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Role of C1q in Efferocytosis and Self-Tolerance — Links With Autoimmunity

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1. Introduction

C1q, the well-known initiator of the classical complement pathway, belongs to a family of soluble pattern recognition receptors (PRRs) called defense collagens comprised of a C-terminal globular region and an N-terminal collagen-like tail. The defense collagens include collectins (collagen containing lectins) such as mannan-binding lectin (MBL), lung surfactant protein A (SP-A), SP-D, CL-K1 CL-L1, conglutinin, collectin-43 and related proteins ficolins. They are capable of recognizing a wide range of microorganisms by binding to their pathogen-associated molecular patterns (PAMPs) [1-3].

C1q differs from the collectins and ficolins members of the defense collagens in that it does not contain a C-type lectin or fibrinogen like recognition domain [4]. Rather, it contains a “jellyroll” beta sandwich fold typical of tumor necrosis factor family [5]. Additionally, C1q is structurally related to some TNF-related proteins such as adiponectin, and the members of CTRP (C1q/TNF-related proteins) family [6], suggesting that C1q could share some “cytokine-like” functions with these molecules.

On the other hand, C1q is classically known for its ability to bind IgG-and IgM-containing immune complexes [7, 8] which initiates the classical complement cascade for microbial killing and phagocytosis.

The traditional view of the biological role of C1q as a first line of innate immunity against pathogens has been reconsidered over the past 15 years with evidences showing that C1q has the ability to sense many altered structures from self, including the pathological form of the prion protein [9, 10], β -amyloid fibrils [11], modified forms of low-density lipoprotein [12, 13] and apoptotic cells [14-16]. Unlike the other complement proteins that are expressed mostly, if not exclusively, in the liver, C1q is predominantly synthesized by myeloid cells such

as macrophages and dendritic cells (DC) and also, in smaller quantities, by a wide range of cell types (reviewed in [17] and paragraph 3.2).

Importantly, C1q is one actor of efferocytosis, which is the mechanism of the clearance of altered self-cells and in particular of apoptotic cells, and is essential for development and to maintain tissue homeostasis [18]. C1q is involved in this process, at least as a physical bridge between the phagocyte and its prey. Numerous C1q-binding molecules at both sides of the phagocytic synapse have been characterized today (summarized in Tables 1 and 2), suggesting a multiligand-binding process even if the consequences of their molecular interplay are not deciphered yet.

Consistent with its importance in the fight against pathogens and as for the other complement proteins, genetic deficiency in C1q leads to a plethora of infections [19]. However, in the case of C1q, deficiency is also strongly correlated with autoimmune diseases, such as systemic lupus erythematosus (SLE) and glomerulonephritis, associated with compromised removal of apoptotic cells (as developed in paragraph 7).

A key element that highlights the non-traditional C1q functions, linking it to autoimmunity, is its capacity to regulate immune cells. This includes a wide range of effects such as regulation of dendritic cells and macrophage polarization, phagocytosis enhancement, stimulation of leukocytes, suppression of T and B cells proliferation. This chapter will provide an update of the C1q functions with an emphasis on its role in autoimmunity.

2. The C1q protein and its classical functions in complement activation

The association with a Ca^{2+} -dependent tetramer comprising two copies of two serine proteases, C1r and C1s [20, 21], allows C1q to trigger the classical complement pathway. C1q is a 460-kDa hexameric protein assembled from six heterotrimeric collagen-like fibers (Figure 1), each being prolonged by a C-terminal globular domain. One heterotrimeric fiber consists of 3 distinct but similar polypeptide chains, A, B, C encoded by 3 genes *C1QA*, *C1QB* and *C1QC* localized on human chromosome 1p, and aligned in the same orientation in the order A-C-B. From a structure-function point of view, the collagen-like domain (cC1q) and the globular heads (gC1q) define two well characterized functional domains. The gC1q domain has the ability to sense and engage an amazing variety of ligands that could be part of surface molecular patterns [20, 22] and is considered as the key to the versatility of C1q function [21, 23, 24]. Until even recently, cC1q specificity has been restricted to the interaction with the associated serine proteases C1r-C1s and with the phagocyte endocytic receptor CRT/CD91(LRP1). As the recognition molecule of the classical pathway, C1q is well known to recognize the IgG and IgM Fc fragments of membrane-bound antibodies, but also of aggregated immune complexes. In addition to this best known “classical” property, C1q recognizes C-reactive protein and other pentraxins bound to pathogens and other surfaces, as well as various molecular motifs on several Gram-negative bacteria and viruses (PAMPs), including gp41 or DNA [20, 25-27]. In most cases, recognition of these non-self ligands, as well as Ig and pentraxins, by C1q triggers activation of the classical complement pathway, thereby contributing to their elimination through enhanced phagocytosis, lysis, and inflammation (Figure 2).

Figure 1. Schematic representation of the assembly of the C1q molecule. C1q is assembled from three polypeptide chains (A, B and C) encoded by 3 different genes (*C1QA*, *C1QB* and *C1QC*). Each chain comprises an N-terminal collagen-like sequence and a C-terminal globular gC1q module, with disulfide bridges linking the N-terminal ends of the A and B chains and two C chains. Each A-B dimer associates with a C chain, resulting in a basic subunit comprised of two disulphide-linked heterotrimeric collagen-like stalks prolonged by globular domains. The association of 3 subunits results in a full-length protein with a typical shape of a bouquet of six flowers, the stalks being held together in their N-terminal half through strong non-covalent interactions and then diverging to form six individual stems, each terminating in a globular head.

Figure 2. Activation of the classical pathway of complement. C1q binds to microbial surface motifs directly or via or other immune molecules (antibodies, pentraxins) through its globular heads. This multivalent binding triggers sequential activation of the C1r and C1s proteases associated to C1q collagen stalks. Activated C1s cleaves complement components C4 and C2, leading to the assembly of the C3 convertase responsible for C3 cleavage and opsonization of the target by C3b. Activation of the complement cascade generates fragments involved in inflammation, enhancement of phagocytosis and ends in target lysis by the membrane attack complex assembled from complement components C5b, C6, C7, C8 and C9. C1 inhibitor (C1-Inh), a member of the serine protease inhibitor (serpin) family, controls both C1 activation and C1s proteolytic activity.

