Virus encoded cytokines and cytokine receptors

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SUMMARY

In order to replicate efficiently within the host, viruses have evolved multiple strategies to evade the host's immune system. In many cases viruses have actually hijacked various components of the host's immune system to ensure their own survival. One such strategy is the expression of virus encoded cytokines and cytokine receptors. Members of the poxvirus and herpesvirus families have been particularly successful with this strategy. The study of virus survival strategies provides important information regarding both virus biology as well as information about the immune system itself.

Key words: Virokines, viroceptors, herpes virus, immunosubversion, poxvirus.

INTRODUCTION

Large DNA viruses, such as the herpesviruses and poxviruses, encode upwards of 100 open reading frames that are predicted to express protein products. Many of these genes encode proteins that have been shown to counteract specifically the host's immune response thus facilitating survival of the virus within immunocompetent hosts (reviewed in Marrack & Kappler, 1994; Smith, 1994; Spriggs, 1996). A number of these proteins were identified by virtue of their homology to known cellular proteins (Murphy, 1993). Other virus open reading frames, however, do not have known corresponding cellular counterparts, suggesting that either these viral open reading frames are unique or the eukaryotic homologue remains unidentified. Although viruses have been viewed in the past simply as vehicles of plague and pestilence, it is now clear that viruses are extensively educated in regard to the innermost workings of the vertebrate immune system and therefore can be viewed as educational tools for the study of immunology. In fact, not only have viruses been adept pupils, but they have systematically exploited components of the host's immune system to overcome the hostile host environment in which they replicate. Since viruses have co-evolved with animal hosts for untold millennia, it is not surprising that they have much to teach us, and we are now realizing that viruses can be used as potential probes for understanding and manipulating the immune system.

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VIRUS ENCODED CYTOKINES AND GROWTH FACTORS

One strategy of immune evasion by viruses is the capture and expression of host cytokines and growth factors. Currently, several viruses are known to express cytokines and growth factors, all of which are restricted to members of the herpesvirus and poxvirus families (Table 1) (also reviewed in Smith 1994; Spriggs, 1994, 1996; Evans, 1996). It has been proposed that the expression of these viral cytokines and growth factors is important for virus survival, although in most cases the rationale for this particular strategy remains poorly understood. The recent identification of additional virus encoded cytokines in molluscum contagiosum (Senkevich *et al*. 1996) and in Kaposi's sarcoma-associated herpesvirus (KSHV) (Moore *et al*. 1996) suggests that more virus encoded cytokines probably remain to be discovered.

Virus encoded IL-10

The first example of a virus encoded cytokine identified was the BCRF1 open reading frame of Epstein–Barr virus (EBV) which encodes a secreted polypeptide with significant homology to interleukin-10 (IL-10) (Baer *et al*. 1984; Moore *et al*. 1990; reviewed in Swaminathan & Kieff, 1995). Epstein–Barr virus is a member of the herpesvirus family and is the causative agent of infectious mononucleosis. In addition, EBV has been associated with various lymphomas such as Burkitt's lymphoma and nasopharyngeal carcinoma. The BCRF1 gene of EBV, designated viral IL-10 (vIL-10), is 70% homologous to murine IL-10 and 84% homologous to human IL-10, indicating that vIL-10 is more closely related to human IL-10 (Moore *et al*. 1990; Vieira *et al*. 1991). IL-10 is a multifunctional cytokine normally

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Table 1. Virus encoded cytokines

Virus	Gene	Host homologue
Herpesviruses		
Epstein–Barr	BCRF1 (vIL-10)	IL-10
Herpesvirus saimiri	ORF13 (vIL-17)	CTLA-8/IL-17
Kaposi's sarcoma-associated virus	K4 and K6	MIP-1α
Kaposi's sarcoma-associated virus	K2 (vIL-6)	IL-6
Poxviruses		
Vaccinia virus[a]	VGF	EGF/TGFα
Myxoma virus[a]	MGF	EGF/TGFα
ORF virus	A2R	VEGF
Molluscum contagiosum	MC148R	MIP-1β

[a] Other poxviruses express VGF/MGF homologues.

produced by Th2 $CD4^+$ helper T cells, and from activated B cells and macrophages, and was originally classified as a cytokine synthesis inhibitory factor (CSIF) because of its ability to inhibit synthesis of a wide variety of cytokines, from macrophages, monocytes, natural killer cells, and T cells (reviewed in deWaal Malefyt *et al*. 1992; Howard & O'Garra, 1992; Moore *et al*. 1993). The high degree of amino acid identity between vIL-10 and the human protein suggests that EBV may have originally captured this gene from its human host in order to enhance virus propagation in IL-10 responsive leukocytes.

