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The contribution of gC1qR/p33 in infection and inflammation

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Abstract

Human gC1qR/p33 is a multi-compartmental and multi-functional cellular protein expressed on a wide range of tissues and cell types including lymphocytes, endothelial cells, dendritic cells, and platelets. Although originally isolated as a receptor for C1q by virtue of its affinity ($K_d = 15\text{--}50\text{ nM}$), and specificity for the globular heads of this molecule, a large body of evidence has now been accumulated which shows that in addition to C1q, gC1qR can serve as a receptor for diverse proinflammatory ligands including proteins of the plasma kinin-forming system, most notably high molecular weight kininogen (HK; $K_d = 9\text{ nM}$). In addition, gC1qR has been reported to recognize and bind a number of functional antigens of viral and bacterial origin. It is its ability to interact with microbial antigens and its potential to serve as a cellular protein for bacterial attachment and/or entry that has been the focus of our laboratory in the past few years. On the surface of activated platelets, gC1qR has been shown to serve as a binding site for *S. aureus* and this binding is mediated by protein A. Since the binding of *S. aureus* to platelets is postulated to play a major role in the pathogenesis of endocarditis, gC1qR may provide a suitable surface for the initial adhesion of the bacterium. Recent data also demonstrate that the exosporium of *B. cereus*, a member of a genus of aerobic, gram-positive, spore-forming rod-like bacilli, which includes the deadly *B. anthracis*, contains a binding site for gC1qR. Therefore, by virtue of its ability to recognize plasma proteins such as C1q and HK, as well as bacterial and viral antigens, cell surface gC1qR not only is able to generate proinflammatory byproducts from the complement and kinin/kallikrein systems, but also can be an efficient vehicle and platform for a plethora of pathogenic microorganisms.

Keywords

gC1qR; infection; inflammation

Introduction

The receptor for the globular heads of C1q, gC1qR/p33, is a ubiquitous and highly anionic cellular protein of 33 kDa that was initially identified and characterized as a receptor for the globular heads of C1q (gC1q) (Ghebrehiwet et al., 1994; Ghebrehiwet and Peerschke, 1998; Ghebrehiwet et al., 2001). Known alternatively as p33, and sometimes as p32 or HABP-1 (hyaluronic acid binding protein-1), it is also - and in fact predominantly - a protein of the mitochondrial matrix. In addition, however, it is distributed in several other cellular compartments, including the ER, the nucleus, and on the cell surface (Ghebrehiwet et al., 1994; Braun et al., 2000; Kittleson et al., 2000; Mahdi et al., 2001; Mahdi et al., 2002). However,

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it is its expression and role on the cell surface that has been the major focus of our laboratory for the past 10 years (Ghebrehiwet et al., 2001).

Although gC1qR is synthesized as a pre-protein of 282 amino acid residues, the mature protein isolated from the cell surface corresponds to residues 74–282, which in turn is identical with the mitochondrial protein (Ghebrehiwet et al., 1994). The 73-aa pre-sequence that is cleaved off to generate the mature protein not only has a possible signal sequence at its N terminus, MLPLLRVCVPRVLG, which might direct the protein to the secretory pathway, but also contains a mitochondrial targeting sequence (MTS). The latter not only can be inferred from computer assessment of secondary structure (the program MitoProt returns $P = 0.97$ for the probability of an MTS in the 1–73 pre-sequence), but has also been demonstrated experimentally by fusing the pre-sequence to green fluorescent protein and demonstrating targeting to the mitochondria (Dedio et al., 1998). The cleavage at Ser73 that forms the mature protein, in the sequence CGSLHT, is the same for mitochondrial and cell-surface gC1qR. Although the evidence is far from conclusive, the implication is that gC1qR expressed on the cell surface is in part processed by the same pathway as the intracellular protein (van Leuwen and O'Hare, 2001). Possible mechanisms by which gC1qR may be retargeted from the predominant mitochondrial pathway to other sites, and the cellular site(s) of precursor cleavage in these cases have been recently explored (van Leuwen and O'Hare, 2001).

Mature gC1qR is extremely acidic with a calculated pI of 4.15. It has one Cys at residue 186 and thus does not have any intrachain-disulfide bonding (Ghebrehiwet and Peerschke, 1998; Ghebrehiwet et al., 2001). It does not dimerize by inter-chain disulfide bonding either: on SDS-PAGE, it migrates as a 33-kDa band under both reducing and non-reducing conditions. However, it behaves as a trimer on gel filtration in non-dissociating conditions (Ghebrehiwet et al., 1994). The noncovalent trimeric structure has since been confirmed by X-ray crystallography (Jiang et al., 1999). The majority of the structure of gC1q-R (p32, p33) has been determined at 2.25 Å resolution (Jiang et al., 1999). The PDB ID is 1P32, available at www.rcsb.org/pdb. Except for the L→M mutation introduced at the N-terminal position 74 for expression purposes, the molecule is identical with the mature form of gC1qR (residues 74–282). The structure is a trimeric doughnut of three identical chains with a partially covered channel of about 10 Å (Jiang et al., 1999). Evidence from our laboratory also suggests that multimer formation is likely a critical process that increases the affinity of gC1qR for multivalent ligands such as C1q and HK (Ghebrehiwet et al., 1998; Ghebrehiwet et al., 2001) and HK (Joseph et al., 1996; Herwald et al., 1996). The gC1qR amino acid sequence contains three consensus N-glycosylation sites at residues 114 (NGT), 136 (NNS), and 223 (NYT) that are utilized; a PKC phosphorylation site at residue 207; a tyrosine kinase recognition site at position 268; and a myristylation site at position 252. An intriguing feature of the mature gC1qR molecule is that its translated amino acid sequence does not predict the presence of either a transmembrane segment or a consensus site for a GPI anchor (Ghebrehiwet et al., 1994; Ghebrehiwet and Peerschke, 1998; Ghebrehiwet et al., 2001). However the lack of a direct conduit into the cell to transduce a signal is circumvented by its ability to signal by proxy. Recent evidence suggests that at least on endothelial cells, gC1qR might be anchored on the cell surface via glycosaminoglycans (unpublished observations) and upon ligand binding transduces its signal by forming a docking/signaling partnership with transmembrane proteins such as $\beta 1$ integrins (Ghebrehiwet et al., 2001; Feng et al., 2002). Since the biological responses mediated by C1q vary from cell to cell (e.g. inhibition of T cells proliferation; endothelial cell activation etc.), the signaling partners that induce each response are likely going to be different in each case.