3. New emerging C1q functions

3.1. A sensor of altered-self structure

One main starting point for revisiting C1q function was probably the work of Korb and Ahearn showing that gC1q binds specifically to apoptotic cells and that binding is important for prevention of autoimmunity in SLE [14]. Since then, numerous experimental proofs have established that C1q is an efficient sensor of self-modifications defined as ACAMPs and DAMPs (for Apoptotic Cells- and Danger-Associated Molecular Patterns, respectively). In contrast to PAMPs, DAMPs and ACAMPs are molecules that can initiate and perpetuate the immune response in a noninfectious inflammatory context. On one way, ACAMPs should be defined as a restricted class of DAMPs which is linked and/or restricted to apoptotic cell membrane modifications. Viewed from another angle, some DAMPs are ACAMPs when they are exposed at the surface of apoptotic cells, the best illustration being probably DNA: it is indeed a mainly intracellular molecule released upon cell damage, but DNA is also anchored at the cell surface in association with histone proteins as a consequence of apoptosis. C1q recognizes the deoxyribose moiety of DNA, through unexpected lectin-like property [28, 29].

At the surface of apoptotic cells, C1q also binds phosphatidylserine (PS), the canonical marker of apoptosis, and calreticulin (CRT) which was characterized as a strong “Eat-me” signal for phagocyte. Growing evidence emphasizes the role of **C1q as a sensor and integrator of the self-alteration** and the list of recognized molecules summed up in Table I (C1q ligands on apoptotic cells), is probably far from being closed. These interactions are mainly mediated by the globular region of C1q (gC1q) that were shown to contain the binding sites for self-molecules specifically exposed on altered-self cells surface. However, as the C1q collagenous tail (cC1q) is known to interact with several membrane receptors (see paragraph 6), widely distributed on various cell types, C1q can enter into a vast array of interactions by binding of its heads or/and its stalks depending of their accessibility in a particular situation. C1q binding to altered-self structures is not dependent on its associated serum proteases. Indeed, purified C1q freed of the C1r₂-C1s₂ tetramer, and the isolated gC1q domains retain its binding capacity [14, 28, 30, 31].

	Interaction with C1q domains	Function(s)	Potential partners in complex(es)	References (first authors, years)
DNA* on early and late ACs	gC1q through deoxy-D-ribose, interaction with C chain (Arg 98, Arg 111, and Asn 113) ¹	Promotion of phagocytosis, dependent on iC3b opsonin and on CD46 regulation ³	Annexin II, factor H, histones	Jiang, H. 1992 [32] Tissot, B. 2003 [33] Palaniyar, N. 2004 [34] Elward, K. 2005 [35] Paidaisi. H, 2008 [28] Garlatti, V. 2010 [29]

	Interaction with C1q domains	Function(s)	Potential partners in complex(es)	References (first authors, years)
Ecto-Calreticulin (CRT)* isolated gC1q binds to CRT on early ACs (possibly preapoptotic)	gC1q and cC1q with CRT globular domain	Bridging AC and phagocyte, Modulation of cytokines release	PS	Paidaisi, H. 2011 [31] Verneret, M. 2014 [36] Donnelly, S. 2006 [37]
PS * characterized on early ACs ²	gC1q through phosphoserine, interaction with C chain (Arg 98 and Arg 111) ¹	ACs recognition, Bridging AC and phagocyte(?) Modulation of PS-dependent signaling(?) Complement activation in the presence of serum	CRT, annexin(s)	Paidaisi, H. 2008 [30] Tan, L. 2010 [38]
Annexin V* most likely on late ACs	ND ⁴	Modulation of ACs uptake(?) Modulation of complement activation(?)	PS, CRT	Martin, M. 2012 [39]
Annexin II* most likely on late ACs	ND ⁴	Modulation of complement activation(?)	DNA, PS, Factor H, Leffler, J. 2010[40] histones	Martin, M. 2012[39]
GAPDH detected on early ACs ²	gC1q	ND on ACs		Terrasse, R. 2012 [41]
CERT(L) most likely on late ACs	gC1q	Complement activation		Bode, G. 2014 [42]

*These ligands are autoantigens targeted in lupus erythematosus. PS, phosphatidylserine; CERT(L), the longer splicing isoform of ceramide transporter protein (CERT); GAPDH, Glyceraldehyde-3-phosphate dehydrogenase;¹ determined by X-ray crystallography;² does not exclude a binding to late ACs;³CD46 was characterized as a “Don’t Eat me” signal (Elward *et al*, 2005, [35]);⁴ full length C1q was used in these studies; (?) probable function that remains to be further investigated; ND, not determined.

Table 1. C1q ligands characterized on apoptotic cells (ACs).

3.2. A modulator of the phagocyte functions

Other biological functions of C1q with particular emphasis on immune responses have been revealed. They include stimulation of leukocyte oxidative response, chemotaxis of monocytes derived cells, including neutrophils, modulation of lymphocytes proliferation, DC differentiation, cytokines expression by phagocytes (reviewed in [17]) and more recently macrophage

M1/M2 polarization [43]. The recent findings that C1q, together with its receptors, is directly involved in the safe removal of self-waste (paragraph 4), also provide strong evidence for a relationship between C1q activity, maintenance of immune tolerance and prevention of autoimmunity (developed in paragraph 7). Importantly, the monocyte lineage includes macrophages and dendritic cells which are key actors for phagocytosis, cytokines production and antigen presentation. This suggests a special place for C1q at the interface of both innate and acquired immunity. Indeed, recent observations by the group of Berhane Ghebrehiwet [44, 45] suggest that C1q functions as a molecular switch dictating the monocyte to dendritic cell transition and arresting DCs in an immature state, which influences the cell response, mainly in a “tolerogenic” way. Although not precisely known, this process seems supported by the oligo heterotrimeric nature of C1q which can bind to gC1q and cC1q receptors which are differentially expressed during monocytes maturation. It should also be mentioned that these interactions are likely differentially modulated by the pathogenic or altered-self nature of gC1q ligands (e. g, PAMPs or DAMPs).

In the same way, C1q influences the M1 pro-inflammatory / M2 pro-resolving macrophage polarization [46]. Specifically, the C1q-stimulated phagocyte is anti-inflammatory (M2-polarized) and this is also observed when C1q-opsonized apoptotic cells are engulfed. In blood, C3 complement component is another actor that, in the presence of C1 (C1q/C1r-C1s complex) helps the clearance of apoptotic debris in an anti-inflammatory manner, through its cleavage fragments C3b and iC3b generated by complement activation. Apoptotic blebs become opsonized by iC3b, which is recognized by complement receptor 3 (CR3, Mac-1) on phagocytes. This induces an immune suppression beneficial to the safe elimination of altered-self structures.

