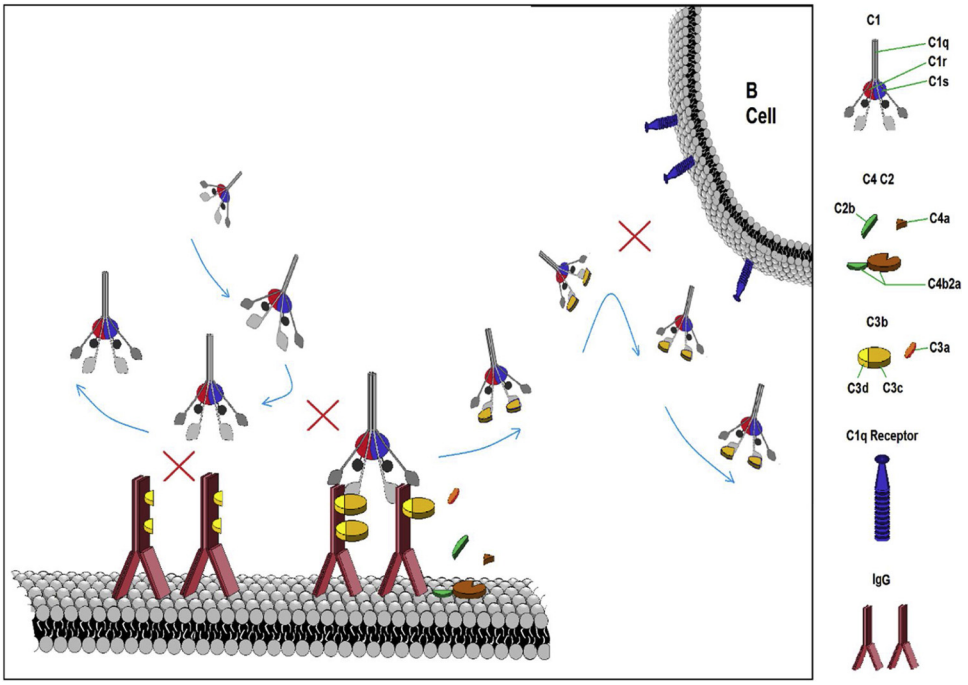

Fig. 10. The regulation of C1q by the activation and binding of C3. During complement activation, C3 is able to bind the globular heads of C1q and the Fc fragment of IgG and subsequently remove C1q bound to an IgG containing immune complex. The resulting fluid-phase C1q-C3c complex may prevent the interaction of cellular ligands that bind the globular heads of C1q. Additionally, the presence of C1r and C1s on the C1q molecule may prevent the interaction of cellular ligands that bind the collagen like region of C1q.

These experiments did not find C1q-C3d or C1q-C4d complex formation during complement activation, and this result follows the work of Fishelson and Muller-Eberhard who demonstrated that purified C1q only bound sheep EC3b and not sheep EC3bi or EC3d (Fishelson and Müller-Eberhard, 1987). However, this data does differ with Wouters et al who reported that C1q-C3d and C1q-C4d complexes were generated during complement activation when using fluid-phase heat aggregated human IgG as the complement activator (Wouters et al., 2005, 2008). In contrast to antigen-antibody immune complexes, Muñoz et al reported that C3 does not bind the C_H2 and C_H3 domains in the Fc region of heat aggregated IgG during complement activation, and this restriction may hinder the ability of C3 to bind and release C1q (Muñoz et al., 1998). To add to this, Ziccardi could not show complete inhibition of C1 activation in normal human serum exposed to heat aggregated IgG unlike antigen-antibody immune complexes or sensitized erythrocytes (Ziccarid, 1986). Larsson et al also performed C1q binding experiments with plate bound heat aggregated IgG and saw unremarkable loss of the C1q signal in concentrated serum (Larsson et al, 1989). Although C3 can bind to the C_H1 domain of IgG, the restrained access of the Fc region of heat aggregated IgG to C3 binding during complement activation may explain the discrepancies with Wouters et al and should be explored further (Vivanco et al., 1999). If antigen-antibody immune complexes are more physiologically relevant in terms of complement activation and regulation, *in vitro* C1q-C3c complex formation may be an important indicator of the ability of serum to effectively opsonize immune complexes serving not only as a complement activation marker but also as a complement regulatory marker since efficient C3 activation controls the amount of C1 activation induced by immune complexes (Manderson et al., 2001, Ziccarid, 1986). To this end, it would be interesting to note if C1q-C3c complexes are released and C3d deposition occurs during HLA antibody testing and the assessment of potential transplantation recipients (Schwaiger et al., 2014; Greenshields and Liwski, 2019).