Comparisons of the cDNA revealed that rat and mouse gC1qR share a degree of identity of 97.6%, whereas both of the rodent sequences are 89.9% identical to the human sequence (Lynch et al., 1997; Lim et al., 1998). The human gene is located on chromosome 17p13.3

(Guo et al., 1997). Hybridization of a full-length gC1qR probe to human genomic DNA digests indicates a simple pattern suggestive of a single copy of the gene in both the human and mouse genomes (Lim et al., 1998). The human and mouse gC1qR genes are essentially similar in their exon/intron organization comprising 6 exons and 5 introns each within a total length of approximately 6 kb, which is spliced into a 1.5 kb mRNA (Guo et al., 1997; Lim et al., 1998; Lim et al., 2001). The first exon encodes the 73-aa pre-sequence, including the putative signal peptide, plus four amino acid residues that comprise the N-terminus of the mature protein (Lim et al., 1998). Crystallography (Jiang et al., 1999) shows that the termini of exons II and VI code for a pair of anti-parallel terminal helices that form the majority of the circumference of the native trimeric doughnut, while the remainder of exon II plus exons III-V code mainly for a highly twisted 7-strand β sheet that makes up the core of the trimeric doughnut (Jiang et al., 1999). The molecule features several conspicuous surface loops that join β strands, and it is largely on these regions we believe some important ligand interactions may occur (Ghebrehiwet et al., 2002). Putative binding sites for the ligands C1q (Ghebrehiwet et al., 1994), and multimeric vitronectin (Lim et al., 1996) have been identified in non-overlapping regions of the domain encoded by exon II, whereas the site for HK has been located in a domain encoded by exon V (Joseph et al., 1996).

Differential cellular localization of gC1qR may dictate its compartment-specific functional role

Although the biological significance of its multi-compartmental distribution is yet to be elucidated, the relevance of gC1qR as an important modulator of ligands both inside and outside the cell is becoming increasingly apparent.

Intracellular gC1qR has been reported to interact specifically with an arginine-rich motif in the C-tails of both hamster α 1B- and α 1D-adrenoreceptors in a manner that controls their expression and cellular localization (Pupo and Minneman, 2003). However, the C-tail of α 1A adrenoreceptor, which lacks a similar motif, was unable to bind gC1qR (Pupo and Minneman, 2003). Similarly, employing the yeast two-hybrid analysis method, precipitation assays using glutathione-S-transferase fusion proteins, and reciprocal immunoprecipitations, it was shown that gC1qR specifically binds to protein kinase μ (PKC μ) (Storz et al., 2000). However, while gC1qR binds to the kinase domain of PKC μ , it does not serve as a substrate (Storz et al., 2000). On the basis of these findings, it was proposed that gC1qR is part of an intracellular receptor that retains PKC μ at intracellular compartments such as mitochondria, and serves as a regulator of its kinase activity (Storz et al., 2000).

In other studies, it was demonstrated that the C-terminal cytoplasmic tail of membrane type-1 metalloproteinase (MT1-MMP), a key enzyme primarily recruited to the leading edge of migrating tumor cells, binds to gC1qR (Rozanov et al., 2002). Although a direct functional link between these two proteins remains to be investigated more thoroughly, this observation suggests that the transient association of gC1qR with the cytoplasmic tail of MT1-MMP is likely to be involved in the mechanisms regulating presentation of the protease at the tumor cell surface where gC1qR expression has been shown to be enhanced in a tumor cell-specific manner (Rubinstein et al., 2004).

The localization of gC1qR gene to chromosome 17p13.3 (Guo et al., 1997), where several tumor suppressor genes deleted in ovarian cancer - distinct from Tp53 - are also located had also prompted us to question whether gC1qR plays a role in carcinogenesis. This hypothesis was put to the test by experiments in which a combinatorial immunoglobulin (Ig) library of 10^{10} clones was first generated from the cDNA of primary breast adenocarcinoma cells (Rubinstein et al., 2004). Following subtractive panning, the library was enriched for Ig (Fab fragment) binding to intact adenocarcinoma cells and the resultant Fab was screened against

a cDNA expression library generated from breast cancer cells. Using this approach, clones were isolated from the cDNA library expressing gC1qR. Sequencing of the gene encoding tumor-associated gC1qR did not reveal any consistent tumor-specific mutations (Rubinstein et al., 2004). However, histochemical staining with anti-gC1qR monoclonal antibody demonstrated marked differential expression of gC1qR in thyroid, colon, pancreatic, gastric, esophageal, and lung adenocarcinomas as compared to non-malignant histologic counterparts (Rubinstein et al., 2004). In contrast, differential expression was not seen in endometrial, renal, and prostate carcinomas. Although gC1qR is expressed in non-malignant breast tissue, its expression increased significantly in breast carcinoma (Rubinstein et al., 2004). The precise relationship of gC1qR to carcinogenesis is as yet unclear. However, the finding of tumor overexpression in this study and the known multivalent binding of gC1qR to not only C1q but also to a variety of circulating plasma proteins, and involvement in cell-to-cell interactions suggest that gC1qR may have a role in tumor metastases and potentially serve in molecule-specific targeting of malignant cells (Rubinstein et al., 2004).