vIL-10 has retained many of the functions of IL-10, such as the inhibition of cytokine synthesis and the stimulation of B cell growth and differentiation (Hsu *et al*. 1990; Go *et al*. 1990; Rousset *et al*. 1992). The inhibitory effects of vIL-10 on the synthesis of both human and murine IFN-γ has been confirmed (Hsu *et al*. 1990), and recombinant EBVs devoid of the BCRF1 gene demonstrate directly that vIL-10 is necessary for the inhibition of IFN synthesis during virus infection (Swaminathan *et al*. 1993). Although vIL-10 has clearly retained several functions of its cellular counterpart, it has not retained the ability to stimulate mast cells (Vieira *et al*. 1991), murine thymocytes and T cells, or induce the expression of MHC class II antigens on murine B cells (Go *et al*. 1990; MacNeil *et al*. 1990). These observations clearly suggest that retention of cytokine synthesis inhibition and B cell stimulation is more important for virus survival. In theory, the ability of vIL-10 to inhibit the production of anti-viral cytokines such as IFN-γ and promote B cell growth should greatly benefit a B cell lymphotropic virus such as EBV. In support of this notion, two other herpesviruses, herpesvirus papio and equine herpesvirus type 2, have also been found to contain vIL-10 homologues (Rode *et al*. 1993; Swaminathan & Kieff, 1995).

Virus encoded CTLA-8/IL-17

Completion of the genomic sequence of Herpesvirus saimiri yielded the identification of 76 putative open reading frames (Albrecht *et al*. 1992). A number of these were demonstrated to contain sequence similarities to cellular proteins, including those involved in host defence or immune modulation, such as complement regulatory proteins, and chemokine receptors (Albrecht *et al*. 1992). When first sequenced, no homologue for the ORF13 gene of Herpesvirus saimiri had been identified, suggesting that ORF13 encoded a virus-specific gene, or that the cellular counterpart was not yet characterized. It became apparent, however, that ORF13 was indeed homologous to a cellular open reading frame when CTLA-8 was cloned from a murine lymphocyte cDNA library (Rouvier *et al*. 1993). All cysteine residues were conserved between the two proteins, as well as a single glycosylation site and five putative phosphorylation sites. Overall, the percent amino acid identity was found to be 57% between CTLA-8 and the protein encoded by ORF13 of Herpesvirus saimiri (Rouvier *et al*. 1993). CTLA-8 is only expressed from a subset of activated T cells and the presence of AU rich repeats in the 3′ untranslated region of the mRNA suggest that expression of the protein is tightly controlled via mRNA degradation, a mechanism utilized for the controlled expression of many cytokines and growth factors (Rouvier *et al*. 1993). Although no function was initially associated with CTLA-8 or ORF13, the acquisition of this gene by Herpesvirus saimiri, which encodes other proteins important for immune evasion, suggested that the protein may be important to counteract host immune mechanisms.

Recently, Yao *et al*. have cloned and characterized a novel receptor that binds both CTLA-8 and the Herpesvirus saimiri homologue (Yao *et al*. 1995*a*). The authors report that this receptor, now designated the interleukin 17 receptor (IL-17R), is widely distributed among various tissues and cell lines, and database searches have revealed no homology with previously characterized sequences, including members of the cytokine receptor family. The extensive distribution of the IL-17R is in direct contrast to the expression of CTLA-8, now referred to as IL-17,

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which reportedly is expressed only in activated T cells (Rouvier *et al*. 1993; Yao *et al*. 1995*a*). Interaction of IL-17 with the receptor results in the activation of NFκ-B, the secretion of IL-6 from fibroblasts and the enhanced proliferation of murine T cells (Yao *et al*. 1995*a*). The Herpesvirus saimiri protein,ORF13, has retained all of these functions, suggesting that these functional properties may confer a selective advantage for the virus.

The identification and cloning of the human IL-17 gene from a $CD4^+$ T cell library revealed a greater degree of homology with ORF13, sharing 72% amino acid sequence identify (Yao *et al*. 1995*b*). Like its murine counterpart, human IL-17 was found to be expressed only in activated T cells. Human IL-17 mediates the secretion of both IL-6 and IL-8 from cells and increases the cell surface expression of the intracellular adhesion molecule ICAM-1 (Yao *et al*. 1995*b*). Studies to elucidate further the functional properties of IL-17 and its receptor should also provide information regarding the functional significance of the viral homologue ORF13, since the exact function of this protein during virus infection is currently unknown.

