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Membrane Defence against Complement Lysis: The Structure and Biological Properties of CD59

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CD59
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Abstract
The complement system is an important branch of the innate immune response, constituting a first line of defence against invading microorganisms which activate complement via both antibody-dependent and -independent mechanisms. Activation of complement leads to (a) a direct attack upon the activating cell surface by assembly of the pore-forming membrane attack complex (MAC), and (b) the generation of inflammatory mediators which target and recruit other branches of the immune system. However, uncontrolled complement activation can lead to widespread tissue damage in the host, since certain of the activation products, notably the fragment C3b and the C5b-7 complex, can bind nonspecifically to any nearby cell membranes. Therefore it is important that complement activation is tightly regulated. Our own cells express a number of membrane-bound control proteins which limit complement activation at the cell surface and prevent accidental complement-mediated damage. These include decay-accelerating factor, complement receptor 1 and membrane cofactor protein, all of which are active at the level of C3/C5 convertase formation. Until recently, cell surface control of MAC assembly had been attributed to a single 65-kD membrane protein called homologous restriction factor (alternatively named C8-binding protein and MAC-inhibiting protein). However a second MAC-inhibiting protein has since been discovered and it is now clear that this protein plays a major role in the control of membrane attack. This review charts the rapid progress made in elucidating the protein and gene structure, and the mechanism of action of this most recently discovered complement inhibitor, CD59.

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Introduction

An understanding of the mechanisms by which cells protect themselves against accidental complement-mediated damage has been achieved quite recently, although the observation that within a given species, complement is relatively inefficient at lysing erythrocytes was made in the first decade of this century [1]. This phenomenon of 'homologous restriction' was later found to operate, at least in part, at the terminal stages of complement activation, since it was manifest under conditions of reactive lysis, involving only the membrane attack complex (MAC) [2]. In 1983, two groups claimed that glycophorin purified from erythrocyte membranes was able to restrict homologous lysis at the levels of C3 deposition and MAC assembly [3, 4], but since then the mechanism by which certain proteins are anchored to the cell surface via covalent linkage to glycosylphosphatidylinositol (GPI) has been identified (5). When isolated with their anchors intact, GPI-anchored proteins can readily reinsert into cell membranes, and it is possible that the above-mentioned preparations of glycophorin (a conventional transmembrane protein) contained GPI-anchored proteins which were unidentified at that time. It is now clear that a number of fluid-phase and membrane-bound control proteins act in concert to regulate complement activation at the C3/C5 convertase stage. One of these proteins, decay-accelerating factor (DAF), was isolated in 1982 and later found to be GPI-linked [6, 7]. In 1986, the first membrane-bound inhibitor of the MAC was described and also shown to be GPI-linked. Called homologous restriction factor (HRF), C8-binding protein (C8bp) and MAC-inhibiting protein (MIP), this 65-kD protein was purified from human erythrocyte membranes and found to inhibit the action of homologous C8 and C9 [8–11]. However, in the absence of any protein sequence data, there is still much to be learned about this molecule. CD59 was the second MAC inhibitor to be discovered, and with its discovery came a surprise: CD59 has proved to be quite unlike other members of the complement control protein superfamily.

Discovery of CD59

The discovery of an 18- to 20-kD erythrocyte membrane protein with inhibitory activity towards the MAC was made independently in a number of laboratories during the late 1980s. The first report came from Sugita et al. [12], who purified the protein from extracts of human erythrocyte membranes. They later termed this protein membrane-attack-complex-inhibitory factor, or MACIF [13]. Reports from the other groups involved gave rise to other names: HRF20, membrane inhibitor of reactive lysis (MIRL), and protectin [14–16]. We identified the protein in this laboratory by virtue of a monoclonal antibody YTH 53.1 raised in Herman Waldmann's laboratory against a leucocyte surface antigen, and characterised its MAC-inhibiting activity [17]. Meanwhile, a novel GPI-anchored leucocyte surface protein reactive with monoclonal antibody MEM-43 was described by Stefanova et al. [18]. At the fourth leucocyte-typing workshop, YTH 53.1 and MEM-43 were clustered together as CD59 antibodies. It was subsequently shown that CD59 is also identical to the protein H19 and to a protein identified in 1984 by the monoclonal antibody LICR-LON-Fib75.1, whose function was then unknown [19–22].

