

# Viral mimicry of the complement system

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The complement system is a potent innate immune mechanism consisting of cascades of proteins which are designed to fight against and annul intrusion of all the foreign pathogens. Although viruses are smaller in size and have relatively simple structure, they are not immune to complement attack. Thus, activation of the complement system can lead to neutralization of cell-free viruses, phagocytosis of C3b-coated viral particles, lysis of virus-infected cells, and generation of inflammatory and specific immune responses. However, to combat host responses and succeed as pathogens, viruses not only have developed/adopted mechanisms to control complement, but also have turned these interactions to their own advantage. Important examples include poxviruses, herpesviruses, retroviruses, paramyxoviruses and picornaviruses. In this review, we provide information on the various complement evasion strategies that viruses have developed to thwart the complement attack of the host. A special emphasis is given on the interactions between the viral proteins that are involved in molecular mimicry and the complement system.

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## 1. Introduction

The complement system, an integral component of innate immunity, is an ancient system of immunological defense. Recent cloning of C3-like molecules in nonvertebrate deuterostomes – for example, sea urchin (Al-Sharif *et al* 1997) and ascidians (Nonaka *et al* 1999) – and in proteosome – for example, mosquito (Levashina *et al* 2001); and identification of a lectin (Sekine *et al* 2001) and lectin-associated serine proteases in ascidians (Ji *et al* 1997) suggest that the complement system has emerged at least 700 million years ago, long before the appearance of immunoglobulins. The complement system has evolved to perform wide range of immune function with a goal to eliminate ‘nocuous’ substances from the body. This elimination is achieved by various methods: (i) Attach-

ment of the complement component C3 to the pathogen surface leads to engulfment of the foreign pathogens. (ii) Formation of the membrane attack complex (MAC, C5b-9) on the pathogen surface results in direct lysis of the pathogen. (iii) Release of anaphylatoxic peptides as a result of complement activation produces the local inflammatory response against pathogen. (iv) The complement system prevents immune precipitation, and help solubilization and clearance of immune complexes from the circulation. (v) It helps in selection of appropriate antigens for a humoral response by tagging them with C3 (Dempsey *et al* 1996). (vi) The complement system is also involved in negative selection of self-reactive B cells (Prodeus *et al* 1998).

Since the complement system has evolved to perform all the above-mentioned functions, it must recognize a

**Keywords.** Complement system; herpesviruses; paramyxoviruses; picornaviruses; poxviruses; retroviruses; viral mimicry

Abbreviations used: CCP, Complement control protein; CCPH, complement control protein homolog; CV, coxsackieviruses; DAF, decay-accelerating factor; EBV, Epstein-Barr virus; EEV, extracellular enveloped virus; gC-2, glycoprotein C-2; HCMV, human cytomegalovirus; HSV-1, herpes simplex virus-1; HTLV-1, human T-cell leukemia virus type-1; MAC, membrane attack complex; MBL, mannose-binding lectin; MCP, membrane cofactor protein; MV, measles virus; ORF, open reading frame; RCA, regulators of complement activation; HVS, herpesvirus saimiri; SCR, short consensus repeat; VCP, vaccinia virus complement control protein; VV, vaccinia virus.

large array of molecular structures, and must adapt to new structures as they emerge. If the system has to fulfill all the above criteria, then it is likely to become relatively non-specific and perhaps might not be able to discriminate between the self and the non-self. Consistent with this premise, it has been reported that complement proteins which are involved in the activation process, though show preferences for certain structures (Sahu *et al* 1994; Sahu and Pangburn 1994, 1995; Kim *et al* 1992; Kinoshita *et al* 1988), do not discriminate between the self and the non-self and have the potential to destroy any cell including the host cells (Sahu and Lambris 2001). This inadvertent complement-mediated damage of the host cells by homologous complement is protected by a family of structurally and functionally related proteins termed as regulators of complement activation (RCA) (Lambris *et al* 1998) (figure 1). These proteins range from 45–540 kDa and are characterized by the presence of common structural motifs – the short consensus repeat (SCR) or complement control protein (CCP) modules. Each SCR is comprised of approximately 60 to 70 amino acids, and are characterized by a conserved motif that includes four disulphide-linked cysteines, prolines, tryptophan and many other hydrophobic residues which together form a bead-like structure. The proteins belonging to the RCA family include both the plasma proteins; for example, factor H and C4-binding protein (C4bp); and membrane proteins such as complement receptor 1 (CR1), decay-accelerating factor (DAF), and membrane cofactor protein (MCP). These proteins act at the level of C3 and C4 and function by dissociating the subunits of C3 and C5 convertases and/or by acting as cofactors for the factor I-dependent cleavage of C3b and/or C4b (Sahu *et al* 2000) (figure 2). In addition, regulation of complement is also achieved by non-RCA proteins like C1 inhibitor, carboxypeptidase N and CD59: which inhibit activated C1s and C1r, anaphylatoxins (C3a, C4a and C5a), and MAC formation, respectively (Sahu and Lambris 2000).

Given that the complement proteins involved in the activation process do not discriminate between self and non-self, it is not unexpected that viruses are susceptible to complement attack. Viruses are obligatory intracellular parasites and depend solely on the host for their survival and propagation. Thus, when viruses invade the host they are exposed to the complement system of the host, which may result in complement-dependant viral lysis, neutralization of the viral particles by opsonization with complement proteins of the host and destruction of the viral particles by phagocytosis. Therefore, the complement system presents a constant threat to viruses for their survival within the host and for successful transmission. During the co-evolution of the viruses with their hosts over a period of several million years, they have developed mechanisms to combat the complement system of

the host to be a successful pathogen. The viruses have adapted various ways to subvert the complement system; either by capturing CCPs from the RCA loci of their host, or by evolving CCPs within their genome. In addition, they take advantage of the complement system and use the host complement receptors to infect various cells (Sahu *et al* 1998a).

