The role of complement inhibitors beyond controlling inflammation

Blom, A. M.

Published in:
Journal of Internal Medicine

DOI:
10.1111/joim.12606

2017

Document Version:
Peer reviewed version (aka post-print)

Link to publication

Citation for published version (APA):
Complement inhibitors beyond controlling inflammation

Anna M. Blom

From Lund University, Department of Translational Medicine, Division of Medical Protein Chemistry, Malmö, Sweden

Correspondence: Anna Blom, Lund University, Department of Translational Medicine, Division of Medical Protein Chemistry, Skane County Council, Inga Maria Nilsson’s Street 53, S-205 02 Malmö, Sweden. Tel: +46 40 33 72 28; anna.blom@med.lu.se

Abbreviations: C3b, activated complement factor 3; C4b, activated complement factor 4; C4BP, C4b-binding protein; CCP, complement control protein; COMP, cartilage oligomeric matrix protein; COPD, chronic obstructive pulmonary disease; CR, complement receptor; CSMD1, CUB and Sushi multiple domains 1; ECM, extracellular matrix; ER, endoplasmic reticulum; GPI, glycosylphosphatidylinositol; MAC, membrane attack complex; MBL, mannose-binding lectin; PNH, paroxysmal nocturnal haemoglobinuria; SUSD4, sushi domain-containing protein 4.

Abstract
The complement system is an arm of innate immunity that aids in the removal of pathogens and dying cells. Due to its harmful, pro-inflammatory potential, complement is controlled by several soluble and membrane-bound inhibitors. This family of complement regulators has been recently extended by the discovery of several new members, and it is becoming apparent that these proteins harbour additional functions. In this review the current state of knowledge of the physiological functions of four complement regulators will be described: cartilage oligomeric matrix protein (COMP), CUB and Sushi multiple domains 1 (CSMD1), sushi domain-containing protein 4 (SUSD4) and CD59. Complement activation is involved in both the development of and defence against cancer. COMP expression is pro-oncogenic, whereas CSMD1 and SUSD4 act as tumour suppressors. These effects may be related in part to the complex influence of complement on cancer but also depend on unrelated functions such as the protection of cells from endoplasmic reticulum stress conveyed by intracellular COMP. CD59 is the main inhibitor of the membrane attack complex and its deficiency leads to complement attack on erythrocytes and severe haemolytic anaemia, which is now amenable to treatment with an inhibitor of C5 cleavage. Unexpectedly, the intracellular pool of CD59 is crucial for insulin secretion from pancreatic β-cells. This finding is one of several relating to the intracellular functions of complement proteins, which until recently were only considered to be present in the extracellular space. Understanding the alternative functions of complement inhibitors may unravel unexpected links between complement and other physiological systems, but is also important for better design of therapeutic complement inhibition.

Keywords: COMP, SUSD4, CSMD1, CD59, breast cancer, diabetes
The complement system and its inhibitors

Complement, a crucial component of the innate immune system, is composed of over 30 proteins arranged in a proteolytic cascade, with effector mechanisms mediated by several specific receptors [1]. Complement is most abundant in blood but all its components are also present in tissues as a result of either diffusion or local transport. The main function of complement is to recognize microbial pathogens and other unwanted material such as dying cells or misfolded molecules [2]. Furthermore, there is extensive cross-talk between complement and, for example, other parts of the innate and adaptive immune systems, coagulation, angiogenesis and tissue repair. Complement is organized in three main pathways (classical, lectin and alternative), each initiated by sensory molecules able to recognize unwanted material amenable to clearance (Fig. 1A). This leads to a series of proteolytic activation steps that generate effector molecules of complement, such as potent anaphylatoxin C5a and fragments of C3 that serve as strong opsonins facilitating phagocytosis. The final step of the complement cascade is formation of the membrane attack complex (MAC), which is able to lyse Gram-negative bacteria and to damage eukaryotic cells. Activation of complement is potentially destructive for tissues and is therefore tightly controlled by several soluble or membrane-bound inhibitors [3]. These inhibitors include the soluble plasma proteins factor H and C4b-binding protein and the cellular receptors CD46, CD55 and CD59. The inhibitors prevent spontaneous complement activation, limit beneficial complement activation to the local site and contribute to the termination of the response. The importance of the inhibitors is clearly exemplified by various diseases such as haemolytic uraemic syndrome or age-related macular degeneration, which are caused by mutations/polymorphisms that impair the function of the inhibitors [4–6]. In recent years, several novel roles of complement inhibitors have been identified that are unrelated to their canonical functions within the complement system. This area is the focus of the present review.