Cell surface gC1qR has also been shown to provide a site for *L. monocytogenes*, a gram-positive bacterial pathogen responsible for severe food-borne, opportunistic infections especially in immunocompromised humans and animals (Braun et al., 2000; Niemann et al., 2004; Pizzaro-Cerdà et al., 2004). A process in which non-phagocytic cells are converted to become phagocytic by activation of signaling pathways mediates entry of listeria into cells. This process in turn, is mediated by interaction between two closely related bacterial surface proteins or virulence factors designated Internalin A (InlA) and InlB with molecules on the surface of the target cell (Braun et al., 2000; Pizzaro-Cerdà et al., 2004). While InlA binds to the adhesion molecule E-cadherin to promote invasion of enterocytes in crossing the intestinal barrier, InlB is postulated to play a significant role in the dissemination and infection of a wide range of cells and tissues by binding to and activation of the receptor tyrosine kinase Met (c-Met), which is also the receptor for hepatocyte growth factor (HGF) (Shen et al., 2000; Jonquieres et al., 2001; Marino et al., 2002). However, in addition to c-Met, InlB also binds to gC1qR (Braun et al., 2000) and heparan sulfate proteoglycans (Jonquieres et al., 2001). The interaction of InlB with gC1qR is specific and dose-dependent, and invasion of listeria can be inhibited by either anti-gC1qR or C1q. Furthermore, transfection of a guinea pig cell line that is non-permissive to listeria infection was made to be permissive by transfection with human gC1qR and the uptake involves tyrosine phosphorylation of the adaptor proteins Gab1, Cbl, and Shc and activation of phosphatidylinositol 3-kinase (Braun et al., 2000). The binding site for gC1qR has been localized to the GW (Gly-Trp) modules (Jonquieres et al., 2001; Marino et al., 2002) and addition of gC1qR to listeria leads to the release of InlB from the bacterial cell surface (Marino et al., 2002). Although the exact role of gC1qR in InlB-mediated uptake still needs to be further defined, its ability to induce signaling is likely to involve an as yet unidentified signaling partner on the target cell surface including possibly c-Met itself.

Hepatitis C virus (HCV) is another pathogen that employs gC1qR to subvert the immune response (Kittleson et al., 2000). HCV infection is a serious and growing threat to human health. Its ability to efficiently establish persistent infection is postulated to be in part due to T cell suppression and is mediated via interaction between the HCV core protein and gC1qR (Yao et al., 2003), in a manner that is similar to the C1q-mediated T cell anti proliferative response reported previously (Chen et al., 1994). The specificity of the C1q- and HCV core-mediated T cell suppression was shown by inhibition studies using anti-gC1qR antibodies or by treatment of the T cells with gC1qR-silencing siRNA (Yao et al., 2003). More recently obtained data suggest that the HCV core/gC1qR interaction arrests T cell cycle progression through stabilization of the cell cycle inhibitor p27^{Kip1}, thus blocking activated T cells for the G₁ to S phase transition and inhibiting T cell proliferation (Yao et al., 2003). Additionally, HCV core isolates from chronic patients were shown to bind gC1qR more efficiently and inhibit T cell

proliferation more than their resolved isolates both in humans and chimpanzees (Yao et al., 2005; Yao et al., 2006).

These examples serve to demonstrate the potential regulatory role of intracellular and cell surface gC1qR, and collectively support the postulate that the differential cellular localization of this molecule may dictate or contribute to its compartment-specific function as a regulator of cellular and microbial proteins.

Interaction of gC1qR with microbial antigens

1. *S. aureus*

Evaluation of the biological significance of gC1qR in health and disease will undoubtedly come from *in vivo* studies; preliminary data obtained from the first attempt to study the role of gC1qR *in vivo* seem to presage this conviction.

We had shown previously that protein A-bearing *S. aureus*, but not the protein A-deficient Wood strain, binds to platelet surface gC1qR (Nguyen et al., 2000). *S. aureus* causes a variety of infections in humans including endocarditis, osteomyelitis, wound sepsis, skin abscesses and keratitis (Sullam, 1994). At the cardiac valve surface, the interaction between *S. aureus* and platelets represents a critical event in the induction of infective endocarditis. To investigate the role of platelet gC1qR in the pathogenesis of *S. aureus* endocarditis, we used animal models for infective endocarditis (IE) developed by Arnold S. Bayer's group at UCLA (Peerschke et al., 2006a). In this rat model, a polyethylene catheter is introduced into the left ventricle of Sprague-Dawley rats to produce sterile thrombotic endocarditis (sterile vegetations) on the aortic valves (Peerschke et al., 2006a). Then anti-gC1qR or control antibody is administered intraperitoneally at the time of catheterization and *S. aureus* infective endocarditis (IE) is produced 48 h later by the IV injection of the bacteria (5×10^4 CFU). The results indicate that infusion of mAb 74.5.2 >60.11 significantly reduces the dissemination of and colonization by *S. aureus* of target organs and tissues (aortic valves, kidneys, and spleen) when compared to untreated or species- and isotype-specific IgG controls as assessed by the reduction in the mean log (cfu)/g ($\pm$ SD). For example, while the mean log (cfu)/g in the kidneys of control animals was 5.04 ± 0.64 ($n = 7$), those that received mAb 74.5.2 had 3.53 ± 1.10 ($n = 10$), whereas those that received mAb 60.11 had 3.49 ± 1.07 ($n = 6$). Animals that received a combination of the two antibodies on the other hand had a mean log (cfu)/g of 0.94 ± 0.50 ($n = 5$). As described earlier (Ghebrehwet et al., 2001), mAb 74.5.2 is an IgG 1k, which is directed against gC1qR residues 204–218, and blocks binding of HK to gC1qR on endothelial cells, whereas mAb 60.11 also an IgG 1k is directed against the C1q binding site on gC1qR corresponding to residues 76–93. The inhibitory activity of both antibodies therefore implies that both the complement and kallikrein kinin generating systems may be involved in modulating the dissemination of *S. aureus* to distal target organs.

2. *B. cereus*

B. cereus is another pathogenic bacterium, which binds gC1qR. It is a member of a genus of gram-positive, spore-forming rod-like bacilli, which includes the deadly *B. anthracis*. Previous studies have shown that gC1qR binds to *B. cereus* spores that have been attached to microtiter plates (Panessa-Warren et al., 2002; Tantral et al., 2004). Studies were therefore undertaken, to examine if cell surface gC1qR plays a role in *B. cereus* spore attachment and/or entry. To this end, monolayers of human colon carcinoma (Caco-2) and lung cells were grown to confluency on 6 mm coverslips in shell vials with gentle swirling in a shaker incubator. Then, 2 μ l of a suspension of strain SB460 *B. cereus* spores (3×10^8 /ml, in sterile water), were added and incubated (1–4 h; 36°C) in the presence or absence of anti-gC1qR mAb-carbon nanoloop conjugates (Panessa-Warren et al., 2002). Examination of these cells by EM revealed that not