## 2. Role of complement system in virus neutralization

It is known over several decades that the complement system of the host gets activated upon interaction with viruses (Wedgewood *et al* 1956). Activation however is not always associated with virus neutralization. As described further in this review, many viruses take advantage of complement activation to infect cells. Activation of the complement system can occur through three distinct pathways: the classical; alternative; or the more recently identified lectin (mannose-binding lectin; MBL) pathways (see figure 2): (i) The classical pathway activation has been demonstrated in the presence of virus-specific antibodies and cross-reacting antibodies (Beebe and Cooper 1981; Beebe *et al* 1983). In addition, this pathway is also activated in the absence of antibodies due to the direct interaction of C1q with viral surface proteins. For example, p15E of oncornavirus (Bartholomew *et al* 1978) and gp41 (Ebenbichler *et al* 1991) and gp120 (Susal *et al* 1994) of human immunodeficiency virus (HIV-1) have been shown to interact with C1q. (ii) The alternative pathway activation is initiated by covalent attachment of metastable C3b (C3b with an intact thioester bond; half life ~ 100  $\mu$ s) to hydroxyl and amino groups present on the viral surface to form an ester, the preferred linkage, or an amide bond. Since its initiation does not require any recognition molecule, in the absence of complement regulators, the pathway can be efficiently activated by enveloped as well as by non-enveloped viruses. Various examples known to activate the alternative pathway include sindbis virus (Hirsch *et al* 1980), simian virus 5 (McSharry *et al* 1981), vesicular stomatitis virus (VSV) (McSharry *et al* 1981) and Epstein-Barr virus (EBV) (Mold *et al* 1988a). (iii) The lectin pathway is initiated as a result of surface carbohydrate recognition by lectins followed by activation of associated serine proteases. Viruses that are known to activate the MBL pathway include a variety of oncolytic viruses (Wakimoto *et al* 2002), hepatitis C virus (Ishii *et al* 2001) and HIV (Ezekowitz *et al* 1989; Saifuddin *et al* 2000).




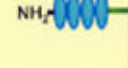


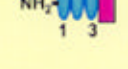
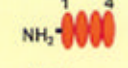



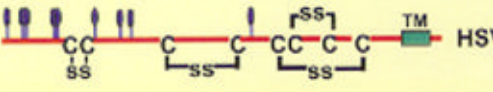
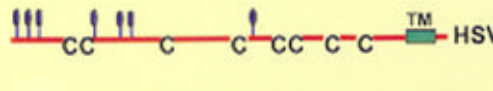
Activation of the complement system in the absence of proper regulation can lead to virus neutralization. The various mechanisms which are known to inactivate viruses are: (i) neutralization by complement dependant

aggregation; (ii) neutralization by opsonization with complement proteins; (iii) neutralization by direct lysis; and (iv) neutralization by phagocytosis (figure 3).

## 2.1 Neutralization by complement dependant aggregation

Neutralization due to aggregation occurs in most enveloped and non-enveloped viruses. Multivalent antibodies

directed against the viral surface proteins induce aggregation and reduction in the total number of infectious units. This type of neutralization has also been demonstrated in polyoma virus where C1q aggregated the antibody coated polyoma virus particle and enhanced neutralization (Oldstone et al 1974). Whether such phenomenon is of any physiological significance is under question since plasma-complement proteins, other than C1q and MBL, are monovalent in nature. In addition, other factors such as virus : antibody ratio and size of the aggregate (due to

Membrane Proteins		Factor I cofactor activity		DAF activity	
		C3b	C4b	C3b,Bb	C4b,2a
   	CR1	+	+	+	+
	CR2	-	-	-	-
	MCP	+	+	-	-
	DAF	-	-	+	+
Soluble Proteins					
  	fH	+	-	+	-
	C4bpα	-	+	-	+
	C4bpβ	-	-	-	-
Viral Proteins					
     	VCP	+	+	+	+
	HVS-mCCPH	ND	ND	ND	ND
	HVS-sCCPH	ND	ND	ND	ND
	HHV-8 CCPH	ND	ND	ND	ND
	HSV gC-1	-	-	+	-
	HSV gC-2	-	-	-	-

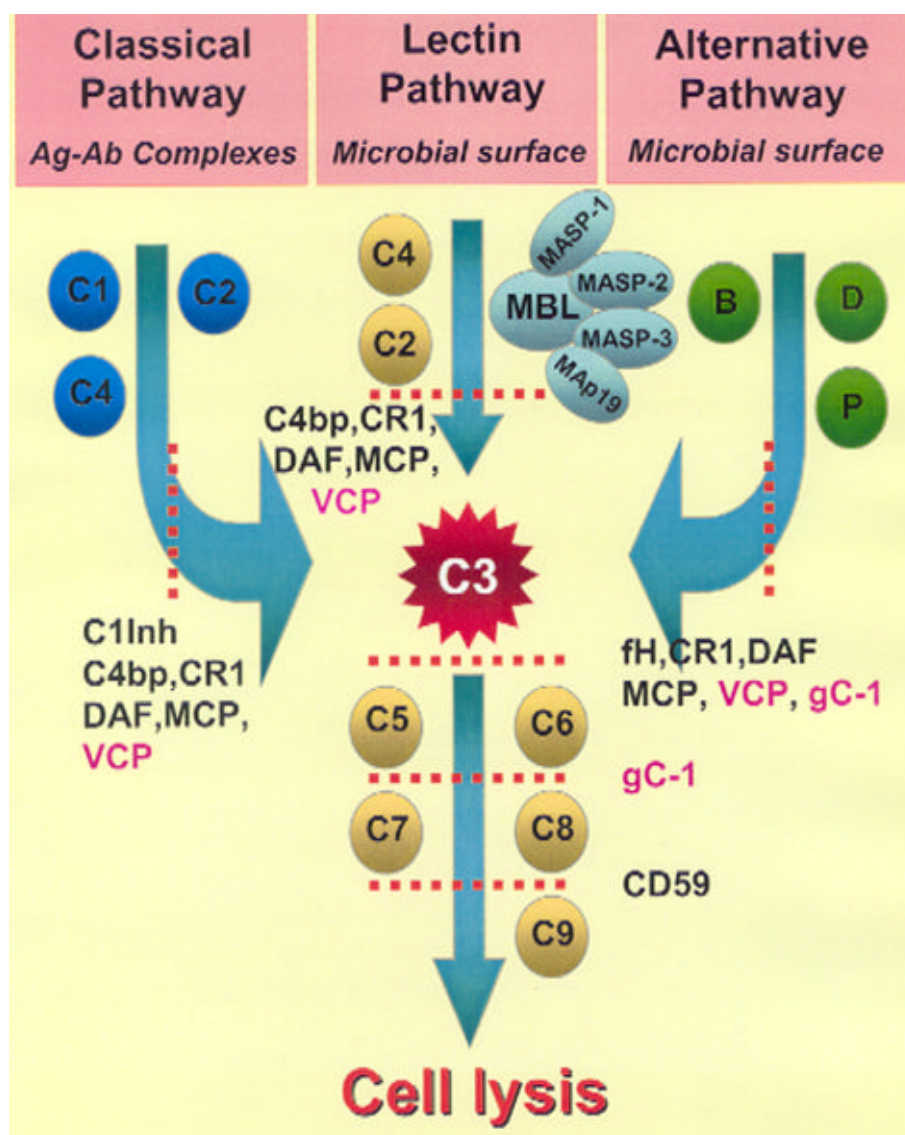
**Figure 1.** Schematic representation of human RCA proteins and viral proteins with complement regulatory activities. The DAF and factor I cofactor activities of these proteins are shown on the right. ND, Not determined.

large size of virus-antibody complexes) would also influence this process (Cooper and Nemerow 1983).

