Vaccinia complement control protein: Multi-functional protein and a potential wonder drug

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Vaccinia virus complement control protein (VCP) was one of the first viral molecules demonstrated to have a role in blocking complement and hence in the evasion of host defense. Structurally it is very similar to the human C4b-BP and the other members of complement control protein. Functionally it is most similar to the CR1 protein. VCP blocks both major pathways of complement activation. The crystal structure of VCP was determined a little over a year ago and it is the only known structure of an intact and complete complement control protein. In addition to binding complement, VCP also binds to heparin. These two binding abilities can take place simultaneously and contribute to its many function and to its potential use in several inflammatory diseases, e.g. Alzheimer's disease (AD), CNS injury, xenotransplantation, etc. making it a truly fascinating molecule and potential drug.

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1. Introduction

The pathogen-host interaction is a dynamic phenomenon which involves generation of defense mechanism by host and its evasion by the pathogen. The success of pathogen and host in the course of evolution depends on the success in their respective roles. Because of this dynamic interaction, the vertebrate host develops complex immune system-specific as well as non-specific. In response to the complex immune system, the pathogens develop different mechanisms to evade host immunity. Viruses are particularly very efficient in evading host immunity (Tortorella *et al* 2000). Diverse array of pathways and molecular targets are used by viruses to evade immune detection and destruction. They do so in many ways using different strategies. One of the used strategies is rapid mutation of genes encoding structural components of the virion that are targets of specific immunity. RNA viruses usually employ this strategy. Another evasion strategy is piracy of immune-response genes from the host – a characteristic feature of DNA viruses (Haig 2001). The virus presumably acquires the DNA encoding the protein from the host and, over an evolutionary period, manipulates the DNA to retain only the most essential domain.

Poxviruses are complex group of highly successful pathogens that infect vertebrates and insects (Moss 1996). They are cytoplasmic, double stranded DNA viruses, which do not undergo a latent stage but instead express an array of viral proteins to evade host-immune response. Poxviruses belonging to different genera employ different immune evasion strategies by expressing different evasion proteins. Some of representative immune evasion proteins are indicated in figure 1. Viral expression of this wide array of proteins suggests that the genes

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Abbreviations used: AD, Alzheimer's disease; BP, binding protein; CP, complement protein; CR1, complement receptor 1; DAF, decay accelerating factor; FGF, fibroblast growth factors; MCP, membrane cofactor protein; SCRs, short consensus repeats; VCP, vaccinia virus complement control protein.

