Kaposi's sarcoma-associated herpesvirus immune modulation: an overview

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the most recently discovered human herpesvirus. It is the aetiological agent of Kaposi's sarcoma (KS), a tumour frequently affecting AIDS patients not receiving treatment. KSHV is also a likely cause of two lymphoproliferative diseases: multicentric Castleman's disease and primary effusion lymphoma. The study of KSHV offers exciting challenges for understanding the mechanisms of virus pathogenesis, including those involved in establishing infection and dissemination in the host. To facilitate these processes, approximately one-quarter of KSHV genes encode cellular homologues or unique proteins that have immunomodulatory roles in cytokine production, apoptosis, cell signalling and the immunological synapse. The activities of these molecules are considered in the present review and the positions of their genes are mapped from a complete KSHV genome sequence derived from a KS biopsy. The understanding gained enables the significance of different components of the immune response in protection against KSHV infection to be evaluated. It also helps to unravel the complexities of cellular and immunological pathways and offers the potential for exploiting viral immunomodulators and derivatives in disease therapy.

Introduction

Establishment of virus infection in higher vertebrates necessitates penetration of the host's complex defence barriers against microbial invasion. Innate immunity provides the first line of defence. Antiviral innate immunity involves several effector mechanisms, including type I interferons (IFNs), phagocytes, natural killer (NK) cells and complement activities (reviewed by Goodbourn et al., 2000; Blue et al., 2004; Lodoen & Lanier, 2005). These mechanisms operate while adaptive immune responses, including humoral and cell-mediated immunity, develop. Innate responses also promote development of adaptive immune responses. To establish infection and disseminate in vivo, viruses have to evade these innate and adaptive immune responses.

Viral strategies of immune evasion

Viral strategies for evading the immune response can be considered as passive or active. In this regard, members of the family Herpesviridae, to which Kaposi's sarcoma-associated herpesvirus (KSHV) belongs, can enter into either of two transcriptional programmes: latency or lytic reactivation. Latency is an example of a passive strategy of immune evasion, when a minimal number of gene products are expressed, thus reducing the number of antigens that can be presented to the immune system and invoke a response. Reactivation is the re-entry to productive, lytic replication from latency. During lytic replication, when the bulk of viral proteins are expressed and are susceptible to immune surveillance, as well as upon virus entry to a cell during de novo infection, active evasion strategies are necessary.

Herpesviruses have the genomic capacity to encode numerous genes that actively modulate immune responses. Many of these genes have cellular homologues and are therefore considered as having been captured by the virus, thereafter evolving separately. Table 1 shows a list of KSHV immunomodulatory genes.

KSHV

KSHV is classified formally as Human herpesvirus 8 in the genus Rhadinovirus of the subfamily Gammaherpesvirinae. Shortly after the discovery of KSHV (Chang et al., 1994), two almost-complete genome sequences became available (Russo et al., 1996; Neipel et al., 1997b). Herein, we report a complete genome sequence (GenBank accession no. AF148805), on which the gene layout shown in Fig. 1 is based. The KHSV genome contains 86 genes, of which at least 22 are potentially immunomodulatory (Table 1) and are the subject of the present review.
KSHV disease associations. KSHV is the aetiological agent of Kaposi’s sarcoma (KS). It is also associated with the pathogenesis of primary effusion lymphoma (PEL, a rare B-cell lymphoma) and multicentric Castleman’s disease (MCD) (Schulz, 1998; Cohen et al., 2005).

KSHV immune modulation. Establishment of KSHV latency in vitro occurs with low efficiency and is dependent upon epigenetic factors such as modification of the chromatin structure (Grundhoff & Ganem, 2004). These observations provided a model in which there is a need for continuous rounds of lytic infection in vivo in order to recruit additional latently infected cells, which would otherwise decline as episomes are lost upon cell division. Lytic replication is also necessary for the initial establishment of infection and dissemination in the host. KSHV proangiogenic and proinflammatory gene products (see below) could provide a microenvironment conducive to host-cell proliferation and necessary for viral persistence, secondarily inducing inflammatory responses and pathogenesis. In this context, the evolutionary pressure driving incorporation of immunomodulatory genes into the KSHV genome can be appreciated: lytic replication, which is necessary to maintain infection and replication of the virus, invokes immune responses that, unless negatively modulated, could yield KSHV clearance and lack of persistence. KSHV latency, at least in the context of KS, is therefore only a partially effective strategy of immune evasion and, although arguably sufficient for persistence in B cells, may be defective for KSHV persistence in endothelial cells (Grundhoff & Ganem, 2004). In this regard, the separation of latent and lytic gene-expression profiles may be less demarcated than originally appreciated, as the transcriptional profiles of some genes overlap with both replicative phases. Examples include K9 (Chen et al., 2000; Poharskaya et al., 2004) and K3 (Taylor et al., 2005). Added complexity was revealed by Krishnan et al. (2004), who reported the concurrent expression of further latent and lytic genes in de novo-infected cells. These genes included some [open reading frames (ORFs) K4, K5, K7 and K11.1/K11] that specify immunomodulatory proteins (Table 1). The import of virion-associated RNAs explains the presence of some (Bechtel et al., 2005). The presence of the remainder is apparently due to their de novo transcription through selective or abortive lytic replication, in which an incomplete lytic cascade of gene expression occurs, but is regulated in ways that we do not yet understand.