## 2.2 Neutralization by opsonization with complement protein

It was believed that complement-mediated lysis operates as the primary mechanism of complement-mediated virus neutralization. However, increasingly it is becoming clear that viral neutralization may occur in the absence of aggregation or lysis. The lack of evidence for lysis in most of the earlier reports and growing list of reports

demonstrating evidence for neutralization without lysis suggest that this may be the major mechanism of viral neutralization. The opsonization-mediated viral neutralization is a direct result of coating of viral surface by complement proteins. These coats are clearly observed in electron microscopic studies (Welsh *et al* 1976; Nemerow and Cooper 1981). Recent studies have clearly demonstrated that coating of viral surface with C3 in case of HIV-1 (Sullivan *et al* 1998) and C5 in case of herpes simplex virus-1 (HSV-1) gC null virus (Friedman *et al* 2000) is essential for viral neutralization. Though there is no direct evidence, complement coating could inhibit



**Figure 2.** Activation pathways of the complement system. The complement system is activated by three distinct pathways: the classical, alternative and lectin pathways. Dotted lines indicate regulation at different steps by complement regulators (black letters) and viral proteins (pink letters).

virus attachment, entry, uncoating, DNA transport to the nucleus, or immediate early gene expression.

### 2.3 Neutralization by direct lysis

Enveloped viruses undergo complement-dependant lysis resulting in the disruption of the viral integrity and irreversible loss of viral activity. In order for lysis to occur, sufficient numbers of MACs must be formed on the viral envelope. This is achieved when a large amount of antibody is deposited on the viral surface which leads to efficient complement activation or when there is a direct activation of the complement in the absence of antibody. Many viruses such as alphavirus, coronavirus, herpesvirus, orthomyxovirus, paramyxovirus and retrovirus families are susceptible to this type of complement-mediated lysis (reviewed in Cooper and Nemerow 1983).

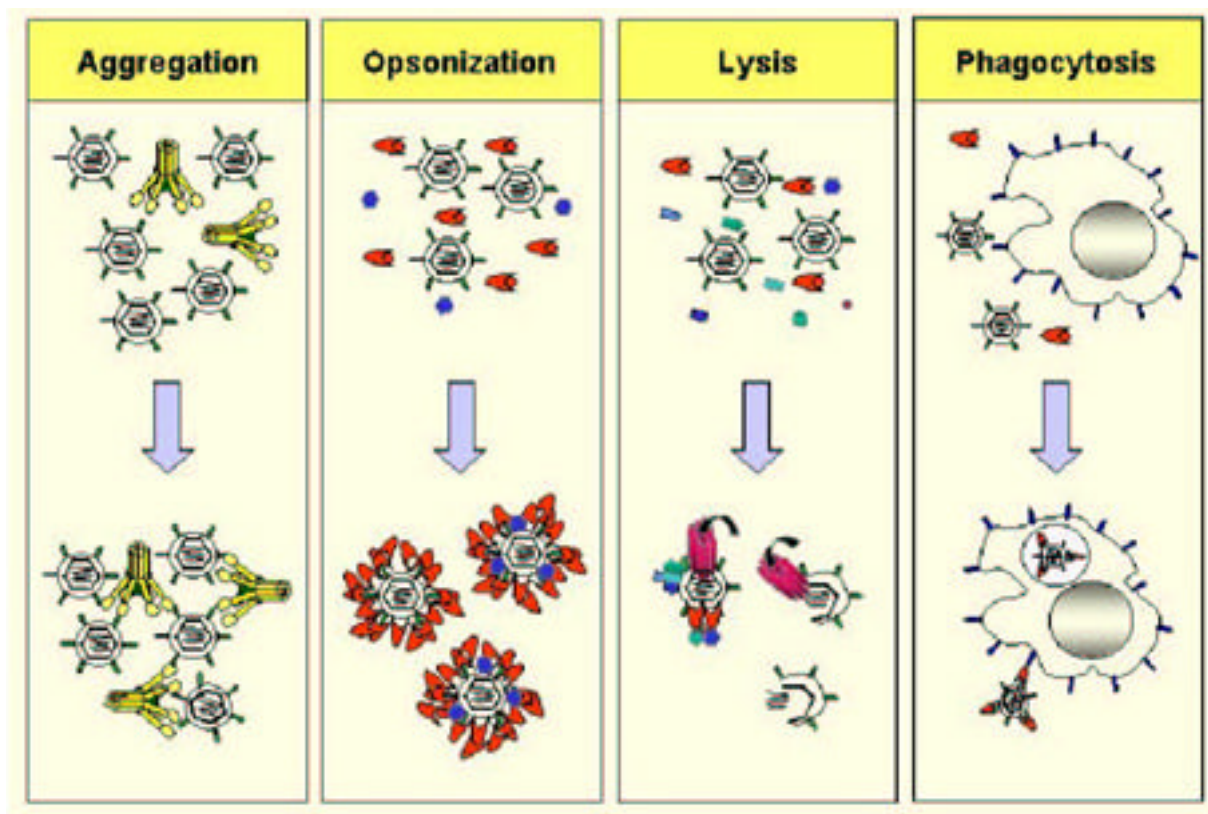
### 2.4 Neutralization by phagocytosis

Complement activation at the surface of the virus leads to covalent attachment of C3b to the viral surface. This

makes opsonized viruses prone to phagocytosis through complement receptors which finally leads to the destruction of the virus. This mechanism is well-documented for herpes simplex and Japanese encephalitis viruses (Kelkar and Gogate 1987; Van Strijp *et al* 1990).