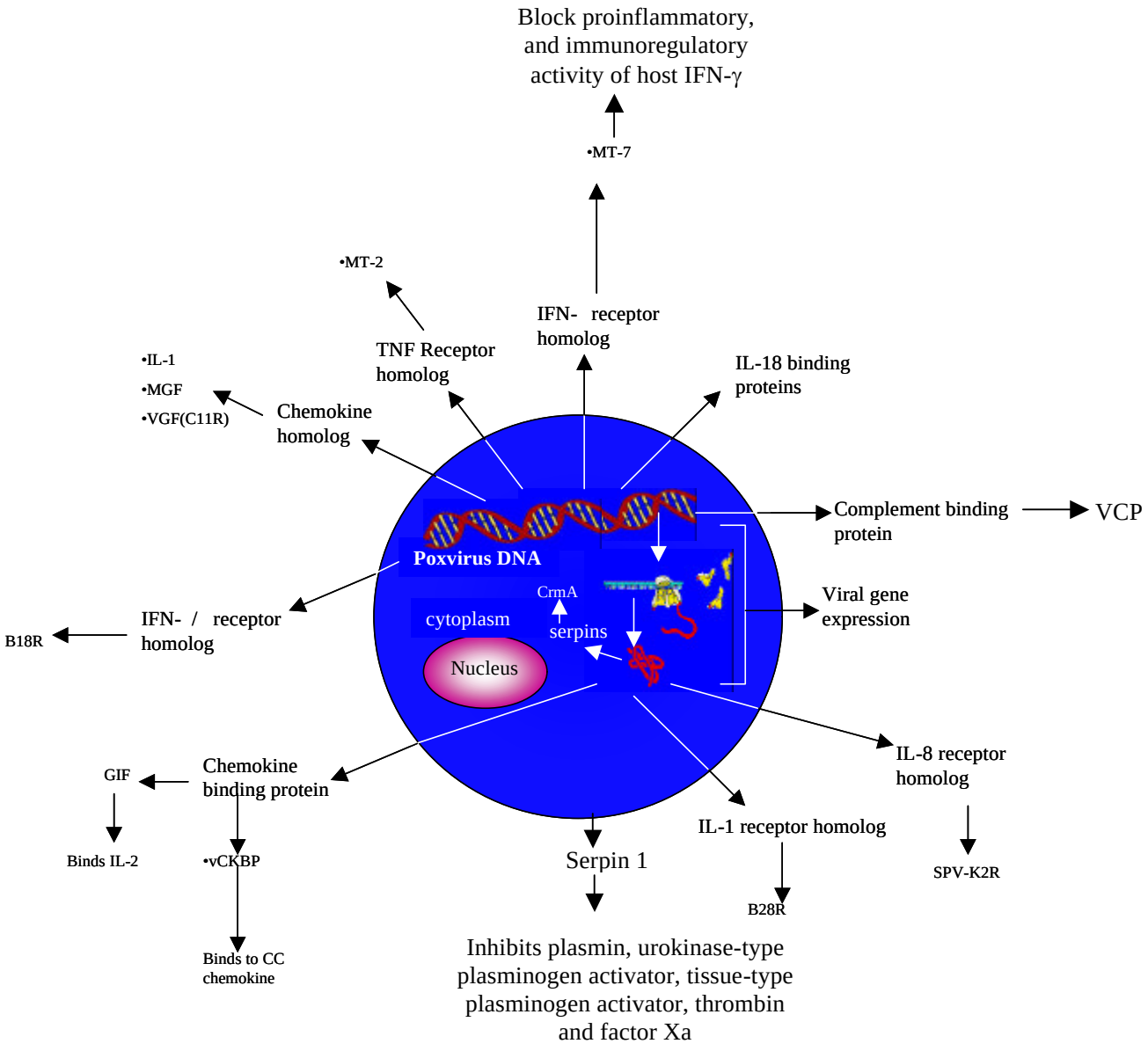

Figure 1. Some of the proteins encoded by poxviruses to evade the host immune system. Molecular mimicry of cytokines and cytokine receptors is a common immune evasion strategy adopted by large DNA viruses like poxviruses. Poxviruses encode soluble proteins that are secreted from infected cells and function as soluble IFNγ receptor (IFNγR) which blocks IFN-γ activity. Poxviruses express four vTNFRs, which have different properties. These vTNFRs are produced at different times during infection, and have different specificities for TNF. The orthopoxvirus cowpox virus (CPV) encodes three vTNFRs: cytokine response modifier B (CrmB), CrmC, and CrmD. Poxviruses also secrete homologs of humoral immune regulators, such as the viral IL-10 and vascular endothelial growth factor, encoded by orf virus, and the viral CC chemokine, encoded by *Molluscum contagiosum* virus. The serpin CrmA (cytokine response modifier A) is expressed order to avoid inflammatory and apoptotic responses of infected host cells. Some viruses produce cytokine homologs called virokines. Myxoma viral growth factor (MGF) is homologs of epidermal growth factor/transforming growth factor a (EGF/TGF-a). Such factors stimulate virus transformed cell growth. *Molluscum contagiosum* encodes three proteins, MC51L, MC53L, and MC54L which are identical to human interleukin-18 binding protein (hIL-18BP), a naturally occurring antagonist of the proinflammatory cytokine IL-18. Another strategy used by poxviruses is via virus-encoded chemokine homologs (vCks) and virus-encoded chemokine-binding proteins (vCkBPs). The vCkBPs are viral chemokine-modulators and function as chemokine scavengers. The pathogenic poxviruses encode for a complement control protein. The vaccinia virus complement control protein or VCP was one of the first soluble microbial proteins postulated to have an active role in the immunomodulation of the host defense.