Taken together, these observations imply the exertion of selective pressure upon KSHV for the evolution of gene products participating in active immune modulation. These proteins are the subjects of the present review.

Table 1. KSHV immunomodulatory proteins

<table>
<thead>
<tr>
<th>KSHV protein</th>
<th>KSHV gene</th>
<th>Cellular homologue</th>
<th>Immunomodulatory function</th>
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<tbody>
<tr>
<td>Modulation of cytokines and cytokine receptors</td>
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<td></td>
<td></td>
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<tr>
<td>ORF45</td>
<td>ORF45</td>
<td>None</td>
<td>Inhibition of IRF-7</td>
</tr>
<tr>
<td>RTA</td>
<td>ORF50</td>
<td>None</td>
<td>Inhibition of IRF-7</td>
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<tr>
<td>vIL-6</td>
<td>K2</td>
<td>IL-6</td>
<td>Cell growth</td>
</tr>
<tr>
<td>vCCL-1, vCCL-2, vCCL-3</td>
<td>K6, K4, K4.1</td>
<td>Macrophage inflammatory proteins</td>
<td>Chemokines</td>
</tr>
<tr>
<td>vGPCR</td>
<td>ORF74</td>
<td>IL-8 receptor</td>
<td>Cell growth</td>
</tr>
<tr>
<td>Kaposin B</td>
<td>K12</td>
<td>None</td>
<td>Increased stability of mRNAs containing AU-rich elements</td>
</tr>
<tr>
<td>Modulation of apoptosis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>vFLIP</td>
<td>K13 (ORF71)</td>
<td>FLIP</td>
<td>Inhibition of apoptosis</td>
</tr>
<tr>
<td>vBcl-2</td>
<td>16</td>
<td>Bcl-2</td>
<td>Inhibition of apoptosis</td>
</tr>
<tr>
<td>vIAP</td>
<td>K7</td>
<td>IAP (survivin)</td>
<td>Inhibition of apoptosis</td>
</tr>
<tr>
<td>Regulation of complement</td>
<td></td>
<td></td>
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<tr>
<td>KCP</td>
<td>ORF4</td>
<td>DAF</td>
<td>Complement regulation</td>
</tr>
<tr>
<td>Deregulation of cell–cell contact</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MIR1, MIR2</td>
<td>K3 (ORF12), K5</td>
<td>None</td>
<td>MHC class I downregulation (E3 ligase)</td>
</tr>
<tr>
<td>K1</td>
<td>K1</td>
<td>ITAM</td>
<td>Downmodulation of B cell-receptor expression</td>
</tr>
<tr>
<td>K15</td>
<td>K15</td>
<td>None</td>
<td>B cell-receptor signalling</td>
</tr>
<tr>
<td>Other immunomodulatory activities</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>vOX2</td>
<td>K14</td>
<td>CD200</td>
<td>Myeloid-cell regulator</td>
</tr>
<tr>
<td>SOX</td>
<td>ORF37</td>
<td>None</td>
<td>Host shutoff</td>
</tr>
</tbody>
</table>
KSHV immunomodulatory proteins

Modulators of cytokines and cytokine receptors

The cytokine network constitutes a communication circuit that links and orchestrates the early innate inflammatory responses and the subsequent developing adaptive immune responses to infections. Cytokines also stimulate the development of haematopoietic cells. The anti-cytokine strategies of viruses inhibit either cytokine production or cytokine activity, for example through repressing signal transduction with cytokine and cytokine-receptor mimics (Alcami, 2003). The KSHV-related diseases, particularly KS, are associated
with deregulation of the inflammatory-cytokine network (Ensoli & Stürzl, 1998; Nicholas, 2003; Milligan et al., 2004), suggesting the capacity of this virus to intervene in normal cytokine responses. The current section focuses on KSHV modulation of cytokine activities.