## 3. Complement system and viral molecular mimicry

As discussed above, the host complement system has the potential to efficiently inactivate all types of viruses. Thus, viruses have devised multiple mechanisms to manipulate and subvert the complement system, and evade the immune system of the host in order to survive as successful pathogens. A few viruses use subtle mechanisms, such as, stay latent in cells and emerge any time later during their lifetime. The lack of expression of any detectable viral antigen during their latent period allows them to evade from all the immune mechanisms including the complement system. However, once reactivated they need to evade the complement attack. Therefore,



**Figure 3.** Complement mediated neutralization of viruses. (1) *Aggregation*: Binding of C1q and MBL may lead to aggregation of viruses. (2) *Opsonization*: Activation of complement may lead to deposition of C3b and C5b on the viral surface. (3) *Lysis*: Activation of complement in the presence or absence of antibody may lead to lysis of viral particles due to formation of C5b-9 (MAC). (4) *Phagocytosis*: Deposition of complement components on viral surface as a result of complement activation may lead to phagocytosis.



along with acute viruses, even latent viruses have developed active strategies to evade the host complement. These strategies include direct inactivation of complement either by encoding complement regulatory proteins or by capturing membrane regulatory proteins from the host and by the use of membrane complement receptors to gain cellular entry.

### 3.1 Evasion by encoding complement control proteins

Two different families of viruses namely the poxviruses and herpesviruses are known to encode for proteins with complement regulatory activities (table 1). Although there are multiple examples in each family, only a few of them have been characterized to significant details.

(i) *Poxviruses*: Viral homologs of complement control proteins have been identified in various members of the poxvirus family e.g. vaccinia, cowpox, rabbitpox, and smallpox viruses (Kotwal and Moss 1988; Martinezpomaes *et al* 1995; Miller *et al* 1997; Rosengard and Ahearn 1998; Sahu *et al* 1998b). The first known and best-studied example is vaccinia virus complement control protein (VCP). This protein received scientific attention when it was discovered that an attenuated mutant of vaccinia virus (VV) does not encode for this protein (Kotwal *et al* 1990).

VCP is a 27 kDa secretory protein encoded by the C3L open reading frame (ORF) of the vaccinia genome. The

primary structure, deduced from the ORF, consists of 263 amino acids, including a 19 amino acid signal peptide. Sequence analysis revealed that it is composed of four tandemly repeating SCRs (Kotwal *et al* 1990) and thus is homologous to members of the RCA family (see figure 1). Efforts have been made to determine the three-dimensional structures of VCP. Analysis of the NMR structures of pairs of VCP modules (SCR 2-3 and SCR 3-4) showed that, each SCR fold into a compact 6 **b**-strand structure and is conformationally similar to SCR 16 of factor H (Wiles *et al* 1997; Henderson *et al* 2001). Importantly, it also revealed that the relative orientation of successive modules differed from one module pair to another. More recently, the crystal structure of the entire VCP molecule has been solved (Murthy *et al* 2001). The salient features of the crystal structure are: (i) SCR modules have the 6 **b**-strand topology; as shown previously by NMR structures. (ii) The “tilt” angles between the long axes of SCR at the SCR 1–2, 2–3 and 3–4 junctions are 60°, 63° and 99°, respectively. Thus, the molecule has an extended structure from SCR 1–3 and a turn between SCR 1–3 and SCR 4. (iii) The “hypervariable” loop of SCR 1 (Lys14-Ala23) projects laterally from VCP close to the N-terminal tip of the molecule. (iv) A two amino acid insertion (Gln42 and Lys43) in SCR 1 forms a **b**-bulge near the interface with SCR 2. (v) A five amino acid insertion (Leu109-Ser114) in SCR 2 forms a loop near the interface with SCR 1.

Initial functional studies revealed that VCP apparently protects the infected cells and the released virions from

**Table 1.** Viruses that encode protein(s) with complement regulatory activity.

Virus family	Virus	Viral protein	Homology	Key feature	References
Poxviruses	Vaccinia	VCP	RCA	Binds C3b and C4b. Accelerates decay of CP and AP C3-convertases. Factor I cofactor for C3b and C4b	Kotwal <i>et al</i> 1990; McKenzie <i>et al</i> 1992; Sahu <i>et al</i> 1998b
	Cowpox	IMP	RCA	Modulates <i>in vivo</i> complement-mediated inflammatory responses	Miller <i>et al</i> 1997
	Variola	SPICE	RCA	Inhibits human complement	Rosengard and Ahearn 1998
Herpesviruses	HSV-1	gC-1	None	Binds native C3, C3b, iC3b and C3c. Accelerates decay of AP C3-convertase. Blocks binding of properdin and C5 to C3b	Friedman <i>et al</i> 1984; Fries <i>et al</i> 1986; Kostavasil <i>et al</i> 1997
	HSV-2	gC-2	None	Binds native C3, C3b, iC3b and C3c	McNearney <i>et al</i> 1987; Kostavasil <i>et al</i> 1997
	EBV	Unknown	Unknown	Accelerates decay of AP C3-convertase. Factor I cofactor for C3b, iC3b, C4b and iC4b	Mold <i>et al</i> 1988b
	HVS	HVSCCPH	RCA	Blocks C3b deposition	Albrecht and Fleckenstein 1992; Fodor <i>et al</i> 1995
		HVSCD59	CD59	Inhibits terminal complement pathway	Albrecht <i>et al</i> 1992; Rother <i>et al</i> 1994