Cartilage oligomeric matrix protein: complement regulator involved in joint diseases, infections and cancer

Cartilage oligomeric matrix protein (COMP) is a large (524 kDa) soluble glycoprotein with limited expression. Until recently the expression of COMP was considered to be restricted mainly to chondrocytes and fibroblasts, particularly under fibrotic conditions for example in scleroderma [7] or liver cirrhosis. In health, COMP is mainly found in cartilage, bone tissue and tendons [8]. Online databases (such as http://www.gtexportal.org) also indicate expression in arteries probably due to expression by vascular smooth muscle cells [9]. COMP belongs to the thrombospondin family (thrombospondin 5) and is composed of five identical monomers linked at the N-terminus by disulphide bridges (Fig. 1B). One monomer of COMP consists of four epidermal growth factor domains, eight thrombospondin type 3 domains and a globular C-terminus. COMP binds to collagen types I and II, aiding their fibrillogenesis, and to other components of the extracellular matrix (ECM) such as collagen types IX, XII and XIV, aggrecan, fibronectin and matrilins [10]. COMP interacts with integrins via its RGD motif, which promotes cell adhesion [11]. The pentameric structure of COMP allows for simultaneous binding of several ligands, for example both cellular receptors and ECM components. COMP is thus an integral part of the cartilage ECM, important for its assembly, integrity and correct function. COMP is released from the cartilage to synovial fluid and blood in joint diseases such as rheumatoid arthritis [12] and osteoarthritis [13], and is thus a useful biomarker for cartilage destruction. Mutations in COMP are linked to skeletal disorders such as pseudoachondroplasia and multiple epiphyseal dysplasia [14].

Recent findings suggest that COMP may have a number of additional physiological and pathological functions. COMP regulates activation of the human complement system [15]. It inhibits the classical and lectin pathways through its ability to bind C1q and mannose-
binding lectin (MBL), the initiators of these pathways (Table 1). It was shown that COMP interacts with the collagenous stalk of C1q thereby disrupting its interaction with C1s/C1r, proteases crucial for further complement activation via cleavage of C4; by contrast, COMP acts as an activator of the alternative pathway through its ability to bind properdin and C3 (Fig. 2A). Accordingly, complexes between COMP and C3b resulting from complement activation are found in synovial fluid and blood of patients with rheumatic diseases [16, 17]. Blood COMP–C3b levels were decreased in patients with rheumatoid arthritis during TNF-α inhibition differently from levels of C-reactive protein, suggesting that formation of COMP–C3b relates to disease features not reflected by general measures of inflammation [16]. These observations are of interest because although it is well established that complement is triggered in joint diseases in which it contributes to pathology, it is less clear which molecular mechanisms are responsible for this unwanted complement activation. COMP is thus an example of an extracellular matrix molecule that, when released from cartilage, has the capacity to trigger complement in synovial fluid probably contributing to ongoing inflammation [18]. Anaphylatoxin C5a released during complement activation in the joint attracts neutrophils and monocytes, which upon activation produce pro-inflammatory cytokines as well as proteases, which degrade cartilage (Fig. 2A).

Based on the ability of COMP to regulate activation of complement, whether COMP may affect bacterial recognition by the system has been questioned. Bacterial pathogens are under tremendous pressure to develop strategies to avoid or attenuate complement attack and many subvert human complement inhibitors for this purpose [19]. In tests of a panel of bacterial pathogens, Moraxella catarrhalis displayed the highest binding of COMP [20]. M. catarrhalis is a respiratory tract commensal that is able to cause otitis media and acute exacerbations in patients with chronic obstructive pulmonary disease (COPD). M. catarrhalis binds COMP efficiently via its ubiquitous surface protein A2 [20]. This interaction results in complement inhibition on the surface of this Gram-negative species followed by an increase in resistance to killing by human serum, i.e. lysis by complement MAC (Fig. 2B). Likewise, the phagocytosis of M. catarrhalis is diminished in the presence of COMP; this is likely to be due to a lower degree of opsonisation with complement. A direct inhibitory effect of COMP on neutrophil phagocytosis of M. catarrhalis was also observed in the absence of serum. This may resemble the mechanism observed for structurally related thrombospondin 1 [21]. Furthermore, COMP reduced bacterial adhesion and uptake by lung epithelial cells thus protecting M. catarrhalis from intracellular killing by these cells. Importantly, immunohistochemical analysis revealed that COMP is present in lungs, alveolar fluid, monocytes/macrophages and endothelium of blood vessels. mRNA analyses revealed upregulation of COMP in lung tissue of smokers with emphysema as well as in patients with idiopathic pulmonary fibrosis, indicating that the observed effects on M. catarrhalis may be particularly relevant in COPD patients [20].