Viral IFN-regulatory factors (vIRFs). The term interferon (IFN) derives from the ability of these cytokines to interfere with virus infection (Isaacs & Lindenmann, 1957). IFNs are a family of multifunctional cytokines that activate transcription of subsets of genes, the induced products of which are responsible for IFN antiviral, anti-proliferative and immunomodulatory properties. There are two types of IFN, I and II (reviewed by Pestka et al., 2004): type I is composed of several classes, but the principal ones that are induced directly by virus infection of many cell types are IFN-α and IFN-β. Although structurally different, they bind to the same cell-surface receptor and represent the first line of innate immune defence against viruses. Antigen-activated T cells also stimulate mononuclear phagocytes to synthesize type I IFNs. The single type II IFN, IFN-γ, is produced by NK cells, but more abundantly by activated T cells; it is important in cell-mediated immunity against intracellular microbes. Thus, IFN-γ is a major component of the adaptive immune response and is involved in upregulation of class I and II major histocompatibility complex (MHC) and other costimulatory molecules, promotion of naïve CD4+ T-helper cell (Th) differentiation into type I cells (Th1), macrophage activation and B-cell antibody class switching into IgG. The Th1 subset of T cells is fundamental for driving optimal cellular responses against intracellular pathogens and, hence, antiviral immune responses, whilst the CD4+ Th2 subset promotes responses such as humoral immunity against extracellular pathogens; each subset may suppress the development or activity of the other (O’Garra & Arai, 2000; McGurk & Mills, 2002).

A new family of IFNs includes interleukin (IL)-28A, IL-28B (IL-28) and IL-29, also called IFN-λ1–3, respectively. Their expression is induced by viral infection and they have antiviral functions (Kotenko et al., 2003; Sheppard et al., 2003). IFN-λs share homology with IFN-α and -β, but signal through their own receptor to IFN-stimulated response element (ISRE)-containing promoters, through formation of the IFN-stimulated gene factor-3 (ISGF-3) transcription complex (reviewed by Vilcek, 2003).

The type I IFNs induce the innate immune response to viral infection, known as the ‘antiviral state’ in both infected cells (through autocrine activities) and uninfected cells (through paracrine activities). The main actions of type I IFNs are inhibition of virus replication, increased expression of class I MHC molecules, development of Th1 cells in humans, inhibition of cell proliferation and promotion of apoptosis (reviewed by Goodbourn et al., 2000). Activation of type I IFN can be considered to result in a defence mechanism that bridges innate and T cell-mediated adaptive immunity.

The regulation of the type I IFN-responsive genes, and the type I IFN genes themselves, involves the IFN-regulatory factor (IRF) family of transcription factors that, in humans, contains at least nine members (reviewed by Nguyen et al., 1997; Harada et al., 1998; Pitha et al., 1998; Tanaka & Taniguchi, 2000), the most important of which are arguably IRF-3 and IRF-7 (Taniguchi & Takeoka, 2002). IRFs bind to cognate DNA sequences that include the ISRE present in the promoters of type I IFN-responsive genes and also to positive-regulatory domains (PRD) I and II of the IFN-β promoter (King & Goodbourn, 1994; Decker et al., 1997; Stark et al., 1998). Fig. 2 presents an outline of the type I IFN signalling pathways.

IFN-γ-dependent gene regulation is also understood in detail, usually involving the phosphorylation and concomitant homodimerization of STAT-1, forming the IFN-γ activation factor (GAF), which translocates to the nucleus and transactivates promoters containing IFN-γ activation sites (GAS) (Stark et al., 1998; Young et al., 2000).

KSHV encodes four vIRF genes with homology to cellular IRFs (Russo et al., 1996; Neipel et al., 1997a). These genes have probably evolved to subvert cellular IRF signalling, but other activities cannot be excluded and may therefore explain why the virus carries so many of these genes. It is also possible that certain of the vIRF genes are expressed preferentially in different cell types or during different stages of the virus life cycle (Dittmer, 2003). The vIRF genes are not unique to KSHV, as rhesus rhadinovirus encodes nine, none of which appears to be spliced (Searles et al., 1999; Alexander et al., 2000). KSHV vIRF1, vIRF2 and vIRF3 have been cloned and characterized functionally, whilst vIRF4 (K10/K10.1) has been detected by gene array (Jenner et al., 2001), Northern blot and RT-PCR analyses (Cunningham et al., 2003), but the protein remains to be characterized. A description of our current understanding of the three characterized vIRF proteins follows.

vIRF-1. K9, specifying the vIRF-1 protein, was the first viral member of the IRF family to be described (Moore et al., 1996). K9 is an unspliced, lytic-cycle gene expressed at a low level in PEL cell lines in those cells in which KSHV reactivation is occurring spontaneously; expression can be enhanced by treatment with TPA (Moore et al., 1996; Sarid et al., 1998; Jenner et al., 2001; Paulose-Murphy et al., 2001; Cunningham et al., 2003). K9 transcripts have been detected in KS biopsies by RT-PCR (Dittmer, 2003), but not by Northern blot analysis (Gao et al., 1997), and analyses of KSHV-associated tumour material revealed that the vIRF-1 protein was only detectable in MCD (Parravicini et al., 2000).