encoding these proteins were acquired during the course of poxvirus host-adaptation (Senkevich *et al* 1996). The host immune-evading proteins include soluble cytokine receptors or binding proteins for tumour necrosis factor (TNF), interleukin-1β (IL-1β), alpha/beta interferon IFN-α/β, IFN-γ (Mossman *et al* 1995; Alcami *et al* 2000), CC chemokines, IL-18, and granulocyte and monocyte colony-stimulating factor and IL-2 (Nash *et al* 1999; Smith *et al* 1997). Poxviruses also secrete homologs of humoral immune regulators, such as the viral IL-10 and vascular endothelial growth factor, encoded by orf virus, and the viral CC chemokine, encoded by *Molluscum contagiosum* virus (Lalani *et al* 2000).

The best example, and one of the first to be documented, for a viral immune-evasion protein, is the vaccinia virus major secretory protein (Kotwal and Moss 1988). This protein is referred to as the vaccinia virus complement control protein (VCP) because of its structural similarity to the family of complement control proteins. Complement system consist of more than 20 plasma and membrane proteins that operate in a sequential manner to eliminate invading microorganism (Reid 1995). The complement system is divided into classical and alternative pathway, both leading to the formation of a common membrane attack complex (MAC) (Muller 1988; Frank and Fries 1989). Peptides produced by the activation of complement cascade stimulates host immune system by different mechanisms. Two of the small peptides-C3a and C5a, which are cleaved from C3 and C5, act as anaphylotoxins which lead to production of reactive oxygen intermediates through respiratory burst in neutrophils and macrophages. Complement components also promote phagocytosis by opsonizing immune complexes. The eukaryotic proteins that regulate complement activation include one receptor and four inhibitors – complement receptor 1 (CR1), factor H, C4b binding protein (C4b-BP), membrane cofactor protein (MCP) and decay accelerating factor (DAF) (Holers *et al* 1985).

2. VCP blocks complement pathway

VCP is a secreted product of vaccinia virus gene *C21L* (the 21st open reading frame starting from the left end of the *Hin*dIII *c* fragment of the genome (Earl and Moss 1989). It contains four short consensus repeats that are most similar in sequence (38% identity) to the first four repeats of C4b-BP (Kotwal and Moss 1988, 1989), one of the inhibitors of classical complement pathway (Barnum 1991). Infected cells secrete VCP after cleavage of signal peptide and can inhibit the classical as well as alternate complement pathway. VCP blocks complement pathway by binding to the third and fourth complement components and by blocking the formation of the C3-convertase, as well as by accelerating the decay of the convertase (Kotwal *et al* 1990; McKenzie *et al* 1992). In addition, VCP has been shown to cleave C3b, in the presence of factor I, to iC3b (Sahu *et al* 1999).

3. VCP is similar to CR1

VCP is a 35 kDa protein which is made up of 243 amino acid residues. Structurally VCP consists of four short consensus repeats (SCRs) which bear 38%, 35%, 31% amino acid identity to C4b-BP, MCP, and DAF, respectively (Earl and Moss 1989; Kotwal *et al* 1990). The amino acid sequence of VCP is consistent with the presence of complement protein (CP) modules, also called SCRs. These CP modules are found in mammalian proteins called the regulators of complement activation (RCA) proteins (Reid *et al* 1995). Studies with deletion mutants have shown that VCP, like CR1, requires all four of its SCRs to bind C3b. The NMR solution structure of VCP has revealed that modules form discreet compactly folded modules, which are joined end to end, forming a fairly rigid rod-like structure (Kirkitadze *et al* 1999; Wiles *et al* 1997). These four domains of VCP show similarities with modules 1–4, modules 8–11 and modules 15–18 of CR1. These regions of CR1 recognize and bind to complement proteins C3b and C4b. So although VCP is structurally more similar to C4bp but its functional profile is more similar to CR1.