The second observation that near physiologic concentrations of serum or purified C1q were required to dissociate or displace nanogram amounts of radiolabeled C1q from immune complexes irrespective of complement activation and the presence of C1r and C1s was originally thought to be artefactual because of the low ionic strength buffer system employed in these experiments. Decades ago, low ionicity was shown to promote the assembly of and to strengthen and maintain the C1 complex thereby preventing autoactivation and dissociation in purified form and inactivation and dissociation by C1 inhibitor in serum (Colten et al., 1968; Sim et al., 1979). For similar reasons, low ionic strength buffers were utilized in the solid-phase immune complex assays here in order to promote the association of serum C1r and C1s with radiolabeled C1q bound to an immune complex. Consequently, classical pathway and C3 activity were enhanced and able to subsequently release C1q into the fluid phase with consistent results. Additionally and equally important for the outcome of these experiments, low ionic strength buffers serve to enhance the binding of C1q to cell receptors for both the collagen like region and globular head domains of the C1q molecule (Chen et al., 1994; Eggleton et al., 1995). It was the inclusion of cell binding experiments after the solid-phase immune complex assays that provided a clearer mechanism of action for the second observation. The experiments together demonstrate simply that C1q can bind one ligand, be released, and then bind another ligand in the absence of C3. This ability to transfer from one ligand to another is highly reminiscent of the classical complement C1 fixation and transfer test developed by Borsos and Rapp (Borsos and Rapp, 1965) that measures the ability of C1 to transfer its ability to activate the classical pathway from one immune complex to another. There was one caveat however in which the transfer step of the test required normal physiologic ionic strength buffer. Low ionic strength buffer abrogated the transfer as a result of the increased strength of the bond between C1 and the antibody of the initial immune complex. A few years later, exceptions were noted after William Linscott demonstrated that the C1 fixation and transfer test does occur at low ionic strength, especially with IgG containing immune complexes (Linscott, 1969a, 1969b). Linscott either lowered the concentration of IgG and the number of C1 binding sites on the immune complex or increased the concentration of C1 in order to completely saturate all available IgG binding sites, and these manipulations allowed C1 to transfer from one immune complex to another in low ionic strength buffer. The experiments in this report show that radiolabeled C1q transfers from an immune complex to a lymphocyte receptor or ligand in the presence of near physiologic concentrations of C1q, or C1, and that this transfer depicts a physiologic mechanism of action similar, if not identical, to the classical complement C1 fixation and transfer test of Borsos and Rapp.

Radiolabeled C1q displaced from immune complexes by near physiologic concentrations of purified C1q retains its ability to effectively bind B and T lymphoblastoid cell lines. Both the globular head domains and the collagen like region of the dissociated C1q molecule are presumed available to interact with receptors and ligands for C1q on these cells. It has been reported however that C1r and C1s in complex with C1q restricts access to C1q receptors specific for the collagen like region yet does not affect the ability of C1q to bind C1q receptors specific for the globular head domains (Ghebrehiwet et al., 1990, 2014). This effect was considered after observing that cell binding was approximately 30–40% less if radiolabeled C1q was dissociated using purified whole C1 rather than purified C1q. Furthermore, an almost identical decrease in radiolabeled C1q binding is observed in C1 containing C3-depleted serum. Both reagents contain near physiologic concentrations of C1r and C1s in addition to C1q, and the transfer of C1s from one C1 complex to another has been reported. Bartholomew and Esser demonstrated that proenzyme C1s can be exchanged in and out of different C1 complexes without a loss in total hemolytic C1 activity (Bartholomew and Esser, 1977) supporting the possibility that C1r and C1s in complex with purified C1 or serum C1 may transfer to radiolabeled C1q and reassemble a new C1 complex. Consequently, radiolabeled C1q in complex with C1r and C1s may bind C1q receptors specific for its globular head domains and not receptors for its collagen like region. In the presence of C3, radiolabeled C1q dissociated from immune complexes in C1q-depleted serum binds poorly to B and T lymphoblastoid cells. Initially, C3 was thought to be the critical mediator that inhibits C1q binding to its respective receptors. However, evidence of C1r and C1s participation is here as well. If radiolabeled C1q is dissociated from immune complexes in C1q-depleted serum and then treated with EDTA before the addition of Raji B cells, the amount of radiolabeled C1q able to bind increases significantly. This increase was also found using Jurkat T cells (results not shown). Because the C1q-C3c complex is stable in EDTA, C1r and C1s have most likely separated from radiolabeled C1q thereby allowing receptors and ligands access to the collagen like region of C1q. Accordingly, the interaction of both the globular head domains and the collagen like region of C1q with their respective cell receptors and ligands are potentially modulated by normal complement activation.