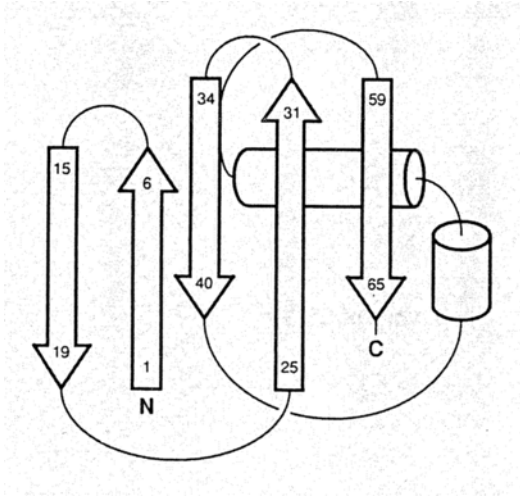

Fig. 1. Global fold of CD59 in solution, determined from NMR data. The first two strands form a separate β-sheet offset from the remaining three strands. The 12 carboxyterminal residues are not shown; they pack between the 3-stranded sheet and the first strand and appear to have no regular secondary structure. The β-sheet structure and connecting topology of the β strands is the same as that found in the snake venom neurotoxins as predicted (146, 147).

Protein and Gene Structure of CD59

CD59 is an 18- to 20-kD membrane glycoprotein which is anchored to the membrane via GPI. Several groups have isolated cDNAs for CD59 [13, 17, 23–25] and identified the N-terminus of the mature protein by protein sequencing [17, 18]. The nucleotide sequence encodes a precursor polypeptide of 128 amino acids in length, with the first 25 residues constituting a signal peptide. The remaining 103 amino acid polypeptide should give rise to a molecular weight of approximately 11-12 kD, but this is lower than the apparent molecular weight of purified CD59 as judged by SDS-PAGE. This discrepancy may be attributable to the post-translational attachment of carbohydrate and the glycolipid anchor, as discussed below. Altogether, the protein sequence contains 10 cysteine residues, suggesting that this small molecule is tightly folded. Using tryptic and chymotryptic fragments of CD59 purified from human urine, the disulphide-bonding pattern has been determined as follows: Cys3–26, Cys6–13, Cys19–39, Cys45–63 (or 64), Cys64 (or 63)–69 [38; R.A. Harrison, pers. commun.]. The three-dimensional structure of the protein component is also being determined using samples of CD59 isolated from urine. Two-dimensional NMR methods have allowed complete assignment of the protein signals and determination of the global fold (fig. 1) [26; M. Fletcher, pers. commun.].

The nucleotide sequence contains a single potential N-glycosylation site at Asn18 (Asn8 is not a candidate for glycosylation since the next residue is a proline). Occupation of this site by carbohydrate has been confirmed by protein sequence analysis of the mature protein, when no signal was detectable at this position, and by amino acid analysis of tryptic peptides labelled with ^{3}H-borohydride to indicate the presence of sugar residues [17, 18, 27]. The carbohydrate attached here is a common biantennary structure and accounts for approximately 25% of the molecular weight of CD59, since enzymatic deglycosylation using N-glycosidases reduces the apparent molecular weight from 18–20 kD to around 14 kD [18, 27–29]. There is no change in mobility on SDS-PAGE after treatment with enzymes which remove O-linked oligosaccharides [18, 27].

The carboxyterminus of CD59 contains a hydrophobic stretch of residues characteristic of proteins which are anchored to the cell membrane via GPI. The release of CD59 from the surface of various cells by treatment with phosphatidylinositol-specific phospholipase C (PIPLC) has confirmed that it is GPI-anchored [17, 18, 30–34]. PIPLC cleaves the

glycolipid anchor between the inositol ring and diacylglycerol embedded in the outer layer of the membrane, thereby releasing the attached protein into the fluid phase (fig. 2). In common with other GPI-linked proteins, on the erythrocyte surface CD59 is resistant to release by PIPLC. Studies on the structure of the GPI anchor of erythrocyte DAF and acetylcholinesterase have shown that this resistance is due to the presence of a third fatty acid chain attached to the inositol ring [35, 36]. Since the nature of the anchor is cell- rather than protein-specific, it is likely that this third fatty acid chain is also present in erythrocyte CD59. Recent evidence suggests that this is the case [37]. GPI-linked proteins are synthesised as precursor proteins which undergo post-translational processing in which a stretch of carboxyterminal amino acids is removed to be replaced with the pre-synthesised glycolipid anchor [5]. In the case of CD59, protein sequencing of tryptic fragments and NMR studies of CD59 purified from urine indicate that the GPI anchor is attached to Asn77 [26, 38].

The gene for CD59 has recently been isolated and found to consist of four exons distributed throughout more than 27 kb of genomic DNA [39, 40]. The first exon, encoding 45 bp of the 5' untranslated region of the mRNA, appears to contain a hotspot for mutation, and three polymorphisms have been identified within this region [39]. Southern blotting gives a simple pattern consistent with a single-gene copy [17, 24, 25]. Northern blotting of RNA from a range of cells and tissues has identified four species of mesage [13, 17, 24, 25], although these can all be accounted for by polyadenylation at successive sites in the sequence, and there is no evidence as yet of alternative splicing [39]. The gene for CD59 is located on the short arm of chromosome 11 and has recently been mapped more precisely to band p13 within 500 kb of another cell surface marker, MIC11 [41, 42]. The gene encoding MIC11 has not been cloned and the relationship of MIC11 to CD59 is not known.