only did the *B. cereus* spores attach to the Caco-2 or lung cells in a manner that was gC1qR-dependent, but that attachment of the spores coincided with up-regulation of gC1qR surface expression. Furthermore, gC1qR expression was often seen adjacent to the spores in association with microvilli (Caco-2 cells) or cytoskeletal projections (lung cells). Interestingly, the exosporium of activated and germinating spores was often decorated with mAb-nanoloops, suggesting the presence of a gC1qR site on this spore structure. These observations were further corroborated by experiments in which *B. cereus* spores, incubated with whole blood, were readily taken up by monocytes and neutrophils in a manner that was partially inhibited by mAb 60.11. The nature of the gC1qR-binding molecule(s) on *B. cereus* spores has not been identified as yet. However, recent published reports (Sylvestre et al., 2002; Rety et al., 2005) show that the crystal structure of BclA (Bacillus collagen-like protein of *anthracis*), the immunodominant protein of the *B. anthracis* spore surface (Sylvestre et al., 2002), bears remarkable similarity to the C1q/TNF family of mammalian proteins (Rety et al., 2005). Since *B. cereus* and *B. anthracis* are genetically closely related and their respective spores possess an exosporium, it is tempting to speculate that *B. cereus* like *B. anthracis*, might possess a BclA-like molecule. The presence of such a molecule would be predicted to facilitate the attachment of the spore to the cell surface through gC1qR and allow germination and/or entry to proceed.

Although these data collectively suggest that gC1qR is involved in the pathogenesis of microbial infections, the veracity of these observations will have to be ascertained by comparing the results obtained in animal models that are gC1qR deficient with those that are gC1qR sufficient. If gC1qR is indeed involved, then the organs of gC1qR deficient animals should have a profoundly reduced dissemination of bacteria, with reduced or less severe inflammation since gC1qR-mediated complement activation and kinin generation would be abrogated. Such animal studies could also shed light into the pathophysiological relevance of the apparently specific interaction between gC1qR and a diverse group of bacterial (e.g. *B. cereus*, *L. monocytogenes*, *S. aureus*,) and viral (e.g. HIV, Epstein Barr Virus, Hepatitis C Virus, etc.) antigens (reviewed in Ghebrehwet et al., 2001).

Contribution of gC1qR to inflammation

1. Complement activation

Previous studies have shown that native gC1qR purified from Raji cell membranes, or highly purified, and enzyme-free recombinant gC1qR can activate the classical pathway as assessed by hemolytic assay or by solid phase ELISA using gC1qR coated plates and monoclonal anti-C4d to detect activation (Ghebrehwet et al., 1994; Peterson et al., 1997; Ghebrehwet et al., 2006). Subsequent experiments showed that the binding site(s) for gC1qR and IgG on the C1q molecule may be identical or perhaps overlap with each other (Peterson et al., 1997) since preincubating serum with gC1qR had a diminished hemolytic activity when further incubated with antibody sensitized sheep erythrocytes. Moreover, ligand blot analysis demonstrated that not only does gC1qR bind preferentially to the A chain of C1q, but also specifically to a synthetic peptide (residues 155–164) of the A chain postulated to contain a putative site for IgG (Marques et al., 1993). More recently, it was also shown that the surface of activated platelets is able to activate the classical pathway of complement by a process that seems to partially involve gC1qR (Peerschke et al., 2006b). These observations imply that at sites of inflammation, where gC1qR is abundantly expressed both in soluble form and on cell surfaces (Peerschke et al., 2004), it has the potential to activate complement and thus exacerbate the existing inflammatory process through the generation of vasoactive peptides not only from the complement system but additionally from the kallikrein/kinin system (KKS).

2. Bradykinin generation

The assembly of the KKS on the endothelial cell surface requires the presence of highly charged surface structures and zinc. The KKS comprises three proteins: namely factor XII, prekallikrein, and high molecular weight kininogen (HK) that are assembled and activated in a manner that is similar to the activation of the classical pathway of complement (Kaplan, 2004a; Silverberg et al., 1980). First, upon binding to a negatively charged cell surface structure, factor XII undergoes autocatalytic activation resulting in the conversion of factor XII to XIIa (Silverberg et al., 1980). Factor XIIa in turn not only proceeds to generate more Factor XIIa from Factor XII in a positive amplification mechanism, but it also converts prekallikrein to kallikrein and the latter in turn digests HK to generate bradykinin (reviewed in Kaplan, 2004a) (Fig. 1). The second substrate for Factor XIIa in plasma is coagulation factor XI and activation of surface bound factor XI by factor XIIa initiates the intrinsic coagulation pathway (Kaplan, 2004a; Kaplan, 2004b; Silverberg et al., 1980). The assembly and interactions of the four proteins is known as contact activation, and the formation of bradykinin is therefore a cleavage product of the initiating step of the cascade (Kaplan, 2004a).

Key to the assembly of the contact activation proteins is of course the existence of highly charged surface structures that allow the binding of HK and/or factor XII on the endothelial cell surface. What had eluded investigators until very recently is the identification and characterization of the cell surface molecule(s) involved. Earlier experiments have shown that the binding of HK to HUVECs (K_d 40–50 nM) is strictly zinc-dependent (25–50 μ M), saturable, and reversible with an estimated 1×10^6 binding sites/cell (Schmaier et al., 1988; van Iwaarden et al., 1988). Furthermore, these experiments showed that the binding is with both the heavy and light chains of HK (Fig. 1) suggesting that the nature of the binding may require two independent sites (Kaplan, 2004a). Since there is no separate site for prekallikrein, the prekallikrein-HK complex is brought to the endothelial cell surface by virtue of HK binding. When the binding of factor XII was studied, it was found to have binding characteristics strikingly similar to those seen with HK including a similar requirement for zinc (Reddigari et al., 1993a; Reddigari et al., 1993b). This suggested that the binding sites for HK and Factor XII might be in close proximity or even overlapping with each other on the cell surface (Reddigari et al., 1993a).