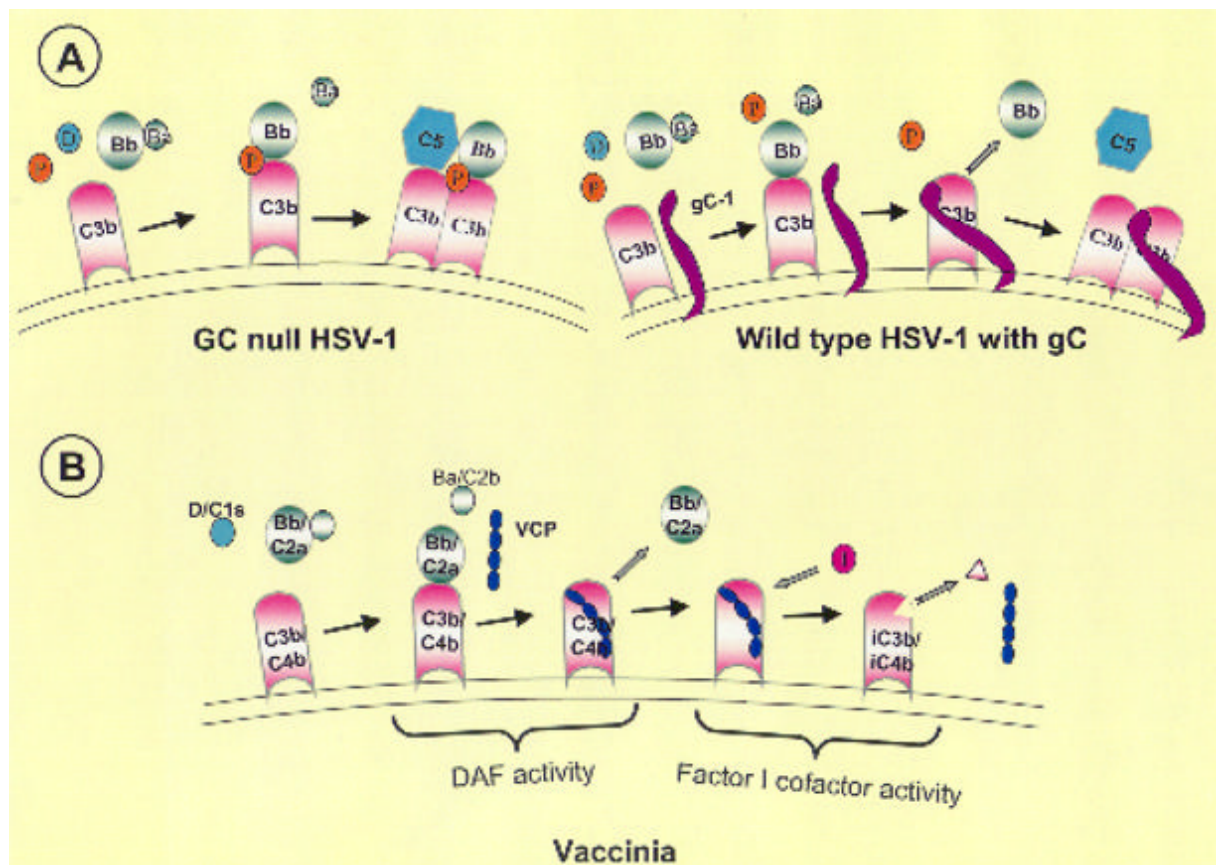
CP, Classical pathway; AP, alternative pathway; IMP, inflammation modulatory protein; SPICE, small pox inhibitor of complement enzymes.

attack by host complement. It was shown that VCP abrogates antibody-dependent complement-mediated neutralization of vaccinia virus (VV) (Isaacs *et al* 1992a). In addition, studies using recombinant VV that do not express VCP have shown that these viruses are attenuated *in vivo* (Isaacs *et al* 1992a). Further, studies to determine the mechanism of complement inactivation of VCP were performed using the culture medium containing secreted VCP and partially purified wild type VCP. In these studies, VCP was shown to inhibit the classical pathway-mediated lysis of sheep erythrocytes, to bind to C3b and C4b, and to accelerate the decay of classical as well as of alternative pathway C3 convertases (Mckenzie *et al* 1992) (figure 4). These studies also suggested that VCP possesses factor I cofactor activities for C3b and C4b, however, the cleavage products were not visualized and thus the nature of cofactor activity could not be determined.

To understand the detailed mechanisms by which VCP inactivates complement, earlier we expressed recombi-

nant VCP using *Pichia* expression system (Sahu *et al* 1998b) and asked: Is VCP as effective as other physiologic complement inhibitors in inhibiting complement activation? Does it discriminate between activator bound and non-activator bound C3b? Is VCP's cofactor activity similar to other cofactor molecules (CR1 and factor H)? Analysis of our data led to following conclusions, (i) VCP is significantly less effective than CR1 in inhibiting classical as well as alternative pathways of complement and factor H in inhibiting the alternative pathway, but is 4-fold more effective than factor H in inhibiting the classical pathway; (ii) unlike factor H, it does not discriminate between activator (rabbit erythrocyte)- and non-activator (sheep erythrocyte)-bound C3b; and (iii) unlike CR1 and factor H, it displays cofactor activity primarily for the first site, which is sufficient to inactivate C3b (Sahu *et al* 1998b) (see figure 4).

Although VCP is composed only of four SCR domains, it binds to C3b as well as C4b and possesses all the com-



**Figure 4.** Complement inactivation mechanisms of gC-1 and VCP. (A) HSV-1 is sensitive to complement-mediated neutralization in the absence of gC. Binding of C5, but not C6-C9 is essential for the neutralization of HSV-1 gC null viruses. Presence of gC in wild type HSV-1 virus accelerates the decay of alternative pathway C3-convertase C3b,Bb and inhibits binding of properdin (a positive regulator of C3b,Bb) and C5 to C3b. (B) VCP secreted by vaccinia virus protects the virions from antibody-dependent complement-mediated neutralization. VCP possesses both DAF- as well as factor I cofactor-activities for C3b and C4b.

plement regulatory activities of CR1 (composed of 30 SCRs). Previously it has been shown that at least three SCRs are essential for C3b/C4b binding in CR1 (Klickstein *et al* 1988; Krych *et al* 1991), while four are needed for C3b binding in factor H (Gordon *et al* 1995; Alsenz *et al* 1984; Sharma and Pangburn 1996). In case of VCP, it has been shown that all the four SCRs are required for binding to C3b (Rosengard *et al* 1999). This led to the belief that the binding sites for C3b and C4b in VCP are overlapping in nature. Our recent laboratory data however suggests that, physically distinct sites on VCP are involved in binding to C3b and C4b (Bernet J and Sahu A, unpublished data).

VCP also contains two distinct putative heparin-binding sites: the first site overlaps between SCRs 1–2; and the second site is located in SCR 4 (Murthy *et al* 2001). Amongst the human complement regulators, factor H and C4bp bind to heparin (Sahu and Pangburn 1993). Importantly, interaction of factor H with heparin is an important step in the regulation of activation of alternative pathway. Whether interaction of VCP with heparin is fortuitous or has any physiological relevance needs further study. Currently our laboratory is focusing on these aspects of VCP.

(ii) *Herpesviruses*: A number of herpesviruses are known to encode for proteins with complement regulatory activities. The known examples are HSV-1 (McNearney *et al* 1987; Friedman *et al* 1996), HSV-2 (McNearney *et al* 1987), EBV (Mold *et al* 1988b), herpesvirus saimiri (HVS) (Albrecht and Fleckenstein 1992), Kaposi's sarcoma-associated herpesvirus (HHV-8) (Russo *et al* 1996), and murine  $\gamma$ -herpesvirus 68 (Virgin *et al* 1997).