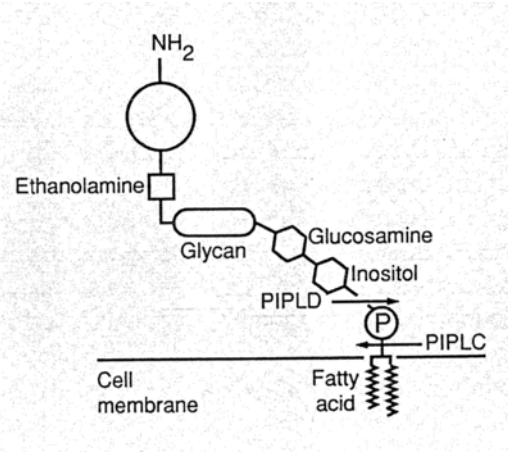

Fig. 2. Composition of the GPI membrane anchor, indicating sites susceptible to cleavage by phospholipases C and D. Reproduced with permission from Robinson et al. [103].

Relationship of CD59 to Other Proteins

The protein sequence of CD59 is unlike that of any other complement component or regulatory protein. This is unusual, since other members of the complement superfamily are closely related structurally and are encoded by genes which are situated together in the regulators-of-complement-activation (RCA) region on chromosome 1 [43]. Searches of sequence databases have revealed that CD59 is approximately 25% homologous to the Ly-6 superfamily of proteins, for which a role in T cell activation has been advocated [44]. Although this degree of homology is not high, it does include alignment of the cysteine residues which are abundant in both proteins,

suggesting that the molecues may have a similar secondary structure. Furthermore, the proteins are of a similar size (Ly-6A/E and Ly-6C have a molecular weight of 16-kD with no N-linked glycosylation) and are both GPI-linked to the membrane. At the genomic level, the exon structure of the two proteins is remarkably similar, although the CD59 gene covers a much wider area with larger intron sequences than are found in Ly-6 [39, 40]. This is further evidence that the two proteins may have a common ancestry. However, it is unlikely that CD59 is the human homologue of Ly-6. Firstly, the tissue distribution of the proteins is different. The most notable example of this is the lack of Ly-6 expression on the surface of murine erythrocytes [45, 46]. Moreover, the Ly-6 proteins are encoded by a family of genes which are inducible by interferon, whereas the CD59 gene is present in a single copy and is not interferon inducible [24]. In addition, an 11- to 17-kD molecule called B5 which may be related to the murine Ly-6 antigens has been identified on sheep lymphocytes, and more recently the sheep homologue of human CD59 has been isolated from sheep erythrocyte membranes [47, 48]. A monoclonal antibody raised against B5 does not cross-react with sheep CD59 [48]. A rat homologue of human CD59 has also been identified, and the N-terminal protein sequence obtained [49]. A comparison of the N-terminal sequences of human, rat and sheep CD59 show a high degree of homology: approximately 60% between rat and human, 40% between rat and sheep, and 50% between sheep and human. Considering the phylogenetic proximity of rat and mouse, it is noteworthy that rat CD59 is more similar to human CD59 than it is to murine Ly-6 (with which it is approximately 30% homologous). Finally, the murine Ly-6 genes map to mouse chromosome 15, whereas the region of human chromosome 11 which carries the CD59 gene is syntenic with mouse chromosome 2 [50–54]. Collectively, the above data argue against the likelihood that the Ly-6 and CD59 antigens are identical. However, their relationship is interesting in light of the proposed role of CD59 in T cell activation (see below).

CD59 is also homologous to a squid glycoprotein (SGP2) and to human urokinase plasminogen activator receptor (HUPAR), two other GPI-linked proteins [55, 56]. The homology between CD59 and HUPAR is particularly interesting since a region within the C9b portion of C9 is homologous to urokinase, the ligand for HUPAR. Another more striking similarity has been discovered between the nucleotide sequence of CD59 and that of a gene recently identified in Herpesvirus saimiri (HVS), designated HVS-15 [57]. The CD59 and HVS-15 sequences are 64% identical at the cDNA level and 48% identical at the level of predicted amino acid sequences. Positions of the cysteine residues are identical, and the putative site for addition of the GPI anchor in CD59 is conserved in HVS-15. In addition, HVS-15 contains a single consensus sequence for N-linked glycosylation. It is likely that HVS acquired is CD59-like gene from its natural host, the squirrel monkey *(Saimiri sciureus)*. A monkey equivalent of CD59 has yet to be descibed, but a cross-hybridising mRNA has been identified in monkey cells by Northern blotting [24]. As yet as protein product of the HVS-15 gene has not been discovered, but such a protein might contribute to the virulence of HVS by aiding immune evasion through interaction with the complement system.