vIRF-1 negatively regulates IFN signalling in the cell. Thus, in reporter-gene studies, vIRF-1 inhibited IFN signalling from type I and type II IFN-responsive reporter genes, although not by a mechanism that involves DNA binding (Gao et al., 1997; Flowers et al., 1998; Zimring et al., 1998). Cellular IRFs contain a conserved N-terminal DNA-binding
Fig. 2. An overview of the type I IFN signalling pathways. The mechanisms behind the regulation of IFN-pathway genes are understood with increasing clarity, at least in response to RNA virus infection. A cellular mechanism senses virus infection and triggers the IFN type I pathway to respond (reviewed by Sen & Sarkar, 2005b), as shown at the left side of this illustration. The induction and assembly of the IFN-β enhanceosome are shown, as it is the prototype complex for understanding IFN-responsive gene activation. It consists of IRF-3, NF-κB and ATF-2/c-Jun. These proteins are expressed constitutively. They are activated in response to infection by post-translational modification and assemble on the promoter at their cis-acting cognate recognition sequences, originally identified as positive-regulatory domains (PRDs) I–IV (Goodbourn et al., 2000). Here they act coordinately, but they can also function separately to induce antiviral gene expression. HMG-I(y), CBP/p300 and RNA polymerase II are then recruited to the IFN-β enhanceosome as the pre-initiation complex evolves. C-terminal phosphorylation of IRF-3 is a pivotal step between cellular sensing of virus infection and the response to that infection. It occurs by a 'virus-activated kinase' (VAK) (Servant et al., 2001; Smith et al., 2001) that promotes translocation of IRF-3 from the cytoplasm to the nucleus. The components of VAK that phosphorylate IRF-3 include the IκB kinase homologues IκB kinase-epsilon (IKKε) and TANK-binding kinase-1 (TBK1) (Fitzgerald et al., 2003; Sharma et al., 2003). The pathway leading to activation of IRF-3 depends on the route of virus entry, which determines whether the viral RNA is 'sensed' either by Toll-like receptor (TLR) 3 or one of the RNA helicases, RIG-I (Yoneyama et al., 2004) or mda5 (Andrejeva et al., 2004). If the recognition event occurs through TLR3, this protein interacts with TRIF via the Toll/IL-1 receptor (TIR) domains in each protein. If recognition occurs through RIG-I or mda5, these proteins interact with IPS1 via their respective caspase-recruitment domains (CARD) (reviewed by Crozat & Beutler, 2004; Levy & Marie, 2004; Sen & Sarkar, 2005b). The choice of pathways by IPS1 is cell type-specific. IRF-3 can also form the double-stranded RNA (dsRNA)-activated factor 1 (DRAF-1) in association with p300 (Weaver et al., 1998) in cells activated through treatment with dsRNA, which positively regulates IFN-stimulated genes (not shown). Whether KSHV induces similar pathways, for example through the production of dsRNA molecules during gene expression, is unknown and the role of TLR3 in sensing virus infection is controversial (Sen & Sarkar, 2005a). However, KSHV targeting of IRF-3 activity, for example by vIRF-2, implicates the involvement in KSHV entry or replication of at least some of the illustrated components of the 'cellular-sensing' mechanism of virus infection. The mechanism could involve TLRs other than TLR3. TLR4 is expressed on the cell surface and uses TRIF and the TRIF-related adaptor molecule (TRAM) to activate IRF3. Other possibilities include TLR2 and TLR9, known to recognize other herpesviruses (e.g. envelope glycoproteins and CpG DNA motifs, respectively) and signal through MyD88, although IRF-3 may still be activated (reviewed by Boehme & Compton, 2004). Once the cell has responded to the infection through the production of type I IFNs, they are secreted and can act in autocrine and paracrine ways to initiate the remainder of the IFN response (right side of illustration). This initiation occurs through the production of IFN-stimulated gene factor (ISGF)-3 and concomitant expression of genes that have IFN-stimulated response element (ISRE)-containing promoters. The products of these genes establish the antiviral state in the infected cell and uninfected bystander cells (reviewed by Stark et al., 1998). This process involves the recruitment and phosphorylation of signal transducer and activator of transcription (STAT)-1 and -2 by IFN receptor-associated tyrosine kinases upon IFN binding. ISGF-3 is formed by the heterodimerization of phosphorylated STAT-1 and -2, followed by their recruitment of p48 (IRF-9). As the ISRE is recognized by IRF-3 and ISGF-3, the genes activated with early kinetics in response to virus infection and those induced by the type I IFNs overlap. IRF-7, inactivated by KSHV ORF45, RTA and possibly LANA-2 (vIRF-3) (see text), is not shown in the figure. This figure was compiled from the reviews by Goodbourn et al. (2000) and Sen & Sarkar (2005a, b).
domain (Escalante et al., 1998) (as well as a C-terminal regulatory region) that is partially conserved in vIRF-1, but apparently does not serve this function. vIRF-1 inhibits IFN induction of responsive genes by suppressing the transcriptional activity of IRF-1 and IRF-3, interacting with them directly or competing for their binding to the transcriptional coactivator p300 (Buryšek et al., 1999b; Lin et al., 2001). The vIRF-1 protein may also inhibit the histone acetyltransferase activity of p300, restricting chromatin remodelling and therefore transcriptional activity of cellular genes, including those encoding cytokines (Li et al., 2000). Nevertheless, it is debatable whether the kinetics of K9 expression in KSHV-infected cells are consistent with an effective anti-IFN response (Pozharskaya et al., 2004). The multifunctional nature of vIRF-1 can be appreciated from other studies indicating that, in addition to its role in inhibiting transcription, this protein can act as a transcriptional activator (Roan et al., 1999).