Because COMP is a crucial component of ECM, whether it could be produced by cancer-associated fibroblasts, analogous to other fibrotic conditions in which such expression is observed, has also been questioned. Indeed, immunohistochemical staining of tissue biopsies from two independent cohorts has shown COMP in fibroblasts and ECM in breast cancer tissue. More surprisingly, COMP was also frequently expressed by epithelial breast tumour cells and such expression strongly correlated with poor patient survival and the presence of metastases independently of other markers [22]. This finding was confirmed in a mouse xenograft model in which human breast cancer MDA-MB-231 cells expressing COMP formed much larger tumours producing more frequent metastases than mock-transfected cells. Further, cells expressing COMP did not change their capacity to adhere and migrate but were more invasive, partly due to expression of larger amounts of metalloproteinase 9 (Fig. 2C). To investigate the molecular mechanisms underlying the strong effect of COMP on breast cancer pathophysiology, COMP-induced changes in gene expression were determined using mRNA
arrays. Subsequent pathway analyses indicated that COMP protects the cancer cells from endoplasmic reticulum (ER) stress. ER stress is caused by accumulation of faulty proteins in the ER and the aim of the ER stress response pathway is to alleviate ER stress by enhancing protein folding and degradation. Accordingly, COMP-expressing cells were protected from apoptosis induced by brefeldin A, a compound that inhibits transport from the ER to Golgi apparatus leading to protein accumulation in the ER. Interestingly, thrombospondin 4, a protein similar to COMP, was recently shown to have a related function [23]. The observed protection from apoptosis can be expected to provide significant growth advantage to cells expressing COMP. Furthermore, mRNA analyses indicated that these cells underwent a metabolic switch, known as the Warburg effect (i.e. use by cancer cells of high rates of glycolysis under aerobic conditions and thus a decrease in the rate of oxidative phosphorylation). This effect was confirmed with in vitro measurements of cell respiration, which showed decreased mitochondrial metabolism in COMP-expressing cells. One advantage of the observed metabolic switch is acidification of the extracellular environment via the production of lactate to which normal cells are much more sensitive than cancer cells. It remains to be elucidated which of the observed effects of COMP are mediated by its intracellular presence in the ER and which are due to the observed binding of COMP to cell surfaces [22], presumably via integrins [11].

This phenomenon whereby COMP acts pro-oncogenically is not likely to be limited to breast cancer as analyses of expression data using the Oncomine database indicated COMP expression also in colorectal, gastric, lung, ovarian and pancreatic cancers. Future investigations are needed to evaluate the usefulness of COMP as a cancer biomarker and a target for treatment in breast cancer and perhaps also in other cancer types.

**CUB and Sushi multiple domains 1: complement inhibitor, tumour suppressor and neuromodulator**

CUB and Sushi multiple domains 1 (CSMD1) is a relatively poorly studied large transmembrane protein of 390 kDa composed of 14 N-terminal CUB domains interspersed with complement control protein (CCP) domains followed by 15 consecutive CCP domains (Fig. 1B). The C-terminal part of CSMD1 includes a single transmembrane domain and a short cytoplasmic tail with a putative tyrosine phosphorylation site implying that CSMD1 may have intracellular signalling potential. CSMD1 is highly expressed in human brain and testes [24], which is similar to the observed expression in central nervous system and epithelial tissues in rat [25]. In mice, expression was detected mainly in the central nervous system but also in visceral fat and ovaries [26]. The gene encoding human CSMD1 encompasses 70 exons located on chromosome 8p23.1 [27].