Crystal structure of VCP and its relationship to its function has been extensively studied by Murthy *et al* (2001). Module 1 and 4 are highly positively charged and module 2 and 3 carry a net negative charge. Studies indicate that interaction of positively charged regions of complement regulators CR1 (Krych *et al* 1998), CR2 (Prodinger *et al* 1998), C4b-BP (Blom *et al* 1999) and f (H) (Soames *et al* 1996) with negatively charged regions on C3/C4 is a major contribution to their interaction. Therefore the charge distribution on VCP molecule indicates that an ionic interaction with C3 may take place through either module 1 and 2 or module 4. But it has been shown that the truncated VCP which is without module 1 does not inhibit complement, indicating that this module is essential for interaction of VCP with C3b (Smith *et al* 2000).

4. VCP has heparin-binding ability

Apart from complement regulation, VCP share another common property with other complement regulators-MCP, DAF and CR1. This common property is cell-surface association, although the mechanism of surface association is different. VCP has a strong heparin-binding ability. It has two heparin-binding sites on

modules 1 and 4, respectively (Reynolds *et al* 2000; Smith *et al* 2000). As stated before, VCP is an extended molecule. Involvement of regions of module 1 in heparin-binding and complement inhibition in VCP indicates that heparin-binding function of module 4 is probably more important in cell-surface association. Murthy *et al* (2001) reported that there is a significant resemblance between the model for VCP interaction with heparin and that for the fibroblast growth factors (FGF). It is noteworthy that FGF is unrelated to VCP. This resemblance of VCP with unrelated proteins like FGF implicates possibilities of wide applicability of heparin-VCP interaction. Many small chemo-attractant cytokines also possess heparin-binding ability. These chemokines bind to heparin along endothelial walls and regulate the localization and migration of leukocytes into the tissue (Lalani and McFadden 1997). So it can be expected that due to its heparin-binding ability, VCP can bind to heparin on endothelial cells and block the attachment of these small chemokine. This way VCP potentially can prevent leukocyte localization and migration. Indeed VCP has been shown to be taken up in a mixed mast/endothelial cell culture and that VCP reduces chemotactic migration of monocytes in the presence or absence of chemokine MIP 1α (Reynolds *et al* 2000).

5. VCP helps virus to evade the host immune system

Viral invasion leads to activation of complement, in the presence or absence of Abs. Activation of complement can lead to neutralization of viruses, phagocytosis of complement coated viral particles, lysis of viral infected cells and inflammation. The vaccinia virus through evolution has developed an efficient strategy to escape neutralization by host immune system (figure 2). The ability of virus to secrete VCP interferes with complement attack and provides a mechanism for resistance of vaccinia virus to host immunity. VCP can prevent antibody-dependent enhance neutralization of vaccinia virus mediated by complement (Issacs *et al* 1992). It binds to both C4b and C3b, which give it ability to regulate both pathways of complement. The presence of VCP in the membrane of vaccinia virus or virus-infected cells provides a second line of defense for the virus. The importance of VCP for the viral virulence is evident from the finding that deletion of the gene encoding VCP results in significant attenuation of virus pathogenecity (Issacs *et al* 1992). In addition, VCP reduces levels of C3a, C4a and C5a, which are proinflammatory chemotactic factors, resulting in reduced cellular influx and inflammation. VCP secretion may be advantageous for the virus in another way. The ability of VCP to block attachment of chemokine may prevent leukocyte localization and migration. As stated earlier it has been shown that VCP inhibits migration of monocytes through blood vessels to the site of infection.

NK cells are significant effector components of the innate immune system, which aid in the initial defense against viral infection both via direct cellular cytotoxicity and by the production of inflammatory cytokines. VCP inhibits the NK mediated killing of the cells (Al-Mohanna *et al* 2001). So VCP may act as the strongest protector of the viral infected cells from the host immune system.