Interestingly, recent studies have also shown that T cell responses to apoptotic cells are regulated by complement in a C3-dependent manner [47, 48]. The group of Marina Botto has demonstrated that C3 bound to dying cells not only facilitates the uptake of apoptotic cells but also influences their intracellular processing, facilitating the MHC II/peptide presentation, and thus dedicates the T cell response to an “apoptotic cell” antigen [48]. This observation contributes to the understanding of how use of the same engulfing machinery yields different responses for a pathogen or an altered-self cell.

Whether C1q alone or C3b-derived opsonins are involved is certainly a key for understanding the cellular response mechanism. Therefore it must be dependent on the local delivery of C1q, i.e. in blood or in the surrounding tissues. Approximately 80% of C1q contained in the plasma is associated with the C1r₂-C1s₂ tetramer to form the C1 complex which is capable to trigger proteolytic processing of other serum complement components, including C3. Since monocyte-derived cells are the most abundant sources of C1q, local production of C1q in the absence of other complement proteins could induce a specific response, independently of the complement proteolytic cascade. The “phagocytic synapse”, which organizes the information that will trigger a specific signaling event, is one of the micro environments where C1q produced by phagocytes may serve as an autocrine signal to induce specific responses. Interestingly, growing evidence is now in favor of C1q production in various tissues, such as endothelial cells [49], neurons [50], fibroblasts [51], osteoclasts [52], which reinforces the view that it may be involved in distinct functions in the absence of serum complement proteins.

3.3. A role in angiogenesis and development

C1q is produced by endothelial cells (ECs) and in the absence of other complement proteins such as the C1r and C1s proteases, C3 and C4, exerts a proangiogenic effect [49]. C1q is very efficient in inducing new vessel formation in wound healing. The globular region of C1q seems to be implicated in this function by binding specifically to the gC1q membrane receptor (gC1qR) expressed by ECs and mainly restricted to the skin ulcer. These observations provide significant insights in favor of a role of C1q in development, tissue repair and also tumor growth. Importantly, this is a new entry to apprehend the role of C1q in inflammatory processes. Angiogenesis is an essential component of inflammatory and it is related to inflammatory pathologies such as rheumatoid arthritis and other systemic autoimmune diseases [53, 54].

C1q produced by cells of the central nervous system (CNS) has been shown to play an important role in remodeling synaptic connections in the developing visual system by tagging unwanted synapses for elimination. These results suggest that complement-mediated synapse elimination may become aberrantly reactivated in neurodegenerative diseases [55]. In addition a dramatic increase of C1q level in the CNS (as much as 300-fold) during normal aging was demonstrated recently [56]. C1q has also been reported to activate Wnt signaling, a pathway involved in development of many organs and implicated in mammalian aging, thereby possibly promoting aging-associated decline in tissue regeneration [57].

4. Efferocytosis

Efferocytosis (from *efferre*, Latin for 'to take to the grave') is now the term admitted to name the mechanism of the clearance of apoptotic cells [58]. Apoptosis occurs throughout life as an essential process during development, tissue homeostasis but also in pathogenic events. Despite the billion of apoptotic cells produced per day in human [59], they were rarely observed in tissue excepted in pathological situations. Efficient removal of apoptotic cells before the onset of necrosis is considered as a pre-requisite for prevention of autoimmune and inflammatory diseases. This fundamental process, which appears to promote immune tolerance to self together with anti-inflammatory effects, is ongoingly studied since at least 15 years at the cellular and molecular levels and is increasingly better characterized. Such knowledge leads to discover signals that play together to modulate the way by which dead cells are recognized, engulfed by phagocytes and also determine the final immunological outcome. Of great interest, it was shown that drug-manipulating apoptosis of tumor cell for therapy could benefit to their elimination possibly in an immunogenic way [60, 61]. This also highlights that the balanced immune response to apoptotic cell death results from a fine regulation of "factors" promoting either immunity or tolerance. In brief, efferocytosis proceeds in successive steps, including (i) the contact between the damaged cell and the phagocyte, that could be affected by release of soluble "Find-me" signals by ACs, (ii) the specific recognition of the target with the organization of the phagocytic synapse, and (iii) the intracellular degradation and processing of the debris that impact the global "immune" response.

Because excellent reviews are available on this subject [18, 62-64], the next paragraphs will only summarize the pivotal steps of efferocytosis, with a particular emphasis on the role of C1q.

4.1. C1q recognizes apoptotic cells rapidly from death induction

If it is now clear that C1q binds to various molecules on “apoptotic surfaces” (see Table 1 and paragraph 3). The “age” of the apoptotic cells (young/early or rather aged/late) concerned by C1q-dependent clearance remains a subject of debate [65-67], essentially because extensive binding to necrotic cells is observed *in vitro*. Various elements must be taken into account to make up its mind on the question. At first, when does C1q bind to the cell? When does the recognized motif become accessible? An interesting element is the nature of the debris that is engulfed by the phagocyte and, importantly, this is clearly dependent on the type of phagocyte. Some phagocytes (mostly the professional phagocytes i.e. macrophages and DCs) are able to eat the apoptotic cell in whole [68, 69], whereas there are other instances where phagocytes engulf smaller cell fragments proceeding from membrane blebbing. This is one of the observations explaining why C1q has been involved in early or late apoptotic cells uptake, depending on the experimental setup. The efficiency of engulfment itself and the kinetics of the apoptosis process are two other factors that could impact the time course of the C1q effect. The presence of serum complement components (i.e. C3 and C4) is known to amplify the C1q effect. At last, an important point is certainly the nature of the ligands recognized by C1q that could impact its function. It is now known that C1q deficiency correlates with autoimmune diseases characterized by an increased number of non-engulfed apoptotic cells together with production of auto-antibodies (paragraph 7). It was also shown that in the case of patients with systemic lupus erythematosus who present auto-antibodies against C1q, anti-C1q specifically targeted C1q bound on early apoptotic cells [70]. These latter two observations strongly argue for an early action of C1q in the course of apoptosis development, nevertheless it does not preclude that C1q could also act on later stages of induced cell death and necrosis.

In favor of a rapid response during the elimination process of apoptotic cells, it was shown by our group and others that the C1q globular region binds cells with non-yet permeabilized plasma membrane. Among the C1q ligands on the outlet face of the plasma membrane, PS, CRT, DNA, annexins and GAPDH are molecules that become accessible or increase significantly at early stage of apoptosis [71]. In particular, we have observed by confocal microscopy and FRET (Fluorescence Resonance Energy Transfer) measurement that CRT/gC1q interaction occurs mainly on HeLa apoptotic cells without detectable membrane blebs and that this interaction decreases on developed blebs [71]. Additionally, in a study performed with Jurkat cells, gC1q binding was detected before the appearance of PS, which is a hallmark of very early stage of the morphologic changes of apoptosis. Importantly, Obeid and coworkers have shown a preapoptotic translocation of CRT to the plasma membrane [60].