The presence of multiple, consecutive CCP domains frequently found in well-established complement inhibitors raised the possibility that CSMD1 may also act as an inhibitor. First, it has been shown that a recombinant, truncated variant of rat CSMD1 has the capacity to inhibit the classical but not the alternative pathway of complement [25]. CSMD1 was shown to attenuate complement at the level of C3b deposition but the underlying molecular mechanism was not investigated. The following study of human CSMD1 revealed that CCP14–28 of CSMD1 expressed together with a transmembrane domain protected Chinese hamster ovary (CHO) cells from complement attack measured as deposition of C3b and C9 from human serum [24]. Accordingly, efficient knockdown of CSMD1 with ribozymes in the human breast cancer cell line T47D caused increased deposition of complement factors on the cell surface. The active domains of CSMD1 were then identified in CCP17–21, which were shown to interact with C4b and C3b and present these complement proteins for degradation by factor I [5]. This is a typical mode of action of CCP-containing complement inhibitors, for example factor H. Further, CSMD1 also inhibits assembly of MAC by preventing incorporation of C7 in the complex.
CSMD1 has for some time been implicated as a tumour suppressor based on the fact that it is frequently deleted in several types of cancer such as breast, head and neck, prostate, colorectal, liver, lung and skin cancers and oral squamous cell carcinoma [28, 29]. Although CSMD1 has been suggested to act as a tumour suppressor in many types of cancer, only recently has direct experimental evidence of such action been provided in melanoma [30] and breast cancer [31]. Expression of CSMD1 in A375 melanoma cells decreased their proliferation and migration and also increased apoptosis. In a xenograft model, expression of CSMD1 led to decreased tumour size with fewer microvessels and better survival [30]. One of the suggested molecular mechanisms underlying the action of CSMD1 may rely on its ability to interact with Smad3 and activate Smad4-dependent signalling. Smad3 and Smad4 are key mediators of the TGF-β signalling pathway, which is well known to play important roles in cell differentiation, growth and apoptosis. Furthermore, normal human breast tissue was found to express CSMD1 at higher levels than breast tumour tissues, and decreased expression of CSMD1 was linked to shorter survival of breast cancer patients [31]. The observation from patients was confirmed in a mouse xenograft model in which expression of CSMD1 in MDA-MB-231 cells blocked the ability of cells to metastasize to secondary sites. It is likely that this was due to the effect on local invasion from primary tumour rather than inhibition of the extravasation to target tissues. Overexpression of CSMD1 in human breast cancer BT-20 and MDA-MB-231 cells inhibited their malignant phenotype including migration, adhesion and invasion. Conversely, attenuation of CSMD1 expression in T47D breast cancer cells with ribosyme yielded the expected opposite phenotype. Although the exact molecular mode of action of CSMD1 has not yet been elucidated, its expression diminished the signalling potential of cells via several pathways and the cells also decreased their stem cell-like properties measured as aldehyde dehydrogenase activity [31]. It does not appear that the tumour suppressor role of CSMD1 is related to its effect on complement as no effect on leukocyte infiltration was detected in the xenograft model. However, such an effect cannot be excluded and there are many examples of tumour cells upregulating expression of complement inhibitors, which has been suggested to be part of their immune evasion strategy. A direct evidence for such a mechanism in lung cancer cells was provided in a mouse model [32]. However, the role of complement in cancer progression is complex because in some cases complement activation may be beneficial for cancer development due to sustained inflammation and an effect of C5a on myeloid-derived suppressor cells [33]. Thus, the role of complement in cancer development must be assessed for each specific tumour type and even then it may vary considerably depending on pathophysiological circumstances.

CSMD1 is highly expressed in brain and genetic studies have linked mutations and polymorphisms in CSMD1 with schizophrenia [34, 35], cognitive function [36] and multiple sclerosis [37]. Interestingly, knockout mice lacking CSMD1 display neuropsychological deficits [26]. It is not yet clear whether this function of CMSD1 is directly related to its ability to regulate complement. This possibility is supported by the fact that complement, and specifically interactions between C3 activation products and complement receptor 3 regulates axonal pruning by phagocytic microglia [38]. This produces the correct structure of neuronal circuits in the development of the visual system. Several complement components are localized to developing synapses where they play a role in synaptic refinement and neuronal connectivity [39]. Based on these observations, it is possible that complement is involved in synaptic elimination in other parts of the brain, which could influence the risk of development of neurodegenerative and psychiatric disorders [40].

**Sushi domain-containing protein 4: complement inhibitor and tumour suppressor**

Sushi domain-containing protein 4 (SUSD4) is another example of a complement inhibitor that simultaneously acts as a tumour suppressor. The 49 kDa protein is structurally very similar to another complement inhibitor CD46, and is composed of four CCP domains
followed by a transmembrane region and cytoplasmic tail with putative phosphorylation sites (Fig. 1B). In humans, another splice form is predicted to exist, which is composed of a soluble protein with three CCP domains followed by a short region with no known homologies (27 kDa). However, we were not able to detect such a form of SUS4D in plasma/spinal fluid or conditioned media of cells transfected with a construct coding for this isoform (unpublished observation). Thus, it is possible that this form of SUS4D is not translated into a functional protein. The gene coding for SUS4D is located at chromosome 1q41, which is not far from the cluster of well-characterized complement inhibitors such as CD46 (1q32.2) or factor H (1q31.3). In humans, expression of the membrane-bound form of SUS4D at the mRNA level was detected to the largest degree in brain, oesophagus, kidneys, breast and prostate [41]. In mice, protein expression was detected in brain, eyes, spinal cord and testes [42]. Deletion of SUS4D has been suggested in some patients with Fryns syndrome [43], an autosomal recessive disorder associated with several developmental malformations. Interestingly, knockdown of SUS4D expression in zebrafish markedly increased mortality and developmental abnormalities [42]. By contrast, SUS4D knockout mice, which we have recently developed, do not present any developmental abnormalities and breed normally even in a homozygous state (unpublished observation).