Tissue Distribution

CD59 is broadly distributed on cells of haemopoietic and non-haemopoietic origin. The expression of CD59 in cells and tissues

has been widely studied using the variety of monoclonal and polyclonal antibodies available, and by Northern blot analysis using the CD59 cDNA. In summary, CD59 is present on leucocytes [17, 18, 25, 30], platelets [58, 59], endothelial and epithelial cells from a number of sources [33, 60–63], skin [30, 62], placental tissues [25, 32, 64], spermatozoa [65], lung, pancreas [25], thyroid cells [66–68], and eye tissues [69]. Table 1 shows the distribution of CD59 in human tissues as assessed by staining with the monoclonal antibody YTH 53.1. Functional activity of CD59 has been demonstrated on human umbilical-vein endothelial cells [33, 61], human amniotic epithelial cells [32], glomerular epithelial cells [63], platelets [58, 59], spermatozoa [65] and thyroid cells [67]. Little or no CD59 expression has been reported for many B cell lines [17, 70], and in CNS tissue [60, 62], although studies of isolated cell types from the CNS have demonstrated expression of CD59 by astrocytes but not oligodendrocytes [71, 72]. The number of CD59 molecules present on the surface of erythrocytes has been estimated as 25,000–50,000 per cell [14, 16].

Soluble forms of CD59 have been detected in urine, saliva, tears, sweat, concentrated cerebrospinal fluid, breast milk, amniotic fluid and seminal plasma [17, 32, 73, 74]. It is not clear whether the soluble forms have been secreted, cleaved off from cell membranes by a phospholipase, or shed from the surface of cells by some other means. CD59 has been purified from urine, amniotic fluid and seminal plasma [17, 32, 74]. The urine form does not reincorporate into cell membranes and is not active. It contains the glycosylinositol moiety of the GPI anchor but has a slightly higher apparent molecular weight than the membrane form, and matches in size CD59 which has had its fatty acid chains removed by PIPLC [17, 38, 75]. NMR studies of CD59 from urine confirm that the lipid is not present, and further indicate that the residual part of the GPI anchor contains only two phosphate groups [26]. This suggests that the lipid component was removed by PIPLD rather than PIPLC (fig. 1). In contrast, the soluble forms of CD59 present in amniotic fluid and seminal plasma retain the GPI anchor, incorporate into cell membranes and retain full functional activity [32, 74].

Table 1. A summary of the distribution of CD59 in human tissues

Tissue	Cells	Expression
Skin	epidermis	++
	endothelia	+/++
Liver	hepatocytes	−
	bile canaliculi	+/+−
	bile duct	+++
	vascular endothelia	+
	Kupffer cells	−
Kidney	glomerular capillaries	+
	glomerular epithelial cells	+−/+
	proximal tubules	−
	distal tubules	+/++
	collecting ducts	+++
Lung	bronchi and bronchioli	++
	alveolar lining	−
Pancreas	exocrine ducts	++
	islets of Langerhans	−
	acinar cells	−
Salivary gland	ductal epithelium	++
	acinar cells	+−/+
Placenta	syncytiotrophoblast	+++
	trophoblast	−/+−
	villous capillaries	
Nervous system	endothelia	++
	oligodendrocytes	−
	astrocytes	−
	nerve axons	−

Reproduced with permission from Meri et al. [62].

The Function of CD59

CD59 appears to be involved in the regulation of two apparently quite distinct immunological phenomena, those of complement-mediated cell lysis and T cell activation. The role of CD59 in complement inhibition has been extensively studied, but recently interest in the interaction between CD59 and T cells has also been gathering momentum. Although some information is available regarding the specific sites on target molecules with which CD59 interacts, still very little is known about which parts of the CD59 molecule are important for biological activity. It will be interesting to discover how this small and highly folded molecule can achieve this apparently dual role.

The Role of CD59 within the Complement System

Inhibition of Complement Lysis

CD59 inhibits complement lysis by preventing the full assembly of the MAC. This was shown initially using two experimental approaches: (1) monoclonal antibodies raised against CD59 were used to enhance the lysis of human cells by human complement [14, 17], and (2) the purified protein was incorporated into guinea pig erythrocytes and shown to inhibit their lysis by human complement [12, 16, 28]. In both approaches the effects of adding either antibody or CD59 were evident even after C5b-7 sites had been preformed on the cells, indicating that CD59 was acting late in the complement pathway at the stage of C8 and C9 incorporation into the MAC.