Three endothelial surface binding sites for HK and factor XII have been identified and described to date. These include gC1qR, the 33 kDa receptor for C1q (Joseph et al., 1996; Herwald et al., 1996), cytokeratin-1 (Hasan et al., 1998; Joseph et al., 1999; Mahdi et al., 2002), and the urokinase plasminogen activator receptor (u-PAR) (Colman et al., 1997). The binding of HK and factor XII to each of these proteins is zinc-dependent, although gC1qR, which binds specifically to the light chain of HK (Kaplan, 2004a), (Fig 2) and not to the heavy chain, may be the most important and predominant site on endothelial cells (Kaplan, 2004a). An HK binding site on gC1qR has been identified on the C-terminal half and corresponds to residues 204–218 (Joseph et al., 1996). Conversely, domain 5 of HK located within the N-terminus of the light chain, and which is rich in histidine and arginine residues, is postulated to contain the site for interaction with gC1qR. Indeed, a 20-amino acid peptide termed HKH20, has been shown to be the site for attachment within domain 5 and this peptide can be used to inhibit the interaction of HK with intact endothelial cells (Hasan et al., 1995). The other site for attachment of HK to endothelial cells is found within domain 3 on the heavy chain, and a peptide containing the binding site, designated LDC27 has been identified although its binding affinity is approximately 100-fold less than the light chain (Kaplan, 2004a; Herwald et al., 1995). Because antibody to gC1qR can immunoprecipitate gC1qR and cytokeratin-1 but not u-PAR, and antibody to u-PAR can immunoprecipitate u-PAR and cytokeratin-1 but not gC1qR, it is postulated that these molecules may be organized on the surface as non-covalently linked and closely associated bimolecular complexes - gC1qR-cytokeratin-1 and u-PAR-

cytokeratin-1 (Joseph et al., 2004;Mahdi et al., 2001). In this configuration, it is postulated that the u-PAR-cytokeratin-1 complex will favour the binding of factor XII, which in turn undergoes an autocatalytic activation process and amplifies the generation of XIIa. The gC1qR-cytokeratin-1 complex on the other hand, would bind the HK-PK bimolecular complex through a high affinity interaction between gC1qR and HK. These interactions are responsible for the assembly of the contact activation on the cell surface thus leading to the generation of bradykinin (Fig. 2).

Bradykinin acts on the B2 receptor (Fig. 2) on the surface of the endothelial cells to cause vasodilation and, this in turn is enhanced by secondary production of vasodilators such as nitric oxide (NO) following stimulation of B2 receptors (Regoli and Barabe, 1980). The formation of bradykinin is generally thought to be responsible for the swelling seen in angioedema but in addition, bradykinin plays a major role in other physiological and pathophysiological processes including hypotension, tumor angiogenesis, and pain (Mori and Nagasawa, 1981;Fields et al., 1983;Greengard and Griffin, 1984;Bhoola et al., 1992). Furthermore, because ACE inhibitors reduce the catabolism of bradykinin thus resulting in increased levels of bradykinin, a major side effect on some patients of ACE-inhibitors is angioedema. ACE inhibitor-mediated angioedema is indistinguishable from hereditary angioedema, with features including swelling of the tongue and potentially lethal laryngeal edema (Erdos and Sloane, 1962;Vleming et al., 1998;Agah et al., 1999;Agostoni et al., 1999;Dean et al., 2001;Molinaro et al., 2002;Tom et al., 2002).

Bradykinin is regulated by digestion with carboxypeptidase N, which removes the C-terminal arginine from bradykinin to yield an eight amino acid peptide, des-arg-9 bradykinin (Sheikh and Kaplan, 1986a;Yang et al., 1967). The second enzyme in plasma, kininase II, on the other hand is concentrated along the pulmonary vascular endothelial cell surface, and is identical to angiotensin-converting enzyme (ACE) (Sheikh and Kaplan, 1986b). This enzyme removes the dipeptide phe-arg from the C-terminus of bradykinin to yield a heptapeptide and a second cleavage removes ser-pro to leave a pentapeptide (Sheikh and Kaplan, 1986b).

Concluding remarks and future outlook

When molecules, especially those that are expressed on the cell surface, have multiple partners, there is always the risk that some of this promiscuity is due to charge-dependent, non-specific interaction and *ergo* has no relevance to any particular biologic function. But we have learned time and again, that the multiplicity of functions associated with one single molecule may in fact be nature's way of ensuring redundancy, especially if the molecule plays a vital role in health and disease. Such a conclusion is far from being warranted, but the data accumulated to date and partly presented here, support the concept that gC1qR is a unique multi-compartmental and multi-functional protein, which plays an important role in infection and inflammation, cancer and autoimmunity. Even the controversy of whether it is or is not on the surface - a controversy that had shrouded its initial discovery - is irrelevant since the molecule is likely to be able to transmit information in both directions across the plasma membrane. What remains to be elucidated is why such an "unconventional" surface molecule has a doughnut shape with remarkably differing surface charges, and how it is attached to the membrane. Furthermore, since the biologic responses and, therefore, signaling mediated by its primary ligands - C1q and HK - will perforce differ from cell to cell, understanding the mechanism involved in partner-recruitment will be crucial. And if therapeutic approaches for the prevention of the pathologic processes mediated by gC1qR are to be attempted, then the veracity of the *in vitro* studies described to date would have to be validated in the future, using suitable animal models in which either the gene is completely deleted or tissue-specific deletion is realized.