Because SUS4D is structurally so similar to the membrane complement inhibitor CD46, which also encompasses four CCP domains followed by a transmembrane region, it was reasonable to hypothesize that SUS4D would inhibit complement. This is indeed the case but the molecular mechanism of action of SUS4D is not what one would initially expect. Whereas CD46 binds C3b and acts at the level of C3 convertases, SUS4D interacts with C1q within the C1 complex and therefore prevents C2 cleavage and formation of the classical C3 convertase, which is composed of C4b and C2a, activated by the C1 complex [41]. Similarly, SUS4D also inhibited lectin pathway activation triggered by MBL. Experiments showing these activities were performed using soluble, Fc- or His-tagged versions of SUS4D. When native, membrane-bound SUS4D was expressed on the surface of CHO cells, it inhibited deposition of C3b from both the classical and alternative pathways. The mechanism underlying the effect of SUS4D on the alternative pathway has not yet been elucidated.

Immunohistochemical staining of several tumour tissues showed SUS4D-positive cells infiltrating tumour tissue in colon, lung and breast cancer. Further, through detailed investigation of breast cancer tissues, these cells were identified as CD4+ and CD8+ T cells [44]. The numbers of such SUS4D-positive T cells found in tumour stroma were positively associated with better survival of patients with breast cancer. Furthermore, CD4+ and CD8+ T cells isolated from peripheral human blood were shown to express SUS4D, and a similar observation was made in mouse T cells (unpublished observation). Thus the role of SUS4D in T cells in particular should be investigated because the similar protein CD46 has an important role in human T cell biology. Thus, CD46 acts as crucial co-receptor in the generation of IL-10-producing T regulatory 1 cells [45]. CD46 controls Th1 activation and regulation via a miR-150-dependent mechanism [46]. One of the identified ligands of CD46 is Jagged1, and CD46 regulates expression of Notch receptors [47]. These processes appear to be disturbed in patients with multiple sclerosis [48], rheumatoid arthritis [49] and systemic lupus erythematosus [50]. Unfortunately, these processes cannot be studied in mouse models because expression of CD46 is limited to the testes in this species. Considerable information was gathered from studies using blood cells from patients lacking CD46 but these cases are exceptionally rare. The discovery of SUS4D raises question whether it may have any similar effects to CD46 in T cell biology and, if so, whether such effects could be studied in mice.

In breast cancer tissues, SUS4D was not only detected in infiltrating T cells but also in epithelial tumour cells. Analysis of two independent cohorts showed that SUS4D-expressing tumours were smaller, more differentiated and less prone to lymph node metastases compared to tumours lacking SUS4D expression. SUS4D was also associated with better patient
survival [44]. An independent study found that SUSD4 was downregulated in the group of breast cancer patients with poor prognosis [51]. This is supported by the finding that expression of SUSD4 in MDA-MB-231 and BT20 breast cancer cell lines decreased cell migration and invasion [44]. Such cells formed smaller colonies when co-cultured with cancer-associated fibroblasts. Taken together, it appears that SUSD4 acts as a tumour suppressor but the molecular mechanism underlying this function remains to be elucidated.

**CD59: inhibitor of complement-mediated lysis and regulator of insulin secretion**

CD59 is the main inhibitor of the MAC, which is a transmembrane pore that causes osmotic lysis of a cell on which it is deposited. Furthermore, even sublytic MAC deposition causes profound changes in various cell types resulting in proliferation, secretion of proinflammatory mediators and thrombosis. CD59 is a small, heavily glycosylated protein of ~18–20 kDa attached to cell membranes via a glycosylphosphatidylinositol (GPI) link [52]. CD59 is widely expressed on most cells and inhibits binding of C9 to C5b-8 by competing for binding to a neoepitope on C8 [53].