(a) *Herpes simplex virus*: Glycoprotein C of HSV-1 (gC-1) (see figure 1) was the first viral-encoded protein to be identified as complement-binding protein and is also the most extensively studied protein amongst all the virally encoded complement regulators (Friedman *et al* 1984; Sahu *et al* 1998a). It is one of the 11 proteins expressed on the virion envelop as well as on the surface of infected mammalian cells (Spear 1985). A similar protein present on HSV-2 is known as glycoprotein C-2 (gC-2) (see figure 1). Both gC-1 and gC-2 are highly homologous in sequence and occupy collinear positions on their respective viral genomes (Zezulak and Spear 1984; Swain *et al* 1985). It is important to note that none of these proteins show either partial or complete homology to proteins of the RCA family. Thus, the mechanisms through which they inactivate complement are distinctly different from RCA proteins.

HSV gC-1 is a 511 amino acid protein including 25 amino acid signal sequence encoded by HSV-1 UL44 gene (Frink *et al* 1983). The protein is highly glycosylated: containing nine potential sites for *N*-linked oligo-

saccharides (*N*-CHO) (Frink *et al* 1983) and numerous *O*-linked oligosaccharides (*O*-CHO) (Johnson and Spear 1983; Dall'Olio *et al* 1985). Although the detailed carbohydrate analysis has not been performed, it has been shown that eight to nine *N*-linked oligosaccharides of expressed gC-1 are occupied by approximately 1 kDa glycans, and the *O*-CHO moieties are primarily located at the N-terminal region (residues 33–123) (Rux *et al* 1996). A complete disulphide linkage pattern has also been determined for gC-1. It contains eight cysteine residues that form four disulphide bonds (Rux *et al* 1996). Glycoprotein C-2 on the other hand, is a 480 amino acid transmembrane-protein with seven potential sites for *N*-CHOs and several sites for *O*-CHOs (Swain *et al* 1985). Like gC-1, it also contains eight cysteine residues. Since cysteine positions are highly conserved between gC-1 and gC-2 it is predicted that a similar disulphide pattern must be present in gC-2 (Rux *et al* 1996).

Studies with gC-null HSV-1 and HSV-2 mutants have clearly established that these molecules provide protection against complement-mediated neutralization (McNearney *et al* 1987; Friedman *et al* 1996; Hidaka *et al* 1991). Examination of interactions of these proteins with complement proteins revealed that both gC-1 and gC-2 bind to native C3 and its proteolytically cleaved fragments C3b, iC3b and C3c (Fries *et al* 1986; Kostavasil *et al* 1997). Glycoprotein C-1, but not gC-2, is also known to inhibit the binding of properdin and C5 to C3b (Hung *et al* 1994; Kostavasil *et al* 1997) (see figure 4). Further, insight into the mechanism of complement inactivation has revealed that though gC-1 does not inhibit the formation of alternative pathway C3 convertase (C3b, Bb) (Kostavasil *et al* 1997), like other regulators of complement activation (factor H, CR1, and DAF), it accelerates the decay of C3b, Bb into its subunits. Glycoprotein C-2 on the other hand is known to stabilize the C3 convertase (Fries *et al* 1986; Eisenberg *et al* 1987) (see figure 4). None of these proteins however mediate the proteolytic inactivation of C3b or C4b by factor I (Fries *et al* 1986).

Structure-function analysis of gCs, using linker insertion and deletion mutants, identified four distinct regions in gC-1 and three separate regions in gC-2 as C3b-binding sites (Seidel-Dugan *et al* 1990; Hung *et al* 1992). These C3b-binding sites are located in the central portion of the gCs. Further, studies using deletion mutants have revealed that the N-terminal region (residues 33–123) of gC-1, which is not involved in binding to C3b, is necessary to inhibit properdin and C5 binding to C3b (Kostavasil *et al* 1997). Thus, it appears that this region sterically hinders the access to properdin and C5-binding sites on C3b. Since all these mechanistic studies were done *in vitro* it was important to evaluate the *in vivo* importance of these domains. Recent *in vivo* study on the role of complement-interacting domains of gC-1 has



clearly shown that the C3-binding domain is more important than the C5/P-blocking domain and is a major contributor to complement evasion (Lubinski *et al* 1999).

From the studies described above it is clear that gCs offer survival advantage to HSV by subverting the complement system. This notion is further supported by the fact that though spontaneous gC knockout of HSV have frequently emerged in cell cultures, they are rarely isolated from human materials (Hidaka *et al* 1990).

(b) *Herpesvirus saimiri*: Analysis of the genome of HVS has identified a gene that encodes for a protein with striking homology to VCP and other members of the RCA protein family (Albrecht and Fleckenstein 1992). The gene encodes for a protein of 360 amino acids with seven consensus sites for N-linked glycosylations, a signal peptide of 20 amino acids, and a transmembrane domain of 23 residues (residues 328–350) which lie at the C-terminus. The region between amino acids 21 to 265 forms four SCR domains.

The HVS complement control protein homolog (CCPH) gene encodes for two different forms of the same protein, the membrane form (mCCPH) and the secretory form (sCCPH), as a result of differential splicing of the primary transcript (Albrecht and Fleckenstein 1992). Both proteins contain all the four SCRs except that the membrane form contains a putative transmembrane domain (see figure 1). The membrane form has already been cloned and the functional data shows that it protects the transfected cells from complement-mediated damage (Fodor *et al* 1995). Although the mechanism of complement inactivation has not been studied, it was reported that mCCPH inhibits the deposition of C3b on transfected cells (Fodor *et al* 1995). Currently our laboratory is attempting to decipher the mechanism by which HVS-CCPH and a similar protein present in HHV-8 inactivate complement.

Apart from CCPHs, HVS is also known to encode for a CD59-like molecule (Albrecht *et al* 1992). In mammals, CD59 protects host cells from the cytolytic action of C5b-9 by tightly binding to C5b-8 complex, and by preventing the incorporation of C9 molecules (Morgan 1999;

Sahu and Lambris 2000). Cloning and functional analysis of this molecule showed that it is a functional homolog of CD59 (Rother *et al* 1994). Thus, unlike other viruses, HVS encodes for two distinct complement control proteins that function at two different steps of complement activation.

(c) *Epstein-Barr virus*: Although the genome of EBV does not contain ORF that has either full or partial sequence similarity to members of the RCA family, it contains protein(s) that has complement regulatory activity. Purified EBV has been shown to possess cofactor activity for factor I mediated cleavage of C3b, iC3b, C4b and iC4b (Mold *et al* 1988b). In addition, EBV also accelerates the decay of alternative pathway C3-convertase C3b,Bb (Mold *et al* 1988b). Though these activities seem similar to human complement regulator CR1, there are significant differences. Unlike CR1, EBV does not bind to C3b and accelerate decay of the classical pathway C3-convertase, C4b,2a (Cooper 1998). To date, no efforts have been made to characterize these proteins.