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References

- Agah R, Bandi V, Gutnurparlli KK. Angioedema: the role of ACE inhibitors and factors associated with poor clinical outcome. *Intensive Care Med* 1999;23:793–796. [PubMed: 9290997]
- Agostoni A, Cicardi M, Cugno M. Angioedema due to ACE inhibitors. *Immunopharmacol* 1999;15:21–25.
- Bhoola KD, Figueroa CD, Worthy K. Bioregulation of kinins: kallikrein, kininogen and kinases. *Pharmacol Rev* 1992;44:1–80. [PubMed: 1313585]
- Braun L, Ghebrehwet B, Cossart P. gC1q-R/p32, a C1q-binding protein is a novel receptor for *Listeria monocytogenes*. *EMBO J* 2000;19:1458–1466. [PubMed: 10747014]
- Chen A, Gaddipati S, Volkman DJ, Peerschke EIB, Ghebrehwet B. Human T cells possess specific receptors for C1q: role in activation and proliferation. *J Immunol* 1994;153:1430–1440. [PubMed: 8046223]
- Colman RW, Pixley RA, Najamunnisa S, Yan W, Wang J, Mazar A, McRae KR. Binding of high molecular weight kininogen to human umbilical vein endothelial cells is mediated via a site within domains 2 and 3 of the urokinase receptor. *J Clin Invest* 1997;100:1481–1487. [PubMed: 9294114]
- Dean DE, Schultz DL, Powers RH. Asphyxia due to angiotensin converting enzyme (ACE) inhibitor mediated angioedema of the tongue during the treatment of hypertensive heart disease. *J Forensic Sci* 2001;46:1239–1243. [PubMed: 11569573]
- Dedio J, Jahnen-Dechent W, Bachmann M, Muller-Esterl W. The multiligand-binding protein gC1q-R, putative C1q receptor, is a mitochondrial protein. *J Immunol* 1998;160:3534–3542. [PubMed: 9531316]
- Erdo EG, Sloane SM. An enzyme in human plasma that inactivates bradykinin and kallidins. *Biochem Pharmacol* 1962;11:585–592. [PubMed: 13890520]
- Feng X, Tonnesen MG, Peerschke EIB, Ghebrehwet B. Cooperation of C1q receptors and integrins in C1q-mediated endothelial cell adhesion and spreading. *J Immunol* 2002;168:2441–2448. [PubMed: 11859136]
- Fields TR, Kaplan AP, Ghebrehwet B. Kinin formation in hereditary angioedema plasma: Evidence against kinin derivation from C2 and in support of "spontaneous" formation of bradykinin. *J Allergy Clin Immunol* 1983;72:54–60. [PubMed: 6222104]
- Ghebrehwet B, Lim BL, Peerschke EIB, Willis AC, Reid KBM. Isolation cDNA cloning, and overexpression of a 33-kDa cell surface glycoprotein that binds to the globular 'heads' of C1q. *J Exp Med* 1994;179:1809–1821. [PubMed: 8195709]
- Ghebrehwet B, Peerschke EIB. Structure and function of gC1q-R a multiligand binding membrane protein. *Immunobiol* 1998;199:225–238.
- Ghebrehwet B, Lim BL, Kumar R, Feng X, Peerschke EIB. gC1q-R/p33, a member of a new class of multifunctional and multicompartmental cellular proteins is involved in inflammation and infection. *Immunol Rev* 2001;180:65–77. [PubMed: 11414365]
- Ghebrehwet B, Jesty J, Peerschke EIB. gC1q-R/p33: structure-function predictions from the crystal structure. *Immunobiol* 2002;205:421–432.
- Ghebrehwet B, Cebada Mora C, Tantral L, Peerschke EIB. gC1qR/p33 serves as a molecular bridge between the complement and contact systems and is an important catalyst in inflammation. *Current Topics in Complement. Adv Exp Med Biol* 2006;586:95–105. [PubMed: 16893067]
- Greengard JS, Griffin JH. Receptors for high molecular weight kininogen on stimulated washed human platelets. *Biochemistry* 1984;23:6863–6869. [PubMed: 6099145]
- Guo N, Weremowicz S, Lynch N, Schwaeble W, Lim BL, Morton CC, Peerschke EIB, Reid KBM, Ghebrehwet B, Sastry KN. Assignment of gC1q-R, the C1q globular domain binding protein (C1qBP) to human chromosome band 17p13.3 by in situ hybridization. *Cytogen Cell Genet* 1997;77:283–284.

- Hasan AAK, Cines DB, Herwald H, Schmaier AH, Müller-Esterl W. Mapping the cell binding site on high molecular weight kininogen domain 5. *J Biol Chem* 1995;270:19256–119261. [PubMed: 7642598]
- Hasan AAK, Zisman T, Schmaier AH. Identification of cytokeratin 1 as a binding protein and presentation receptor for kininogens on endothelial cells. *Proc Natl Acad Sci USA* 1998;95:3615–3620. [PubMed: 9520414]
- Herwald H, Hasan AAK, Godovac-Zimmermann J, Schmaier AH, Müller-Esterl W. Identification of an endothelial cell binding site on kininogen domain D3. *J Biol Chem* 1995;270:14634–14642. [PubMed: 7540175]
- Herwald H, Dedio J, Kellner R, Loos M, Müller-Esterl W. Isolation and characterization of the kininogen-binding protein p33 from endothelial cells. Identity with the gC1q receptor. *J Biol Chem* 1996;271:13040–13047. [PubMed: 8662673]
- Jiang J, Zhang Y, Krainer A, Xu RM. Crystal structure of p32, a doughnut-shaped acidic mitochondrial matrix protein. *Proc Natl Acad Sci USA* 1999;96:3572–3577. [PubMed: 10097078]
- Jonquieres R, Pizzaro-Cerdà J, Cossart P. Synergy between the N- and C-terminal domains of InlB for efficient invasion of non-phagocytic cells by *Listeria monocytogenes*. *Mol Microbiol* 2001;42:955–965. [PubMed: 11737639]
- Joseph K, Ghebrehiwet B, Peerschke EIB, Reid KBM, Kaplan AP. Identification of the zinc-dependent endothelial cell binding protein for high molecular weight kininogen and factor XII: identity with the receptor which binds to the globular "heads" of C1q (gC1q-R). *Proc Natl Acad Sci USA* 1996;93:8552–8557. [PubMed: 8710908]
- Joseph K, Ghebrehiwet B, Kaplan AP. Cytokeratin-1 and gC1q-R mediate high molecular weight kininogen binding to endothelial cells. *Clin Immunol* 1999;92:246–255. [PubMed: 10479529]
- Joseph K, Tholanikunnel BG, Ghebrehiwet B, Kaplan AP. Interaction of high molecular weight kininogen binding proteins with each other. *J Thromb Haemost* 2004;91:61–70.
- Kaplan, AP. Mechanisms of bradykinin formation. In: Greaves, MW.; Kaplan, AP., editors. *Urticaria and Angioedema*. Marcel Dekker Inc; NY: 2004a. p. 51-72.
- Kaplan, AP. C1 inhibitor deficiency. In: Greaves, MW.; Kaplan, AP., editors. *Urticaria and Angioedema*. Marcel Dekker Inc; NY: 2004b. p. 303-320.
- Kittleson DJ, Chianese-Bullock KA, Yao ZQ, Braciale TJ, Hahn YS. Interaction between complement receptor gC1q-R and hepatitis C virus core protein inhibits T-lymphocyte proliferation. *J Clin Invest* 2000;106:1239–1249. [PubMed: 11086025]
- Lim BL, Preissner K, Ghebrehiwet B, Leigh LEA, Reid KBM. The binding protein for globular "heads" of complement C1q, gC1q-R: Functional expression and characterization as a novel vitronectin binding factor. *J Biol Chem* 1996;271:26739. [PubMed: 8900153]
- Lim BL, White RA, Hummel GS, Mak SC, Schwaeble WJ, Reid KBM, Peerschke EIB, Ghebrehiwet B. Characterization of the murine gene for gC1q-BP (gC1q-R), a novel cell protein that binds the globular heads of C1q, vitronectin, high molecular weight kininogen and factor XII. *Gene* 1998;209:229–237. [PubMed: 9524273]
- Lim BL, Tye AJ, Guo N, Sastry KN, Peerschke EIB, Ghebrehiwet B. The human gC1q-R/p33 gene (C1qBP): Genomic organization and promoter analysis. *J Biol Chem* 2001;276:17069–17075. [PubMed: 11278463]
- Lynch NJ, Reid KBM, van den Berg RH, Daha MR, Leigh LEA, Lim BL, Ghebrehiwet B, Schwaeble WJ. The murine homologues of gC1qBP, a 33 kDa protein that binds to the globular 'heads' of C1q. *FEBS Lett* 1997;418:111–114. [PubMed: 9414106]
- Mahdi F, Shariat-Madar Z, Todd RF III, Figueroa CD, Schmaier AH. Expression and Localization of cytokeratin 1 and urokinase plasminogen activator receptor on endothelial cells. *Blood* 2001;97:2342–2350. [PubMed: 11290596]
- Mahdi F, Shariat-Madar Z, Figueroa CD, Schmaier AH. Factor XII interacts with multiprotein assembly of urokinase plasminogen activator receptor, gC1q-R and cytokeratin 1 on endothelial cell membranes. *Hemost Thromb Vasc Biol* 2002;99:3585–3596.
- Marino M, Banerjee M, Jonquieres R, Cossart P, Ghosh P. GW domains of the *Listeria monocytogenes* invasion protein InlB are SH3-like and mediate binding of host ligands. *EMBO J* 2002;21:5623–5634. [PubMed: 12411480]