Paroxysmal nocturnal haemoglobinuria (PNH) is caused by a clonal mutation in bone marrow progenitor stem cells impairing the ability of cells to produce GPI anchors and thus leading to a lack of CD55 and CD59, among other GPI-linked proteins, on their surface [54]. This renders erythrocytes particularly vulnerable to complement-mediated lysis and thus haemoglobinuria, anaemia and thrombosis. It appears that this effect is mainly dependent on deficiency of CD59 with much less influence from CD55 [55]. Accordingly, PNH can now be successfully treated with eculizumab, an antibody that targets C5 and prevents its cleavage and activation leading otherwise to the formation of MAC. The clinical symptoms of PNH are in good agreement with the notion that CD59 is a main complement inhibitor that protects erythrocytes from lysis. Further, there are rare patients with complete CD59 deficiency who suffer not only from chronic haemolysis but also from severe, recurrent, acute, predominantly motor, demyelinating neuropathy already in childhood. These children can also be treated successfully with eculizumab, showing that at least some of the symptoms result from excessive, uncontrolled complement activation [56].

Recently, evidence for a novel role of CD59 in the development of diabetes and its complications has been provided. Diabetes is associated with macrovascular disease leading to atherosclerosis (coronary heart disease, stroke) and microvascular disease damaging the retina (blindness), kidneys (renal failure) and peripheral nerves (neuropathy). One of the main reasons for the organ damage in diabetes is sustained hyperglycaemia leading to formation of advanced glycation end products, which adversely affect the function of many proteins. This is also the case for CD59, which becomes inactivated by glycation [57]. This together with enhanced complement activation induced by hyperglycaemia activates pathways of intracellular signalling, yielding release of proinflammatory and prothrombotic cytokines as well as growth factors [58]. In support of loss of the protective role of CD59 due to glycation, colocalisation between glycated CD59 detected using a specific neoepitope antibody and MAC deposition was detected in target organs of diabetic complications [59]. Erythrocytes from diabetic patients with high levels of glycated haemoglobin and thus poorly controlled disease were more sensitive to MAC-mediated lysis than cells from healthy individuals [59]. This is consistent with reversible mild haemolytic anaemia reported in patients with poorly controlled diabetes. Increased MAC deposition was detected in kidneys of patients suffering from diabetic nephropathy [60]. Furthermore, mice lacking CD59 on an ApoE/- background displayed accelerated atherosclerosis with occlusive coronary disease, vulnerable plaques and premature death [61], which was further accelerated by a lack of CD59 when diabetes was induced in these mice [62].

Recently, it was demonstrated that CD59 is even more involved in the pathogenesis of diabetes than so far anticipated [63]. Pancreatic islets, including β-cells, express large
amounts of CD59 (Fig. 3). This expression is decreased in pancreatic islets isolated from several mouse and rat models of diabetes [64]. Treatment of isolated human islets with high glucose or diabetogenic cytokine IL-1β also decreased expression of CD59. Surprisingly, downregulation of CD59 expression using siRNA largely decreased the ability of rat clonal β-cells (INS-1 cells) to secrete insulin upon stimulation with glucose [64]. Removal of surface-bound CD59 with phospholipase C, which cleaves GPI anchors, had no effect on insulin secretion indicating that intracellular CD59 was involved in insulin release. Whether this CD59 is GPI linked to intracellular membranes remains to be elucidated. Currently, no alternative splice forms of CD59 lacking GPI have been found, but such a search is complicated by the fact that many antibodies generated against GPI-linked proteins, including CD59, lose their ability to bind their targets once the GPI link is removed [65]. Therefore, the putative, intracellular, soluble form of CD59 may have been long overlooked. The mechanism of the CD59 effect on insulin secretion was related to its ability to associate with intracellular soluble NSF attachment protein receptor (SNARE) proteins, VAMP2 and Syntaxin-1 [64]. Syntaxin-1 is present in the cell surface membrane whereas VAMP2 localizes to insulin granules. Interaction between these proteins occurs in high-glucose conditions allowing the release of insulin to the extracellular environment. It remains unclear whether CD59 interacts directly with these two proteins or another component of the SNARE complexes that include the two proteins. CD59 was also found to associate with insulin granules, particularly upon stimulation with glucose. Under glucose stimulation, colocalisation between VAMP2 and Syntaxin-1 increased and so did colocalisation between CD59 and either of the two SNARE proteins. This effect was lost when CD59 expression was attenuated with siRNA. Further, cells with lower CD59 expression had fewer fusion events between insulin granules and cell membrane as measured using live imaging total internal reflection fluorescence microscopy [64]. In support of the role of CD59 in influencing insulin granule interactions with the cell membrane, rather than affecting glucose uptake, and the subsequent changes in signalling, metabolism and membrane potential, stimulation of INS-1 cells with K⁺ did not correct defects in insulin secretion caused by downregulation of CD59 by siRNA.