It is now clear that CD59 itself becomes incorporated into the partially formed MAC. It binds to C5b-8 (but not to C5b-7) and interferes with the subsequent binding and polymerisation of multiple C9 molecules within the MAC [16, 76]. Although CD59 does not bind to native C8 and C9, it has been shown to interact with denatured C8 and C9 that have been transferred onto nitrocellulose after SDS-PAGE, and with C8 and C9 that have been immobilised on plastic, suggesting that sites for binding to CD59 are exposed on these molecules after some conformational change has taken place. The specific binding sites have been narrowed down to the α chain of C8 and the 'b' domain of C9 [77]. These are the sites on C8 and C9 which have been reported to interact with one another, the C9b portion of C9 being the putative membrane-binding domain [78–80].

In the presence of CD59, the number of C9 molecules incorporated into the MAC is restricted to a C9:C8 ratio of 1.5 instead of 3.5 [16]. The single C9 molecule bound under these conditions is tightly incorporated into the CD59-C5b-8 complex, since it is not displaceable by other C9 molecules in the fluid phase, unlike C9 which has loosely bound at 4°C [81]. However, it cannot be fully unfolded and inserted into the membrane since lysis is blocked. Using guinea pig erythrocytes as targets, it was found that adding CD59 after a single C9 molecule had been allowed to bind at 4°C could not prevent the subsequent lysis achieved by warming the cells to 37°C. However, nucleated cells are less susceptible to complement-mediated lysis than are erythrocytes, and more than one C9 molecule per MAC is necessary to achieve cell lysis [82]. In these circumstances it may be possible that CD59 can block lysis after one C9 molecule has bound, by binding to it and thereby blocking the addition and polymerisation of further C9 molecules. A model for the mechanism of action of CD59 in inhibiting complement lysis is shown in figure 3.

A glycolipid anchor is necessary for CD59 to insert into cell membranes in vitro and thereby exert an effect in complement inhibition assays. However, it is not necessary for

the binding of CD59 to C8 and C9 which have been adsorbed onto plastic or nitrocellulose [77]. Therefore the GPI anchor is unlikely to play any direct role in the interaction between CD59 and the MAC. It has been speculated that possession of a GPI anchor might confer increased lateral mobility within the membrane, which could be advantageous for quickly reaching sites of complement activation. There is no evidence at present for a non-GPI-anchored form of CD59.

The role of the carbohydrate residue attached at Asn18 in the functional activity of CD59 is more complex. Enzymatically deglycosylated CD59 retained the capacity to bind to C8 and C9 adsorbed onto plastic [27], whereas this same treatment resulted in an almost complete loss of activity when CD59 was tested for inhibition of complement lysis after incorporation into chicken erythrocytes [27]. Similarly, recombinant CD59 expressed in CHO cells suffered an equivalent loss of activity when the cells were grown in an α-mannosidase inhibitor which restricts the membrane glycoprotein to a high-mannose type [27]. In our hands, recombinant CD59 expressed on the surface of insect cells conferred a high level of protection against lysis by human complement, despite the fact that the nature of the glycosylation carried out in these cells is not exactly comparable to that in mammalian cells [83]. Together, these data suggest that although the carbohydrate residue at Asn18 in CD59 may not be necessary for the actual binding to C8 and C9, the attachment of a complex carbohydrate here may influence the orientation of CD59 on the membrane, and this may govern its ability to interact with C8 and C9 in the assembling MAC. The binding site for C8 and C9 on CD59 appears to lie near the N-terminus, since a fragment of CD59 consisting of three peptides, residues 1–14, 15–31 and 39–41, linked together by disulphide bonds, has been