6. Applications of VCP as a therapeutic agent

Because of the above mentioned properties VCP has strong potential as a therapeutic agent. Its smaller size as compared to other complement modulating proteins and its physically robust characteristics (Smith *et al* 2000) makes VCP a better candidate for therapeutic purposes. As stated earlier, VCP has close resemblance to the host proteins (C4bBP, MCP and DAF) which means that it will not be immunogenic and very little antibodies will be formed against it. The heparin-binding ability increases the effectiveness of VCP as an agent for treatment of diseases involving complement system. For a protein to be a good candidate for therapeutic agent, it is expected that the protein should be released over a period so that its *in vivo* retention time is high. VCP is taken up by mast cell granules, which could be then taken up by endothelial cells (Reynolds *et al* 2000). Because of this uptake, VCP can be released over a longer period, which will result in sustained inhibition of complement activity *in vivo*. It has been demonstrated that VCP could have a possible application treatment of Alzheimer's disease (AD) (Daly and Kotwal 1998). The β-amyloid protein, a neurotoxic peptide implicated in the pathogenesis of AD (Yan *et al* 1997), activates both classical and alternative complement pathways VCP has been shown to block complement activation by the β-amyloid protein.

Using a zymosan-induced mouse model of multiple organ dysfunction syndrome (MODS) (Mahesh *et al* 1999), it has been shown that MIP-1α and C5 play a critical role in modulating cellular changes associated with lethality in a zymosan model of MODS. As stated earlier, VCP can inhibit complement as well as chemotactic migration of monocytes in the presence or absence of MIP 1α, which opens the prospect of VCP being used as a therapeutic agent for MODS. The role of VCP to reduce the damage to the neuronal tissue in the case of both brain and spinal cord injury has also been studied. The lateral fluid-percussion (FP) model of head injury in rats leads to an intense local inflammatory reaction and subsequent tissue destruction, which is mediated by

activation of complement system (Keeling *et al* 2000). Therefore VCP could reduce this damage by blocking the complement activation. VCP, through its heparin-binding ability, can cover the tissue and stop the formation of MAC on cell membrane by already activated complement components. Another promising application of VCP would be in xenotransplantation. Humans, have high levels of pre-existing naturally circulating antibodies XNA that predominantly recognize the epitope Gal (1,3-Gal) on the surface of cells of the donor's tissue. These antibodies deposit on the endothelial cell surface of xenografts and lead to activation of the classical pathway of the