Furthermore, vIRF-1 has transforming activity; it reduced the inducible cyclin-dependent kinase inhibitor (CDKI) p21WAF1/CIP1 and transformed NIH3T3 cells to become tumorigenic in nude mice (Gao et al., 1997). Further evidence in support of an oncogenic role is the fact that vIRF-1 suppresses the transcription and pro-apoptotic activities of p53 (Nakamura et al., 2001; Seo et al., 2001); it also facilitates degradation of p53 by the proteasome (Shin et al., 2006). The involvement of vIRF-1 in immune escape, as well as modulating the cell cycle, is supported by its inhibition of Fas ligand (CD95L) expression and concomitant T-cell antigen receptor (TCR)/CD3-mediated activation-induced cell death (Kirchhoff et al., 2002). Moreover, by binding to and suppressing the activity of the smad3 and smad4 transcriptional components of the TGF-β signalling pathway, vIRF-1 suppresses this ‘anti-tumour’ cellular-defence strategy (Seo et al., 2005).

vIRF-2. The first functional studies of this protein were performed with a 163-residue protein encoded by K11.1, the first exon of vIRF2 (Buryšek et al., 1999a). This protein bound to a consensus NF-κB-binding site, but not to the ISRE, and suppressed IRF-1- and IRF-3-driven activation of an IFN-α reporter promoter in cells infected with Newcastle disease virus. In pull-down assays, this fragment of the vIRF-2 protein also interacted with cellular IRF-1 and weakly with p300/CREB-binding protein (CBP), p65, IRF-2 and IFN consensus sequence-binding protein (ICSBP)/IRF-8; it did not bind IRF-3. This group went on to show that K11.1 is a 20 kDa protein that exerts its anti-IFN effect in part by binding to, and suppressing, double-stranded RNA-activated protein kinase R (PKR) (Buryšek & Pitha, 2001). Other workers showed that, like vIRF-1, K11.1 inhibited apoptosis by transcriptional repression of CD95L (Kirchhoff et al., 2002).

vIRF2 is now known to encode an inducible, 2-2 kbp, spliced transcript representing the two exons K11.1 and K11 (Jenner et al., 2001; Cunningham et al., 2003) from which full-length vIRF-2 protein is translated. Others have also found vIRF-2 to be inducible (Sarid et al., 1998; Paulose-Murphy et al., 2001; Fakhari & Dittmer, 2002). In contrast, some suggested that expression of this gene is constitutive (Buryšek et al., 1999a; Buryšek & Pitha, 2001). Our functional studies indicate that the full-length vIRF-2 protein inhibits both IRF-3- and type I IFN-driven signalling pathways, as well as signalling induced by IFN-α family members (Fuld et al., 2006). These functions are consistent with the expression of vIRF2 being detectable as early as 2 h (the earliest time point studied) following experimental infection of cells (Krishnan et al., 2004) and with vIRF-2 mitigating the innate IFN response during KSHV de novo cell infection. IRF-3 deregulation by viruses is not unprecedented; other examples include, but are not limited to, Hepatitis C virus (Foy et al., 2003) and Bunyamwera virus (Weber et al., 2002).

vIRF-3. This protein is encoded by a transcript spliced from K10.5 and K10.6 (Lubyova & Pitha, 2000; Jenner et al., 2001; Rivas et al., 2001; Cunningham et al., 2003). As with vIRF2, there is controversy in the literature as to whether vIRF3 is inducible in PEL cell lines: two groups claim that it is (Lubyova & Pitha, 2000; Jenner et al., 2001) and three that it is not (Rivas et al., 2001; Fakhari & Dittmer, 2002; Cunningham et al., 2003). However, only two published studies have documented the function of the vIRF-3 protein. It was observed initially that vIRF-3 decreased the transcription of the type I IFN genes by targeting IRF-3 and IRF-7 (Lubyova & Pitha, 2000). Subsequently, this group found that vIRF-3 transactivated genes under the transcriptional control of IRF-3 and IRF-7 (Lubyova et al., 2004), thus contradicting the concept that the vIRF proteins inhibit the IFN response.