There are numerous potential receptors and ligands for C1q that exist on or near the surface of lymphocytes, and the number of ligands may increase after treatment with human serum. To identify and block these C1q binders, especially in a low ionic strength environment, is beyond the capability of this investigation. Despite this shortfall, C1q binding to the Raji B lymphoblastoid cell line was examined rather by the mechanism of C1q dissociation from immune complexes and the effects of C1r / C1s and C3 on the availability of the collagen like region and the globular head domains of C1q. Table 1 first highlights that radiolabeled C1q dissociated from immune complexes in purified C1q most effectively binds Raji B cells and radiolabeled C1q dissociated in C1q-depleted serum, with both active C1r/C1s and C3, least effectively binds Raji B cells. The difference in Raji cell binding between radiolabeled C1q dissociated in purified C1q and radiolabeled C1q dissociated in purified C1 reveals the effect of C1r / C1s on the transfer of radiolabeled C1q. As stated earlier, C3-depleted serum, also a provider of C1r /C1s, imitates purified C1 with a similar reduced binding. Both reagents allow for the determination of the amount of radiolabeled C1q dissociated in purified C1q that is bound to receptors and ligands for the collagen like region of C1q. Alternatively, the difference in Raji B cell binding between radiolabeled C1q dissociated in C3-depleted serum and radiolabeled C1q dissociated in C1q-depleted serum determines the inhibitory effect of serum C3 and thus the amount of radiolabeled C1q bound to receptors for the globular head domains of C1q since both reagents supply C1r / C1s to prevent receptor access to the collagen like region. Interestingly, EDTA equilibrated the effects of all four reagents by increasing the binding of radiolabeled C1q dissociated in C1q-depleted serum and decreasing the binding of radiolabeled C1q dissociated in C3-depleted serum. It is noteworthy that EDTA augments the binding of radiolabeled C1q dissociated in reagents without C3 suggesting that this reduction in binding to the Raji cell line may be specific for receptors and ligands for the globular head domains of C1q. Moreover, EDTA does not completely replicate the same result using Jurkat T cells possibly indicating different and varying levels of receptors and ligands on any given cell line (results not shown).

Table 1
Determination of the availability of C1q domains that bind Raji B lymphoblastoid cells.

Radiolabeled C1q Binding to RAJI B Lymphoblastoid Cells [cpm]		
Radiolabeled C1q Dissociated from Immune Complexes in:	DVBS + +	EDTA-DVBS
C1q-depleted serum [C1r / C1s, C3]	461 cpm	3849 cpm
C3-depleted serum [C1q, C1r / C1s]	5630 cpm	3985 cpm
Purified HuC1 [C1q, C1r / C1s]	5697 cpm	3397 cpm
Purified HuC1q [C1q only]	9654 cpm	3931 cpm
Calculations:		**gC1q or cC1q Availability?**
Purified C1q [C1q only] – C1q-depleted serum [C1r / C1s, C3] =	9193 cpm	both available
Purified C1q [C1q only] – Purified C1 [C1q, C1r / C1s] =	3957 cpm	cC1q available
Purified C1q [C1q only] – C3-depleted serum [C1q, C1r / C1s] =	4024 cpm	cC1q available
C3-depleted serum [C1q, C1r / C1s] – C1q-depleted serum [C1r / C1s, C3] =	5169 cpm	gC1q available
EDTA (Average of 4 reagents tested) =	3790 cpm	cC1q available