### 3.2 Evasion by acquiring host complement control proteins

Complement regulatory proteins such as MCP (CD46), DAF (CD55) and CD59 are expressed on the mammalian cell surface to protect the host cells from the bystander effects of complement activation (Sahu and Lambris 2000; Sun *et al* 1999). During maturation by budding, a number of enveloped viruses such as human cytomegalovirus (HCMV), a herpesvirus, human T-cell leukemia virus type 1 (HTLV-1), HIV-1 and simian immunodeficiency virus (SIV) all belonging to the family of retroviruses, and VV, a poxvirus, capture one or more of the host's cellular complement regulatory proteins and use them to evade the complement attack (table 2).

Studies by Cooper and his coworkers have shown that the complement regulatory activity in the HCMV virion is functionally similar to the regulatory effect of MCP and DAF and that this activity was blocked by the addi-

**Table 2.** Viruses that acquire host complement control proteins.

Virus family	Virus	Acquired proteins	References
Poxvirus	Vaccinia	MCP, DAF, and CD59	Vanderplasschen <i>et al</i> 1998
Herpesvirus	HCMV	MCP, DAF and CD59	Cooper 1998
Retroviruses	HTLV-1	DAF and CD59	Spear <i>et al</i> 1995
	HIV-1	DAF, CD59, and factor H	Marschang <i>et al</i> 1995; Stoiber <i>et al</i> 1996; Schmitz <i>et al</i> 1995
	SIV	MCP, DAF, CD59	Montefiori <i>et al</i> 1994

tion of specific monoclonal antibodies to MCP and DAF (Cooper 1998). Similarly, DAF and CD59 were also found to be associated in HTLV-1 viral preparations. Removal of these proteins from the viral envelope increased the sensitivity to complement-mediated inactivation, while reconstitution by MCP and DAF restored the resistance to the complement attack (Spear *et al* 1995).

HIV-1 acquires complement control proteins DAF and CD59 into their membrane while budding from the host cells and gains protection against complement mediated cytolysis (Marschang *et al* 1995; Schmitz *et al* 1995; Stoiber *et al* 1996). In addition to the primary HIV-1 isolates, these host cell regulatory proteins were also found to be present in the HIV-1 infected cells. Importantly, the degree of protection to infected cells against host complement attack was found to be dependant on the expression levels of DAF and CD59 (Saifuddin *et al* 1995). Studies with phosphoinositol phospholipase C (PI-PLC), which removes the GPI-linked DAF and CD59 from the membrane, showed that removal of these proteins rendered the virus more susceptible to the complement attack (Saifuddin *et al* 1995). It is believed that in addition to DAF and CD59, factor H also protects HIV-1 from complement due to its interaction with the viral envelope proteins (Stoiber *et al* 1997). Consistent with this notion factor H was shown to interact with gp41 and gp120 (Pinter *et al* 1995a,b; Stoiber *et al* 1995). Further, it was also shown that removal of factor H from the sera results in lysis of free virus (Stoiber *et al* 1996).

As described in the previous section, VV encodes a secretory complement regulatory protein VCP, which controls complement activation in fluid phase. However, it would be advantageous for the virus to encode a membrane-bound complement regulatory protein, which would inhibit the complement activation focused on the viral surface and the surface of infected cells. Thus we pro-

posed that B5R, which shows sequence similarity to complement control proteins (Schwartz *et al* 1985; Goebel *et al* 1990; Engelstad *et al* 1992), and is also present on the outer envelope of the virus (Takahashi-Nishimaki *et al* 1991; Engelstad *et al* 1992; Isaacs *et al* 1992b), might confer resistance to extracellular enveloped virus against complement (Sahu *et al* 1998b). When we examined the functional activity of expressed B5R we found that like VCP it lacked factor I cofactor activity (Sahu A, Lambris J D and Isaacs S N, unpublished observation). Later it became clear that complement resistance to extracellular enveloped virus (EEV) was not imparted by B5R, instead, human RCA proteins acquired by EEV provide resistance against complement (Vanderplasschen *et al* 1998).

### 3.3 Complement receptor as a tool for cellular entry

Viruses from at least five different families are known to interact with complement receptors to aid their entry into cells (table 3). These viruses bind to complement receptors either through the proteins they encode or through the C3 fragments attached to their surface as a result of complement activation.

(i) *Epstein-Barr virus*: One of the important and well-studied examples is the EBV, which infects B cells and epithelial cells through CR2 (Fingerroth *et al* 1984). The ligand responsible for binding to CR2 is a major EBV glycoprotein, gp350/220 (Nemerow *et al* 1987, 1989; Tanner *et al* 1987). Like C3d (a physiological ligand of CR2), gp350/220 also interacts with the first two SCRs of CR2 (Lowell *et al* 1989; Molina *et al* 1991). One of the binding sites in C3d is located between residues 1201 and 1214 (PGKQLYNVEATSYA) (Lambris *et al* 1985). A sequence similar to this C3d sequence has also been identified

**Table 3.** Viruses that use complement receptors for cellular entry.

Virus family	Virus	Ligand in viruses	Complement receptor	References
Herpesviruses	EBV	gp350/220	CR2	Fingerroth <i>et al</i> 1984; Nemerow <i>et al</i> 1987; Tanner <i>et al</i> 1987
	HHV-6	Unknown	MCP	Santoro <i>et al</i> 1999
Paramyxovirus	MV	Hemagglutinin	MCP	Naniche <i>et al</i> 1993; Dorig <i>et al</i> 1993; Manchester <i>et al</i> 1994
	Echoviruses	Unknown	DAF	Bergelson <i>et al</i> 1994; Clarkson <i>et al</i> 1995; Powell <i>et al</i> 1997
Retrovirus	Coxsackieviruses	Unknown	DAF	Bergelson <i>et al</i> 1995; Shafren <i>et al</i> 1995
	HIV	<sup>a</sup>	CR1, CR2, CR3	Robinson <i>et al</i> 1988; Stoiber <i>et al</i> 2001; Spear <i>et al</i> 2001

<sup>a</sup>Unlike other viruses, binding of HIV to complement receptors is due to the presence of C3 fragments on its surface and not due to the direct interaction of its surface proteins with complement receptors.