4.2. C1q enhances phagocytosis

Despite numerous studies demonstrating the effect of C1q on phagocytosis, a clear understanding of the molecular events which support its functions is still lacking. Even if the

enhancement of uptake was initially reported, it proved to be modest in most cases. Various factors that could modulate this effect have to be taken into account. First, efferocytosis is a very redundant process, involving a large number of molecules, which could partially compensate each other. Second, it depends on either the apoptotic cell or the phagocyte type and third it is conditioned by the cell microenvironment, i.e. the presence of serum proteins. In accordance with the observation that C1q by itself binds ACAMPs, the enhancement of uptake is observed independently from its ability to activate the classical complement pathway. However it was also clearly demonstrated that complement protein iC3b, a proteolytic inactive product of the cleavage of C3, could opsonize the target, resulting in enhanced phagocytosis due to iC3b recognition by phagocyte receptors (CR3) [35]. It cannot also be excluded that in some cases other complement opsonins such as C4b could also be involved.

4.2.1. C1q bridges the phagocyte and its prey

It was first proposed that C1q binds the prey through its globular regions and the phagocyte through its collagen stalks, essentially because CRT with its co-receptor CD91 (LRP1) at the phagocyte surface was characterized as a receptor for the collagen part of C1q, and because C1q binds directly to pathogens as well as damaged self-cells surface via its globular heads. This model is undoubtedly challenged by the increasing number of molecules and receptors known to recognize the globular and/or the collagen part of C1q, which could be exposed at both faces of the phagocytic synapse (reviewed in Tables 1 and 2).

4.2.2. C1q aggregates motifs and potentially helps synapse formation

By its capacity to recognize a large number of molecular motifs, mainly supported by the C1q globular region versatility (paragraph 5) but also by the collagen region, C1q has the potential to aggregate molecules in close proximity to help the phagocytic synapse formation in a way similar to the organization of the immunologic synapse. Beyond its role as a molecular bridge, a large number of studies demonstrate that C1q activates signals stimulating the cell responses, thereby providing evidence for its function as a “transmitter” molecule.

4.2.3. C1q induces cell signaling

An important primary observation was that C1q serves as an activation ligand for cells from the monocyte lineage. Indeed, it was shown that fluid phase or immobilized C1q binds to various membrane receptors, including $\beta 1$ and $\beta 3$ integrins and other not yet characterized molecules [72-74]. This binding correlates with various intracellular signaling events, such as integrin activation and platelet aggregation, cell migration and tissue remodeling, and also generation of NF-kappa B complexes, most obviously linked with anti-inflammatory effects and thus directly to efferocytosis. In addition, several independent investigators have analyzed the role of C1q on dendritic cells and proposed that C1q interaction with cC1qR(s), but also with gC1qR/p33 in cooperation with the DC-SIGN receptor, are responsible for the modulation of DC maturation and consequently for the “nonimmunogenic” presentation of self-antigens [75, 76]. Notably, DCs which express elevated levels of C1q co-localized with DC-SIGN-gC1qR

are functionally immature, whereas inducing DC maturation with LPS and inflammatory cytokines decreases expression of both C1q and its receptors. A recent work has revealed a shared signaling pathway for C1q and adiponectin in murine macrophages, through the demonstration that C1q utilizes 5' adenosine mono-phosphate-activated protein kinase (AMPK) to induce MER tyrosine kinase which is a receptor known to regulate efferocytosis [77]. Remarkably, various phagocyte receptors that are linked to efferocytosis have been shown to bind C1q, but so far the consequences of these interactions remain elusive. They include a scavenger receptor expressed by endothelial cell and also in some subsets of DCs (SREC-I/SCARF1), which binds C1q dependently of its interaction with PS on the apoptotic cell surface [51], the leukocyte-associated Ig-like receptor 1 (LAIR1) [78], but also the CD91(LRP1)-CRT complex (see paragraph 6).

4.2.4. *C1q modulates cytokine production*

It is now unambiguously demonstrated that C1q promotes an anti-inflammatory response and thus probably contributes to the global tolerogenic effect triggered by apoptotic cells. A major contribution was provided by Andrea Tenner and coworkers, who have analyzed the C1q effect on cytokine response during ingestion of apoptotic cells by the various monocyte derived-cells (monocytes, macrophages and DCs) [79]. This effect is mainly supported by increase of the IL-10 anti-inflammatory cytokine, inhibition of the pro-inflammatory NF-kappa B transcription factor [74] and modulation of interferon (IFN)-alpha [80, 81]. It however depends either on the maturation state of the phagocyte or on the nature of the apoptotic cell. In addition, the recent characterization of CRT as a major "Eat-me" signal that enhances phagocytosis of apoptotic cells [60, 82] had shed new light on the "signaling" potentiality of C1q, by showing that it could also trigger an immunogenic response in reaction to proapoptotic/anticancer drugs. CRT is a ligand of both the collagen stems and the heads of C1q and is present on each side of the phagocytic synapse. We have demonstrated the direct interaction between CRT and gC1q [71] on early apoptotic cells and also that decreasing CRT exposure impacts C1q binding [31], together with a modulation of cytokine released by macrophages that have engulfed these cells. We also showed that a deficiency of CRT induces contrasting effects on cytokine release by THP-1 macrophages, by increasing interleukin (IL)-6 and monocyte chemotactic protein 1/CCL2 and decreasing IL-8. Remarkably, these effects were greatly reduced when apoptotic cells were opsonized by C1q, which counterbalanced the effect of CRT deficiency. Most notably, these data emphasize the dual role of C1q on uptake and on signaling events during the elimination of apoptotic cells and highlight the crucial role of C1q in tissue homeostasis in controlling the inflammatory phagocyte status. However, the molecular mechanisms that control this effect remain to be fully elucidated. Interestingly, C1q and CRT share PS as a common ligand, itself characterized as a signal involved in the non-immunogenic handling of apoptotic cells. Other serum PS binding molecules have been shown to regulate C1q function such as factor H and beta 2-glycoprotein 1 [38, 83]. Annexins (II, V) which are PS binding molecules were also reported to interact with C1q [39]. All these observations suggest that these immunomodulatory proteins could interact inside complexes involving both faces of the phagocytic synapse.