The data in this report ultimately reinforce the importance of the strength of the bond between C1q and IgG within an immune complex. In a low ionic strength environment, this bond is strong and resistant to dissociation, and it requires the activation of two complement pathways or the presence of 50–100 times the concentration of C1q that is bound to an IgG immune complex to disrupt this bond in these experiments. On the contrary, Nilsson demonstrated that the signal of C1q present in normal human serum is minimized within minutes after incubation in a normal ionic strength buffer system (Nilsson, 2001). This laboratory has performed very similar experiments with the same results, and C1q-C3c complexes were also found in a similar time frame (results not shown). However, using this same method but under low ionic strength conditions, the signal for C1q remained over the course of an hour (results not shown). This contradicts the experiments in this report that demonstrate normal serum can still effectively dissociate radiolabeled C1q already bound to an immune complex and generate C1q-C3c complexes. Since it was found that near physiologic concentrations of C1q can also dissociate radiolabeled C1q from immune complexes, it is plausible that low iconicity favors the dissociation of and replacement of C1q by excess C1q over the dissociation of C1q by the activation and binding of C3, which ultimately leaves C1q on the immune complex in the method described by Nilsson. To support this possibility, low iconicity has been shown to disrupt the ability of complement to solubilize immune complexes, a C3 dependent mechanism, and the inability of C3 to compete and disrupt the IgG-C1q bonds may reflect its inability to solubilize an immune complex (Baatrup et al., 1984). A normal ionic strength environment therefore favors C3 control that ultimately inhibits further C1q binding and C1 activation by the initial complement activating immune complex.

It must be reiterated that Nilsson could not find a relationship between C3 activation and C1q release from immune complexes under physiologic ionic strength conditions (Nilsson, 2001). Preliminary studies to the work presented in this report also observed that under normal physiologic ionic strength conditions the amount of radiolabeled C1q bound to solid-phase immune complexes incubated in heat inactivated serum or buffer alone significantly drifted downwards during an hour of incubation. This instability was one more reason low

ionic strength buffer replaced normal physiologic strength buffer for these experiments. Hence, it may be difficult to distinguish the mechanisms of C1q release under normal physiologic ionic strength conditions. Nevertheless, the experiments in this report infer that the increased strength between C1q and antibody under low ionic strength conditions allows these two mechanisms of C1q dissociation to be observed concomitantly.

C1q dissociation experiments were extended to patient serum samples with acquired angioedema type II with C1 inhibitor deficiency hypothesizing that low hemolytic titers of C4 and C2 and the low antigenic levels of C1q would disrupt both mechanisms of C1q dissociation. Both patient samples tested were indeed defective in the ability to dissociate C1q from immune complexes in either $DVBS^{++}$ or EDTA-DVBS, and with the exception of heat inactivated serum, these serum samples exhibited the least amount of C1q dissociation observed in these experiments. It is interesting to postulate that in such patients C1q that binds an immune complex remains on an immune complex attached by its globular heads, and that this bond may strengthen and become more resistant to dissociation over time (Kaul and Loos, 1997). Since these patients also have low levels of C1 inhibitor, the C1q bound may also contain C1r and C1s. For these reasons, receptors and ligands for C1q may have reduced access to the C1q bound on these complement activating immune complexes.

Lastly, it was an unanticipated finding that C3-depleted serum is able to eliminate C1q from immune complexes as effectively as C1q-depleted serum. This ability to completely remove radiolabeled C1q from an immune complex did not occur in CVF-treated normal serum or serum depleted in other classical or alternative pathway proteins. Radiolabeled C1q, now free to interact with another ligand, was shown to be replaced by unlabeled serum C1q, which will most likely be displaced by yet another C1q. The absence of C3 may allow unrestricted access to the globular head domains of C1q and thus provide an optimal environment for uncontrolled C1q binding and transfer to multiple receptors and ligands. Again, it is well established that free C1q has numerous noncomplement functions involved in both pro-inflammatory and anti-inflammatory reactions, and receptors and ligands for C1q play major roles in many of these functions. Experiments to distinguish whether such functions are related to receptors for the globular heads of C1q, receptors for the collagen like region of C1q, or receptors for both and whether access or blockade of a specific receptor will augment the immune mechanism desired may prove difficult but nonetheless informative about these important mechanisms of action.

Bridging the work of many dedicated complement system investigators and the research presented here, a major function of C3 during complement activation by IgG containing immune complexes is to bind and dissociate from IgG the C1q that initially bound and initiated complement activation. This mechanism of negative feedback elaborates upon the work of Lachmann and Ziccardi that describes the regulation of C1 activation and further complement activation induced by immune complex formation. The role of C3 however expands to include the regulation of C1q binding to receptors and ligands for C1q. This work suggests that the receptors for the globular heads of C1q are the most affected by C3, and C3 may therefore serve to mediate the noncomplement functions of C1q that occur via these receptors. Equally important, it may be prudent in certain immune conditions to interfere with the ability of C3 to regulate C1q binding to its respective receptor for its globular head domains and allow C1q to perform its non-complement functions (Kandov et al., 2017; Ling et al., 2018; Kandov et al., 2018). The evolving relationship between C1q and C3 emphasizes that although studies in complement deficient animals are beneficial, the absence of one complement protein may profoundly affect the activity of other complement proteins and in turn promote or deter innate or adaptive immune functions that would not otherwise occur in the presence of an intact and interconnected complement system.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

None.