in gp350/220, suggesting that the C3d and EBV binding sites on CR2 are either identical or conformationally related. A series of studies based on monoclonal antibody competition, peptide mapping, and site-directed mutagenesis have determined that the CR2 binding site on gp350/220 lies within residues 1-470 of the viral glycoprotein. Specifically, a monoclonal antibody (72A1) that binds to this region has been shown to inhibit gp350/220 binding to CR2 and viral entry into host cells (Nemerow *et al* 1987; Tanner *et al* 1988). Further, a nine amino acid peptide (residues 21-30, EDPGFFNVEI) that binds to CR2 has been identified within this region of the molecule (Nemerow *et al* 1987), and deletion of residues 28 and 29 (VE) have been shown to abolish the binding of gp350/220 to CR2 (Tanner *et al* 1988). Recently, kinetics of binding of gp350/220 to CR2 has been studied and compared to other physiological ligands (iC3b and C3d) of CR2 (Sarrias *et al* 2001). It was observed that unlike binding of C3 fragments to CR2, binding of gp350/220 to CR2 follows a simple 1 : 1 binding model. Importantly the apparent  $K_D$  value obtained for gp350/220-CR2 interaction was 45 nM compared to 6.2  $\mu$ M obtained for iC3b-CR2 and 4.3  $\mu$ M obtained for C3d-CR2 interactions (Sarrias *et al* 2001). The high affinity of gp350/220 for CR2 compared to C3 fragments suggests that it would effectively compete out the C3 fragments from the receptor. This subtle strategy may help the virus to better infect the B cells.

(ii) *Measles virus*: Measles virus (MV) is another example which uses MCP as a receptor to initiate infection (Dorig *et al* 1993; Naniche *et al* 1993; Manchester *et al* 1994). The MV entry is mediated by the interaction of its surface glycoprotein H with MCP (Nussbaum *et al* 1995; Devaux *et al* 1996), which triggers F-induced fusion between the virus and the cell membrane. The region of MCP that interacts with the hemagglutinin has been mapped to the first two SCR domains (Iwata *et al* 1995; Manchester *et al* 1995). The crystal structure of the first two SCRs of MCP (Casasnovas *et al* 1999) along with antibody binding data and site-directed mutagenesis studies of MCP (Buchholz *et al* 1997; Manchester *et al* 1997) indicate that H interaction extends from the top of the first SCR to the bottom of second SCR. The interaction sites are located at the top of SCR 1 and at the base of SCR 2. In addition, a highly hydrophobic loop that protrudes at the base of the first SCR contains residues important for virus binding (Casasnovas *et al* 1999). It is believed that the *N*-glycan of the SCR 2, which was found to be important for viral binding (Maisner *et al* 1996), stabilizes the conformation of virus binding region (Casasnovas *et al* 1999). It is important to mention here that identification of MCP as a MV receptor led to the development of transgenic mouse

models, which immensely aided in *in vivo* studies on MV pathogenesis (reviewed in Manchester and Rall 2001). In addition to MV, human herpesvirus 6 is also known to utilize MCP as a cellular receptor (Santoro *et al* 1999).

(iii) *Echoviruses and coxsackieviruses*: A number of picornaviruses use DAF as a cell surface receptor. These include echoviruses and coxsackieviruses (CV) (Bergelson *et al* 1994; Shafren *et al* 1995, 1997a). The interesting feature among these viruses is that even the closely related viruses use different SCRs of DAF for binding. For example, SCRs 2-4 are utilized by echovirus 7 (Clarkson *et al* 1995; Powell *et al* 1997) and CV B3 (CVB3) (Bergelson *et al* 1995), while SCRs 1 and 2 are sufficient for binding of CV A21 (CVA21) (Shafren *et al* 1997a). Affinity determination using surface plasmon resonance indicated that unlike gp350/220-CR2 interaction ( $K_D = 45$  nM), the interaction of echoviruses with DAF is a low affinity interaction ( $K_D = 3.0$   $\mu$ M) (Lea *et al* 1998). It is increasingly getting clear that DAF by itself may not be sufficient for mediating productive infection and may require an accessory molecule. For example, in case of CVB3, it has been shown that putative CVB3 cellular receptor complex include DAF and CV-adenovirus receptor protein and both these receptors are essential for viral entry (Shafren *et al* 1997b). Similarly, it has been shown that association of DAF with intercellular adhesion molecule 1 is essential for cellular entry of CVA21 (Shafren *et al* 1997a). Thus, the current belief is that DAF primarily facilitates binding of the virus to the host cells and this in turn enables the virus to interact with the second receptor/molecule, which is the key step in cellular entry.

(iv) *Human immunodeficiency virus*: There is considerable evidence showing activation of complement by HIV (Ebenbichler *et al* 1991; Spear *et al* 1991). Activation of the complement system either in the presence or in the absence of antibody results in opsonization of the HIV surface with C3b molecules, which may undergo proteolytic cleavage to form iC3b and C3dg fragments. Thus, opsonized viral particles have the ability to interact with complement receptors such as CR1, CR2 and CR3. In late 1980's, it was shown that treatment of HIV with antibody and complement results in enhanced HIV infection (Robinson *et al* 1988). Currently, several groups are trying to delineate the role of complement and complement receptors in HIV infection (reviewed in Cooper 1998; Spear *et al* 2001; Stoiber *et al* 2001). It is generally believed that binding of opsonized HIV particles to complement receptors either help in enhancement of CD4-dependant entry and infection or result in effective transfer of these particles to T cells.

#### 4. Concluding remarks

There exists a continuous interplay between the host's complement system and pathogens. Viruses come in contact with complement and are prone to complement attack during both the stages of their life cycle: (i) while they are present outside the cell, and (ii) during their intracellular phase, due to the presence of viral proteins on the surface of infected cells which are known to activate complement. Thus, for extracellular survival as well as for propagation, viruses must develop mechanism(s) to evade complement attack. Understanding these mechanisms are difficult since *in vitro* studies in tissue culture as well as studies performed using animal models may not reflect the true situation. Nevertheless, studies performed thus far clearly indicate that viruses have devised multiple strategies to manipulate and subvert the complement system. Some viruses encode proteins that modulate and inhibit the host's complement function, while others pickup the host complement regulatory proteins to do the same, and still others use complement receptors to gain cellular entry. Current efforts are directed at identification of the vital structural determinants of immune evasion molecules that are important in interacting with complement proteins. These studies would not only allow us to better understand the structural features of immune evasion molecules important in its biology, but would also identify a significant control point in the molecule that would direct the development of pharmacological ligands to neutralize these viruses.

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