The vIRF-3 protein has also been named latency-associated nuclear antigen 2 (LANA-2), consistent with its expression kinetics and cellular location and in order to distinguish it from the ORF73-encoded LANA (Rivas et al., 2001). These authors found LANA-2 in the nuclei of B cells of PEL and MCD; it was not expressed in KS and they showed that it inhibited p53-induced transcription and apoptosis.

ORF45 and RTA. The ORF45 virion-associated (tegument) protein blocks phosphorylation, nuclear translocation and therefore the function of IRF-7 (Zhu et al., 2002; Zhu & Yuan, 2003). ORF45 inhibits the activation of type I IFNs and their genes during viral infection, as IRF-7 is a fundamental component orchestrating the IFN response (Honda et al., 2005). Its introduction into the cell as part of the virion ensures that ORF45 is available at the very earliest stages of infection, when deregulating IRF-7 activity is key. It may complement the activities of the vIRF proteins, which are expressed during virus replication. In addition, as cellular IRFs are multifunctional, also being involved in cell-cycle regulation, apoptosis and tumour suppression or promotion (Taniguchi & Takaoka, 2002), the role of ORF45 in regulating IRF-7 activity may not be limited to negatively regulating type I IFNs. The apparent
importance to KSHV of the ability to modulate IRF-7 activity is evident from the observation that the RTA protein also negatively regulates IRF-7, by targeting it for proteasome-mediated degradation (Yu et al., 2005).

vIL-6 (K2). Human IL-6 is a multifunctional cytokine with effects on a wide variety of cell types, particularly those involved in both innate and adaptive immunity. Overexpression of IL-6 has been implicated in the pathology of a number of proliferative diseases, including multiple myeloma, Castleman’s disease and some autoimmune diseases, such as rheumatoid arthritis, psoriasis, post-menopausal osteoporosis and colitis. In response to microbes and to other cytokines, particularly IL-1 and tumour necrosis factor (TNF), IL-6 is synthesized by mononuclear phagocytes, vascular endothelial cells, fibroblasts and other cells. The receptor for IL-6 consists of a cytokine-binding protein, IL-6Rz (gp80), and a signal-transducing subunit, gp130. Receptor engagement by any member of the IL-6 family results in homodimerization of gp130 or heterodimerization of gp130 and gp80. The ensuing JAK/STAT signalling pathway (reviewed by Hodge et al., 2005) in turn induces the synthesis of acute-phase proteins, contributing to the systemic effects of inflammatory reactions during innate immunity. In adaptive immunity, IL-6 may be involved in the development of some autoimmune diseases; also, it stimulates the growth of B lymphocytes that have differentiated into antibody producers. In addition, human IL-6 has anti-apoptotic activity in some cells, mediated by upregulation of apoptotic antagonists such as BCL-XL (Schwarze & Hawley, 1995; reviewed by Hodge et al., 2005).

The role of IL-6 in KS and MCD had been suspected prior to the discovery of KSHV. Thus, IL-6 enhanced the proliferation of KS cells in culture and elevated levels of IL-6 correlated with disease development in MCD (Miles et al., 1990; Burger et al., 1994). Hence, the discovery of a KSHV homologue (vIL-6) of this cytokine, encoded by K2 and sharing 25% identity with human IL-6 (Moore et al., 1996; Neipel et al., 1997b; Nicholas et al., 1997b; Burger et al., 1998), implicated the viral cytokine in KSHV pathogenesis. However, the kinetics of expression are complex: although KSHV vIL-6 is expressed constitutively in PEL cell lines in vitro and is minimally inducible (Parravicini et al., 2000), in biopsy material, the expression profile is restricted to haematopoietic cells (Moore et al., 1996; Staskus et al., 1999). Thus, vIL-6 is rarely detectable in KS, expressed in <5% of cells in PEL tumours and present in up to 25% of LANA-1-positive cells from MCD (Cannon et al., 1999; Staskus et al., 1999; Parravicini et al., 2000). In other studies, vIL-6 acted as an angiogenic factor through the induction of vascular endothelial growth factor (VEGF) (Aoki et al., 1999; Hideshima et al., 2000; Mullberg et al., 2000) and was abundant in the effusions of PEL (Aoki et al., 2000). Many myeloma cells secrete IL-6 as an autocrine growth factor, which led to the erroneous aetiological link between KSHV and multiple myeloma. The first in vivo evidence for immune modulation per se by vIL-6 was provided by a model of peritoneal inflammation that mimics bacterial peritonitis, in which administration of vIL-6 inhibited chemokine-driven recruitment of neutrophils (Fielding et al., 2005).

Unlike human IL-6, vIL-6 binds directly to the shared human cytokine-signalling receptor gp130, independently of gp80 (Molden et al., 1997; Chatterjee et al., 2002). However, vIL-6 can also form a hexameric vIL-6-gp80-gp130 complex with enhanced signalling potency (Boulanger et al., 2004), activating the STAT and MAPK signalling pathways (Osborne et al., 1999).