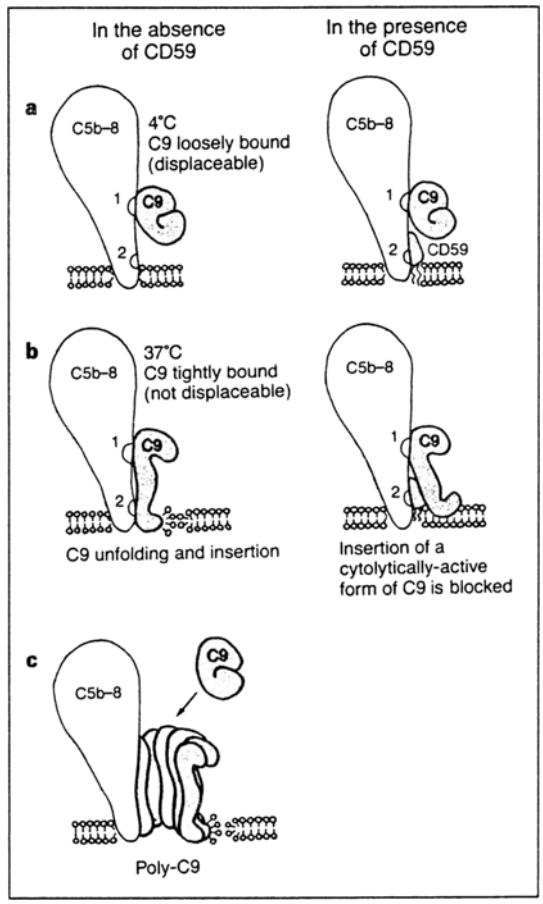

Fig. 3. Schematic representation of the proposed mechanism of CD59 function. Two hypothetical binding sites on C5b-8 for C9 (sites 1 and 2) are envisaged. **a** At 4 °C, C9 becomes loosely bound at site 1, and may be displaced. **b** After warming to 37 °C, C9 starts unfolding during which it interacts with a second site in C5b-8 and becomes integrated in the membrane. **c** Membrane damage ensues after exposure of (partly hydrophobic) new interaction sites with the lipid bilayer and by formation of transmembrane channels. In the presence of CD59 the initial binding of C9 to C5b-8 occurs (**a**), but the subsequent insertion of C9 and interaction with site 2 on C5b-8 is prevented. Inhibition of interaction between C5b-8 and C9 considered to be due to blocking of site 2 on C5b-8 by CD59. The firm association of the 'first' C9 molecule with the C5b-8-CD59 complex could be achieved by the interaction of CD59 with C9 whereby the recruitment of additional C9 molecules into the complex is prevented. Reproduced with permission from Meri et al. [16].

reported to interact with the terminal complement complex C5b-8 formed in the fluid phase [V. Feriani, pers. commun.].

Homologous Restriction

Complement of a given species is relatively inefficient at lysing cells from the same species. This phenomenon is known as homologous restriction, and has been attributed to the actions of a number of membrane-bound complement regulatory proteins which are proposed to be more efficient at inhibiting homologous than heterologous complement. On human cells, these include DAF, HRF and CD59. The ability of CD59 to inhibit complement from species other than the human has been tested in a number of laboratories. CD59 does not inhibit the lytic activity of guinea pig complement, due to an inability to interact with C8 and C9 from this species [84–87]. Reports concerning its interaction with rabbit complement vary [17, 28, 31]. CD59 appears to have greater inhibitory activity towards baboon complement than it does towards human complement, and intermediate levels of inhibition have been observed with complement from a variety of other species, for example dog and sheep [85]. Although it has been reported that CD59 does not inhibit lysis by rat complement, others have found that this is not the case [86, and our unpubl. obs.]. The rat homologue of CD59 also exhibits a degree of species selectivity, inhibiting rat, rabbit and human complement best, whilst failing to inhibit complement from horse, goat and guinea pig [49]. Sheep CD59 appears to be non-selective in the species of target complement, inhibiting most species to varying degrees [48]. Evidently, homologous restriction is a graded rather than an all-or-none phenomenon. Many of the above studies have been carried out using purified CD59 incorporated into guinea pig or chicken erythrocytes. CD59 incorporated into erythrocytes from other species appears not to be active [16, C.W. van den Berg and B.P. Morgan, pers. commun.]. It may be that the effect of a relatively small amount of incorporated CD59 is masked by any endogenous inhibitor already present on the cell surface. Therefore the interaction of CD59 with C8 and C9 from heterologous species may be best addressed using liposomes as targets for lysis rather than heterologous cells, to eliminate possible interference by endogenous complement inhibitors.

CD59 and Perforin-Mediated Lysis

Perforin isolated from cytotoxic T lymphocyte and natural killer cells shows limited structural homology to the terminal complement components, and forms lytic pores in cell membranes analogous to those formed by the MAC [89]. It has been proposed that HRF is capable of inhibiting perforin-mediated lysis [90, 91]. However, this has been disputed on the grounds that perforin lysis is not homologously restricted, and that cells which lack HRF are not unduly sensitive to perforin-mediated lysis [92–94]. The possibility that CD59 might inhibit perforin lysis has also been investigated and it was found that CD59 incorporated into guinea pig erythrocytes or Raji cells (which have little CD59 of their own) offered no protection against lysis mediated by lymphokine-activated killer cells, or by purified human or mouse erforin [95, 96]. This rules out a role for CD59 in the protection of cytotoxic cells against the effects of their own perforin.

A Role for CD59 in T Cell Activation?