Virus encoded chemokines

Molluscum contagiosum virus is a relatively benign poxvirus that infects humans and causes dermal wart-like lesions. DNA sequence analysis of the genome has revealed the presence of 163 putative open reading frames of which 59 were found to be unique (not present in the genome of other poxviruses) (Senkevich *et al*. 1996). Different members of the poxvirus family are known to contain a diverse array of genes that function to evade the host immune response, but surprisingly many of these anti-immune genes have no obvious counterpart within the genome of molluscum contagiosum virus. Several of the open reading frames of molluscum contagiosum are, however, homologous to cellular genes with known function, including the open reading frame MC148R which shares amino acid identity with the C-C chemokine macrophage inflammatory protein 1β (MIP-1β) (Senkevich *et al*. 1996). Chemokines are a family of immune modulatory chemoattractant cytokines that mediate the chemotaxis of various leukocytes (reviewed in Baggiolini, Dewald & Moser; 1994; Schall & Bacon, 1994). The predicted protein encoded by MC148R has retained the correct cysteine positioning important for overall structure of the protein, but lacks a significant segment of the N-terminal region. Since studies have demonstrated that engineered chemokines that lack this N-terminal region can bind to chemokine receptors but are unable to trigger an intracellular signal (Clark-Lewis *et al*. 1995), the authors speculate that MC148R functions as a chemokine inhibitor by competing directly with cellular chemokines for binding to chemokine receptors and preventing the subsequent activation of leukocytes (Senkevich *et al*. 1996). This type of a strategy is not foreign to members of the poxvirus family, since various other secreted viral proteins seems to function by preventing activation of the immune system, but in a very different manner (discussed in later sections of this review). However, molluscum contagiosum was the first virus, and currently the only poxvirus, reported to encode a gene with homology to a cellular chemokine which may contribute to virus survival.

A recent report has revealed the presence of two chemokine homologues in the genome of Kaposi's sarcoma-associated herpesvirus (KSHV), also designated human herpesvirus 8 (HHV-8) (Moore *et al*. 1996). KSHV is a member of the gamma herpesvirus family which also includes EBV and Herpesvirus saimiri, both of which encode multiple proteins involved in immune evasion. Open reading frames K6 and K4 of KSHV are predicted to encode 10·5 kDa proteins with approximately 40% amino acid identity to human MIP-1α. Although nothing is currently known about the function of K4, the K6 open reading frame encodes a functional chemokine which can interact with chemokine receptors and subsequently inhibit infection of HIV, which utilizes the CCR5 receptor for cellular entry (Moore *et al*. 1996). In contrast to the chemokine encoded by the MC148R open reading frame in molluscum contagiosum, the K4 and K6 open reading frames in KSHV seem to be intact and are not missing the N-terminal region. This suggests that the chemokines encoded by KSHV may in fact trigger a signal upon interaction with the cellular receptor, but this remains to be demonstrated experimentally. The rationale for the expression of a virus encoded functional chemokine is currently unknown, but since the virus has presumably acquired this gene from the host, it seems likely that it may somehow function to facilitate virus survival *in vivo*. It is possible that chemokine/chemokine receptor interaction may modify the host cell environment in order to benefit the virus. For example, the binding of a virus encoded chemokine to the normal cellular receptor may inappropriately induce chemotaxis or cellular activation that favours virus survival.

Virus encoded IL-6

In addition to the presence of virus encoded chemokines, genomic sequencing of KSHV revealed the presence of an open reading frame with homology to interleukin-6 (IL-6), including the presence of four cysteine residues which are necessary for structural conservation (Moore *et al*. 1996). IL-6 is a multifunctional cytokine that is an important growth factor for various cell types and stimulates B-cell differentiation (reviewed in Hirano *et al*. 1990).

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The KSHV K2 open reading frame encodes a secreted 23 kDa protein, designated viral IL-6 (vIL-6), that has retained at least one of the functions of IL-6. Supernatants from Cos-7 cells that express vIL-6 have been shown to support growth of an IL-6-dependent myeloma cell line (Moore *et al*. 1996). Thus, despite the overall low sequence homology, the K2 open reading frame encodes a functional cytokine capable of stimulating leukocyte cell growth. Tissue sections from patients infected with KSHV demonstrated the presence of vIL-6 associated with haematopoietic lymph node cells, particularly in areas containing abundant B cells (Moore *et al*. 1996), suggesting that vIL-6 may play a role in KSHV pathogenesis by stimulating B cells *in vivo*. In fact, various reports suggest a link between KSHV infection of patients and B cell lymphomas (Cesarman *et al*. 1995; Gessain *et al*. 1995; Soulier *et al*. 1995; Corbellino *et al*. 1996). Since the IL-6 receptors is present on a wide variety of cell types (Taga *et al*. 1987), it is likely that vIL-6 interacts with this receptor and stimulates cell growth to enhance KSHV replication and survival.