5. How C1q interactions modulate its function

As stated above, both cC1q and gC1q can interact with different molecular partners, which modulate C1q function. For example, the C1q classical function is mediated by the cC1q-associated C1r and C1s proteases, whereas the cC1q interaction with the phagocyte endocytic receptor CRT-CD91(LRP1) is believed to occur only in the absence of the proteases. More intriguingly, the gC1q domain has the ability to recognize a wide variety of ligands, but the resulting gC1q-mediated C1 binding does not automatically activate the complement pathway as illustrated in figure 3 [21, 23, 24]. How can the nature of the ligand-binding surface modulate such an activation? We can very briefly sum up a current hypothesis as follows: the catalytic domain of the C1r protease lies inside the C1q cone in a 'resting dimeric' configuration preventing its spontaneous auto-activation, as strongly suggested by the combined use of electron microscopy and X-ray structural studies [84]. Therefore, a mechanical signal (or heating) is required to trigger C1 activation [23, 24, 85]). The mobile collagen stems of the C1q molecule can accommodate different positions and transmit molecular distortions to the proteases when binding to a target surface (Fig. 3). The magnitude of the corresponding mechanical stress will vary according to the nature and relative position of the molecular motifs on the surface; it will also be influenced by the position of its binding site on gC1q [29].

Figure 3. Differential C1 activation in response to various binding surfaces. A) Differential C1 activation by IgG-containing immune complexes, heparin and DNA (from Garlatti *et al.* 2010 [29]). The non-immune self-ligands DNA and heparin induce almost no complement activation in the presence of physiological concentrations of C1-inhibitor. B) Schematic molecular interpretation in terms of differential activating mechanical stress induced by C1q binding. C) Larger view of the C1 model showing the positions of C1q flexible hinges, of the inner C1r dimeric catalytic domains, and of the proposed immune complex binding site on the B chain (IC) and the non-immune self-ligand binding site on the C chain (ni).

6. C1q receptors on phagocytes

The identity of C1q receptors and their role in C1q-dependent efferocytosis have been a matter of controversy for many years and are still not fully elucidated. These receptors include cC1qR, gC1qR, C1qRp, CR1, the $\alpha 2\beta 1$ integrin and CD91, and several new receptors described during the past two years, including RAGE, CR3, DC-SIGN and LAIR-1. As will be seen below, certain of these receptors interact with each other and the interaction with C1q might involve ternary complexes.

Paradoxically, the first two receptors identified for C1q collagen-like and globular regions (**cC1qR**/collectin receptor and **gC1qR**, respectively) [86, 87] turned out to be multifunctional, multi-compartmental proteins normally present in the endoplasmic reticulum (cC1qR/calreticulin/CRT) and in mitochondria (gC1qR/p33), but detected at the surface of a wide variety of cells. Since both proteins neither have transmembrane domain nor membrane anchor, they likely function as co-receptors in association with cell surface transmembrane proteins such as CD91/LRP1 (CRT) and DC-SIGN (gC1qR), which have recently been shown themselves to bind C1q [76, 88]. Both gC1qR and cC1qR have also been proposed to form a signaling complex with integrin $\beta 1$ on endothelial cells [89], but the possible binding of C1q to this complex was not investigated.

Two other receptors were described later, a receptor called **C1qRp** (C1q receptor that enhances phagocytosis) [90], further identified as the C-type lectin receptor CD93, and complement receptor 1 (CR1/CD35) [91]. It should be mentioned that CD93 is not considered any more as a C1q receptor due to the lack of direct interaction of the protein with C1q [92]. **CR1**, also called the immune adherence receptor, was initially identified as a receptor for complement fragments C4b and C3b, acting as a regulator of complement activation. CR1 is expressed on numerous cells, including erythrocytes, eosinophils, neutrophils, monocytes, macrophages, B-lymphocytes, some T cells subsets, follicular dendritic cells and glomerular podocytes (reviewed in [93]). CR1 on red blood cells (ECR1) has been shown to transport complement-tagged particles and immune complexes to the spleen and the liver for phagocytosis by resident macrophages. A marked decline in ECR1 has been observed in autoimmune diseases such as SLE [94]. CR1 was also identified as a receptor for C1q and other defense collagens such as MBL and ficolins [91, 95, 96]. However the involvement of C1q-CR1 interaction in phagocytosis has not been demonstrated so far. This receptor is a multi-modular type I membrane glycoprotein composed of an extracellular stretch of 30 complement control protein (CCP) modules, organized into four long homologous repeats (LHR-A, -B, -C and -D) of 7 CCPs each, a transmembrane domain and a short cytoplasmic tail (43 amino acids). The cytoplasmic tail of CR1 contains two PDZ (postsynaptic density protein, Drosophila disc large tumor suppressor and zonula occludens-1) motifs, allowing interaction of ECR1 with Fas-associated phosphatase 1, a scaffolding protein with tyrosine phosphatase activity [97]. LHR-A contains the major C4b binding site whereas LHR-B and LHR-C contain homologous C4b/C3b binding sites. C1q, MBL and ficolin-2 have been shown to bind LHR-D and their interaction with CR1 proposed to involve major ionic interactions between their collagen stalks and CCP24 and/or CCP25 of CR1 ([96] and unpublished data).

Integrin $\alpha 2\beta 1$ is known as a receptor for extracellular matrix components, including collagen, and is involved in inflammation and immunity (for a review on $\beta 1$ integrins [98]. It is expressed on many cell types such as epithelial cells, endothelial cells, fibroblasts and hematopoietic cells, including platelets and specific subsets of leukocytes [99]. This integrin has been shown to interact with C1q and other members of the collectin family such as MBL and surfactant protein-A. The C1q- $\alpha 2\beta 1$ interaction has been suggested to play a role in mast cell activation and cytokine secretion [99, 100]. However, its role on other cell types and its potential function in ACs clearance have not been investigated yet. Integrin $\alpha 2\beta 1$ has been shown to interact with calreticulin at the surface of various cells, including platelets [101]. It has been proposed to interact with the collagen-like part of C1q and collectins through its α_2 inserted (I) domain, which is involved in collagen binding [100].

