 DOI 10.1099/vir.0.18709-0

Review

Virus complement evasion strategies

Herman W. Favoreel, Gerlinde R. Van de Walle, Hans J. Nauwynck and Maurice B. Pensaert

Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

Correspondence
Hans Nauwynck
hans.nauwynck@rug.ac.be

The immune system has a variety of tools at its disposal to combat virus infections. These can be subdivided roughly into two categories: 'first line defence', consisting of the non-specific, innate immune system, and 'adaptive immune response', acquired over time following virus infection or vaccination. During evolution, viruses have developed numerous, and often very ingenious, strategies to counteract efficient recognition of virions or virus-infected cells by both innate and adaptive immunity. This review will focus on the different strategies that viruses use to avoid recognition by one of the components of the immune system: the complement system. Complement evasion is of particular importance for viruses, since complement activation is a crucial component of innate immunity (alternative and mannan-binding lectin activation pathway) as well as of adaptive immunity (classical, antibody-dependent complement activation).

INTRODUCTION

Complement consists of an interacting set of enzymes (e.g. proteases), which, upon activation, gives rise to a cascade of reactions that result finally in the destruction of invading micro-organisms and infected cells. The complement system can be activated in three ways, represented schematically in Fig. 1.

The classical activation pathway of complement involves binding of complement protein C1q either to antibody–antigen complexes or, occasionally, directly to the surface of certain pathogens. Direct C1q binding to viral surfaces has been demonstrated for retroviruses such as human immunodeficiency virus (HIV – via gp41) and human T-cell leukaemia virus (HTLV) as well as for human cytomegalovirus (HCMV)-infected cells (Cooper *et al.*, 1974; Ebenbichler *et al.*, 1991; Ikeda *et al.*, 1998; Spiller & Morgan, 1998). C1q belongs to the family of collectins, proteins containing both lectin domains and collagen-like domains. It has six globular heads linked together by the collagen-like tails and forms, together with C1r and C1s, the C1 complex (Fig. 1A). Binding of more than one of the C1q heads to an antibody–antigen complex or the surface of a virion or infected cell causes a conformational change in the C1 complex, activating C1r. The active form of C1r cleaves C1s to generate an active serine protease, which, in turn, cleaves C4 as a first step in the complement cascade (Fig. 1A).

The second pathway of complement activation, the mannan-binding lectin (MBL) pathway, shows strong similarities to the classical pathway. MBL is a protein very similar to C1q. Like C1q, MBL is a six-headed collectin that interacts with two MBL-associated serine proteases (MASP-1 and -2, closely related to C1s and C1r) to form the MBL complex (Fig. 1B). MBL binds to several monosaccharides, especially mannose, and the quaternary structure of the MBL complex allows for high avidity binding to repetitive carbohydrate ligands present on many pathogen surfaces. On vertebrate cells, however, these carbohydrates are covered by other sugar groups, especially sialic acids, that inhibit binding of MBL. MBL binding leads to a conformational change in the complex, which activates the associated proteases and results in C4 cleavage. MBL binding to the virion surface has been shown for HIV (gp120) (Haurum *et al.*, 1993) and the MBL complement activation pathway is thought to be implicated in controlling several virus infections, including hepatitis B virus, hepatitis C virus and influenza virus infections (Reading *et al.*, 1997; Sasaki *et al.*, 2000; Hakozaki *et al.*, 2002).

Activation of the third activation pathway, the alternative pathway, is a default process, consisting of a spontaneous and indiscriminate deposition of complement factor C3b on surfaces of host cells or foreign particles (Fig. 1C). C3b is produced at a significant rate in plasma by spontaneous hydrolysis of the abundantly present C3. After deposition of C3b, complement activation will proceed unless down-regulated by specific mechanisms (see below).

Activation of the classical, MBL or alternative pathway results in cleavage and activation of C3, followed by cleavage of C5, which initiates activation of the terminal pathway comprising the formation of membrane attack complexes (MACs) (Fig. 1D). These MACs can be imagined as ring-shaped structures with a central pore; these

Published ahead of print on 22 October 2002 as DOI 10.1099/vir.0.18709-0.

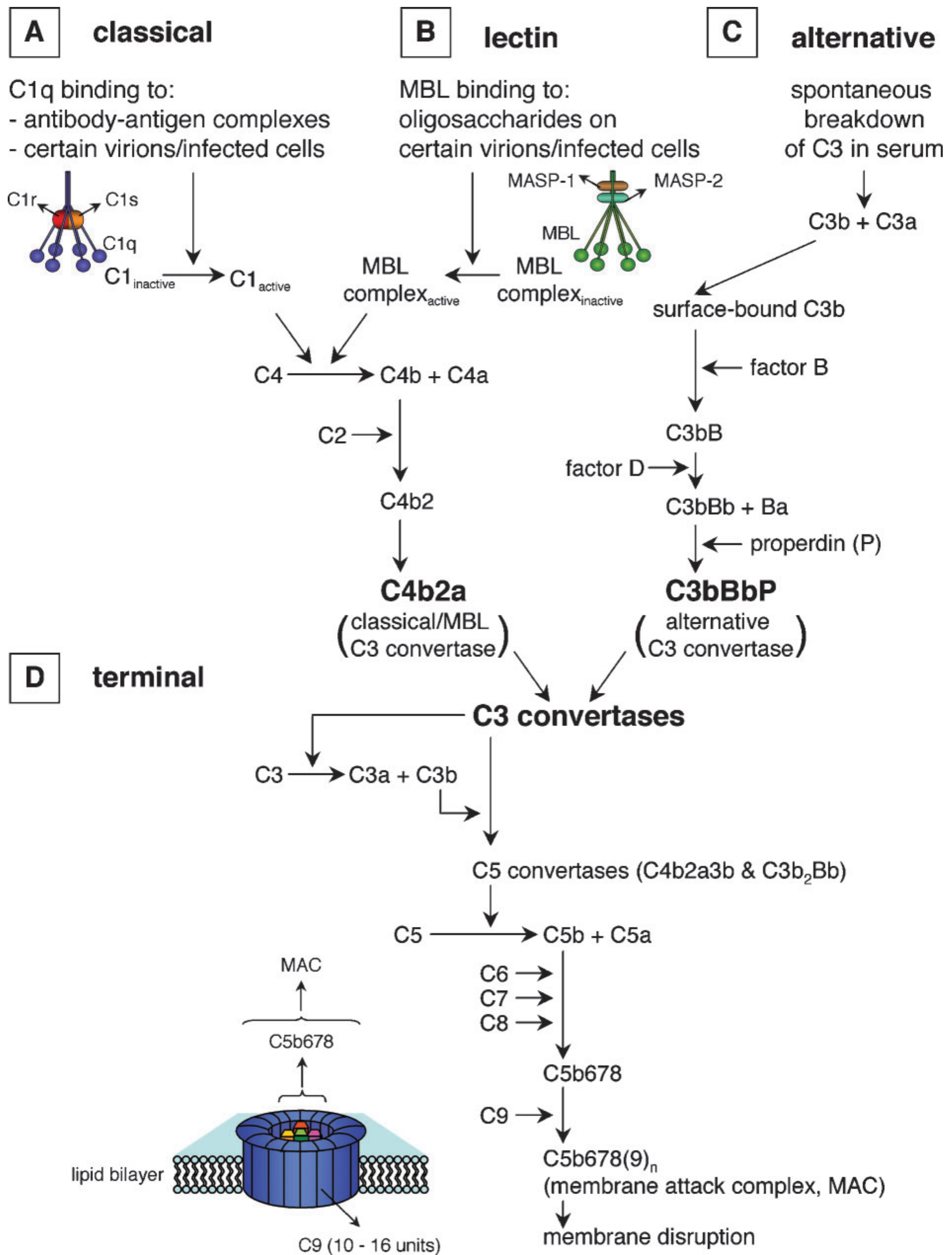

Fig. 1. Activation of the complement cascade via the classical (A), lectin (B) or alternative (C) pathway results in the initiation of the terminal complement pathway (D), leading to the formation of membrane attack complexes.

structures are incorporated into the lipid bilayer of cells and result in osmotic disruption of the cell (Janeway *et al.*, 2001). Complement-mediated control of virus infections is not restricted to MAC-mediated destruction of infected cells or enveloped virions. Activation of any one of the three complement pathways results in the production of several anaphylatoxins (C3a, C4a and C5a). The effects of these molecules lead to the recruitment of antibody, complement and leukocytes to the site of infection. Also, opsonization of virions and infected cells by the deposition of C3b may lead to phagocytic uptake by leukocytes. Furthermore, interaction of complement components with virion surfaces on itself has been reported to be neutralizing for certain viruses (Ikeda *et al.*, 1998; Kase *et al.*, 1999).

Complement activation is a potentially dangerous system and is therefore very carefully regulated. Normal mammalian cells are protected from complement-mediated destruction by the activities of complement-regulating proteins. An overview of the complement regulators together with their functions is given in Table 1 and Fig. 2. These proteins have been considered generally to be species restrictive, although there is increasing evidence that at least some complement regulators, such as DAF (decay-accelerating factor) and CD59, can act in heterologous systems (Van den Berg & Morgan, 1994; Rushmere *et al.*, 1997; Harris *et al.*, 2000; Perez de la Lastra *et al.*, 2000). Several, but not all, of the complement regulators make part of the so-called 'regulators of complement activation' (RCA). True RCA proteins are encoded by genes located in the RCA gene cluster and are composed largely of tandem arrays of a short consensus repeat (SCR). These SCRs contain approximately 60–70 amino acids and are characterized by a motif with four conserved disulphide-bonded cysteines (Pangburn, 1986). Factor H is the most important RCA to control activation of the alternative complement pathway. The character of the surface on which the spontaneously cleaved complement protein C3b is deposited determines whether C3b will be activated further or will be neutralized by binding to factor H. Interaction of factor H with sialic acids or neutral or anionic polysaccharides on the cell surface will enhance its binding to C3b, thereby inhibiting further activation of the alternative pathway by accelerating the decay of C3b (Meri & Pangburn, 1990). The surfaces of many bacterial cells, certain tumour cells and certain virus-infected cells do not promote binding of factor H to C3b. C3b is then capable of binding to factor B, resulting in activation of the alternative pathway.