Acknowledgements

The authors wish to thank Blas Laurrari, M.D. for his schematic diagram - The regulation of C1q by the activation and binding of C3. Michael M. Frank passed away on August 1, 2019 during the submission of this manuscript, which is dedicated to his memory and influence.

References

Bartholomew, R.M., Esser, A.F., 1977. The first complement component evidence for an equlibrium between Cls free in serum and Cls bound in the C1 complex. J. Immunol. 119, 1916–1922.
Baatrup, G., Peterson, I., Svehag, S.-E., Jepson, H.H., Brandslund, I., 1984. Solubilization of immune complexes in complement factor deficient sera and the influence of temperature, ionic strength and divalent cations on the solubilization reaction. J. Clin. Lab. Immunol. 14, 185–189.
Boackle, S.A., Morris, M.A., Holers, V.M., Karp, D.R., 1998. Complement opsonization is required for presentation of immune complexes by resting peripheral blood B cells. J. Immunol. 161, 6537–6543.
Borsos, T., Rapp, H.J., 1965. Hemolysin titration based on fixation of the activated first component of complement evidence that one molecule of hemolysin suffices to sensitize an erythrocyte. J. Immunol. 95, 559–566.
Brasher, G.W., Starr, J.C., Hall, F.F., Spieckerman, A.M., 1975. Complement component analysis in angioedema: diagnostic value. Arch. Dermatol. 111, 1140–1142.
Brasher, G.W., 1976. C1q levels in hereditary angioedema. J. Allergy Clin. Immunol. 59, 263–265.
Chen, A., Gaddipati, S., Hong, Y., Volkman, D.J., Peerschke, E.I.B., Ghebrehiwet, B., 1994. Human T cells express specific binding sites for C1q: role in T cell activation and proliferation. J. Immunol. 153, 143–1440.
Colten, H.R., Borsos, T., Rapp, H.J., 1968. Reversible loss of activity of the first component of complement (C′1) as a function of ionic strength. J. Immunol. 100, 799–807.
Dempsey, P.W., Allison, M.E.D., Akkaraju, S., Goodnow, C.C., Fearon, D.T., 1996. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. Science 247, 348–350.
Eggleton, P., Ghebrehiwet, B., Sastry, K.N., Coburn, J.P., Zaner, K.S., Reid, K.B.M., Tauber, A.I., 1995. Identification of gC1q-binding protein (gC1q-R) on the surface of human neutrophils. J. Clin. Invest. 95, 1569–1578.
Fishelson, Z., Müller-Eberhard, H.L., 1987. Regulation of the alternative pathway of human complement by C1q. Mol. Immunol. 24, 987–993.
Ghaia, R., Waters, P., Roumenina, L.T., Gadjeva, M., Kojouharova, M.S., Reid, K.R., Sims, R.B., Kishore, U., 2007. C1q and its growing family. Immunobiology 212, 253–266.
Ghebrehiwet, B., Habicht, G.S., Beck, G., 1990. Interaction of C1q with its receptor on cultured cell lines induces an anti-proliferative response. Clin. Immunol. Immunopathol. 54, 148–160.
Ghebrehiwet, B., Hosszu, K.K., Valentino, A., Peerschke, E.I.B., 2012. The C1q family of proteins: insights into the emerging non-traditional functions. Front. Immunol. 3, 52–60.
Ghebrehiwet, B., Hosszu, K.K., Valentino, A., Yan, J., Peerschke, E.I.B., 2014. Monocyte expressed macromolecular C1 and C1q receptors as molecular sensors of danger implications in SLE. Front. Immunol. 5, 278–286.
Ghebrehiwet, B., Hosszu, K.K., Peerschke, E.I.B., 2017. C1q as an autocrine and paracrine regulator of cellular functions. Mol. Immunol. 84, 26–33.
Golan, M.D., Thomas Hitschold, T., Loos, M., 1981. The reconstitution of human C1, the first complement component: binding of C1r and C1s to C1q influences the C1q conformation. FEBS Lett. 128, 281–285.
Greenshields, A.L., Liwski, R.S., 2019. The ABCs (DRDQDPs) of the prozone effect in single antigen bead HLA antibody testing: lessons from our highly sensitized patients. Hum. Immunol. 7, 478–486.
Hawksworth, O.A., Li, X.X., Coulthard, L.G., Wolvetang, E.J., Woodruff, T.M., 2017. New concepts on the therapeutic control of complement anaphylatoxin receptors. Mol. Immunol. 89, 36–43.
Hess, C., Kemper, C., 2016. Complement-mediated regulation of metabolism and basic cellular processes. Immunity 45, 240–254.
Hester, C.G., Frank, M.M., 2002. The dissociation of C1q from immune complexes and the regulation of C1 by the activation of C3. In: Presented at the XIX International Complement Workshop. Palermo, Italy September 22–26, 2002.
Kaul, M., Loos, M., 1997. Dissection of C1q capability of interacting with IgG: time-dependent formation of a tight and only partial reversible association. J. Biol. Chem. 272, 33234–33244.
Kandov, E., Pednekar, L., Hosszu, K.H., Nanda, J., Galanakas, D.K., Preissner, K.T.,