CD91/LRP1 (or α_2 -macroglobulin receptor) is a multifunctional endocytic and cell-signaling receptor of the low density lipoprotein (LDL) receptor family, expressed on both professional (macrophages and dendritic cells) and non-professional phagocytes (epithelial, endothelial cells, fibroblasts, microglia...). It is composed of two non-covalently linked α (85 kDa, C-terminal, membrane-spanning) and β (515 kDa, N-terminal, extracellular) chains. The latter is involved in binding to a wide variety of ligands, including lipoproteins, extracellular matrix proteins or protease/inhibitor complexes [102]. The binding versatility is supported by the modular structure of LRP1 composed of four ligand-binding clusters of LDL receptor type A (LA, also called complement-like) repeats, β -propellers (YWTD repeats) and epidermal growth factor (EGF) modules. Binding of the various ligands was shown to involve mainly clusters II and IV. The cytoplasmic tail of LRP1 consists of 100 amino acids and exhibits diverse potential endocytosis and signaling motifs, including two NPXY motifs, one YXXL motif and two dileucine motifs. It has been shown to interact with GULP, an adaptor protein involved in engulfment of apoptotic cells.

LRP1 was proposed to use CRT as a coreceptor for engulfment of C1q-opsonized targets, but also to serve as a docking platform for CRT-dependent recognition of dying cells by C1q. However, it has been reported that LRP1 is not always required for C1q-dependent enhancement of AC phagocytosis, suggesting involvement of other C1q receptors [103]. In addition, no direct interaction between isolated LRP1 and CRT has been reported yet while a direct interaction between the LRP1 and C1q molecules has been described, which involves both the collagen-like stalks and the globular heads of C1q [88]. This indicates that the role of LRP1 and CRT in C1q-dependent efferocytosis is more complex than anticipated and needs further clarification.

SREC-I (scavenger receptor (SR) expressed by endothelial cells I, also called **SCARF1** or **SR-F1**) is a class F type scavenger receptor expressed on endothelial cells, macrophages and dendritic cells, that binds various ligands including modified LDL, heat-shock proteins and fungal pathogens. It is characterized by an extracellular domain composed of 6-7 EGF/EGF-like modules, a transmembrane domain and a long (388 amino acids) C-terminal cytoplasmic tail containing serine/proline and glycine rich segments. SREC-I has been shown very recently to play a crucial role in the recognition and engulfment of ACs and in preventing autoimmunity. Most strikingly, this function is mediated through SREC-I interaction with C1q bound

to PS exposed at the surface of AC [51]. This C1q-SREC-I efferocytosis pathway, although not fully non-redundant, contributed to AC clearance up to 70%, depending on the phagocyte type, again suggesting involvement of different pathways, depending on cell types and tissue environment. Interestingly, SREC-I has been described as an endocytic receptor for CRT [104], but no interaction was detected recently using isolated proteins [51]. SREC-I has also been shown to trans-interact through its ectodomain with SREC-II, a related type F SR [105], that however does not recognize typical SR ligands and is not involved in C1q-dependent efferocytosis [51]. Identification of protein domains and amino acid residues critical in C1q-SREC-I interaction, the potential existence of trimolecular complexes with CRT, and the signaling pathway triggered by SREC-I binding to C1q-opsonized ACs, remain to be investigated at both molecular and functional levels.

RAGE (receptor for advanced glycation end-products (AGEs) or **SR-J**) is a 45 kDa receptor of the immunoglobulin (Ig) superfamily comprising an extracellular domain of 3 Ig-like domains (V, C1 and C2 type), a transmembrane domain and a short C-terminal cytoplasmic tail (41 amino acids). RAGE oligomerization is believed to play a role in ligand recognition and signal transduction, which is mediated through association with cytoplasmic adaptor proteins [106]. RAGE is expressed on monocytes, macrophages and dendritic cells, and acts as a pattern recognition receptor for endogenous danger signals including AGEs, high mobility group box 1 (HMGB1), β -amyloid fibrils, S-100 protein and DNA, known to play a pathogenic role in chronic inflammatory diseases [107]. It has also been shown to participate in efferocytosis by acting as a PS receptor [108, 109] and proposed to contribute to the resolution of inflammation. This dual “friend or foe” role appears to depend on the cell context and environment [107]. Of note, soluble forms of RAGE (sRAGE), produced by alternative mRNA splicing or ectodomain shedding, were shown to act as decoys for RAGE ligands [110]. RAGE was shown recently to bind to the globular heads of C1q and to enhance C1q-mediated phagocytosis of ACs, a process suggested to involve formation of a receptor complex with **CR3** (see next paragraph) [111]. The RAGE domain and the amino acid residues of both partners participating in the receptor-C1q interaction remain to be determined. Interestingly, RAGE has been shown recently to bind collagen *in vitro* [112].

The phagocytic receptor **CR3** (alias Mac-1, integrin $\alpha_M\beta_2$, CD11bCD18) is an integrin expressed on most immune cells including neutrophils, dendritic cells and macrophages. It binds to a wide range of ligands including complement C3 fragments C3b/iC3b, extracellular matrix proteins, adhesion molecules (ICAM-1 and -2) and microbial motifs, most of them binding to the inserted (I) domain of the α chain. CR3 inside-out activation leads to a high affinity ligand binding state (extended conformation) allowing target uptake and triggering phagocytosis and signaling [113]. CR3 has been shown to interact extracellularly with other C1q receptors including LRP1 [114] and RAGE [111]. In addition, a direct interaction between CR3 and C1q was reported recently [111]. The receptor and C1q domains involved in the interaction are not known. Interestingly, CR3 has also been shown recently to bind collagens *in vitro* [115].

DC-SIGN (DC-specific intercellular adhesion molecule (ICAM)-3 grabbing non integrin/CD209) is a C-type lectin receptor with a short N-terminal cytoplasmic tail (37 amino acids) containing recycling and internalization motifs, a transmembrane domain and a C-terminal

extracellular domain comprising an extended tetrameric neck region surmounted by a cluster of 4 Ca^{2+} -dependent carbohydrate recognition domains (CRDs). It is expressed by DCs and specific macrophage populations and is a receptor involved in multiple functions including adhesion, pathogen recognition and antigen presentation [116]. Interactions between neutrophils and DCs are mediated through interaction of DC-SIGN with CR3 [117]. DC-SIGN was reported recently to bind C1q, and the interaction proposed to involve the IgG binding site of C1q globular heads and the Ca^{2+} -binding pocket of the lectin domain of DC-SIGN [76]. In addition, C1q and gC1qR were shown to associate with DC-SIGN on the surface of immature DCs and to regulate DC differentiation and function. Interestingly, SIGN-R1, a DC-SIGN homolog expressed on mouse splenic marginal zone macrophages, was shown recently to enhance ACs clearance through interaction with C1q and subsequent complement activation [118]. However, the potential capacity of DC-SIGN to activate the classical complement pathway remains to be investigated.