VIRUS EVASION STRATEGIES

Viruses have developed different strategies to evade complement-mediated destruction, summarized in Table 2 and Fig. 3. First, viruses belonging to the families *Herpesviridae* and *Coronaviridae* interfere with the classical complement activation pathway by avoiding complement binding to antibody–antigen complexes, either by removing (shedding or internalization) these antibody–antigen complexes from the cell surface of the infected cell or by the expression of Fc receptors. Second, some poxviruses and herpesviruses encode and express proteins with functional similarities to RCA proteins and other complement regulators and can thereby protect their lipid envelopes and the membranes of the cells they have infected. Some of these virus complement-interfering proteins show genetic similarities to the known complement regulators, while other complement-interfering proteins do not. Third, viruses belonging to the families *Poxviridae*, *Herpesviridae*, *Retroviridae* and *Togaviridae* can incorporate host complement

Table 1. Physiological control of complement activation

Complement regulator			
Abbreviation	**Full name**	**Function**	**Distribution**
C1-Inh	C1 inhibitor	Prevents spontaneous activation of C1	Soluble
	Factor I	Cleaves C3b and C4b to inactive fragments	Soluble
CD46, MCP	Membrane co-factor protein	Co-factor for the cleavage of C3b and C4b by factor I	Membrane-bound
CD35, CR1	Complement receptor 1	Inhibits formation and accelerates decay of both classical and alternative C3 convertases Co-factor for the cleavage of C3b and C4b by factor I	Membrane-bound
C4-bp	C4-binding protein	Accelerates decay of C4b2a	Soluble
	Factor H	Accelerates decay of C3b Regulates C5 convertase activity of C3b	Soluble/associated with membranes
CD55, DAF	Decay-accelerating factor	Inhibits formation and accelerates decay of classical and alternative C3 convertase	Membrane-bound/ soluble (significant?)
	S protein (vitronectin)	Binds C5b67 and thereby inhibits binding of this complex to the membrane	Soluble
CD59	Protectin	Inhibits MAC formation	Membrane-bound

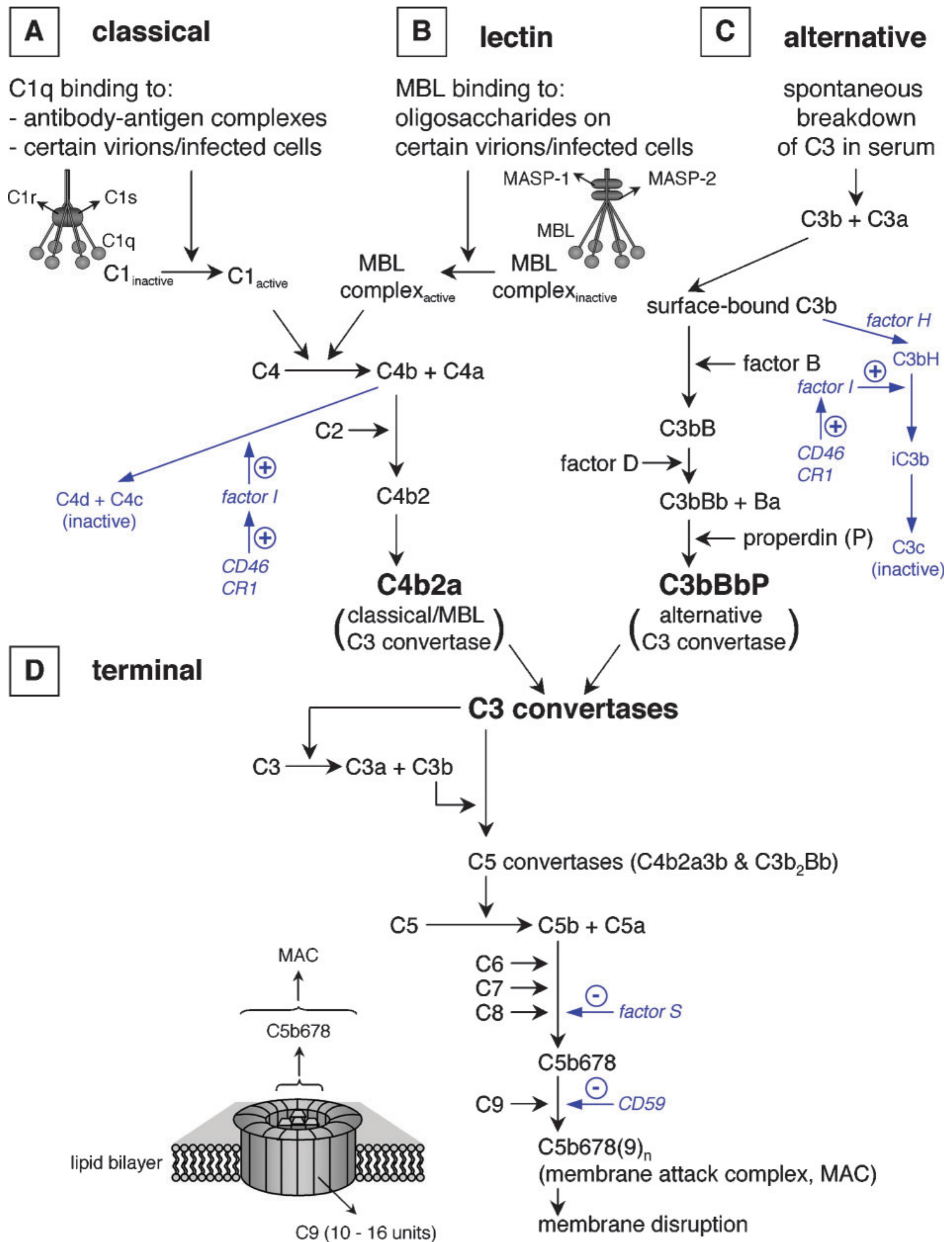

Fig. 2. Physiological regulation of the complement cascade. Host proteins that interfere with different steps of the complement cascade are indicated in blue.

Table 2. Virus complement evasion

(a) Avoidance of complement binding to antibody–antigen complexes

Viral protein/gene responsible			
Abbreviation	**Full name**	**Function**	**Virus**
gE–gI	Glycoproteins E and I	Shedding of viral protein–antibody complexes	PRV
gB and gD	Glycoproteins B and D	Internalization of viral protein–antibody complexes	PRV
gE–gI	Glycoproteins E and I	Fc receptor activity	HSV, VZV and PRV
UL119–UL118		Fc receptor activity	HCMV
TRL11/IRL11		Fc receptor activity	HCMV
Fcr-1		Fc receptor activity	MCMV
S	Spike protein	Fc receptor activity	MHV, BCV and TGEV

(b) Virus mimicry of complement regulators

Virus complement regulator			
Abbreviation	**Full name**	**Function**	**Virus**
VCP	Vaccinia virus complement control protein	Binds with C4b and C3b Co-factor for factor I	Vaccinia virus
SPICE	Smallpox inhibitor of complement enzymes	Binds with C4b and C3b Co-factor for factor I	Variola virus
IMP	Inflammation modulatory protein	Downregulates chemotactic proteins C3a, C4a and C5a	Cowpox virus
CCPH	Complement control protein homologue	Inhibits formation and accelerates decay of classical and alternative C3 convertases	HVS
gC1	Glycoprotein C1	Binds human C3b Accelerates alternative C3 convertase decay Inhibits C5 and P binding	HSV-1
gC2	Glycoprotein C2	Binds human C3b Accelerates alternative C3 convertase decay	HSV-2
gC	Glycoprotein C	Binds species-specific C3b	PRV, BHV-1 and EHV-1 and -2
EBV protein	Unknown EBV protein	Accelerates alternative C3 convertase decay/Co-factor for factor I	EBV

(c) Virion incorporation or upregulation of cellular complement regulators

Mechanism responsible	Function	Virus
Unknown	Upregulation of CD55 and CD46	HCMV
Unknown – lipid raft association?	Incorporation of cellular complement regulators/Incorporation of sialic acids	PRV, vaccinia virus, HIV, HTLV and Sindbis virus

control proteins in their viral envelope and/or upregulate expression of these proteins in infected cells.

Avoidance of complement binding to antibody–antigen complexes

Besides resulting in inefficient activation of the classical complement pathway, interference with antibody–antigen complexes on virions or infected cells may have numerous implications, such as impaired phagocytosis and reduced natural killer cell activation. Some viruses belonging to the families *Herpesviridae* and *Coronaviridae* are known to express Fc receptors. Expression of such an Fc receptor may result in (i) binding of non-immune immunoglobulin G (IgG) to the Fc receptor, expressed on virus or virus-infected cells, by which access of virus-specific immune IgG is sterically hindered (Dowler & Veltri, 1984) and (ii) 'antibody bipolar