Kishore, U., Peerschke, E.I., Ghebrehiwet, B., 2017. The dual roles of membrane and soluble forms of gC1qR and C1q in the breast cancer microenvironment. J. Immunol. 198 (1 Supplement) 75.5.
Kandov, E., Kaur, A., Kishore, U., Ping, J., Williams, J., Peerschke, E.I.B., Ghebrehiwet, B., 2018. C1q and C1q receptors (gC1qR and cC1qR) as potential novel targets for therapy against breast cancer. Curr. Trends Immunol. 19, 59–76.
Kitamura, H., Teshima, H., Day, N.K., 1978. The presence of active C1 (C1) on peripheral human lymphocytes. Vox Sang. 35, 197–206.
Kishore, U., Ghai, R., Greenbough, T.J., Shrive, A.K., Bonifati, D.M., Gadjeva, M.G., Waters, P., Kojouharova, M.S., Chakraborty, T., Agrawal, A., 2004. Structural and functional anatomy of the globular domain of complement protein C1q. Immunol. Lett. 95, 113–128.
Kojouharova, M., Reid, K.B.M., Gadjeva, M., 2010. New insights into the molecular mechanisms of classical complement activation. Mol. Immunol. 47, 2154–2160.
Kouser, L., Madhukaran, S.P., Shastri, A., Saraon, A., Ferluga, J., Al-Mozaini, M., Kishore, U., 2015. Emeriging and novel functions of complement protein C1q. Front. Immunol. 6, 1–19.
Lachmann, P.J., 1966. A possible homeostatic mechanism in complement fixation: inhibition of the activity of fixed C'_1 by the fixation of C'_{3a}. Nature 210, 140–141.
Larsson, A., Sjöquist, J., 1989. Binding of complement components C1q, C3, C4 and C5 to a model immune complex in ELISA. J. Immunol. Meth. 119, 103–109.
Ling, G.S., Crawford, G., Buang, N., Bartok, I., Tian, K., Thielens, N.M., Bally, I., Harker, J.A., Ashton-Rickardt, P.G., Rutschmann, S., Strid, J., Botto, M., 2018. C1q restrains autoimmunity and viral infection by regulating CD8+ T cell metabolism. Science 360, 558–563.
Linscott, W.D., 1969a. The effects of ionic strength, temperature, and antibody class and avidity on fixation and transfer of the first component of complement. J. Immunol. 102, 993–1001.
Linscott, W.D., 1969b. Complement: heterogeneity of binding of the first component of complement as a function of antibody concentration. J. Immunol. 102, 1322–1326.
Liszewski, M.K., Elvington, M., Kulkarni, H.S., Atkinson, J.P., 2017. Complement's hidden arsenal: new insights and novel functions inside the cell. Mol. Immunol. 84, 2–9.
Lu, J., The, B.K., Wang, L., Wang, Y., Tan, Y.S., Lai, M.C., Reid, K.B.M., 2008. The classical and regulatory functions of C1q in immunity and autoimmunity. Cell. Mol. Immunol. 5, 9–21.
Lu, J., Kishore, U., 2017. C1 complex: an adaptable proteolytic module for complement and non-complement functions. Front. Immunol. 8, 592–602.
Manderson, A.P., Pickering, M.C., Botto, M., Walport, M.J., Parish, C.R., 2001. Continual low-level activation of the classical complement pathway. J. Exp. Med. 194, 747–756.
Muñoz, E., Vidarte, L., Casado, M.T., Pastor, C., Vivanco, F., 1998. The C_H1 domain of IgG is not essential for C3 covalent binding – importance of the other constant domains as targets for C3. Int. Immunol. 10, 97–106.
Nayak, A., Pednekar, L., Reid, K.B.M., Kishore, U., 2011. Complement and non-complement activating functions of C1q: a prototypical innate immune molecule. Innate Immun. 18, 350–363.