LAIR-1 (leukocyte-associated immunoglobulin-like receptor-1/CD305) is part of the family of inhibitory immune receptors and is expressed at the surface of both myeloid and lymphoid immune cells. LAIR-1 contains a single extracellular C2-type Ig-like domain, a transmembrane domain and a cytoplasmic tail of 101 amino acids with two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) (reviewed in [119]). When phosphorylated, ITIM motifs can bind the SH2 domain of several SH2-containing phosphatases, leading to down-regulation of immune cell activation. LAIR-1 is a receptor for membrane and matrix collagens and its interaction with collagens is dependent on the presence of hydroxyproline residues [120]. LAIR-1 and its soluble homologue LAIR-2 have been shown to interact with several defense collagens, including C1q, MBL and surfactant protein D through their collagen-like regions [78, 121, 122]. LAIR-2 has been reported to inhibit the classical and MBL-dependent lectin complement pathways [121]. LAIR-1 engagement by C1q has been shown to trigger phosphorylation of LAIR-1 ITIMs in monocytes, which likely represents a mechanism involved in maintaining immunological tolerance [78, 123].

It has been classically considered that C1q binds to the apoptotic cell surface through its globular heads, which leaves the collagen-like stems available for interaction with the C1q receptor at the surface of phagocytes. As mentioned above, recently published results challenge this hypothesis since (i) LRP1 and CRT bind both functional C1q regions and may be present on both AC and phagocyte surfaces and, (ii) two recently identified C1q receptors, RAGE and DC-SIGN, bind the globular regions of C1q. Therefore alternative hypotheses about C1q orientation in the phagocytic synapse should be investigated, taking into account the hexameric structure of C1q, possibly allowing simultaneous interaction of the globular heads with both AC and phagocyte surfaces. Since many receptors seem to function as macromolecular complexes, simultaneous interaction of the C1q molecule with two receptors at the phagocyte surface may also be envisaged, as proposed for the globular heads of C1q within the gC1qR/C1q/DC-SIGN complex [76].

Another important feature of C1q receptors lies in the existence of soluble counterparts, that may arise from alternative gene splicing (soluble RAGE variants, DC-SIGN isoforms), as a result of gene duplication (LAIR-2), or after receptor proteolytic shedding from the membrane

(as reported for LRP1 and CR1). These soluble proteins might act as decoy receptors by competing with membrane-bound receptors for ligand binding and preventing subsequent signal transmission, thereby playing a role in regulation of C1q-dependent clearance of altered self.

Names (CD number)	Receptor type	Ectodomain composition	C1q specificity	Main self ligands /partners	Functional relevance to autoimmunity
gC1qR, C1qBP, p33, p32	Mitochondrial protein	"doughnut-like" trimer	gC1q	DC-SIGN, CRT β1 integrin	Gene deletion in mouse: lethality
cC1qR, calreticulin, CRT	ER lectin-like chaperone	Lectin-like + "arm"	gC1q cC1q	MBL, ficolins, CD91	Gene deletion in mouse: lethality
CR1 (CD35)	Regulator of complement activation	4 long homologous repeats of CCP modules	?	C3b, C4b MBL, ficolins	Marked decline in CR1 observed on erythrocytes from SLE patients
LRP1 α2M receptor (CD91)	LDL receptor	4 clusters of LA, WTD and EGF-like modules	gC1q cC1q	Lipoproteins, ECM proteins, CRT, CR3, protease-inhibitor complexes	MΦ and DCs selective LRP1 KO mice: impaired efferocytosis
α2β1 (CD49bCD29)	β1 integrin	integrin	cC1q gC1q?	Collagen, laminin MBL, SP-A, CRT	KO mice: profound defect in the innate immune response
SREC-I, SCARF1, SR-F	Scavenger receptor	6/7 EGF-like modules	?	oxLDL, HSP CRT	KO mice: autoimmunity, deficient AC uptake
RAGE, SR-J	Scavenger receptor	3 IgG-like modules (V-C1-C2)	gC1q	HMGB1, AGE, PS, CR3	KO mice: deficient AC uptake
CR3, Mac1, αMβ2 (CD11bCD18)	β2 integrin	integrin	?	iC3b, sRAGE, LRP1, collagen	Reduced efferocytosis in CD11b-lupus associated variant
DC-SIGN (CD209)	C-type lectin receptor	Tetramer; neck + C-lectin domain	gC1q cC1q?	ICAM-3, gC1qR	SIGNR1 KO mice: delayed AC clearance
LAIR-1 (CD305)	Inhibitory immune receptor	Ig-like domain (C2)	cC1q gC1q?	MBL, SP-D, collagens	defective expression and function in B lymphocytes from SLE patients

Table 2. C1q receptors on phagocytes

7. C1q in autoimmune disease

Numerous studies suggest that C1q has a protective role in lupus erythematosus, an autoimmune disease resulting largely in dysregulation of the adaptive immunity, i.e; loss of tolerance and generation of autoreactive T-cells [124]. Other observations, however, argue in favor of a facilitating role of C1q in SLE (systemic lupus erythematosus) correlating with tissue damage due to complement activation. Indeed, the complement cascade generates pro-inflammatory products such as C3a and C5a but could also contribute to acquired-C1q deficiency. These two hypotheses are not exclusive and in any case this highlights the pivotal function of C1q at the interface of the innate and adaptive immunity.

It is well established that complete genetic deficiency of C1q is the strongest risk factor for development of SLE [125]. More than 90 % of individuals who are homozygous for a mutation impairing the expression of one of the *C1Q* genes (A, B or C) developed severe clinical manifestations common for SLE (cutaneous lupus and photosensitivity, nephritis). It was also reported that partial genetic deficiency, subtle genetic variations (e.g. point mutations that could modify the assembly or function of the C1q molecule) or auto antibodies against C1q [126-128] and to the C1q receptor CRT are associated with SLE [129, 130]. Importantly, targeting genes for C1q in mice resulted in disease susceptibility similar to that observed in human, even if the mice genetic background was shown to contribute to the disease [125, 131, 132]. Of note, deficiencies of the early components of the classical complement pathway predispose to SLE in a hierarchical fashion (C1q>C1rs>C4->C2). In brief, this indicates that C1q deficiency is the most important factor whereas C3 is not critical. A feature that could link the C1q deficiency to the adaptive immune response is its role in prevention of immune precipitation. C1q is essential in keeping antigen-antibody complexes soluble and for their removal from the circulation through the CR1 receptor, thereby preventing inflammatory tissue injury [133]. Another C1q function of significance in autoimmune processes likely resides in its involvement in the removal of apoptotic cells. In agreement with this hypothesis, C1q-deficient mice developed a proliferative glomerulonephritis associated with the presence of multiple apoptotic cell bodies, and apoptotic cells are also observed in lymph node germinal centers in some SLE patients with severe disease [134]. Accordingly, most of the relevant autoantigens in SLE, which are nuclear and cytoplasmic molecules [135], are also found at the surface of apoptotic cells or blebs [136]. As previously mentioned, it is interesting to note that among the autoantigens in various forms of lupus are calreticulin, an "Eat-me" signal recognized by the gC1q domain also acting as a cC1q receptor on phagocyte, or C1q itself when bound to apoptotic surfaces.