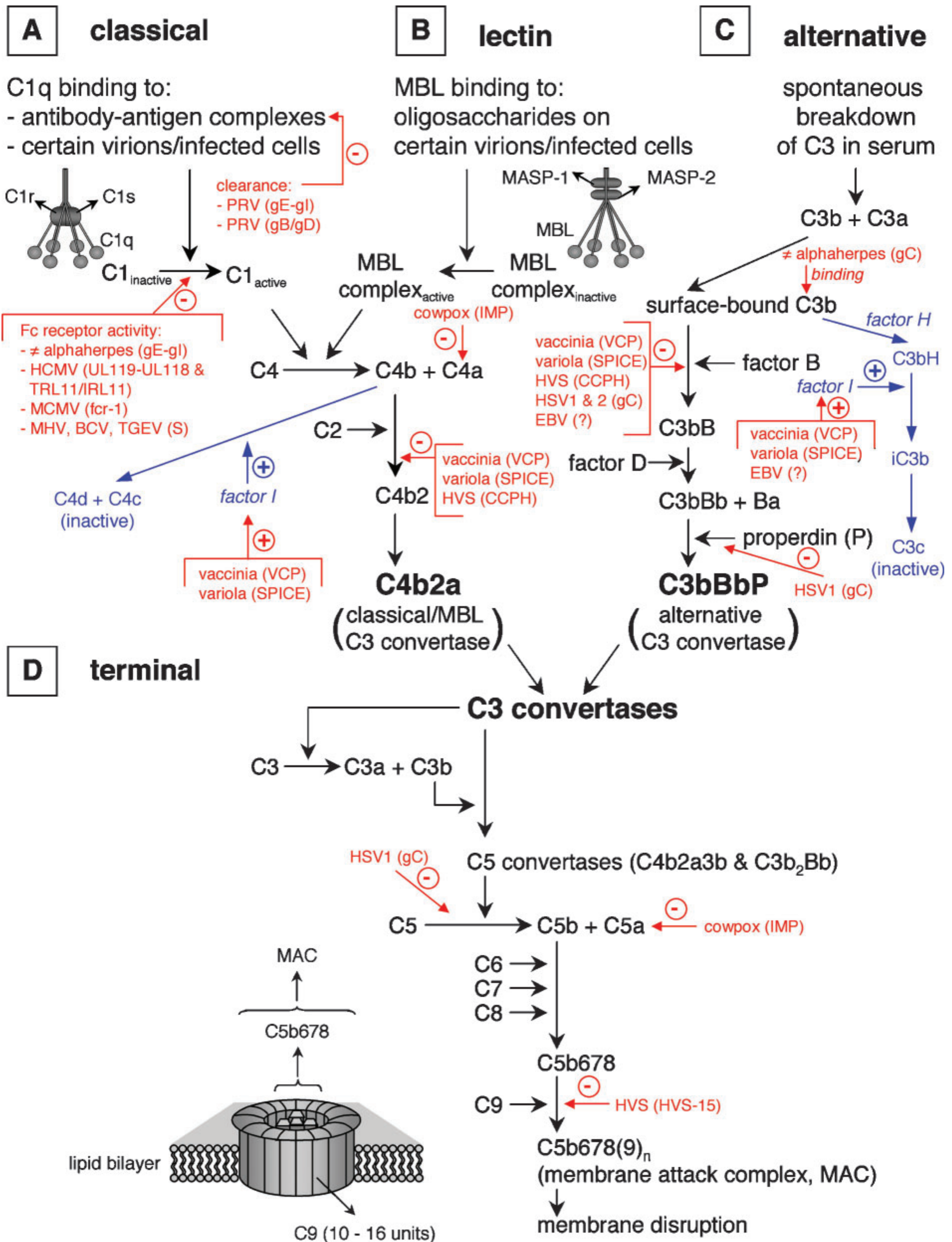

Fig. 3. Virus interference with the complement cascade. Viral proteins that interfere with the complement cascade or promote the activity of physiological complement regulators are indicated in red. Physiological complement regulation is shown in blue (see Fig. 2).

bridging', consisting of simultaneous binding of the hypervariable regions of an antibody to viral proteins in the viral envelope or on the surface of infected cells, and of the Fc side of the same antibody to viral Fc receptors (Frank & Friedman, 1989), resulting in inefficient activation of antibody-dependent components of the immune system, including classical complement activation.

Another mechanism of interference with binding of complement to antibody–antigen complexes consists of virus-mediated clearance of these complexes from the surface of virus-infected cells, as has been shown for the alphaherpesvirus pseudorabies virus (PRV) (Favoreel *et al.*, 1997, 1999b).

Herpesviridae

Alphaherpesvirinae. The presence of an Fc receptor for IgG on the plasma membrane of herpes simplex virus (HSV)-infected cells and in virion preparations isolated from HSV-infected cells has been described several years ago (Watkins, 1964; Westmoreland & Watkins, 1974). Further characterization of this receptor revealed that the viral glycoprotein gE was responsible for IgG binding (Para *et al.*, 1982). Moreover, it was shown that the IgG-binding domain of gE is located between amino acids 235 and 380 and that this immunoglobulin-like domain shares amino acid similarity to mammalian Fc receptors (Dubin *et al.*, 1994). However, gE of HSV-1, transfected and expressed in mouse cells, was found not to bind radiolabelled IgG (Johnson & Feenstra, 1987). Therefore, it was suggested that gE by itself could not act as an IgG Fc receptor. Another glycoprotein was detected, originally designated g70 and later renamed gI, which was able to bind IgG in conjunction with gE (Johnson & Feenstra, 1987; Johnson *et al.*, 1988).

The HSV Fc receptor allows the virus to avoid complement-mediated lysis via two mechanisms. First, Adler *et al.* (1978) demonstrated that polymeric non-immune IgG protected HSV-1-infected cells from complement-mediated lysis and lysis by sensitized lymphocytes. Dowler & Veltri (1984) showed that monomeric non-immune IgG or purified Fc fragments could protect HSV virions from antibody neutralization. This mechanism of protection consists presumably of a sterically hindered access of immune IgG or Fc-dependent effector cells to the virus or virus-infected cells by bound non-immune IgG. Other investigators have performed research on a second mechanism of complement evasion due to the Fc receptor activity, first described by Frank & Friedman (1989) and called antibody bipolar bridging in analogy to a physiological process described for several Fc receptor bearing leukocytes (Benichou & Voisin, 1987). Antibody bipolar bridging by HSV consists of binding of immune IgG with its hypervariable region end to a viral envelope or cell surface protein and with its Fc end to the HSV Fc receptor gE–gI. This phenomenon has been shown to protect virus from complement- and antibody-dependent neutralization (Frank & Friedman, 1989).

Using mutants of HSV deleted in gE and/or gI, Dubin *et al.* (1990) demonstrated that gE alone is sufficient for the binding of polymeric IgG as a low-affinity Fc receptor and that the gE–gI complex is required to obtain a higher-affinity Fc receptor that is able to bind monomeric IgG. These observations suggest that gE has intrinsic IgG Fc-binding activity, which is intensified by its interaction with gI. This implies that the low affinity receptor gE alone may be sufficient to protect cells from complement-mediated lysis and antibody-dependent cellular cytotoxicity by bipolar binding of immune IgG, whereas the high affinity receptor gE–gI may offer protection by binding of non-immune IgG, resulting in sterically hindered access of immune IgG or effector cells (Dubin *et al.*, 1991).

More recently, the gE–gI Fc receptor activity of HSV has been demonstrated to mediate immune evasion of the virus *in vivo* (Nagashunmugam *et al.*, 1998). To this end, an HSV mutant was constructed in which four amino acids were inserted into the immunoglobulin-like domain of gE, abolishing gE Fc receptor function without affecting other gE functions. Comparing this mutant in mice to wild-type HSV revealed that the Fc receptor activity of gE enables HSV to evade antibody and complement attack *in vivo* (Nagashunmugam *et al.*, 1998; Lubinski *et al.*, 2002).

For varicella-zoster virus (VZV), it has been demonstrated by haemadsorption and immunofluorescence techniques that, starting at 6 h post-infection, cells express a receptor with specificity for the Fc portion of human and rabbit IgG (Ogata & Shigeta, 1979). Ishak *et al.* (1984), however, failed to detect the expression of such a receptor using similar techniques. Litwin *et al.* (1990) used biotinylated non-immune human IgG to clarify this issue and like Ogata & Shigeta (1979), they could demonstrate Fc receptor activity and moreover, showed that this activity was not due to one of the known cellular Fc gamma receptors. Further research revealed that the two viral glycoproteins gpI and gpIV, the equivalents of HSV gE and gI, form the VZV Fc receptor (Litwin *et al.*, 1992). In contrast to HSV gE and gI, the amino acid sequences of gpI and gpIV did not show regions of amino acid similarity with the human cellular Fc receptors, as determined by extensive computer-assisted analysis (Litwin & Grose, 1992; Litwin *et al.*, 1992).

The third member of the *Alphaherpesvirinae* subfamily known to display Fc receptor activity is PRV, a swine alphaherpesvirus, again with the gE–gI complex being responsible for the IgG-binding effect (Favoreel *et al.*, 1997). Recently, the role of this gE–gI Fc receptor activity in antibody-dependent complement evasion has been investigated *in vitro* and it was demonstrated that cells infected with a PRV gE–gI-null mutant were significantly more susceptible towards antibody-dependent, complement-mediated cell lysis compared to cells infected with wild-type PRV

(G. R. Van de Walle, H. W. Favoreel, H. J. Nauwynck and M. B. Pensaert, unpublished observations).

PRV has also developed a second strategy to interfere with complement binding to antibody–antigen complexes, consisting of virus-mediated clearance of these complexes from the surface of infected cells. This clearance was described first for PRV-infected swine kidney cells and consisted of clustering, polarization and shedding of the antibody–antigen complexes from the cell surface (Favoreel *et al.*, 1997). Viral glycoprotein gE was found to have a dual role during this process. First, the gE-mediated Fc receptor function was found to be necessary for efficient clustering of antibody–antigen complexes (Favoreel *et al.*, 1997). Second, two tyrosine amino acid residues in the cytoplasmic tail of gE were found to be crucial for efficient polarization of the clustered antibody–antigen complexes, possibly by mediating a signal transduction event (Favoreel *et al.*, 1999a). Clearance of antibody–antigen complexes from the cell surface could also be demonstrated in PRV-infected monocytes (Favoreel *et al.*, 1999b), the natural carrier cell of PRV in the blood of vaccinated animals (Nauwynck & Pensaert, 1992). Here, clearance consisted of clustering and subsequent internalization of the antibody–antigen complexes. Using PRV mutants, viral glycoproteins gB and gD were shown to be indispensable for efficient internalization (Favoreel *et al.*, 1999b; Van de Walle *et al.*, 2001). Recently, it was demonstrated that a single tyrosine residue in the cytoplasmic tail of gB is crucial for efficient functioning of gB in the internalization process, possibly by linking gB to endocytosis adaptor protein complexes (AP-2) as a first step in the formation of clathrin-coated vesicles (Favoreel *et al.*, 2002). Although the internalization process in PRV-infected monocytes is fast and efficient, starting within minutes after antibody addition, it remains to be determined whether this time span is short enough not to allow the complement cascade to lyse the infected cells. Nevertheless, allowing the antibody-induced internalization of antibody–antigen complexes to proceed in PRV-infected monocytes has been shown recently to protect these cells from efficient antibody-dependent complement-mediated lysis *in vitro* (G. R. Van de Walle, H. W. Favoreel, H. J. Nauwynck and M. B. Pensaert, unpublished observations).