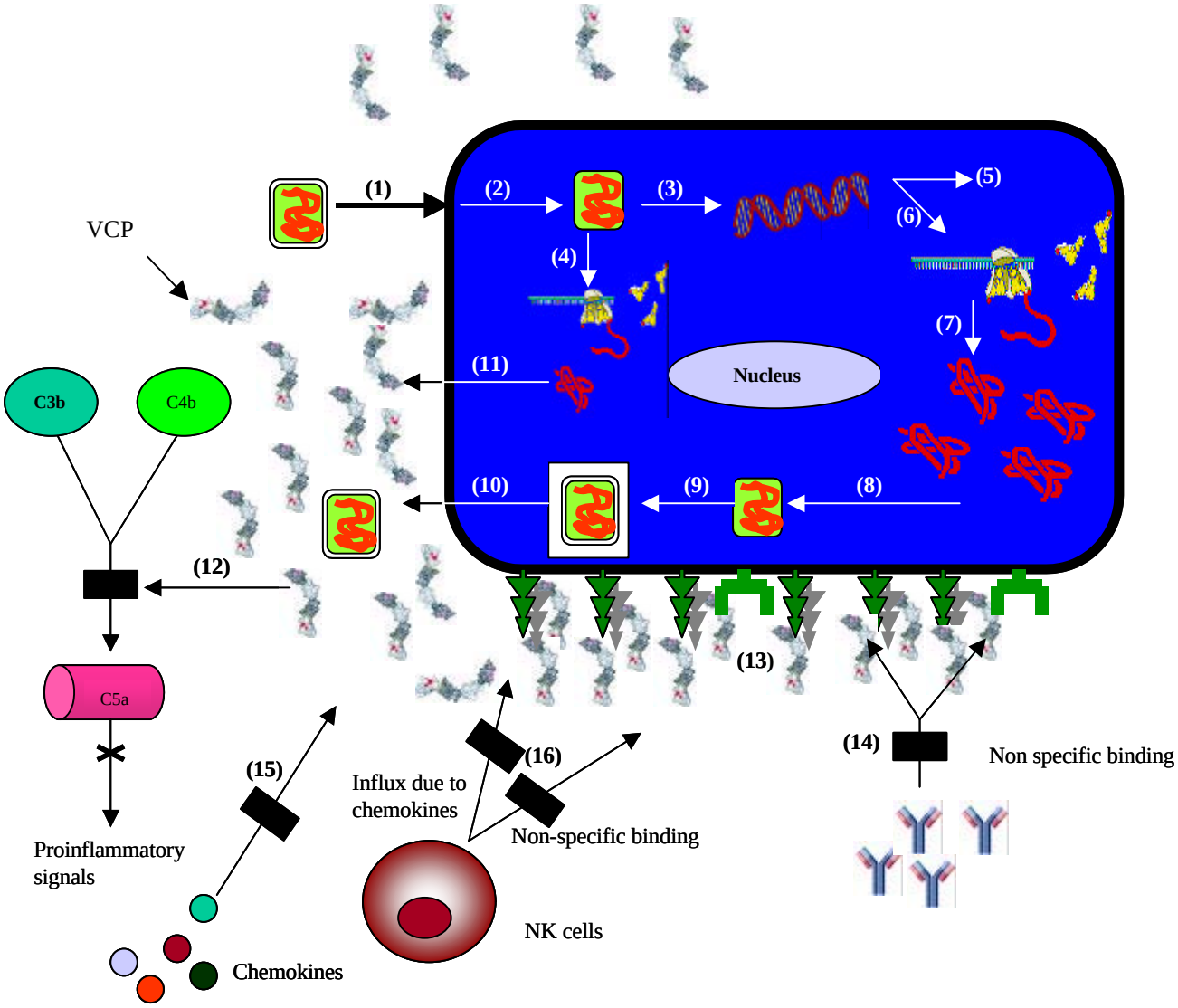

Figure 2. Role of VCP in viral host evasion. The virus attaches to the cells (1) and penetrates the cell membrane with the release of core into the cytoplasm (2). Early mRNA is synthesized from the core (4) and is translated into various proteins, including immunomodulatory proteins like VCP (11). After uncoating (3), the DNA undergoes replication (5). The intermediate and late genes are transcribed (6) and translated to respective proteins (7). Assembly and maturation proceeds to form infectious intracellular mature virions (8). The virions are further processed in the golgi where they gain extra membrane (9). The mature virions are released from the cell (10). VCP provides a favourable microenvironment at the site of infection (11–15) as the virus goes through its life cycle (12–16). VCP binds to C3b and C4b complement proteins (12) and in this way is able to reduce levels of C3a, C4a and C5a, which are proinflammatory chemotactic factors. This reduction in proinflammatory chemotactic signals reduces the cellular influx and inflammation. VCP binds to the heparin on the cell surface (13) and inhibits immune complex formation in a non-specific manner (14). Since the cell can be covered by VCP so the interaction between different chemokines and there respective receptive on the cell surface can be blocked (15). By binding to the heparin on cell surface VCP also blocks NK-cell mediated killing by blocking cell-cell interaction (16).

complement system (Al-Hussein *et al* 2001). This activation of complement system is a major problem in xenograft transplantation because it results in hyperacute rejection (HAR). VCP has been shown to block interaction between pig aortic endothelial cells (PAECs) and cytotoxic cells, including naïve neutrophils and natural killer (NK) cells (Al-Mohanna *et al* 2001). The role of VCP is immunosuppresent in xenotransplantation is being extensively studied.

In summary, VCP and its orthopoxviral homologs are fascinating molecules, which enable orthopoxviruses to overcome the inflammatory response elicited by the host. Understanding the structure and structure-function relationship as well as the mechanism of action of VCP allows us an opportunity to employ VCP in treatment of disease in which complement plays a damaging role.