- Marques G, Anton LC, Barrio E, Sanchez A, Ruiz S, Gavilanes F, Vivanco F. Arginine residues of the globular regions of human C1q involved in the interaction with immunoglobulin G. *J Biol Chem* 1993;268:10393–10402. [PubMed: 8486696]
- Molinaro G, Cugno M, Perez M. ACE-inhibitor-associated angioedema is characterized by a slower degradation of desarginine(9)-bradykinin. *J Pharmacol Exp Ther* 2002;303:232–237. [PubMed: 12235256]
- Mori K, Nagasawa S. Studies on human high molecular (HMW) kininogen. II Structural changes in HMW kininogen by the action of human plasma kallikrein. *J Biochem* 1981;89:1465–1473. [PubMed: 6168636]
- Nguyen T, Ghebrehwet B, Peerschke EIB. *Staphylococcus aureus* protein A recognizes platelet gC1q-R/p33: A novel mechanism for staphylococcal interactions with platelets. *Infect Immun* 2000;68:2061–2068. [PubMed: 10722602]
- Niemann HH, Schubert WF, Heinz DW. Adhesins and invasins of pathogenic bacteria: a structural view. *Microbes Infect* 2004;6:101–112. [PubMed: 14738899]
- Panessa-Warren, BS.; Wong, S.; Ghebrehwet, B.; Tortora, GT.; Warren, J. Carbon nanotube membrane probes: immuno-labelling by LM, TEM & FESEM. In: Voelkl, E.; Piston, D.; Gauvin, R.; Lockley, A.; Bailey, G.; McKernan, S., editors. *Microscopy and Microanalysis*. 8. Cambridge University Press; NY: 2002. p. 726-727.
- Peerschke EIB, Minta JO, Zhou SZ, Bini A, Gotlieb A, Colman RW, Ghebrehwet B. Expression of gC1qR/p33 and its major ligands in human atherosclerotic lesions. *Molec Immunol* 2004;41:759–766. [PubMed: 15234555]
- Peerschke EI, Bayer AS, Ghebrehwet B, Xiong YQ. gC1qR/p33 blockade reduces *Staphylococcus aureus* colonization of target tissues in an animal model of infective endocarditis. *Infect Immun* 2006a;74:4418–4423. [PubMed: 16861627]
- Peerschke EIB, Yin W, Grigg SE, Ghebrehwet B. Blood platelets activate the classical pathway of human complement. *Thromb Haemost* 2006b;4:2035–2042.
- Peterson K, Zhang W, Lu PD, Keilbaugh SA, Peerschke EI, Ghebrehwet B. The C1q binding membrane proteins cC1q-R and gC1q-R, are released from activated cells. Subcellular localization and immunochemical characterization. *Clin Immunol Immunopathol* 1997;84:17–26. [PubMed: 9191880]
- Pizzaro-Cerdà J, Sousa S, Cossart P. Exploitation of host cell cytoskeleton and signalling during *L. monocytogenes* entry into mammalian cells. *C R Biologies* 2004;327:115–123. [PubMed: 15060982]
- Pupo AS, Minneman KP. Specific interactions between gC1q-R and alpha 1-adrenoreceptor subtypes. *J Recept Signal Transduct Res* 2003;23:185–195. [PubMed: 14626446]
- Reddigari SR, Shibayama Y, Brunnee T, Kaplan AP. Human Hageman factor (factor XII) and high molecular weight kininogen compete for the same binding site on human umbilical vein endothelial cells. *J Biol Chem* 1993a;268:11982–11987. [PubMed: 8505323]
- Reddigari SR, Kuna P, Miragliotta GF, Shibayama Y, Nishikawa K, Kaplan AP. Human high molecular weight kininogen binds to human umbilical vein endothelial cells via its heavy and light chains. *Blood* 1993b;81:1306–1311. [PubMed: 8443392]
- Regoli D, Barabe J. Pharmacology of bradykinin and related kinins. *Pharmacol Rev* 1980;32:1–46. [PubMed: 7015371]
- Rety S, Salamitou S, Garcia-Verdugo I, Hulmes DJS, Le Hagarat F, Chaby R, Lewit-Bentley A. The crystal structure of the *Bacillus anthracis* spore surface protein BclA shows remarkable similarity to mammalian proteins. *J Biol Chem* 2005;280:43073–43078. [PubMed: 16249180]
- Rozanov DV, Ghebrehwet B, Ratnikov BI, Monosov EZ, Deryugina EI, Strongin A. The cytoplasmic tail peptide sequence of membrane type-1 matrix metalloproteinase (MT-MMP) directly binds to gC1q-R, a compartment-specific chaperone-like regulatory protein. *FEBS Lett* 2002;571:51–57. [PubMed: 12220632]
- Rubinstein DB, Stortchevoi A, Boosalis M, Ashfaq R, Ghebrehwet B, Peerschke EIB, Calvo F, Gillaume T. Receptor for the globular heads of C1q (gC1q-R; p33; Hyaluronan-binding protein) is preferentially expressed by adenocarcinoma cells. *Internl Cancer Res* 2004;110:741–750.