CD59 is present on the surface of both T cells and accessory cells and may play a role in T cell activation. One of the earliest descrip-

tions of CD59 (H19) reported its involvement together with CD58 (LFA-3) in the rosetting of human T cells and erythrocytes, and (on the surface of accessory cells) in the process of T cell activation [19]. These results were supported by later studies using CHO cell transfectants expressing CD58, CD59, or both, and it was shown that CD59 could bind to CD2 at a site that was distinct from but overlapping with the binding site for CD58 [97, 98]. However, a more recent report has disputed these findings, claiming that CD48 but not CD59 is the second ligand for CD2 [99], and others have suggested that CD59 binds to activated B cells but not to T cells [100]. Further investigations will be necessary to clarify this and establish whether or not a physiological ligand for CD59 does exist.

CD59 on the T cell surface has been implicated in the process of signal transduction and cell stimulation. Cross-linking of CD59 on human T cells induced a rise in intracellular free calcium, inositol phosphate production, IL2 production and cell proliferation [101]. These responses were dependent upon the coexpression of the CD3/TCR complex. Interestingly, only the antibodies YTH 53.1 and MEM-43 which are directed against one of the two reported epitopes on CD59 were able to mediate this effect. An antibody (H19) against the other epitope, which inhibits rosette formation and blocks monocyte-dependent T cell activation by anti-CD3, was unable to stimulate T cells directly by cross-linking CD59 at the cell surface. In contrast, antibodies against both epitopes have been shown to abrogate complement-inhibitory activity [17, 20].

Many other GPI-anchored proteins have been implicated in the process of signal transduction and cell activation in both lymphoid and myeloid cells through the effects achieved by cross-linking them at the cell surface [reviewed in ref. 102]. In all cases tested it was found that the possession of a GPI anchor was a prerequisite for this effect to be realised [103–105]. This phenomenon is generally believed to mimic the physiological events that occur when a GPI-anchored protein meets its natural ligand in vivo. Clearly not all GPI-linked molecules that can be induced by cross-linking to induce cell activation in vitro necessarily do so in vivo. However, the above findings have stimulated great interest in the mechanism by which GPI-anchored molecules tethered to the outer leaflet of bilayer can transmit signals to the inside of the cell. Recent studies have shed some light on this: a number of GPI-anchored molecules have been found to associate with one another in clusters in the membrane, together with protein tyrosine kinase activity [105–109]. This has been demonstrated for CD59, which occurs in a non-covalent complex with DAF, other GPI-linked molecules and a rotein tyrosine kinase activity in the membrane of HPB-All cells [106, 110]. However, since the single case of an isolated CD59 deficiency is not associated with any deficiency in immunity (see below), the likelihood of an in vivo role for CD59 in T cell activation remains open to question.

CD59 and Disease

Complement-mediated tissue injury has been implicated in the pathogenesis of a number of inflammatory and autoimmune diseases. Increased CD59 expression has been reported on glomerular endothelial and epithelial cells in diffuse lupus nephritis [111] and on thyroid follicular epithelial cells in Graves' disease and Hashimoto's thyroiditis [66–68], suggesting that CD59 expression is increased in the face of ongoing complement attack or in response to other stimuli. Indeed, expression of CD59 in thyroid cells has been

shown to be upregulated by cytokines such as IL1, tumour necrosis factor and interferon-γ and by thyroid-stimulating hormone [66-68]. Conversely, decreased expression of CD59 and DAF has been observed on the surface of CD4+ and CD8+ T lymphocytes from HIV-infected patients, and may contribute to the increased sensitivity of these cells to complement-mediated lysis [112, 113].

WAGR syndrome (Wilms' tumour, aniridia, genito-urinary anomalies, mental retardation) is associated with a series of deletions and translocations involving band p13 of chromosome 11. This is the region on chromosome 11 to which the CD59 gene has been mapped, and indeed the gene is deleted in some WAGR individuals [42]. Control of complement lysis has not been extensively investigated in cases of WAGR syndrome, although haemolytic anaemia is not normally reported. Now that an association with WAGR syndrome and deletion of the CD59 gene has been found, further investigation may provide some insight into the role(s) of CD59 in vivo. A case of a complete, inherited deficiency of CD59 associated with haemolytic disease is discussed in the following section.