References

Alcami A, Symons J A and Smith G L 2000 The vaccinia virus soluble alpha/beta interferon (IFN) receptor binds to the cell surface and protects cells from the antiviral effects of IFN; *J. Virol*. **74** 11230–11239

Al-Hussein K, Al-Mukhalafi Z, Pyle H R, Parhar R S, Al-Mohanna F and Lee J 2001 Detection of xenoantibodies using a simple flow cytometric assay; *Xenotransplantation* **8** 172–175

Al-Mohanna F, Parhar R and Kotwal G J 2001 Vaccinia virus complement control protein is capable of protecting xenoendothelial cells from antibody binding and killing by human complement and cytotoxic cells; *Transplantation* **71** 796–801

Barnum S R 1991 C4b-binding protein, a regulatory protein of complement; *Immunol. Res*. **10** 28–42

Blom A M, Webb J, Villoutreix B O and Dahlback B 1999 A cluster of positively charged amino-acids in the C4BP α-chain is crucial for C4b binding and factor I cofactor function; *J. Biol. Chem*. **274** 19237–19245

Daly J and Kotwal G J 1998 Pro-inflammatory complement activation by the Aβ peptide of Alzheimer's disease is biologically significant and can be blocked by vaccinia virus complement control protein; *Neurobiol. Aging* **19** 619–627

Earl P L and Moss B 1989 Vaccinia virus; in *Genetics maps* (ed.) S J O'Brian (New York: Cold Spring Harbor Lab), 5th edition, pp 1·138–1·148

Frank M M and Fries L F 1989 Complement; in *Fundamental immunology* (ed.) W E Paul (New York: Raven) pp 679–701

Haig D M 2001 Subversion and piracy: DNA viruses and immune evasion; *Res. Vet. Sci*. **70** 205–219

Holers V M, Cole J L, Lubin D M, Seya T and Atkinson J P 1985 Human C3b- and C4b-regulatory proteins: a new multigene family; *Immunol. Today* **6** 188–192

Issacs S N, Kotwal G J and Moss B 1992 Vaccinia virus complement-control protein prevents antibody-dependant complement-enhanced neutralization of infectivity and contributes to virulence; *Proc. Natl. Acad. Sci. USA* **89** 628–632

Keeling K L, Hicks R R, Mahesh J, Billings B B and Kotwal G J 2000 Local neutrophil influx following lateral fluid-percussion brain injury in rats is associated with accumulation of complement activation fragments of the third component (C3) of the complement system; *J. Neuroimmunol*. **105** 20–30

Kirkitadze M D, Henderson C, Price N C, Kelly S M, Mullin N P, Parkinson J, Dryden D T F and Barlow P N 1999 Central modules of the vaccinia virus complement control protein are not in extensive contact; *Biochem. J*. **344** 167–175

Kotwal G J and Moss B 1988 Vaccinia virus encodes a secretory polypeptide structurally related to complement control proteins; *Nature* (*London*) **335** 176–178

Kotwal G J and Moss B 1989 Vaccinia virus encodes two proteins that are structurally related to members of the plasma serine protease inhibitor superfamily; *J. Virol*. **63** 600–696

Kotwal G J, Issacs S N and McKenzie R 1990 Vaccinia virus major secretory protein inhibits the classical complement cascade; *Science* **250** 827

Krych M, Hauhart R and Atkinson J P 1998 Structure function analysis of the active sites of complement receptor type I; *J. Biol. Chem*. **273** 8623–8629

Lalani A S and McFadden G 1997 Secreted poxvirus chemokine binding proteins; *J. Leukoc Biol*. **62** 570–576

Lalani A S, Barrett J W and McFadden I 2000 Modulating chemokines: more lessons from viruses; *Immunol. Today* **21** 100–106

Mahesh J, Daly J, Cheadle W G and Kotwal G J 1999 Elucidation of the early events contributing to zymosan-induced multiple organ dysfunction syndrome using MIP-1alpha, C3 knockout, and C5–deficient mice; *Shock* **12** 340–349