Virus encoded growth factors

The capture and expression of cellular growth factors by numerous poxviruses has been well documented (reviewed in McFadden, Graham & Opgenorth, 1995*b*). To date, virus encoded proteins that mimic the activities of epidermal growth factor (EGF), transforming growth factor-α (TGFα) and vascular endothelial growth factor (VEGF) have been identified. The first example of a virus encoded growth factor was found serendipitously when the vaccinia virus 19K open reading frame, now designated vaccinia growth factor (VGF), was sequenced (Bloomquist, Hunt & Barker, 1984; Brown *et al*. 1985; Reisner, 1985). VGF displays significant homology to EGF and TGFα including the conservation of 6 essential cysteine residues. Additional secreted EGF-like proteins are encoded by other poxviruses, including variola virus (Massung *et al*. 1993*a*, 1994), Shope fibroma virus (Chang *et al*. 1987), and myxoma virus (Upton *et al*. 1987*b*). Virus encoded growth factors exploit the cellular system by binding to cellular growth factor receptors and inappropriately stimulating cell growth. Deletion of both VGF and the myxoma virus growth factor (MGF) results in attenuation *in vivo* (Buller *et al*. 1988; Opgenorth *et al*. 1992), indicating that the presence of VGF and MGF confer a selective advantage for the virus in the host. In order to restore virus virulence, the MGF gene of myxoma virus can be replaced with either VGF or rat TGFα, further supporting the theory that MGF and VGF are *bona fide* functional growth factors (Opgenorth *et al*. 1993). In addition to the EGF-like growth factors encoded by members of the poxvirus family, orf virus, another member of the poxvirus family, expresses a protein with sequence homology to mammalian VEGF. Supernatants from orf infected cells, display mitogenic activity suggesting that the A2R open reading frame encodes a functional VEGF homologue (Lyttle *et al*. 1994). Since VEGF is an important regulator of angiogenesis, the expression of VEGF from orf virus infected cells is consistent with the presence of lesions at the primary site of infection that are characterized by capillary proliferation and oedema (Ferrara *et al*. 1992; Lyttle *et al*. 1994). To date, mimics of EGF and VEGF growth factors that favour virus replication have only been observed in members of the poxvirus family; however, the presence of these growth factors in other viruses is a distinct possibility.

VIRUS ENCODED CYTOKINE RECEPTORS

The acquisition of genes encoding cytokine receptors by poxviruses and their subsequent expression from infected cells has been extensively documented (reviewed in Pickup, 1994; Alcami & Smith, 1995*b*; McFadden *et al*. 1995*a*). The term 'viroceptor' was coined to describe soluble virus-encoded cytokine receptors that are expressed from virus infected cells and function by binding to host cell cytokines preventing interaction of host cytokines with cellular receptors (Upton *et al*. 1991). Currently, five such soluble viroceptors have been described in poxviruses (Table 2). In addition, both herpesviruses and poxviruses express proteins with significant homology to transmembrane cytokine receptors (reviewed in Ahuja, Gao & Murphy, 1994; Murphy, 1994; Schall *et al*. 1995) and this review will discuss both types of viroceptors.

Virus encoded TNF receptor homologues

The first example of a viroceptor was discovered in Shope fibroma virus (SFV), a poxvirus that causes benign subcutaneous fibromas in rabbits (Upton *et al*. 1987*a*; Smith *et al*. 1990; reviewed in Smith & Goodwin, 1995; McFadden, Schreiber & Sedger, 1996). Sequence analysis initially revealed significant homology between SFV-T2 and the nerve growth factor receptor. It later became apparent, when the type 1 and type 2 tumour necrosis factor (TNF) receptors were cloned and sequenced, that T2 was indeed a virus encoded homologue of the TNF type 1 receptor that was able to bind TNFα and lymphotoxin (Smith *et al*. 1990, 1991). Like its cellular homologue, the T2 protein contains a cysteine-rich region and a signal sequence, but no obvious transmembrane domain, suggesting that T2 is in fact a secreted TNF receptor capable of binding and sequestering TNF. Further studies revealed the

Table 2. Virus encoded cytokine receptors

Virus	Gene	Host homologue
Soluble cytokine receptors[a]		
Myxoma virus	M-T2	TNF receptor
Cowpox virus	CrmB, C	TNF receptor
Myxoma virus	M-T7	IFN-γ receptor
Vaccinia virus	B15R	IL-1β receptor
Vaccinia virus	B18R	IFN-$\alpha\beta$ receptor
Myxoma virus	M-T1	Unknown
Membrane-associated receptors		
Herpesvirus samiri	ECRF3	Chemokine receptor
Human cytomegalovirus	US28	Chemokine receptor
Swinepox virus	K2R	Chemokine receptor
Capripox virus	Q2/3L	Chemokine receptor

[a] Related cytokine receptor homologues are encoded in other poxviruses.