CD59 Deficiency and Paroxysmal Nocturnal Haemoglobinuria

Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired haemolytic disease in which affected cells are abnormally sensitive to complement-mediated lysis [reviewed in ref. 114]. The disease arises through somatic mutation in a pluripotent stem cell of the haematopoietic system, leading to a clone of abnormal cells involving erythrocytes, granulocytes, monocytes, platelets and sometimes lymphocytes. The molecular defect(s) underlying PNH has not yet been identified, but appears to lie in the biochemical pathways responsible for synthesis and attachment of glycolipid anchors: affected cells lack all GPI-linked proteins on their surfaces, including acetylcholinesterase, LFA-3 and HUPAR [115–118] as well as the complement control proteins DAF, HRF and CD59 [15, 21, 118–121], whereas the proteins themselves or the mRNAs that encode them can be detected intracellularly [122–124]. Recent progress in understanding the steps involved in the biosynthesis of the GPI core has facilitated a more precise localisation of the defect in PNH [125–127]. Patients with PNH suffer other clinical manifestations besides haemolysis, which may be associated with the absence of other GPI-linked proteins [reviewed in ref. 128].

The abnormal sensitivity of PNH erythrocytes to complement lysis was at first attributed to the lack of DAF on the cell membrane [129]. However, it was also known that PNH cells were abnormally sensitive to C5b-9-induced lysis, in which DAF, acting at the stage of the C3 convertase, could not be involved [130, 131]. When HRF and CD59 were discovered and found to be GPI-linked, it became clear that an absence of all three complement inhibitors contributed to the heightened sensitivity of PNH cells to complement lysis. However, the discovery of isolated deficiencies of DAF and CD59 have since given some indication of the relative importance of these inhibitors in protection against complement attack. Individuals with an isolated deficiency of DAF (the Inab phenotype) are asymptomatic [132, 133]. In contrast, the single reported case of an isolated and complete CD59 deficiency due to two single-base deletions in the CD59 gene is associated with a PNH-like syndrome involving haemolytic anaemia and thrombosis [134–137]. This suggests that it is the absence of CD59 in PNH that is the most significant fac-

tor in enhancing complement sensitivity, although the absence of other complement control proteins can only serve to exacerbate the condition.

Future Prospects and Therapeutic Implications

With a greater understanding of the role played by control proteins in the regulation of complement activity, it is possible to envisage circumstances in which manipulation of these inhibitors could be of great use therapeutically. Soluble forms of complement receptor proteins are already in use as anti-inflammatory and immunosuppressive agents [reviewed in ref. 138]. Future therapeutic strategies may also include the use of membrane-bound inhibitor proteins which could be incorporated into the surfaces of cells which express little or none of their own. CD59 would be particularly useful in disease conditions in which the MAC has been implicated, for example in multiple sclerosis. Interestingly, cultured rat oligodendrocytes are highly sensitive to complement-mediated damage and appear to express little if any CD59 on their surfaces [71], suggesting that in vivo they may be particularly vulnerable as targets of complement attack. The incorporation of human CD59 into rat oligodendrocytes in vitro was found to protect them against lysis by human complement, and to a lesser extent by rat complement [88]. However, for this approach to be feasible in vivo, a more specific method of targeting CD59 will have to be devised, since proteins with a glycolipid anchor will incorporate non-specifically into any nearby cell membrane. Following the discovery that CD59 in seminal plasma is naturally associated with bilamellar vesicles and can readily be transferred from these into guinea pig cell membranes, the use of lipid vesicles as a vehicle for delivering GPI-linked proteins has recently been suggested [74].

There is a growing interest in the part played by complement in hyperacute rejection of xenografts. Xenotransplantation is becoming increasingly attractive as a solution to the problem of organ shortage. However, naturally occurring antibodies against xenoantigens mediate rejection by inducing complement-mediated destruction of the donor organ endothelial cells [reviewed in ref. 139]. Measures aimed at introducing human complement inhibitors into foreign cells by genetic engineering are therefore generating considerable interest [140–142]. Underlying this is the assumption that complement control proteins on the foreign organ will be poor inhibitors of the human complement system. It may turn out, however, that endogenous inhibitors on xenotransplanted organs can interact quite adequately with human complement, and this warrants further investigation.

Many micro-organisms which are successful human pathogens are able to evade destruction by the human immune system, including complement [reviewed in ref. 143, 144]. Proteins with complement regulatory activity have been identified in a number of micro-organisms, and in particular a CD59-like molecule has been reported on the surface of *Schistosoma mansoni* [145]. In this laboratory we are investigating the presence and potential role of complement control proteins on the surface of virus particles, including those of HIV. The identification of such proteins on the surface of pathogenic microorganisms could be beneficial therapeutically, for, providing they differ sufficiently from those of their host, they represent potential targets for vaccine development.

Concluding Remarks

This review outlines the discovery of CD59, the latest addition to the family of complement control proteins, and charts the progress made in understanding its structure and function. Although recent investigations have answered many questions, new ones have arisen regarding the potential interaction between CD59 and the T cell system, the significance of the relationship between CD59 and the Ly-6 family of proteins, and perhaps more interestingly, the apparent lack of any structural relationship between CD59 and other members of the complement system. It will be interesting to see how this story unfolds.

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