McKenzie R, Kotwal G J and Moss B 1992 Regulation of complement activity by vaccinia virus complement control protein; *J. Infect. Dis*. **166** 1245

Moss B 1996 Poxviridae: The viruses and their replication; in *Fields virology* (ed.) B N Fields (Lippincott: Raven) vol. 2, pp 2637–2671

Mossman K, Upton C, Buller R M and McFadden G 1995 Species specificity of ectromelia virus and vaccinia virus interferon-γ binding proteins; *Virology* **208** 762–769

Muller-Eberhard H J 1988 Molecular organization and function of the complement system; *Annu. Rev. Biochem*. **57** 321–347

Murthy K H M, Smith S A, Ganesh V K, Judge K W, Mullin N, Barlow P N, Ogata C M and Kotwal G J 2001 Crystal structure of a complement control protein that regulates both pathways of complement activation and binds heparan sulfate proteoglycans; *Cell* **104** 301–311

Nash P, Barrett J, Cao J X, Hota-Mitchell S, Lalani A S, Everett H, Xu X M, Robichaud J, Hnatiuk S, Ainslie C, Seet B T and McFadden G 1999 Immunomodulation by viruses: the myxoma virus story; *Immunol. Rev.* **168** 103–120

Prodinger W M, Scwendinger M G, Schoch J, Kochle M, Harcher C C and Dierich M P 1998 Characterization of C3dg binding to a recess formed between consensus repeats 1 and 2 of complement receptor type 2; *J. Immunol*. **161** 4604–4610

Reid K B M 1995 The complement system: A major effector mechanism in humoral immunity; *Immunologist* **3** 206–211

Reynolds D N, Keeling K L, Molestina R, Srisatjaluk R, Butterfield J H, Ehringer W, Justus D E and Kotwal G J 2000 heparin binding activity of vaccinia virus complement control protein confers additional properties of uptake by mast cells and attachment to endothelial cells; in *Advances in animal virology* (ed.) S Jameel (Villarreal: Science Publishers) pp 337–342

Senkevich T G, Bugert J J, Sisler J R, Koonin E V, Darai G and Moss B 1996 Genome sequence of human tumorigenic pox-

virus: prediction of specific host response-evasion genes; *Science* **273** 813–816

Smith S A, Mullin N P, Parkinson J, Shchelkunov S N, Totmenin A V, Loparev V N, Srisatjaluk R, Reynolds D N, Keeling K L, Justus D E, Barlow P N and Kotwal G J 2000 Conserved surface-exposed K/R-X-K/R motifs and net positive charge on poxvirus complement control proteins serve as putative heparin-binding sites and contribute to inhibition of molecular interactions with human endothelial cells: a novel mechanism for evasion of host defense; *J. Virol*. **74** 5659–5666

Smith G L, Symons J A, Khanna A, Vanderplasschen A and Alcami A 1997 Vaccinia virus immune evasion; *Immunol. Rev*. **159** 137–154

Soames C J, Day A J and Sim R B 1996 Prediction from sequence comparisons of residues of factor H involved in the interaction with complement component C3b; *Biochem. J*. **315** 523–531

Tortorella D, Gewurz B E, Furman M H, Schust, D J and Ploegh H L 2000 Viral Subversion of the Immune System; *Annu. Rev. Immunol*. **18** 861–926

Wiles A P, Shaw G, Bright J, Percelez A, Campbell L D and Barlow P N 1997 NMR studies of a viral protein that mimics the regulators of complement activation; *J. Mol. Biol*. **272** 253–265

Yan S D, Fu J, Soto C, Chen X, Zhu H, Al-Mohanna F, Collison K, Zhu A, Stern E, Saido T, Tohyama, M, Ogawa, S, Roher A and Stern D 1997 An intracellular protein that binds amyloid-beta peptide and mediates neurotoxicity in Alzheimer's disease; *Nature* (*London*) **389** 689–695