Thus, in terms of pathogenesis, the lack of gp80 requirement for signalling suggests that vIL-6 has broader cell specificity than its human equivalent, potentially substituting for or synergizing with IL-6 and contributing to KSHV-associated MCD and perhaps PEL by promoting cell survival, driving proliferation and preventing apoptosis (Nicholas et al., 1997b). At least for MCD, this hypothesis is consistent with the established relationship between elevated IL-6 and MCD (reviewed by Waterston & Bower, 2004). However, in the absence of abundant vIL-6 expression in KS lesions, it seems unlikely that it contributes significantly to the pathogenesis of this tumour.

Viral macrophage inflammatory proteins (vMIPs, vCCLs). The chemokines form a large family of cytokines that coordinate leukocyte movement and regulate the recruitment of leukocytes to the site of inflammation (reviewed by Rot & von Andrian, 2004). At least 43 human chemokines have been identified, excluding isoforms. They mediate their biological effects by binding to and signalling through a family of G protein-coupled receptors, which numbers 19 so far. The response common to most cells carrying receptors following their ligation by the majority of chemokines is that of chemotaxis.

KSHV ORFs K6, K4 and K4.1 encode three chemokine homologues (Russo et al., 1996; reviewed by Nicholas, 2005), formerly referred to as viral macrophage inflammatory proteins (vMIP-I, vMIP-II and vMIP-III) and now known as vCCL-1, vCCL-2 and vCCL-3, respectively. They are lytic-cycle genes (Nicholas et al., 1997a; Jenner et al., 2001; Paulose-Murphy et al., 2001). KSHV is not the only virus to have evolved mechanisms for interacting with the chemokine network; other examples include the use of chemokine receptors as coreceptors for cell infection by human immunodeficiency virus (HIV) (Fauci, 1996), the production of soluble decoy proteins that sequester chemokines by certain poxviruses (Graham et al., 1997) and murine gammaherpesvirus-68 (MHV-68) (Parry et al., 2000), and the expression of a chemokine homologue by human cytomegalovirus (Penfold et al., 1999; reviewed by Lalani et al., 2000; Murphy, 2001; Nicholas, 2005). The KSHV chemokines share up to 41% identity at the amino acid sequence level with the CC chemokine macrophage inflammatory protein (MIP)-1z, whilst vCCL-1 and vCCL-2 are related...
more closely to each other, sharing 48% amino acid identity, than they are to vCCL-3 (Moore et al., 1996; Boshoff et al., 1997). Thus, the cellular homologues from which these viral genes arose probably have yet to be identified (McGeoch, 2001).

Cells of the Th1 and Th2 subsets express different chemokine receptors, hence the predominant chemokine profile can polarize the Th response and consequently the effector T-lymphocyte response. The CC chemokine receptors CCR4 and CCR8, and perhaps CCR3, are thought to be involved in the chemotaxis and polarization of Th2 lymphocytes (reviewed by Cosmi et al., 2001) and the KSHV vCCLs are preferential agonists for these receptors (reviewed by Nicholas, 2005). vCCL-2, but not vCCL-1, activated and triggered the chemotaxis of eosinophils and Th2-like T cells by engaging CCR3 (Boshoff et al., 1997; Weber et al., 2001). Moreover, vCCL-2 functioned as an antagonist of CCR1 and CCR5, inhibiting the recruitment of Th1-like lymphocytes. Consistent with these data, the authors observed a predominance of Th2-type, CCR3+ cells in KS lesions (Weber et al., 2001). Likewise, vCCL-1 is an agonist for CCR8, although vCCL-2 is a dominant antagonist (Sozzani et al., 1998; Dairaghi et al., 1999), and vCCL-3 is an agonist of CCR4 (Stine et al., 2000). Overall, these results indicate that, as Th2-cell chemokine-receptor agonists, the KSHV vCCLs can polarize the adaptive immune response toward a predominantly Th2-type (i.e. humoral) response at sites of KSHV infection, potentially reducing the efficacy of the antiviral response.

Aside from their apparent immunomodulatory roles, the vCCLs have at least two other activities. First, vCCL-2 inhibited infection of CD4+ cells by dual-tropic, syncytium-inducing HIV strains via CCR5 and CXCR4 and more strongly via CCR3 (Boshoff et al., 1997; Kledal et al., 1997). Second, all three vCCLs are angiogenic in the chick chorioallantoic-membrane assay (Boshoff et al., 1997; Stine et al., 2000). Thus, they may contribute to the development of KSHV-associated diseases (Boshoff et al., 1997; Stine et al., 2000; Liu et al., 2001), cooperating with other growth factors such as the potently angiogenic VEGF that is abundant in KS, where it may function as an autocrine and paracrine growth factor (Cornali et al., 1996; Masood et al., 1997; Nakamura et al., 1997), not least because expression of the gene encoding its receptor is abundant in KS lesions (Brown et al., 1996).