Betaherpesvirinae. Back in 1976, evidence was obtained for the existence of a receptor on HCMV-infected cells that could react specifically with the Fc region of human IgG (Rahman *et al.*, 1976). Further research showed that the HCMV-induced Fc receptor had affinity for all subclasses of human IgG and that the reaction site was located in the CH2 domain of the Fc fragment on human IgG (Mackowiak & Marling-Cason, 1987). The fact that this receptor was not only located on the cell surface and in the cytoplasm of HCMV-infected cells but also on the HCMV virion itself was demonstrated by Stannard & Hardie (1991). Further research using murine monoclonal antibodies directed against the known human IgG Fc receptors revealed that the HCMV-induced Fc receptor was distinct from cellular Fc receptors (MacCormac & Grundy, 1996). Recent studies have shown that the HCMV genome in fact encodes two Fc gamma receptors, one with a molecular mass of 63–68 kDa, encoded by a spliced UL119/UL118 mRNA, and the other with a molecular mass of 34 kDa, encoded by TRL11/IRL11 (Lilley *et al.*, 2001; Atalay *et al.*, 2002). Comparison of the sequences with different cellular Fc receptors suggests that both viral Fc receptors have different ancestors and functions (Atalay *et al.*, 2002).

For murine cytomegalovirus (MCMV), the *fcr-1* early gene has been shown to encode an 88 kDa Fc receptor (Thäle *et al.*, 1994). Mutants deleted in *fcr-1* were constructed to investigate the biological role of the MCMV-induced Fc receptor (Crnkovic-Mertens *et al.*, 1998). These mutants displayed comparable *in vitro* growth kinetics as those of wild-type MCMV but a reduced growth in various organs. However, a similar reduced replication of the *fcr-1*-deletion mutant was observed when inoculating antibody-deficient mice, indicating that the reduced replication was not due to an increase in antibody-mediated clearance of the virus (Crnkovic-Mertens *et al.*, 1998).

Coronaviridae

The spike peplomer protein of three important members of the family *Coronaviridae*, mouse hepatitis virus (MHV), bovine coronavirus (BCV) and transmissible gastroenteritis virus (TGEV), has been shown to display Fc receptor activity towards IgG of its natural host (Oleszak *et al.*, 1993). MHV, BCV and human coronavirus (HCV) on the one hand and TGEV on the other hand belong to two distinct antigenic subgroups of the *Coronaviridae*. A third antigenic subgroup contains the infectious bronchitis virus (IBV) as an important member. For HCV and IBV, however, Fc receptor activity of the spike protein could not be demonstrated (Oleszak, 1994; Oleszak *et al.*, 1995).

Virus mimicry of complement regulators

Some viruses can protect their viral lipid envelopes and the membranes of the cells they infect from complement lysis by encoding proteins with functional similarities to complement control proteins, inhibitors of the complement cascade system. A number of distinct proteins from several viruses have been identified to have complement regulatory activity, some of them bearing structural or genetic similarities to the known complement control proteins, others showing no relationship with the cellular complement regulators.

(a) Virus complement-interfering proteins with genetic similarities to cellular complement regulators

Poxviridae

Vaccinia virus is the prototype of the family *Poxviridae* and it is known that vaccinia virus encodes for, and vaccinia virus-infected cells secrete, an abundant soluble protein called 'vaccinia virus complement control protein' (VCP),

with an inhibitory activity for both classical and alternative activation of complement (Kotwal *et al.*, 1990). This protein contains four SCRs, similar to the first four repeats of the RCA C4-binding protein, and was shown to bind C4b and C3b and to act as a co-factor of complement-regulating factor I (McKenzie *et al.*, 1992). This protein has been demonstrated to be an important virulence factor, since a VCP-null mutant produced much smaller skin lesions compared to wild-type virus when inoculated in rabbits (Isaacs *et al.*, 1992). Besides VCP, vaccinia virus also encodes another protein with amino acid similarity to RCA, the membrane-bound glycoprotein B5R (Engelstad *et al.*, 1992; Takahashi-Nishimaki *et al.*, 1991).

Other poxviruses, including variola virus and cowpox virus, also encode for complement control proteins that are structurally and functionally related to RCAs. Variola virus is a virulent member of the *Poxviridae* and infects only humans. Very recently, the complement control protein of this virus has been characterized and designated 'small pox inhibitor of complement enzymes' (SPICE) (Rosengard *et al.*, 2002). It was demonstrated that SPICE also possesses factor I co-factor activity, as described for vaccinia virus VCP, but is nearly 100-fold more potent than VCP at inactivating human C3b and 6-fold more potent at inactivating C4b (Rosengard *et al.*, 2002).

For cowpox virus, the orthologue of the vaccinia virus VCP was designated 'inflammation modulatory protein' (IMP) (Howard *et al.*, 1998; Kotwal *et al.*, 1998). The main function of this IMP seems to be limiting macrophage infiltration upon infection by downregulating the production of complement proteins with chemotactic activity (C3a, C4a and C5a). *In vivo* studies in mice showed that an IMP-null virus caused severe tissue damage compared to the parental strain, indicating that IMP is able to preserve the tissue at the site of infection, thereby preserving the virus habitat (Howard *et al.*, 1998; Kotwal *et al.*, 1998).

Recently, potential regulators of the complement system have been identified for other poxviruses, such as myxoma virus (M144R protein), Yaba-like disease virus and swinepox virus (Afonso *et al.*, 2002; Barrett *et al.*, 2001; Lee *et al.*, 2001), based on amino acid sequence similarities with mammalian and vaccinia virus VCPs. Further research will be necessary to deduce the function(s) these proteins may fulfil in complement-mediated lysis.

Herpesviridae

Gammaherpesvirinae. For the lymphotropic herpesvirus saimiri (HVS), two open reading frames (ORFs) with sequence similarity to complement regulators have been identified: ORFs 4 and 15 (Albrecht *et al.*, 1992a). The product of ORF4 has been designated 'complement control protein homologue' (CCPH) and shows a high amino acid similarity to the RCA protein DAF (Albrecht & Fleckenstein, 1992). A functional role for this CCPH has been confirmed by the demonstration that cells transfected with CCPH inhibited C3 convertase activity, effectively reduced cell surface deposition of the complement component C3b and had an increased resistance to lysis by human complement (Fodor *et al.*, 1995). The protein encoded by HVS ORF15, designated HVS-15, shares a high amino acid sequence similarity with the complement regulator CD59 (protectin) (Albrecht *et al.*, 1992b). Transfection studies with HVS-15 showed that complement regulatory activity occurred after C3b deposition on the cell surface, indicating terminal complement inhibition. Furthermore, it was demonstrated that HVS-15 is not very species restrictive, in contrast to its cellular counterpart protectin (Rother *et al.*, 1994), although homologous restriction of protectin (CD59) is controversial (Van den Berg & Morgan, 1994; Rushmere *et al.*, 1997).

Murine gammaherpesvirus 68 (MHV-68) is a virus related to the primate gammaherpesviruses. It was observed that an ORF conserved among these viruses encodes a protein containing four SCRs, similar to the ones found in mammalian RCAs (Virgin *et al.*, 1997). Kapadia *et al.* (1999) showed that this MHV-68 ORF is a late gene, that the encoded protein is expressed in membrane-bound and soluble isoforms and that the protein downregulates both classical and alternative pathways of murine complement activation.

(b) Virus complement-interfering proteins without genetic similarities to cellular complement regulators

Herpesviridae

Alphaherpesvirinae. HSV-1 and -2, together with VZV, PRV, bovine herpesvirus-1 (BHV-1) and equine herpesvirus-1 and -4 (EHV-1 and -4), are well-studied members of the *Alphaherpesvirinae* subfamily. They all encode the conserved viral glycoprotein gC, a non-essential glycoprotein known to play a role in virus attachment, release and virulence (Schreurs *et al.*, 1988; Mettenleiter *et al.*, 1990; Herold *et al.*, 1991). Besides that, gC of the different members (with the exception of VZV) (Smiley *et al.*, 1985) has also been reported to bind C3b, the pivotal component of the alternative complement cascade (Friedman *et al.*, 1984; Huemer *et al.*, 1993).

Glycoprotein gC of HSV-1 and -2 (designated gC1 and gC2, respectively) are both able to bind human C3b. However, on infected cells, only gC1 can act as a receptor for C3b, implying potentially important differences between the glycoproteins of these two HSV types (Friedman *et al.*, 1984; Hung *et al.*, 1992). A lower affinity of gC2 towards C3b cannot account for the lack of C3b receptor activity in HSV-2-infected cells, since, on the contrary, it was demonstrated recently that gC2 has a 10-fold higher affinity towards C3b compared to gC1 (Rux *et al.*, 2002).

A relatedness of gC1 and gC2 with the cellular RCA complement receptor 1 (CR1) has been reported but now seems controversial. Structural relatedness with CR1 was suggested by the observation that a monoclonal antibody that blocked

binding of CR1 to C3b could also block binding of gC1 to C3b, as well as by regions with amino acid similarities found in gC1, gC2 and CR1 (Kubota *et al.*, 1987; Seidel-Dugan *et al.*, 1990). However, Hung *et al.* (1992) reported that gC1 with mutations in three of the four cysteines in the supposed SCR motif of region III showed wild-type C3b-binding capacity, indicating that region III is probably not an SCR-like motif. Therefore, these authors did not support the concept of a structural relationship between the SCR of CR1 and gC1. Furthermore, a functional relationship between gC1 and CR1 was found to be only partial. Both CR1 and gC1 accelerate the decay of the alternative pathway C3 convertase but, unlike CR1, gC1 does not accelerate the decay of the classical pathway C3 convertase nor does it possess co-factor activity for RCA factor I (Fries *et al.*, 1986).