Mice harbour two genes encoding CD59 with the two forms of CD59 protein (CD59a and CD59b) sharing over 60% amino acid identity. CD59a is widely expressed and knockout mice lacking this form of CD59 show a PNH-like phenotype [66]. CD59b is highly expressed in the testes [67], and reproductive abnormalities such as decreased sperm count and morphological sperm abnormalities have been reported in mice lacking CD59b, particularly in older mice [68]. In mouse pancreatic islets comparable expression of mRNA for both forms of CD59 was detected using quantitative PCR [64], suggesting that double knockout mice are required to study the effect of CD59 on insulin secretion in vivo. Such mice have been developed and show an impaired ability to regulate glucose levels in blood (unpublished observation).

Of interest, a role for CD59 in T cells has also been suggested. In T cells, cross-linking of CD59 with specific antibodies led to increased signalling and calcium flux, and phorbol 12-myristate 13-acetate treatment of these cells caused proliferation and IL-2 production [69]. Stimulation of CD59 was then shown to result in TCRζ/ZAP70 phosphorylation [70], and CD59 was suggested to interact with CD2 although this finding was challenged in other studies [71]. By contrast, in CD59a knockout mice, CD8+ T cell responses were normal whereas CD4+ T cells showed enhanced proliferation [72]. The suppressive effect of CD59 on T cell activation in mice was independent of the complement system. There may be a difference in CD59 function between humans and mice but PNH patients show impaired T cell responses [73]. CD59 is localized to lipid rafts, similar to other GPI-linked proteins such as CD55 or Thy-1, and similar effects on T cell activation were shown for many of these proteins [74, 75]. Thus it is unclear whether CD59 has a direct, specific effect on T cell
activation or whether its influence is mediated by modulation of lipid rafts [76], which are crucial for formation of the immunological synapse.

**Concluding remarks**
Evidence is emerging that complement inhibitors are involved in many physiological and pathological systems in the body. Some of these functions are related to the effects of complement activation products on other systems, whereas others are entirely independent of complement. Importantly, these novel findings indicate that some complement inhibitors such as COMP, SUSD4 and CSMD1 could be used as biomarkers in breast cancer. Some of our novel observations could lead to the development of new therapies for cancer and diabetes.

**Acknowledgments**
Our studies of the roles of complement regulators COMP, CD59, CSMD1 and SUSD4 were funded by the Swedish Research Council, Cancerfonden, The Wallenberg Foundation and grants for clinical research (ALF). The author thanks Dr. Ben King for critical comments of this review and language correction.

**Conflict of interest statement**
The author has no conflict of interest to declare.
Figures

Fig. 1 Overview of the human complement system showing sites of inhibition by cartilage oligomeric matrix protein (COMP), sushi domain containing protein 4 (SUSD4), CUB and Sushi multiple domains 1 (CSMD1) and CD59 with a schematic representation of their domain structures. A) The complement system can be triggered by initiating molecules such as C1 complex, mannose-binding lectin (MBL) and ficolins which are all associated with specific proteases. The alternative pathway undergoes spontaneous activation through hydrolysis of thiolester bond in C3. Further activation of any pathway leads to assembly of C3 convertases, which generates activated C3b, further converted to iC3b, the main opsonin interacting with CR3. In the next step, C5 convertases generate the potent anaphylatoxin C5a, as well as C5b, which initiates formation of the membrane attack complex (MAC). B) COMP is a soluble homopentamer while CD59 is attached to cell surfaces via a glycosylphosphatidylinositol (GPI) anchor. SUSD4 and CSMD1 are transmembrane proteins with short intracellular domains.

Fig. 2 Role of cartilage oligomeric matrix protein (COMP) in joint diseases, infections and cancer. A) COMP is an extracellular matrix protein crucial for cartilage structure. In joint diseases, COMP is released from cartilage due to proteolytic degradation. COMP then activates the alternative complement pathway, forming COMP–C3b complexes that can be detected in synovial fluid and blood of patients with joint diseases such as rheumatoid arthritis and osteoarthritis. Complement activation in synovium leads to release of anaphylatoxins that attract neutrophils and monocytes, which then secrete pro-inflammatory cytokines and proteases further contributing to tissue damage. B) The ability of COMP to inhibit the classical complement pathway is exploited by the lung pathogen Moraxella catarrhalis. Binding of COMP to this bacterium leads to decreased opsonisation with fragments of C3b and a reduction in lysis by the membrane attack complex (MAC). This resistance to killing by human serum allows M. catarrhalis to survive and proliferate. C) Breast cancer cells express COMP, and high COMP expression correlates with poor patient survival and metastases. COMP is secreted by the cells whereby it rebinds to their surface, and is also present in the endoplasmic reticulum (ER) of the cells. As a result of COMP expression, breast cancer cells express higher levels of metalloproteinase 9 (MMP9), which increases their metastatic potential. Further, the cells are protected from apoptosis induced by ER stress and undergo metabolic changes (the Warburg effect). Together, these changes allow the cells to proliferate.