- Schmaier AH, Kuo A, Lundberg D, Murray S, Cines DB. Then expression of high molecular weight kininogen on human umbilical vein endothelial cells. *J Biol Chem* 1988;263:16327–16333. [PubMed: 2460446]
- Sheikh IA, Kaplan AP. Studies of the digestion of bradykinin, lysyl bradykinin and des-arg9 bradykinin by angiotensin converting enzyme. *Biochem Pharmacol* 1986a;35:1951–1957. [PubMed: 3013204]
- Sheikh IA, Kaplan AP. Studies of the digestion of bradykinin, lysyl bradykinin and kinin degradation products to carboxypeptidase A, B, and N. *Biochem Pharmacol* 1986b;35:1957–1963. [PubMed: 3718539]
- Shen Y, Naujokas M, Park M, Ireton K. Internalin B internalization of *Listeria* is mediated by Met receptor tyrosine kinase. *Cell* 2000;103:501–510. [PubMed: 11081636]
- Silverberg M, Dunn JT, Garen L, Kaplan AP. Autoactivation of human Hageman factor. *J Biol Chem* 1980;255:7281–7286. [PubMed: 7391081]
- Storz P, Hausser A, Link G, Dedio J, Ghebrehwet B, Pfizenmaier K, Johannes FJ. Protein kinase C μ is regulated by the multifunctional chaperon protein p32. *J Biol Chem* 2000;275:24601–24607. [PubMed: 10831594]
- Sullam PM. Host-pathogen interaction in the development of bacterial endocarditis. *Curr Opin Infect Dis* 1994;7:304–309.
- Sylvestre P, Couture-Tosi E, Mock M. A collagen-like surface glycoprotein is a structural component of the *Bacillus anthracis* exosporium. *Molec Microbiol* 2002;45:169–178. [PubMed: 12100557]
- Tantral L, Panessa-Warren BJ, Tortora GT, Wong SS, Warren JB, Ghebrehwet B. The process of *B. cereus* spore attachment induces upregulation of cell surface gC1q-R/p33. *Molec Immunol Abstr* 2004;41:333.
- Tom B, Dendorfer A, deVries R. Bradykinin potentiation by ACE inhibitors: a matter of metabolism. *Br J Pharmacol* 2002;137:276–284. [PubMed: 12208785]
- van Iwaarden F, deGroot PG, Bouma BN. The binding of high molecular weight kininogen to endothelial cells. *J Biol Chem* 1988;263:4698–4703. [PubMed: 2832403]
- van Leuwen HC, O'Hare P. Retargeting of the mitochondrial protein p32/gC1qR to a cytoplasmic compartment and the cell surface. *J Cell Sci* 2001;114:2115–2123. [PubMed: 11493647]
- Vleming W, van Amsterdam JG, Stricker BH, de Wildt DJ. ACE inhibitor-induced AE. Incidence, prevention and management. *Drug Safety* 1998;18:171–188. [PubMed: 9530537]
- Yang HYT, Erdos EG. Second kininase in human blood plasma. *Nature* 1967;215:1402–1403. [PubMed: 6055465]
- Yao ZQ, Eisen-Vandervelde A, Ray S, Hahn YS. HCV core/gC1q-R interaction arrests T cell cycle progression through stabilization of cell cycle inhibitor p27Kip1. *Virology* 2003;314:271–282. [PubMed: 14517080]
- Yao ZQ, Waggoner SN, Cruise MW, Hall C, Xie X, Oldach DW, Hahn YS. SOCS1 and SOCS3 are targeted by hepatitis C virus core/gC1qR ligation to inhibit T cell function. *J Virol* 2005;79:15417–15429. [PubMed: 16306613]
- Yao ZQ, Shata MT, Tricoche N, Shan MM, Brotman B, Pfahler W, Hahn YS, Prince AM. gC1qR expression in chimpanzees with resolved and chronic infection: Potential role of HCV core/gC1qR-mediated T cell suppression in the outcome of HCV infection. *Virology* 2006;346:324–337. [PubMed: 16368125]

Abbreviations

gC1qR

33kDa cellular protein, which binds to the globular heads of C1q

Fig 1. Schematic representation of the domain structures of HK and HKa. The numbers inside the boxes represent the domains of HK. HK binds to cell surfaces as a complex with prekallikrein (PK), which does by itself bind to cells. HK binds to cells via domain 3 (cytokeratin 1) and domain 5 (gC1qR). Kallikrein (generated by XIIa from PK) cleaves domain 4 at two sites within the disulfide bridge to convert HK to HKa and in the process releases the nonapeptide, bradykinin.

Binding of FXII and assembly of HK-PK complex on ECs

Fig 2.

Hypothetical model of the binding of FXII and the HK-PK complex to endothelial cells. In this partial model, FXII is shown to bind to the cytokeratin 1-uPAR complex, whereas HK/PK complex on the PK/HK complex binds to the gC1qR-cytokeratin 1 complex. Both bindings have an absolute requirement of zinc. FXIIa converts PK to kallikrein and the latter cleaves HK (see Fig. 1) to generate bradykinin, which in turn binds to its receptor, BK-B2 to cause vascular permeability.