By expressing gC1 and gC2 in a baculovirus expression system, Kostavasili *et al.* (1997) found that gC1, but not gC2, not only binds C3b but also inhibited binding of two complement components (properdin and C5) to C3b. Properdin (also designated P) is an important factor of alternative complement activation by increasing the half-life of the alternative C3 convertase from 5 up to 30 min, whereas C5 is an important component of terminal complement activation by being a part of the MAC (Fig. 1). For both proteins, binding to C3b is essential for their function. The transmembrane segment of gC1 was found to be required for the inhibition of properdin binding, but not C5 binding, to C3b (Kostavasili *et al.*, 1997). The gC1-mediated inhibition of C5 binding to C3b is likely to be the result of sterically hindered access for the C5-binding site to C3b upon binding of gC1 to C3b, rather than a competition of gC1 for the same binding site on C3b as C5. Thus, gC1 has two structural domains that are involved in modulating complement activation: one binds C3b and is located in the central region of the molecule from residues 124 to 366 and the other which is required for blocking properdin binding to C3b and is located near the NH_2 terminus from residues 33 to 133 (Hung *et al.*, 1994). The C3b-binding capacity of gC1 has been demonstrated to mediate complement evasion of the virus *in vivo* (Lubinski *et al.*, 1998, 1999, 2002). Further *in vivo* research by this group using deletion mutants lacking one or both domains of gC1 involved in modulating complement activation indicated that the C3b-binding domain of gC1 was much more important during complement evasion than the domain responsible for blocking properdin binding to C3b (Lubinski *et al.*, 1999). Interestingly, it was shown recently that an HSV mutant carrying mutations in the C3b-binding domain of gC as well as in the IgG-binding domain of gE (see above) was much more sensitive to antibody and complement attack than the single mutated strains, suggesting synergistic effects by acting at multiple steps in the complement cascade (Lubinski *et al.*, 2002).

The role of gC in complement evasion for other viruses of the subfamily *Alphaherpesvirinae* has not been studied as extensively as for HSV. PRV, BHV-1, EHV-1 and EHV-4 gC orthologues are known to bind C3b of the complement cascade (with highest affinity to C3 of the natural host), presumably resulting in inhibition of further downstream events (Huemer *et al.*, 1992, 1993, 1995).

Gammaherpesvirinae. Epstein–Barr virus (EBV), a well-studied member of the subfamily *Gammaherpesvirinae*, has also been reported to interfere with complement activation. It was observed that when serum was incubated with purified EBV, C3 present in the serum was cleaved to inactive C3c (Mold *et al.*, 1988). Since CR1, an RCA with factor I co-factor activity and necessary for such cleavage of C3, is not normally present in serum, EBV was thought to be responsible for this cleavage activity and was therefore tested for possible factor I co-factor activity. It was indeed demonstrated that purified EBV virions, like CR1, accelerate the decay of the alternative pathway C3 convertase and serve as a co-factor for the complement regulatory protein factor I (Mold *et al.*, 1988). However, the EBV protein cannot accelerate the decay of the classical pathway C3 convertase like CR1 does (Mold *et al.*, 1988). The EBV envelope protein responsible for this complement regulatory function has not yet been identified and searching the sequences of the known envelope proteins has not revealed any SCRs.

Virion incorporation or upregulation of cellular complement control factors

Some viruses can borrow the host cellular complement control factors by incorporating them into their viral envelope or can induce an upregulation of these factors on the membranes of the cells they infect. Exactly how complement control proteins are incorporated in the viral envelope remains unclear. Recent studies have led to strong indications that budding of a variety of enveloped viruses, such as HIV, Ebola virus, influenza virus and measles virus, does not happen randomly at the plasma membrane but at specific microdomains enriched in cholesterol and sphingolipids, called lipid rafts (Scheiffele *et al.*, 1999; Vincent *et al.*, 2000; Ono & Freed, 2001; Bavari *et al.*, 2002). Glycosyl phosphatidyl inositol (GPI)-anchored complement control proteins such as CD55 and CD59 have been shown to associate with such lipid rafts (Hannan & Edidin, 1996). Although further research is necessary to explore this hypothesis, evidence obtained recently that lipid raft disruption decreases the amount of CD59 on the HIV envelope (Nguyen & Hildreth, 2000) makes it tempting to speculate that one of the mechanisms of incorporation of complement control (and other cellular) proteins in virion envelopes perhaps comprises the preferential budding of viruses at lipid rafts.

Poxviridae

Vaccinia virus produces two morphologically and antigenically distinct infectious forms of virions, designated ‘intracellular mature virus’ and ‘extracellular enveloped virus’ (Appleyard *et al.*, 1971). Structurally, the extracellular enveloped virus consists of an intracellular mature virus

with an additional outer membrane containing proteins that are absent from the intracellular mature virus (Tooze *et al.*, 1993; Schmelz *et al.*, 1994). At least ten proteins are associated with this outer envelope and six of them are known to be encoded by vaccinia virus genes. One of them is B5R, which has been discussed already in the previous section. In a study by Vanderplasschen *et al.* (1998), the resistance of extracellular enveloped virus and intracellular mature virus to complement neutralization in the absence of immune antibodies (alternative complement pathway) was investigated. It was demonstrated that the extracellular enveloped virus but not the intracellular mature virus is resistant to complement activation. This was not a result of one of the vaccinia virus-encoded proteins present on the membrane of extracellular enveloped virus, including B5R, but was shown to be caused by incorporation of the host complement regulators CD46, CD55 and CD59 into the envelope of the extracellular enveloped virus. This incorporation of complement regulators could be very advantageous for vaccinia virus, because the virus has a wide host range and by this mechanism, progeny virus will always carry complement regulators adapted to the complement of its host.

Herpesviridae

Alphaherpesvirinae. In a recent study, Maeda *et al.* (2002) demonstrated that PRV grown in a porcine cell line was protected against the actions of porcine complement, whereas the same PRV strain grown in a rabbit cell line was extremely sensitive to lysis by porcine complement. This resistance of the porcine cell line-derived PRV was thought to occur via incorporation of complement regulators in the viral envelope from the host cell. However, the complement control proteins responsible for this protection have not been identified so far.

Betaherpesvirinae. HCMV-infected cells are known to be susceptible to lysis by complement only for a short period following acute infection, suggesting that, at later stages of infection, infected cells must be protected from complement-mediated destruction (Betts & Schmidt, 1981; Middeldorp *et al.*, 1986). Since an infection with HCMV results in an upregulation of different cellular host genes, the hypothesis was put forward that HCMV enhances the expression of host-encoded complement inhibitors. Spiller *et al.* (1996) investigated this hypothesis by measuring cell surface expression of complement regulators on HCMV-infected cells by means of flow cytometry. They found that the expression of two RCAs, CD55 and CD46, was increased up to 8-fold following infection with HCMV. This increase was not observed upon infection with adenovirus or HSV, indicating that upregulation was not a generalized response to virus infection. Functional studies demonstrated that the upregulation of CD55 suppressed the activity of the alternative pathway C3 convertases and increased resistance to complement-mediated lysis. The mechanism responsible for this upregulation remains to be determined but it has been hypothesized that an early HCMV gene product induces the transcription of genes of the RCA gene cluster (Spiller *et al.*, 1996).

Retroviridae

The best-studied member of the family *Retroviridae* is indisputably HIV. HIV is able to exploit complement molecules for its own benefit since opsonization of HIV has been shown to allow the virus to enter cells via complement receptors (reviewed by Stoiber *et al.*, 2001). Besides this complement-mediated enhancement of infection, HIV has developed a mechanism to resist complement-mediated destruction by incorporating the complement regulators CD55, CD59 and CD46 in its membrane during virus release; this has been shown to increase the complement resistance of HIV (Saifuddin *et al.*, 1995, 1997).

HTLV-I has been demonstrated also to incorporate complement control proteins CD55 and CD59 from the host cell into its envelope (Spear *et al.*, 1995). Studies with phosphatidylinositol-specific lipases, which remove the glycosyl phosphatidylinositol-anchored CD55 and CD59, showed that absence of these regulatory proteins increased the susceptibility of HTLV-1 towards complement-mediated lysis (Spear *et al.*, 1995).

Togaviridae

This family of enveloped, positive-stranded RNA viruses consists of different genera. One of them is the genus *Alphavirus* and members of this genus infect neurons in the brain and spinal cord, causing acute encephalomyelitis in a variety of mammals. An important member is Sindbis virus, which infects mice and is commonly used as a model for alphavirus-induced encephalomyelitis.

As mentioned already, C3b deposition occurs spontaneously and indiscriminately on the cell surface of host cells, thereby promoting alternative complement activation unless C3b activity on the cell surface is neutralized during this initial random attack. Sialic acids seem to be crucial surface components in protecting autologous cells from activation of the alternative complement pathway by making cell-bound C3b accessible to inhibition by RCA factor H (Meri & Pangburn, 1990). It has been suggested that Sindbis virus takes advantage of the sialic acids present on the host cells, since it was found that the complement resistance of Sindbis virions was correlated with the amounts of sialic acid that are expressed on the cells in which the virus was grown. A large number of sialic acid residues present on the viral envelope, adopted during budding from the plasma membrane, may render bound C3b on the envelope accessible to inhibition by the soluble RCA factor H (Hirsch *et al.*, 1981, 1983).