Nilsson, U., 2001. Deposition of C3b iC3b leads to the concealment of antigens, immunoglobulins and bound C1q in complement activating immune complexes. Mol. Immunol. 38, 151–160.
Okada, M., Utsumi, S., 1989. Role for the third constant domain of the IgG H chain in activation of complement in the presence of C1 inhibitor. J. Immunol. 142, 195–201.
Pepys, M.B., 1976. Role of complement in the induction of immunological responses. Transplant. Rev. 32, 93–120.
Schwaiger, E., Wahrmann, M., Bond, G., Eskandary, F., Böhmig, G.A., 2014. Complement component C3 activation: the leading cause of the prozone phenomenon affecting HLA antibody detection on single-antigen beads. Transplantation 12, 1279–1285.
Sim, R.B., Arlaud, G.J., Colomb, M.G., 1979. C1 inhibitor-dependent dissociation of human complement component C1 bound to immune complexes. Biochem. J. 179, 449–457.
Sörman, A., Zhang, L., Ding, Z., Heyman, B., 2014. How antibodies use complement to regulate antibody responses. Mol. Immunol. 61, 79–88.
Takahashi, M., Czop, J., Ferreira, A., Nussenzweig, V., 1976. Mechanism of solubilization of immune aggregates by complement. Implications for immunopathology. Transplant. Rev. 32, 121–139.
Takata, Y., Tamura, N., Fujita, T., 1984. Interaction with C3 with antigen-antibody complexes in the process of solubilization of immune precipitates. J. Immunol. 132, 2531–2537.
Thielens, N.M., Tedesco, F., Bohlson, S.S., Gaboriaud, C., Tenner, A.J., 2017. C1q: a fresh look upon an old molecule. Mol. Immunol. 89, 73–83.
Thorton, B.P., Větvička, V., Ross, G.D., 1994. Natural antibody and complement-mediated antigen processing and presentation by B lymphocytes. J. Immunol. 152, 1727–1737.
Verschoor, A., Karsten, C.M., Broadly, S.P., Laumonnier, Y., Köhl, J., 2016. Old dogs-new tricks: immunoregulatory properties of C3 and C5 cleavage fragments. Immunol. Rev. 274, 112–126.
Vivanco, F., Muñoz, E., Vidarte, L., Pastor, C., 1999. The covalent interaction of C3 with IgG immune complexes. Mol. Immunol. 36, 843–852.
Wouters, D., Wiessenberg, H.D., Hart, M., Bruins, P., Voskuyl, A., Daha, M.R., Hack, C.E., 2005. Complexes between C1q and C3 or C4: novel and specific markers for classical complement pathway activation. J. Immunol. Methods 298, 35–45.
Wouters, D., Brouwer, M.C., Daha, M.R., Hack, C.E., 2008. Studies on the haemolytic activity of circulating C1q-C3/C4 complexes. Mol. Immunol. 45, 1893–1899.
Ziccardi, R.J., Cooper, N.R., 1977. The subunit composition and sedimentation properties of human C1. J. Immunol. 118, 2047–2052.
Ziccardi, R.J., 1982. A new role for C-1-inhibitor in homeostasis: control of activation of the first component of human complement. J. Immunol. 128, 2505–2508.
Ziccardi, R.J., Tschopp, J., 1982. The dissociation properties of native C1. Biochem. Biophys. Res. Commun. 107, 618–623.
Ziccardi, R.J., 1984. The role of immune complexes in the activation of the first component of human complement. J. Immunol. 132, 283–288.
Ziccarid, R.J., 1986. Control of C1 activation by nascent C3b and C4b: a mechanism of feedback inhibition. J. Immunol. 136, 3378–3383.