presence of similar genes in myxoma virus and malignant rabbit fibroma virus (MRV) (Upton *et al*. 1991), two poxviruses that induce lethal diseases in European rabbits. In order to investigate the role of the myxoma virus T2 gene in virus virulence, both copies of the gene were disrupted. When the resulting recombinant virus was used to infect European rabbits, the majority of rabbits were able to recover from the infection in contrast to rabbits infected with the wild type virus (Upton *et al*. 1991), indicating that the M-T2 protein is necessary for virus pathogenesis *in vivo*. Related open reading frames have been observed in other poxviruses including variola virus (Massung *et al*. 1993*a*, 1994), and various strains of vaccinia virus, although the vaccinia genes appear to be disrupted (Goebel *et al*. 1990; Howard, Chan & Smith, 1991). In addition, the genome of cowpox virus contains two distinct open reading frames, crmB and crmC, with homology to TNF receptors (Hu, Smith & Pickup, Smith & Pickup, 1994; Smith *et al*. 1996). CrmB is the cowpox virus equivalent of M-T2, while crmC encodes a distinct TNF receptor homologue that is secreted late during infection and only interacts with TNF and not lymphotoxin (Smith *et al*. 1996).

Purified M-T2 protein, generated from a vaccinia virus engineered to over-express M-T2, inhibited rabbit TNFα-mediated lysis of L929-8 cells, but was unable to inhibit lysis mediated by either human or murine TNFα (Schreiber & McFadden, 1994). Thus, M-T2 exhibits narrow species specificity and specifically binds and inhibits rabbit TNFα. Furthermore, Scatchard analysis has demonstrated that M-T2 binds rabbit TNFα with an affinity similar to the mammalian TNF receptors (Schreiber, Rajarathnam & McFadden, 1996). M-T2 is secreted from infected cells as both a 80 kDa disulphide linked dimer and a 40 kDa monomer. Both forms can interact with TNFα, but the dimer appears to provide better protection against TNFα-mediated cell lysis (Schreiber *et al*. 1996). A series of deleted and truncated M-T2-expressing recombinant viruses have demonstrated that the N-terminal cysteine-rich domain, the region homologous with other members of the TNF superfamily, is essential for binding rabbit TNFα (Schreiber & McFadden, 1996). In addition, it appears that the C-terminal 132 amino acids, which are unique to M-T2, are necessary for the efficient secretion of the protein, since deletion results in the intracellular retention of M-T2 (Schreiber & McFadden, 1996).

While M-T2 has definitively been shown to be important for the inhibition of cellular lysis due to TNF, it appears that M-T2 may have a dual function in infected cells. Although myxoma virus can replicate normally in infected $CD4^+$ T lymphocytes, infection of lymphocytes with the M-T2 mutant virus results in the induction of cellular DNA fragmentation, a characteristic of cellular apoptosis (Macen *et al*. 1996). Furthermore, experiments have shown that the ability of M-T2 to inhibit apoptosis does not correlate with TNF binding since truncated versions of the protein which cannot bind or inhibit TNF are still able to inhibit apoptosis in virus infected T cells (Schreiber, Sedger & McFadden, 1997). While many viruses encode proteins that are essential for protection against cellular apoptosis (reviewed in White, 1996), M-T2 appears to be the first protein with this unique dual function. Other poxvirus encoded M-T2 homologues have not been tested experimentally for this dual activity, but it is possible that other classes of viral immunomodulatory proteins are multifunctional as well.

Virus encoded IFN-γ receptor homologues

The protein encoded by the M-T7 open reading frame from myxoma virus was the first example of a secreted virus protein designed to circumvent the affects of IFN-γ prior to cytokine/receptor engagement (reviewed in Mossman *et al*. 1995*a*, 1997). Since IFN-γ was originally characterized as a cytokine with potent anti-viral activity, it is not surprising that viruses have evolved strategies to overcome the affects of this cytokine. N-terminal amino acid sequence analysis and subsequent DNA sequence analysis of the major 37 kDa secreted protein from myxoma virus infected cells revealed the existence of a virus encoded protein with approximately 30% amino acid identity to human and murine IFN-γ receptors (Upton, Mossman & McFadden, 1992). Homology is only evident within the ligand-binding domain and the positioning of eight cysteine residues throughout this domain.

The M-T7 protein functions as a *bona fide* soluble IFN-γ receptor that can both bind and inhibit the activity of rabbit IFN-γ (Upton *et al*. 1992). M-T7 binds rabbit IFN-γ with high affinity ($1{\cdot}2 \times 10^{-9}$ M), but not human or murine IFN-γ, reflecting the close

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evolutionary relationship of a rabbit virus with its natural host (Mossman, Upton & McFadden, 1995*c*). An identical result was also observed for the myxoma virus TNF receptor homologue which also displays strict species specificity for ligand recognition (Schreiber & McFadden, 1994). In order to evaluate directly the role of M-T7 *in vivo*, a recombinant myxoma virus containing a deletion in the M-T7 open reading frame was used to infect European rabbits (Mossman *et al*. 1996). Rabbits infected with the recombinant virus remained essentially disease free and recovered fully from infection, in stark contrast to rabbits infected with the parental M-T7 containing virus. Thus, the presence of a virus encoded IFN-γ binding protein contributes directly to virus virulence *in vivo* (Mossman *et al*. 1996).