CONCLUSION

Over the past millennia, evolution has forced several viruses to develop intriguingly ingenious and diverse mechanisms to avoid or delay destruction by the immune response. The complement system, although indisputably of significant importance during the adaptive immune response, evolved originally as part of the elder innate immune system and, as

discussed already, may aid in clearing a virus infection in various ways. These characteristics of the complement system, together with its sophisticated and carefully regulated multi-step activation and suppression, may have fashioned the diversity of virus-encoded proteins that mediate interference with efficient complement activation.

Several viruses have, throughout their evolution, captured cellular genes that are beneficial for their replication or spread. Therefore, it may not be too surprising that, based on their sequence similarity, a lot of the viral genes encoding proteins that interfere with complement activation most likely originate from cellular genes encoding cellular complement regulators. Besides virus capture of cellular *genes*, it has been shown for several enveloped viruses that capture of *proteins*, in other words, the incorporation of cellular complement regulators in their envelope during budding, also provides an efficient means of complement evasion. The exact mechanism of specific acquisition of cellular membrane-associated complement regulators remains rather poorly understood but, at least for some of these processes, may depend on membrane rafts. These cholesterol- and sphingolipid-enriched microdomains in the plasma membrane have been demonstrated to be rich in GPI-anchored complement regulators as well as to function as preferential budding platforms for several enveloped viruses.

Interference with complement-mediated destruction, together with other immune evasion strategies, has helped viruses to extend their lifespan in humans and animals, gaining them more time to spread to uninfected hosts. In addition, for viruses such as cowpox virus, it has been shown that virus interference with the complement cascade may in fact be beneficial for both the virus and the host, limiting tissue damage caused by an otherwise overexaggerated complement activation upon infection. Furthermore, several viruses interact with the complement system to mediate virus entry. Some proteins involved in complement regulation have been shown to be viral receptors (e.g. CD46 for measles virus, CD55 for certain picornaviruses and complement receptor 2 for EBV), whereas C3b-mediated opsonization of HIV has been shown to facilitate attachment and entry (Fingeroth *et al.*, 1984; Naniche *et al.*, 1993; Dorig *et al.*, 1993; Bergelson *et al.*, 1994; reviewed by Stoiber *et al.*, 2001).

The several intriguing interactions between viruses and the complement cascade (and other immune responses) established over time not only provide insights in how evolution has shaped viruses to adapt to host defence mechanisms, and vice versa, but also aid in understanding the complex interplay between the different components of the immune system.

REFERENCES

Adler, R., Glorioso, J. C., Cossman, J. & Levine, M. (1978). Possible role of Fc receptors on cells infected and transformed by herpesvirus: escape from immune cytolysis. *Infect Immun* **21**, 442–447.

Afonso, C. L., Tulman, E. R., Lu, Z., Zsak, L., Osorio, F. A., Balinsky, C., Kutish, G. F. & Rock, D. L. (2002). The genome of swinepox virus. *J Virol* **76**, 783–790.

Albrecht, J. C. & Fleckenstein, B. (1992). New member of the multigene family of complement control proteins in herpesvirus saimiri. *J Virol* **66**, 3937–3940.

Albrecht, J. C., Nicolas, J., Biller, D. & 7 other authors (1992a). Primary structure of the herpesvirus saimiri genome. *J Virol* **66**, 5047–5058.

Albrecht, J. C., Nicolas, J., Cameron, K. R., Newman, C., Fleckenstein, B. & Honess, R. W. (1992b). Herpesvirus saimiri has a gene specifying a homologue of the cellular membrane glycoprotein CD59. *Virology* **190**, 527–530.

Appleyard, G., Hapel, A. J. & Boulter, E. A. (1971). An antigenic difference between intracellular and extracellular rabbitpox virus. *J Gen Virol* **13**, 9–17.

Atalay, R., Zimmermann, A., Wagner, M., Borst, E., Benz, C., Messerle, M. & Hengel, H. (2002). Identification and expression of human cytomegalovirus transcription units coding for two distinct Fc gamma receptor homologs. *J Virol* **76**, 8596–8608.

Barrett, J. W., Cao, J. X., Hota-Michell, S. & McFadden, G. (2001). Immunomodulatory proteins of myxoma virus. *Semin Immunol* **13**, 73–84.

Bavari, S., Bosio, C. M., Wiegand, E. & 7 other authors (2002). Lipid raft microdomains: a gateway for compartmentalized trafficking of Ebola and Marburg viruses. *J Exp Med* **195**, 593–602.

Benichou, G. & Voisin, G. A. (1987). Antibody bipolar bridging: isotype-dependent signals given to guinea pig alveolar macrophages by anti-MHC alloantibodies. *Cell Immunol* **106**, 304–317.

Bergelson, J. M., Chan, M., Solomon, K. R., St John, N. F., Lin, H. & Finberg, R. W. (1994). Decay-accelerating factor (CD55), a glycosylphosphatidylinositol-anchored complement regulatory protein, is a receptor for several echoviruses. *Proc Natl Acad Sci U S A* **91**, 6245–6249.

Betts, R. F. & Schmidt, S. G. (1981). Cytolytic IgM antibody to cytomegalovirus in primary cytomegalovirus infection in humans. *J Infect Dis* **143**, 821–826.

Cooper, N. R., Jensen, F. C., Welsh, R. M., Jr & Oldstone, M. B. (1974). Lysis of RNA tumor viruses by human serum: direct antibody-independent triggering of the classical complement pathway. *J Exp Med* **144**, 970–984.

Crnkovic-Mertens, I., Messerle, M., Milotic, I., Szepan, U., Kucic, N., Krmpotic, A., Jonjic, S. & Koszinowski, U. H. (1998). Virus attenuation after deletion of the cytomegalovirus Fc receptor gene is not due to antibody control. *J Virol* **72**, 1377–1382.

Dorig, R. E., Marcil, A., Chopra, A. & Richardson, C. D. (1993). The human CD46 molecule is a receptor for measles virus (Edmonston strain). *Cell* **75**, 295–305.

Dowler, K. W. & Veltri, R. W. (1984). *In vitro* neutralization of HSV-2: inhibition by binding of normal IgG and purified Fc to virion Fc receptor (FcR). *J Med Virol* **13**, 251–259.

Dubin, G., Frank, I. & Friedman, H. M. (1990). Herpes simplex virus type 1 encodes two Fc receptors which have different binding characteristics for monomeric immunoglobulin G (IgG) and IgG complexes. *J Virol* **64**, 2725–2731.

Dubin, G., Socolof, E., Frank, I. & Friedman, H. M. (1991). Herpes simplex virus type 1 Fc receptor protects infected cells from antibody-dependent cellular cytotoxicity. *J Virol* **65**, 7046–7050.

Dubin, G., Basu, S., Mallory, D. L. P., Basu, M., Tal-Singer, R. & Friedman, H. M. (1994). Characterization of domains of herpes

simplex virus type 1 glycoprotein E involved in Fc binding activity for immunoglobulin aggregates. *J Virol* **68**, 2478–2486.

Ebenbichler, C. F., Thielens, N. M., Vornhagen, R., Marschang, P., Arlaud, G. J. & Dierich, M. P. (1991). Human immunodeficiency virus type 1 activates the classical pathway of complement by direct C1 binding through specific sites in the transmembrane glycoprotein gp41. *J Exp Med* **174**, 1417–1424.

Engelstad, M., Howard, S. T. & Smith, G. L. (1992). A constitutively expressed vaccinia gene encodes a 42-kDa glycoprotein related to complement control factors that forms part of the extracellular virus envelope. *Virology* **188**, 801–810.

Favoreel, H. W., Nauwynck, H. J., Van Oostveldt, P., Mettenleiter, T. C. & Pensaert, M. B. (1997). Antibody-induced and cytoskeleton-mediated redistribution and shedding of viral glycoproteins, expressed on pseudorabies virus-infected cells. *J Virol* **71**, 8254–8261.

Favoreel, H. W., Nauwynck, H. J. & Pensaert, M. B. (1999a). Role of the cytoplasmic tail of gE in antibody-induced redistribution of viral glycoproteins expressed on pseudorabies virus-infected cells. *Virology* **259**, 141–147.

Favoreel, H. W., Nauwynck, H. J., Halewyck, H. M., Van Oostveldt, P., Mettenleiter, T. C. & Pensaert, M. B. (1999b). Antibody-induced endocytosis of viral glycoproteins and major histocompatibility complex class I on pseudorabies virus-infected monocytes. *J Gen Virol* **80**, 1283–1291.

Favoreel, H. W., Van Minnebruggen, G., Nauwynck, H. J., Enquist, L. W. & Pensaert, M. B. (2002). A tyrosine-based motif in the cytoplasmic tail of pseudorabies virus glycoprotein B is important for both antibody-induced internalization of viral glycoproteins and efficient cell-to-cell spread. *J Virol* **76**, 6845–6851.

Fingeroth, J. D., Weis, J. J., Tedder, T. F., Strominger, J. L., Biro, P. A. & Fearon, D. T. (1984). Epstein–Barr virus receptor of human B lymphocytes is the C3d receptor CR2. *Proc Natl Acad Sci U S A* **81**, 4510–4514.

Fodor, W. L., Rollins, S. A., Bianco-Caron, S., Rother, R. P., Guilmette, E. R., Burton, W. V., Albrecht, J. C., Fleckenstein, B. & Squinto, S. P. (1995). The complement control protein homolog of herpesvirus saimiri regulates serum complement by inhibiting C3 convertase activity. *J Virol* **69**, 3889–3892.

Frank, I. & Friedman, H. M. (1989). A novel function of the herpes simplex virus type I Fc receptor: participation in bipolar bridging of antiviral immunoglobulin G. *J Virol* **63**, 4479–4488.

Friedman, H. M., Cohen, G. H., Eisenberg, R. J., Seidel, C. A. & Cines, D. B. (1984). Glycoprotein C of herpes simplex virus 1 acts as a receptor for the C3b complement component on infected cells. *Nature* **309**, 633–634.

Fries, L. F., Friedman, H. M., Cohen, G. H., Eisenberg, R. J., Hammer, C. H. & Frank, M. M. (1986). Glycoprotein gC of herpes simplex virus 1 is an inhibitor of the complement cascade. *J Immunol* **137**, 1636–1641.