The other major effect of C1q modulation of immune responses concerns its function in regulating cells from the immune system, independently from the binding to apoptotic bodies and the uptake mechanism. This includes the role of C1q on the function of the neutrophil. In particular, calreticulin is released from activated neutrophils [137] and, as it was previously shown that C1q-CRT interaction modulates the cytokine release by macrophages, it might be hypothesized that this could interfere with neutrophil-mediated inflammatory processes. Of interest, we have shown that CRT recognizes proteinase 3 (PR3), a member of the family of

neutrophil-derived serine proteases, released from azurophilic granules but also present in secretory vesicles and membrane-exposed on viable and apoptotic neutrophils [138]. PR3 is a pro-inflammatory factor whose membrane expression can potentiate chronic inflammatory diseases such as anti-neutrophil cytoplasmic antibodies systemic vasculitis (AAV) or rheumatoid arthritis [139]. In addition, the role of C1q on the biology of the monocyte-derived cells and particularly DCs is probably of primary importance [140], because the presence of C1q influences the DC and macrophage responses mainly in a “tolerogenic” way [46, 141]. The interaction of C1q with B and T cells [142-144], which modulates cell proliferation, has also been shown to participate both in the activation and inhibition of T cells, and in the negative selection of B autoreactive-cells through mechanisms that remain to be elucidated.

8. How manipulating C1q could help autoimmune disease treatment, hopes and difficulties

Undoubtedly, the aforementioned observations strongly highlight that C1q has a central role in maintaining tissue homeostasis. In this respect, the fact that complement deficiencies, including C1q, are relatively rare according to national and supranational registries (1 to 10 % of all primary immunodeficiencies) [145] together with the observation that an abnormal C1q content generally induces autoimmunity, is one element that also argues for a critical role of this protein. In our opinion, its function in efferocytosis by helping the rapid elimination of cell debris together with its impact on the response developed by the phagocyte makes it a prime target for resolving autoimmune diseases. Indeed, it can be hypothesized that restoring an efficient engulfment of apoptotic cells would decrease the number of not properly cleared dead cells and thus diminish the release of intracellular autoantigens which are strongly correlated to chronic systemic autoimmune disease. Accordingly, apoptotic cell-based therapy has been developed to limit the graft rejection in transplantation [146]. Because all defects in apoptotic cell clearance are not systematically related to immune disease (CD14 or MBL deficiencies for example) [147, 148], this stresses the dual function of C1q in the uptake process and in modulating the phagocyte signaling response in a tolerogenic way, thus reinforcing its therapeutic potential.

However the fact that C1q reacts with a large spectrum of molecules and membrane receptors which will likely induce multiple, possibly antagonist functions adds complexity for its potential use in therapy. A 2001 pilot study including 8 patients suggested that SLE might be treated successfully by immunoadsorption of plasma with a C1q column [149]. The parameters of SLE clinical disease activity improved upon removing from the circulation molecules that bind to and consume C1q, i. e. immune complexes, anti-C1q autoantibodies and inflammatory proteins such as pentraxins. More recently, administration of plasma in a SLE patient with complete C1q deficiency has been therapeutically successful [150] with reestablishment of complement haemolytic activity and absence of anti-C1q antibodies, suggesting that targeting C1q/C1q pathway could be a valid therapeutic option.

The fact remains that a better understanding of the molecular details that control the C1q interaction with its targets is a primary need. One major breakthrough for future strategies was clearly the recent success in production of a recombinant full length C1q molecule [151]. This now allows the production of mutated forms of the molecule to map the amino acid residues involved in the recognition of its different targets and receptors. Importantly, this opens the way to produce C1q variants devoid of a particular interaction or sensing property, as exemplified by the lysines B61 and C58 mutants of the collagen stalks which are both unable to trigger complement activation but retain other C1q binding functions. Recombinant C1q devoid of binding capacity for antibodies recognition motifs could represent an efficient tool to disconnect C1q binding to apoptotic bodies from pathways involved in pathogen recognition or in the clearance of immune complexes. Mutated forms targeting binding to specific receptors such as LRP1/CD91 and CRT, DC-SIGN, SCARF1, gC1qR present on various monocytes-derived cells and DCs would provide powerful tools to modulate their specific functions discussed previously. More than ever, producing engineered C1q paves the way to target efferocytosis in order to gain in or to restore a tolerogenic and efficient elimination of apoptotic cells.

9. Conclusion

Over the past 15 years, the status of the C1q molecule has changed from its classical primary role in the initiation of the classical complement pathway, acting in the first line of the immune defense against pathogens, to **an essential molecule for maintaining tissue homeostasis**, functioning at the crossroad between innate and adaptive immunity. This relatively new finding is undoubtedly one explanation why the primitive C1q status resisted over time. Thanks to an incredible number of studies, not all reported in this chapter, C1q is now known for its involvement in a multiplicity of immunologic functions and other tissue homeostasis-related mechanisms that, when defective, could induce autoimmune and inflammatory diseases as well as cancer. This was emphasized by the discovery of its roles in several steps of the safe clearance of altered-self cells which elicits a global tolerogenic immune response. In our opinion, these properties of C1q arise from its multivalent heterotrimeric structure which supports its amazing ability to recognize and to interpret multiple molecular motifs that, taken individually, would possibly not be sufficient to yield a comprehensive signal. C1q appears to act either as a sensor or a transmitter of subtle changes that must be considered for a balanced appropriate response in numerous physiological processes, including immunity and inflammation.

This remarkable property to interact with a very large number of targets and receptors, involved in various biological processes, is also the major difficulty for its use in therapy. In this respect the recent success in the production of functional full-length recombinant C1q opens new avenues. Indeed, it paves the way for its engineering, first to decipher its molecular mechanisms of action, and second for developing new strategies by specifically targeting one C1q pathway, without undesirable side effects associated with other pathways. **We are only at the beginning of what is possible to imagine or of imagining what is possible.**

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