IFN-γ receptor homologues have been reported in a wide variety of poxviruses (Massung *et al*. 1994; Alcami & Smith, 1995*a*; Mossman *et al*. 1995*b*). In contrast to the myxoma virus encoded IFN-γ receptor, IFN-γ receptor homologues expressed by other poxviruses, particularly members of the orthopoxvirus genus, demonstrate a broader species specificity (Alcami & Smith, 1995*a*; Mossman *et al*. 1995*b*). The evolutionary origin of myxoma virus has been firmly established in the South American rabbit (Fenner & Ratcliffe, 1965); however, the evolutionary origin of most orthopoxviruses is not as clearly established, leading to the suggestion that the broad specificity demonstrated by these virus encoded IFN-γ binding proteins may reflect a varied evolutionary history involving the replication of virus in several species (Alcami & Smith, 1995*a*, 1996*a*; Mossman *et al*. 1995*b*). Intriguingly, IFN-γ binding proteins encoded by members of the orthopoxviruses have retained only 6 of 8 conserved cysteine residues in the ligand-binding domain, which may explain the altered ligand binding properties displayed by these proteins.

Virus encoded IL-1 receptor homologues

Two groups have independently demonstrated that the B15R open reading frame in vaccinia virus (strain WR) encodes a secreted glycoprotein that specifically interacts with IL-1β (Alcami & Smith, 1992; Spriggs *et al*. 1992; reviewed in Alcami & Smith, 1995*c*). Similar to the myxoma virus encoded TNF and IFN-γ receptor homologues, the B15R protein is homologous to the external portion of the cellular IL-1 type II receptor, and does not contain a transmembrane region. Although the cellular IL-1 receptor is able to bind both IL-1α, IL-1β and IL-RA (the naturally occurring receptor antagonist), the virus equivalent has clearly lost these functions and only retains the ability to interact with IL-1β (Alcami & Smith, 1992), suggesting that the inhibition of IL-1β is of particular importance during poxvirus infection. Other poxviruses also have been shown to encode IL-1β-binding proteins, including cowpox virus and myxoma virus (Alcami & Smith, 1992; Spriggs *et al*. 1992; and K. Graham, K. Mossman & G. McFadden, unpublished). Alcami & Smith demonstrated that the vaccinia virus encoded IL-1β binding protein bound IL-1β with high affinity similar to that of cellular IL-1 receptors, and that cells infected with vaccinia virus (WR) secreted large quantities of the protein. In fact, the presence of B15R protein can effectively inhibit binding of IL-1β to cellular IL-1 receptors (Alcami & Smith, 1992), a result consistent with the proposed activity of a *bona fide* viroceptor.

Recombinant vaccinia viruses devoid of the B15R gene were constructed by 2 groups and were found to have conflicting pathogenic effects *in vivo*, probably due to variable routes of inoculation (Alcami & Smith, 1992; Spriggs *et al*. 1992). Mice infected by the intracranial route with the B15R deletion virus exhibited diminished mortality compared to infection with the parental virus indicating that B15R is important for virus virulence (Spriggs *et al*. 1992). In contrast, mice infected by the intranasal route with the B15R deletion virus displayed no alteration in mortality, but did display an accelerated disease profile with 70% of infected mice dying earlier than those infected with the parental virus (Alcami & Smith, 1992). In a subsequent paper, Alcami and Smith report that mice infected with the B15R deletion virus respond to infection with increased fever (Alcami & Smith, 1996*b*), which may account for the accentuated disease symptoms observed after intranasal inoculation of mice with the B15R deletion virus. In addition, virus-induced fever can be inhibited by antibodies specific for murine IL-1β (Alcami & Smith, 1996*b*), clearly demonstrating IL-1β is responsible for the induction of fever and that the presence of B15R is important for virus modulation of fever during infection.