Hakozaki, Y., Yoshiba, M., Sekiyama, K. & 8 other authors (2002). Mannose-binding lectin and the prognosis of fulminant hepatic failure caused by HBV infection. *Liver* **22**, 29–34.

Hannan, L. A. & Edidin, M. (1996). Traffic, polarity, and detergent solubility of a glycosylphosphatidylinositol-anchored protein after LDL-deprivation of MDCK cells. *J Cell Biol* **133**, 1265–1276.

Harris, C. L., Spiller, O. B. & Morgan, B. P. (2000). Human and rodent decay-accelerating factors (CD55) are not species restricted in their complement-inhibiting activities. *Immunology* **100**, 462–470.

Haurum, J. S., Thiel, S., Jones, I. M., Fischer, P. B., Laursen, S. B. & Jensenius, J. C. (1993). Complement activation upon binding of mannan-binding protein to HIV envelope glycoproteins. *AIDS* **7**, 1307–1313.

Herold, B. C., WuDunn, D., Soltys, N. & Spear, P. G. (1991). Glycoprotein C of herpes simplex virus type 1 plays a principal role in the adsorption of virus to the cells and in infectivity. *J Virol* **65**, 1090–1098.

Hirsch, R. L., Griffin, D. E. & Winkelstein, J. A. (1981). Host modification of Sindbis virus sialic acid content influences alternative complement pathway activation and virus clearance. *J Immunol* **127**, 1740–1743.

Hirsch, R. L., Griffin, D. E. & Winkelstein, J. A. (1983). Natural immunity to Sindbis virus is influenced by host tissue sialic acid content. *Proc Natl Acad Sci U S A* **80**, 548–550.

Howard, J., Justus, D. E., Totmenin, A. V., Schelkunov, S. & Kotwal, G. J. (1998). Molecular mimicry of the inflammation modulatory proteins (IMPs) of poxviruses: evasion of the inflammatory response to preserve viral habitat. *J Leukoc Biol* **64**, 68–71.

Huemer, H. P., Larcher, C. & Coe, N. E. (1992). Pseudorabies virus glycoprotein III derived from virions and infected cells binds to the third component of complement. *Virus Res* **23**, 271–280.

Huemer, H. P., Larcher, C., van Drunen Littel-van den Hurk, S. & Babiuk, L. A. (1993). Species selective interaction of *Alphaherpesvirinae* with the 'unspecific' immune system of the host. *Arch Virol* **130**, 353–364.

Huemer, H. P., Nowotny, N., Crabb, B. S., Meyer, H. & Hubert, P. H. (1995). gp13 (EHV-gC): a complement receptor induced by equine herpesviruses. *Virus Res* **37**, 113–126.

Hung, S. L., Srinivasan, S., Friedman, H. M., Eisenberg, R. J. & Cohen, G. H. (1992). Structural basis of C3b binding by glycoprotein C of herpes simplex virus. *J Virol* **66**, 4013–4027.

Hung, S. L., Peng, C., Kostavasili, I., Friedman, H. M., Lambris, J. D., Eisenberg, R. J. & Cohen, G. H. (1994). The interaction of glycoprotein C of herpes simplex virus types 1 and 2 with the alternative complement pathway. *Virology* **203**, 299–312.

Ikeda, F., Haraguchi, Y., Jinno, A., Iino, Y., Morishita, Y., Shiraki, H. & Hoshino, H. (1998). Human complement component C1q inhibits the infectivity of cell-free HTLV-I. *J Immunol* **161**, 5712–5719.

Isaacs, S. N., Kotwal, G. J. & Moss, B. (1992). Vaccinia virus complement-control protein prevents antibody-dependent complement-enhanced neutralization of infectivity and contributes to virulence. *Proc Natl Acad Sci U S A* **89**, 628–632.

Ishak, R., Andiman, W. A. & Tucker, G. (1984). Absence of IgG Fc receptors on varicella-zoster virus-infected cells. *J Med Virol* **13**, 261–267.

Janeway, C. A., Travers, P., Walport, M. & Shlomchik, M. (2001). *Immunobiology – The Immune System in Health and Disease.* New York: Garland Publishing.

Johnson, D. C. & Feenstra, V. (1987). Identification of a novel herpesvirus simplex virus type 1-induced glycoprotein which complexes with gE and binds immunoglobulin. *J Virol* **61**, 2208–2216.

Johnson, D. C., Frame, M. C., Ligas, M. W., Cross, A. M. & Stow, N. D. (1988). Herpes simplex virus immunoglobulin G Fc receptor activity depends on a complex of two viral glycoproteins, gE and gI. *J Virol* **62**, 1347–1354.

Kapadia, S. B., Molina, H., van Berckel, V., Speck, S. H., & Virgin, H. W., IV (1999). Murine gammaherpesvirus 68 encodes a functional regulator of complement activation. *J Virol* **73**, 7658–7670.

Kase, T., Suzuki, Y., Kawai, T. & 7 other authors (1999). Human mannan-binding lectin inhibits the infection of influenza A virus without complement. *Immunology* **97**, 385–392.

Kostavasili, J., Sahu, A., Friedman, H. M., Eisenberg, R. J., Cohen, G. H. & Lambris, J. D. (1997). Mechanism of complement inactivation by glycoprotein C of herpes simplex virus. *J Immunol* **158**, 1763–1771.

Kotwal, G. J., Isaacs, S. N., McKenzie, R., Frank, M. M. & Moss, B. (1990). Inhibition of the complement cascade by the major secretory protein of vaccinia virus. *Science* **250**, 827–830.

Kotwal, G. J., Miller, C. G. & Justus, D. E. (1998). The inflammation protein (IMP) of cowpox virus drastically diminishes the tissue damage by down-regulating cellular infiltration resulting from complement activation. *Mol Cell Biochem* **185**, 39–46.

Kubota, Y., Gaither, T. A., Cason, J., Oshea, J. J. & Lawley, T. J. (1987). Characterization of the C3 receptor induced by herpes simplex virus type 1 infection in human epidermal, endothelial, and A431 cells. *J Immunol* **138**, 1137–1142.

Lee, H. J., Essani, K. & Smith, G. L. (2001). The genome sequence of Yaba-like disease virus, a yatapoxvirus. *Virology* **281**, 170–192.

Lilley, B. N., Ploegh, H. L. & Tirabassi, R. S. (2001). Human cytomegalovirus open reading frame TRL11/IRL11 encodes an immunoglobulin G Fc-binding protein. *J Virol* **75**, 11218–11221.

Litwin, V. & Grose, C. (1992). Herpesviral Fc receptors and their relationship to the human Fc receptors. *Immunol Res* **11**, 226–238.

Litwin, V., Sandor, M. & Grose, C. (1990). Cell surface expression of the varicella-zoster glycoproteins and Fc receptor. *Virology* **178**, 263–272.

Litwin, V., Jackson, W. & Grose, C. (1992). Receptor properties of two varicella-zoster virus glycoproteins, gpI and gpIV, homologous to herpes simplex virus gE and gI. *J Virol* **66**, 3643–3651.

Lubinski, J. M., Wang, L., Soulika, A. M. & 7 other authors (1998). Herpes simplex virus type 1 glycoprotein gC mediates immune evasion *in vivo*. *J Virol* **72**, 8257–8263.

Lubinski, J. M., Wang, L., Mastellos, D., Sahu, A., Lambris, J. D. & Friedman, H. M. (1999). *In vivo* role of complement-interacting domains of herpes simplex virus type 1 glycoprotein gC. *J Exp Med* **190**, 1637–1646.

Lubinski, J. M., Jiang, M., Hook, L. & 7 other authors (2002). Herpes simplex virus type 1 evades the effects of antibody and complement *in vivo*. *J Virol* **76**, 9232–9241.

MacCormac, L. P. & Grundy, J. P. (1996). Human cytomegalovirus induces an Fc gamma receptor (Fc gammaR) in endothelial cells and fibroblasts that is distinct from the human cellular Fc gammaRs. *J Infect Dis* **174**, 1151–1161.

Mackowiak, P. A. & Marling-Cason, M. (1987). Immunoreactivity of cytomegalovirus-induced Fc receptors. *Microbiol Immunol* **31**, 427–434.

McKenzie, R., Kotwal, G. J., Moss, B., Hammer, C. H. & Frank, M. M. (1992). Regulation of complement activity by vaccinia virus complement-control protein. *J Infect Dis* **166**, 1245–1250.

Maeda, K., Hayashi, S., Tanioka, Y., Matsumoto, Y. & Otsuka, H. (2002). Pseudorabies virus (PRV) is protected from complement attack by cellular factors and glycoprotein C (gC). *Virus Res* **84**, 79–87.

Meri, S. & Pangburn, M. K. (1990). Discrimination between activators and nonactivators of the alternative pathway of complement: regulation via a sialic acid/polyanion binding site on factor H. *Proc Natl Acad Sci U S A* **87**, 3982–3986.

Mettenleiter, T. C., Zsak, L., Zuckermann, F., Sugg, N., Kern, H. & Ben-Porat, T. (1990). Interaction of glycoprotein gIII with a cellular heparinlike substance mediates adsorption of pseudorabies virus. *J Virol* **64**, 278–286.

Middeldorp, J. M., Jongsma, J. & The, T. H. (1986). Killing of human cytomegalovirus-infected fibroblasts by antiviral antibody and complement. *J Infect Dis* **153**, 48–55.

Mold, C., Bradt, B. M., Nemerow, G. R. & Cooper, N. R. (1988). Epstein–Barr virus regulates activation and processing of the third component of complement. *J Exp Med* **168**, 949–969.

Nagashunmugam, T., Lubinski, J., Wang, L., Goldstein, L. T., Weeks, B. J., Sundaresan, P., Kang, E. H., Dubin, G. & Friedman, H. M. (1998). *In vivo* immune evasion mediated by the herpes simplex virus type 1 immunoglobulin G Fc receptor. *J Virol* **72**, 5351–5359.