Fig. 3 Role of CD59 in insulin secretion. β-cells take up glucose and activate ion channels, which leads to depolarization of the plasma membrane and calcium influx. Then, interactions between soluble NSF attachment protein receptor (SNARE) proteins dock the insulin granules to the plasma membrane and insulin is released. CD59 is typically anchored by a glycosylphosphatidylinositol (GPI) link to the plasma membrane and concentrated to lipid rafts. However, an intracellular form of CD59, perhaps lacking GPI, colocalizes with insulin granules and SNARE complexes. When CD59 expression is attenuated, glucose-dependent insulin secretion is dramatically decreased.
References
domain 42 convertase. protein 4 (SUSD4) inhibits complement by disrupting the formation of the classical C3 interactions in the developing and mature CNS.

CNS synapse elimination.

circuits in an activity and complement genome and CSMD2 with cognitive function.

CSMD1 and CSMD2 associate to schizophrenia.

immune response by complement.

reduces in vivo tumor growth. Complement factor H sensitizes non-high tumour grade and poor survival in invasive ductal breast carcinoma.

A375 melanoma cells through activation of the Smad pathway. Apoptosis 2012; 17: 927-37.


A thrombospondin containing protein 4.


**Table 1** Functions of the complement inhibitors COMP, CSMD1, SUSD4 and CD59 and the diseases associated with either partial/complete deficiency of or deleterious mutations in these proteins

<table>
<thead>
<tr>
<th></th>
<th>Effect on the complement system</th>
<th>Other functions</th>
<th>Deficiency/deleterious mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMP</td>
<td>Inhibits classical/lectin pathways by binding to stalks of C1q and MBL</td>
<td>Required for proper assembly, integrity and function of the ECM</td>
<td>Pseudoachondroplasia and multiple epiphyseal dysplasia</td>
</tr>
<tr>
<td></td>
<td>Activates alternative pathway by binding C3 and properdin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSMD1</td>
<td>Inhibits all complement pathways by acting on C3 convertases and presenting C3b/C4b for degradation to factor I</td>
<td>Tumour suppressor in breast cancer</td>
<td>Deleted in many types of cancer Schizophrenia Impairment of cognitive function</td>
</tr>
<tr>
<td></td>
<td>Inhibits MAC by preventing incorporation of C7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUSD4</td>
<td>Inhibits the classical pathway via binding to C1q</td>
<td>Tumour suppressor in breast cancer</td>
<td>Not reported</td>
</tr>
<tr>
<td></td>
<td>Inhibits alternative pathway (mechanism not elucidated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD59</td>
<td>Inhibits MAC by preventing incorporation of C9</td>
<td>Required for insulin secretion due to interaction with SNARE proteins Regulates T cell activation</td>
<td>Paroxysmal nocturnal haemoglobinuria (clonal defect in bone marrow cells) Neuropathy (full deficiency)</td>
</tr>
</tbody>
</table>

ECM, extracellular matrix; MAC, membrane attack complex; MBL, mannose-binding lectin; COMP, cartilage oligomeric matrix protein; CSMD1, CUB and Sushi multiple domains 1; SUSD4, sushi domain containing protein 4.
**Figure 1**

A. Diagram illustrating the complement system pathways and the role of CSMD1 in regulating C3 activity. The classical pathway, lectin pathway, and alternative pathway are depicted, showing the formation of C3-convertase and C5-convertase. CSMD1 interacts with C3-convertase and C5-convertase, leading to opsonization with iC3b and MAC-mediated cell lysis.

B. Close-up view of the cell membrane showing the localization of proteins such as SUSD4, CD59, CSMD1, and other domains (CCP, CUB, EGF, globular, thrombospondin) involved in the complement system.
Complement activation, replication, colonization, C5a, iC3b, MAC, COMP, phagocytic uptake, clearance, lysis, Bone, synovium, cartilage, COMP-C3b, inflammatory cytokines, proteases, Moraxella, binding of COMP, complement activation, replication, colonization, lysis, phagocytic uptake, clearance, MMP9, invasion, glucose, pyruvate, lactate, breast cancer cell, proliferation, reduced ER stress.