Virus encoded IFN-$\alpha\beta$ receptor homologues

Although the importance of type I interferon for recovery from vaccinia virus infection has been well documented (Buller & Palumbo, 1991), the existence of a soluble poxvirus encoded protein that inhibits type I interferon was only recently identified (Colmanonici *et al*. 1995; Symons, Alcami & Smith, 1995). The B18R gene of vaccinia virus (strain WR) encodes a 60–65 kDa protein that is present both on the surface of infected cells and in extracellular supernatants (Ueda, Morikawa & Matsuura, 1990; Alcami & Smith, 1992; Morikawa & Ueda, 1993). The soluble B18R protein binds human IFNα2 with high affinity and inhibits both the binding of human IFN to its cellular receptor (Colmanonici *et al*. 1995; Symons *et al*. 1995) and subsequent signal transduction (Colmanonici *et al*. 1995). Since B18R does

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not contain any obvious transmembrane region, yet can be detected on the surface of uninfected cells, Colmanonici *et al*. suggest that B18R binds to the cell surface after secretion. B18R present on the cell surface of infected cells can also interact with type I IFN suggesting the existence of an additional protective mechanism (Colmanonici *et al*. 1995). In addition, B18R reportedly interacts with an unknown protein on the surface of cells with saturable kinetics (Colmanonici *et al*. 1995), suggesting that the interaction is specific. The elucidation of the B18R cellular binding partner may provide significant information in regards to IFN biology. This characteristic sets B18R apart from other described virus encoded cytokine receptor homologues, since none of the others seem to be present as both soluble and cell associated forms. In contrast to soluble cytokine receptors encoded by myxoma virus, the B18R protein exhibits broad species specificity and is able to bind bovine, rat, mouse, and rabbit interferon type 1 with varying affinities, in addition to human IFN type 1. Several other poxviruses secrete similar proteins that inhibit type 1 IFN, including various strains of vaccinia, cowpox, buffalopox, elephantpox, camelpox, rabbitpox (Symons *et al*. 1995), and ectromelia virus (Colmanonici *et al*. 1995). Additionally, variola virus contains an open reading frame which is homologous to B18R (Massung *et al*. 1993*a*, 1994). Despite the fact that B18R has a much lower affinity for murine IFNβ than human IFNβ (150-fold lower) the recombinant vaccinia virus devoid of the B18R gene is attenuated in mice, indicating that B18R is a functionally important immune modulator within infected animals (Symons *et al*. 1995).

Virus encoded chemokine receptors

DNA sequence analysis of the genomes of two herpesviruses, Herpesvirus saimiri and human cytomegalovirus, have revealed the presence of several putative seven-transmembrane serpentine receptors that are related to mammalian chemokine receptors (reviewed in Ahuja *et al*. 1994; Murphy, 1994; Schall *et al*. 1995). The ECRF3 gene of Herpesvirus saimiri and the US28 gene of human cytomegalovirus encode proteins that share approximately 30% amino acid identity with identified chemokine receptors. Both genes have been shown to encode functional chemokine receptors that bind various members of the chemokine family and subsequently trigger calcium mobilization within the cell (Ahuja & Murphy, 1993; Neote *et al*. 1993; Gao & Murphy, 1994). The chemokine family can be divided into three subgroups based on the position of conserved cysteine residues. The α chemokines (C-X-C) include IL-8, NAP-2, and GRO/MGSA, while the β chemokines (C-C) include MCP-1, MIP-1α, MIP-1β, and RANTES, and the (C) chemokines contain only lymphotactin. ECRF3 has been shown to interact with only members of the α chemokine subgroup (Ahuja & Murphy, 1993), while US28 only interacts with members of the β chemokine subgroup (Gao & Murphy, 1994). The rationale behind the capture of a chemokine receptor that specifically interacts with only α chemokines by Herpesvirus saimiri and β chemokines by human cytomegalovirus is not known, and further studies to unravel this relationship will undoubtedly provide critical information in regards to both virus and chemokine biology. Since both ECRF3 and US28 are able to trigger a calcium flux in response to chemokine binding it seems likely the piracy and expression of these functional receptor genes may somehow enhance virus survival, however the precise function of virus encoded chemokine receptors is currently unknown. In support of this, a number of other viruses contain putative transmembrane serpentine molecules that may also interact with members of the chemokine family. These include two additional open reading frames in human cytomegalovirus (Chee *et al*. 1990), three open reading frames in equine herpesvirus type 2 (Telford *et al*. 1995), two open reading frames in human herpesvirus type 6 (Gompels *et al*. 1995), one open reading frame in KSHV (human herpesvirus type 8) (Cesarman *et al*. 1996) and two open reading frames in members of the poxvirus family, capripox and swinepox (Massung, Jayarama & Moyer, 1993*b*; Cao, Gershon & Black, 1995). The K2R open reading frame of swinepox virus and the Q2/3L open reading frame of capripox virus share significant homology with the US28 gene. Since many poxvirus encoded cytokine receptors act to simply bind and sequester cytokines, it will be particularly important to ascertain whether or not the poxvirus encoded transmembrane chemokine receptors simply act as a decoy or transduce a signal similar to the US28 and ECRF3 proteins. In addition, the role of these proteins in virus pathogenesis needs to be evaluated via gene disruption and *in vivo* analysis.