Naniche, D., Varior-Krishnan, G., Cervoni, F., Wild, T. F., Rossi, B., Rabourdin-Combe, C. & Gerlier, D. (1993). Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. *J Virol* **67**, 6025–6032.

Nauwynck, H. J. & Pensaert, M. B. (1992). Abortion induced by cell-associated pseudorabies virus in vaccinated sows. *Am J Vet Res* **53**, 489–493.

Nguyen, D. H. & Hildreth, J. E. (2000). Evidence for budding of human immunodeficiency virus type 1 selectively from glycolipid-enriched membrane lipid rafts. *J Virol* **74**, 3264–3272.

Ogata, M. & Shigeta, S. (1979). Appearance of immunoglobulin G Fc receptor in cultured human cells infected with varicella-zoster virus. *Infect Immun* **26**, 770–774.

Oleszak, E. L. (1994). Molecular mimicry between Fc receptors and viral antigens. *Arch Immunol Ther Exp* **42**, 83–88.

Oleszak, E. L., Perlman, S., Parr, R., Collisson, E. W. & Leibowitz, J. L. (1993). Molecular mimicry between S peplomer proteins of coronaviruses (MHV, BCV, TGEV and IBV) and Fc receptor. *Adv Exp Med Biol* **342**, 183–188.

Oleszak, E. L., Kuzmak, J., Hogue, B., Parr, R., Collisson, E. W., Rodkey, L. S. & Leibowitz, J. L. (1995). Molecular mimicry between Fc receptor and S peplomer protein of mouse hepatitis virus, bovine corona virus, and transmissible gastroenteritis virus. *Hybridoma* **14**, 1–8.

Ono, A. & Freed, E. O. (2001). Plasma membrane rafts play a critical role in HIV-1 assembly and release. *Proc Natl Acad Sci U S A* **98**, 13925–13930.

Pangburn, M. K. (1986). *Immunobiology of the Complement System*, pp. 45–62. Edited by G. D. Ross. New York: Academic Press.

Para, M. F., Baucke, R. B. & Spear, P. G. (1982). Glycoprotein gE of herpes simplex virus type 1: effects of anti-gE on virion infectivity and on virus-induced Fc-binding receptors. *J Virol* **41**, 129–136.

Perez de la Lastra, J. M., Harris, C. L., Hinchliffe, S. J., Holt, D. S., Rushmere, N. K. & Morgan, B. P. (2000). Pigs express multiple forms of decay-accelerating factor (CD55), all of which contain only three short consensus repeats. *J Immunol* **165**, 2563–2573.

Rahman, A. A., Teschner, M., Sethi, K. K. & Brandis, H. (1976). Appearance of IgG (Fc) receptor(s) on cultured human fibroblasts infected with human cytomegalovirus. *J Immunol* **117**, 253–258.

Reading, P. C., Morey, L. S., Crouch, E. C. & Anders, E. M. (1997). Collectin-mediated antiviral host defense of the lung: evidence from influenza virus infection of mice. *J Virol* **71**, 8204–8212.

Rosengard, A. M., Liu, Y., Nie, Z. & Jimenez, R. (2002). Variola virus immune evasion design: expression of a highly efficient inhibitor of human complement. *Proc Natl Acad Sci U S A* **99**, 8808–8813.

Rother, R. P., Rollins, S. A., Fodor, W. F., Albrecht, J. C., Setter, E., Fleckenstein, B. & Squinto, S. P. (1994). Inhibition of complement-mediated cytolysis by the terminal complement inhibitor of herpesvirus saimiri. *J Virol* **68**, 730–737.

Rushmere, N. K., Tomlinson, S. & Morgan, B. P. (1997). Expression of rat CD59: functional analysis confirms lack of species selectivity and reveals that glycosylation is not required for function. *Immunology* **90**, 640–646.

Rux, A. H., Lou, H., Lambris, J. D., Friedman, H. M., Eisenberg, R. J. & Cohen, G. H. (2002). Kinetic analysis of glycoprotein C of herpes simplex virus types 1 and 2 binding to heparin, heparan sulphate, and complement component C3b. *Virology* **294**, 324–332.

Saifuddin, M., Parker, C. J., Peeples, M. E., Gorny, M. K., Zolla-Opazner, S., Ghassemi, M., Rooney, I. A., Atkinson, J. P. & Spear, G. T. (1995). Role of virion-associated glycosylphosphatidylinositol-linked proteins CD55 and CD59 in complement resistance of cell line-derived and primary isolates of HIV-1. *J Exp Med* **182**, 501–509.

Saifuddin, M., Hedayati, T., Atkinson, J. P., Holgiun, M. H., Parker, C. J. & Spear, G. T. (1997). Human immunodeficiency virus type 1 incorporates both glycosyl phosphatidylinositol-anchored CD55 and CD59 and integral membrane CD64 at levels that protect from complement-mediated destruction. *J Gen Virol* **78**, 1907–1911.

Sasaki, K., Tsutsumi, A., Wakamiya, N., Ohtani, K., Suzuki, Y., Watanabe, Y., Nakayama, N. & Koike, T. (2000). Mannose-binding lectin polymorphisms in patients with hepatitis C virus infection. *Scand J Gastroenterol* **35**, 960–965.

Scheiffele, P., Rietveld, A., Wilk, T. & Simons, K. (1999). Influenza viruses select ordered lipid domains during budding from the plasma membrane. *J Biol Chem* **274**, 2038–2044.

Schmelz, M., Sodeik, B., Ericsson, M., Wolffe, E. J., Shida, H., Hiller, G. & Griffiths, G. (1994). Assembly of vaccinia virus: the second wrapping cisterna is derived from the trans Golgi network. *J Virol* **68**, 130–147.

Schreurs, C., Mettenleiter, T. C., Zuckermann, F., Sugg, N. & Ben-Porat, T. (1988). Glycoprotein gIII of pseudorabies virus is multifunctional. *J Virol* **62**, 2251–2257.

Seidel-Dugan, C., Ponce de Leon, M., Friedman, H. M., Eisenberg, R. J. & Cohen, G. H. (1990). Identification of C3b-binding regions on herpes simplex virus type 2 glycoprotein C. *J Virol* **64**, 1897–1906.

Smiley, M. L., Hoxie, J. A. & Friedman, H. M. (1985). Herpes simplex virus type 1 infection of endothelial, epithelial and fibroblast cells induces a receptor for C3b. *J Immunol* **134**, 2673–2678.

Spear, G. T., Lurain, N. S., Parker, C. J., Ghassemi, M., Payne, G. H. & Saifuddin, M. (1995). Host cell-derived complement control proteins CD55 and CD59 are incorporated into the virions of two unrelated enveloped viruses. Human T cell leukemia/lymphoma virus type 1 (HTLV-1) and human cytomegalovirus (HCMV). *J Immunol* **155**, 4376–4381.

Spiller, O. B. & Morgan, B. P. (1998). Antibody-independent activation of the classical complement pathway by cytomegalovirus-infected fibroblasts. *J Infect Dis* **178**, 1597–1603.

Spiller, O. B., Morgan, B. P., Tufaro, F. & Devine, D. V. (1996). Altered expression of host-encoded complement regulators on human cytomegalovirus-infected cells. *Eur J Immunol* **26**, 1532–1538.

Stannard, L. M. & Hardie, D. R. (1991). An Fc receptor for human immunoglobulin G is located within the tegument of human cytomegalovirus. *J Virol* **65**, 3411–3415.

Stoiber, H., Kacani, L., Speth, C., Würzner, R. & Dierich, M. P. (2001). The supportive role of complement in HIV pathogenesis. *Immunol Rev* **180**, 168–176.

Takahashi-Nishimaki, F., Funahashi, S., Miki, K., Hashizume, S. & Sugimoto, M. (1991). Regulation of plaque size and host range by a vaccinia virus gene related to complement system proteins. *Virology* **181**, 158–164.

Thäle, R., Lucin, P., Schneider, K., Eggers, M. & Koszinowski, U. H. (1994). Identification and expression of a murine cytomegalovirus early gene coding for an Fc receptor. *J Virol* **68**, 7757–7765.

Tooze, J., Hollinshead, M., Reis, B., Radsak, K. & Kern, H. (1993). Progeny vaccinia and human cytomegalovirus particles utilize early endosomal cisternae for their envelopes. *Eur J Cell Biol* **60**, 163–178.

Van den Berg, C. W. & Morgan, B. P. (1994). Complement-inhibiting activities of human CD59 and analogues from rat, sheep, and pig are not homologously restricted. *J Immunol* **152**, 4095–4101.

Vanderplasschen, A., Mathew, E., Hollinshead, M., Sim, R. B. & Smith, G. L. (1998). Extracellular enveloped vaccinia virus is resistant to complement because of incorporation of host complement control proteins into its envelope. *Proc Natl Acad Sci U S A* **95**, 7544–7549.

Van de Walle, G. R., Favoreel, H. W., Nauwynck, H. J., Van Oostveldt, P. & Pensaert, M. B. (2001). Involvement of cellular cytoskeleton components in antibody-induced internalization of viral glycoproteins in pseudorabies virus-infected monocytes. *Virology* **288**, 129–138.

Vincent, S., Gerlier, D. & Manie, S. N. (2000). Measles virus assembly within membrane rafts. *J Virol* **74**, 9911–9915.

Virgin, H. W., IV, Latreille, P., Wamsley, P., Hallsworth, K., Weck, K. E., Dal Canto, A. J. & Speck, S. H. (1997). Complete sequence and genomic analysis of murine gammaherpesvirus 68. *J Virol* **71**, 5894–5904.

Watkins, J. F. (1964). Adsorption of sensitized sheep erythrocytes to HeLa cells infected with herpes simplex virus. *Nature* **202**, 1364–1365.

Westmoreland, D. & Watkins, J. F. (1974). The IgG receptor induced by herpes simplex virus: studies using radioiodinated IgG. *J Gen Virol* **24**, 167–178.