Virus encoded soluble chemokine-binding proteins

In addition to membrane-bound chemokine receptors, poxviruses also encode soluble chemokine-binding proteins. The first example of a virus encoded soluble chemokine-binding protein was observed when purified M-T7, the myxoma virus IFN-γ receptor homologue, was found to bind promiscuously to various chemokines from the C-C, C-X-C, and C subgroups in an *in vitro* chemical crosslinking assay (Lalani *et al*. 1997). The initial indication that M-T7 may have additional functions was observed when rabbits infected with the M-T7 deletion virus were found to contain elevated levels of reactive leukocytes in secondary lymphoid organs and a marked disruption of immune cell migration

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into infected lesions (Mossman *et al*. 1996). It was postulated that the M-T7 protein may possess additional biological activities since these functions have not been attributed to IFN-γ *in vitro*. Purified M-T7 interacts with chemokines from a broad range of species including rabbit, human and murine. Furthermore, experiments with various IL-8 analogues containing deletions demonstrated that the C-terminal heparin-binding domain of IL-8 was essential for binding to M-T7 (Lalani *et al*. 1997), indicating that M-T7 interacts non-specifically with chemokines via a common heparin-binding domain. Further evidence supports this observation since heparin can compete for the binding of M-T7 to chemokines, but not for IFN-γ (Lalani *et al*. 1997). In this respect M-T7 may function similarly to the Duffy antigen on erythrocytes which also binds promiscuously to members of at least two chemokine subgroups (Horuk *et al*. 1994). Thus it is proposed that M-T7 may function *in vivo* to disrupt chemokine gradients necessary for inflammatory cell migration, in addition to its ability to bind and sequester IFN-γ.

In a systematic approach to identify other secreted myxoma virus proteins that interact with cytokines, it was discovered that myxoma virus encodes a second distinct chemokine-binding protein encoded by the M-T1 open reading frame (Graham *et al*. 1997). Similar chemokine-binding activity was also observed in supernatants from a number of other poxviruses, including Shope fibroma virus, rabbitpox virus, cowpox virus, raccoonpox virus, and vaccinia virus (strain Lister). It seems likely that vaccinia virus (strain Copenhagen) also expresses a functional homologue, but not vaccinia virus strains Wyeth or Tian Tan (Patel *et al*. 1990). Since no detectable chemokine-binding activity could be observed in vaccinia virus (strain WR) and since vaccinia virus strain Lister contains a gene C23L/B19R that is truncated in vaccinia virus strain WR but is homologous to the M-T1 gene in myxoma virus, the M-T1 open reading frame provided a likely candidate for chemokine-binding activity. Supernatants from cells infected with a recombinant vaccinia virus strain WR engineered to express M-T1, exhibited chemokine-binding activity indicating that the secreted M-T1 protein was capable of chemokine binding (Graham *et al*. 1997). In addition, supernatants from cells infected with a recombinant rabbitpox virus containing a deletion in the homologous gene demonstrated no chemokine-binding activity (Graham *et al*. 1997). Purified M-T1 protein interacts with members of both the C-X-C and C-C chemokine families and binds RANTES with high affinity (Kd = 73 nM). Database searches have revealed no similarity between any non-viral genes and the M-T1/35 kDa family, indicating that these are either a unique set of virus proteins or the eukaryotic homologue has not yet been identified.

FUTURE CONSIDERATIONS

One intriguing recent development is the observation that some virus encoded proteins with immune modulation capabilities appear to have multiple functions. This is supported by recent evidence that several poxvirus proteins with previously characterized specificities are able to interact with other components of the immune system. The first documented case of this phenomenon is the presence of a single secreted protein from Tanapox infected cells that is able to bind multiple cytokines, including IL-2, IL-5, and IFN-γ (Essani *et al*. 1994). The second example of a dual functional protein was discovered when the purified myxoma virus IFN-γ homologue, M-T7, was able to interact with various members of the chemokine superfamily in addition to rabbit IFN-γ (Lalani *et al*. 1997). Finally, the observation that the presence of the myxoma virus TNF receptor homologue, M-T2 not only inhibits extracellular TNF but is also necessary for the intracellular inhibition of apoptosis in virus infected $CD4^+$ T cells supports the notion that many virus proteins may exhibit more than one function (Macen *et al*. 1996; Schreiber *et al*. 1997).

The realization that viruses are important for elucidating fundamental mechanisms of the immune system comes at a time when scientists are debating the future destruction of the remaining stocks of variola virus, the causative agent of smallpox (Massung *et al*. 1993*a*). Smallpox virus was systematically eradicated from the human population by a worldwide vaccination programme and the last known case occurred in 1977. In light of the fact that such highly pathogenic viruses, and particularly poxviruses, have so much still to teach us about the human immune system, it is instructive to consider whether the irrevocable loss of variola virus as a potential tool to investigate immunology might be a high price to pay for the presumptive security that its destruction provides.

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