Viral G protein-coupled receptor (vGPCR). Another homologue of the chemokine system encoded by KSHV is the vGPCR encoded by ORF74. This protein shares similarity with members of the CXC chemokine-receptor family, especially the IL-8 (or CXCL8) receptor, and, like them, is a seven transmembrane-spanning protein (Cesarman et al., 1996; Russo et al., 1996). This viral-receptor homologue is constitutively active, signalling independently of agonist (Arvanitakis et al., 1997), but signalling can be increased in a chemokine-dependent manner (Gershengorn et al., 1998). Interestingly, vGPCR binds both CC and CXC chemokines (Arvanitakis et al., 1997). In HEK 293 epithelial cells, vGPCR activated two of the three major mitogen-activated protein kinase pathways: p38 MAPK and JNK/SAP (not ERK), which are activated by angiogenic and inflammatory cytokines (Bais et al., 1998). vGPCR immortalized endothelial cells through constitutive VEGF receptor-2 expression and activation of the downstream phosphatidylinositol 3-kinase (PI3K)/AKT anti-apoptotic pathway by a VEGF-mediated autocrine loop (Bais et al., 2003). It also induced VEGF-associated KS-like lesions in transgenic animals (Yang et al., 2000).

Taken together, these data suggest that vGPCR is a major determinant of KSHV pathogenesis, having broad signalling activity that transactivates pro-inflammatory and proangiogenic cytokine and growth-factor gene expression (Pati et al., 2001; Schwarz & Murphy, 2001). In part, this activity is mediated through NF-xB, activator protein-1 (AP-1) and nuclear factor of activated T cells (NFAT) via the small G protein Rac1 (Montaner et al., 2004). Clearly, vGPCR orchestrates complex and cell-type-dependent signalling pathways, as demonstrated by work in PEL cells, where it activated AP-1, NFAT and CREB in part through the ERK-1/2 MAPK pathway (Cannon & Cesarman, 2004).

However, vGPCR is an early lytic-cycle protein, whereas KSHV is predominantly latent in KS and lytic replication leads to host shutoff (see below). Therefore, the role of vGPCR in viral pathogenesis, particularly in KS, is controversial (Cesarman et al., 2000). A possible explanation for this paradox is that the putative low level of continuous lytic replication provides sufficient vGPCR to effect pathogenesis (Grundhoff & Ganem, 2004). Furthermore, vGPCR may negatively regulate the latent to lytic switch in KSHV replication, whilst still exerting paracrine effects on infected and uninfected cells (Cannon et al., 2006).

Nevertheless, KSHV has evolved to use the IL-8R signalling cascade in an agonist-independent manner, as agonist (IL-8) is one of the major mediators of the inflammatory response. Whilst the intracellular-signalling consequences of IL-8 may benefit survival of KSHV-infected cells, as determined by studies of vGPCR, abundant levels of this pro-inflammatory chemokine may be disadvantageous to KSHV, perhaps explaining why IL-8 production is suppressed by another KSHV immune modulator, vOX2 (Rezaee et al., 2005) (see below).
different, upstream, GC-rich, direct-repeat sequences termed DR1 and DR2. The number of DR1 and DR2 repeats varies between different KSHV isolates, resulting in variable sizes of the K12 transcript (Sadler et al., 1999). Complex translational regulation of the K12 transcript, including initiation from non-AUG (i.e. CUG) codons, yields a minimum of three protein species, of which kaposin B is the most abundant, at least in the PEL cell line BCBL-1, and encoded by the sequences upstream of K12, but not by K12 itself. Kaposin C is a chimaera of DR1, DR2 and K12, whilst kaposin A is the predicted product of K12, which would need to be initiated from an AUG codon in the absence of an apparent internal ribosome entry site (Sadler et al., 1999).

Kaposin A might be involved in cell transformation (Muralidhar et al., 1998; Tomkowicz et al., 2005), perhaps by activating the ERK-1/2 MAPK signalling pathway (Kliche et al., 2001). However, kaposin B has a distinct immunomodulatory function: increasing the expression of certain cytokines by stabilizing their mRNAs (McCormick & Ganem, 2005). Kaposin B binds to and activates the kinase MK2 in the nucleus through its reiterated DR2 repeats. MK2 kinase is a target of the p38 MAPK pathway and, when activated, inhibits the decay of AU-rich elements (AREs) in 3'-untranslated regions of mRNAs. Inflammatory signals (that activate the p38 MAPK pathway) induced nuclear export of both proteins (MK2 and kaposin B) together, resulting in increased levels of two proteins encoded by ARE-containing mRNAs: IL-6 and granulocyte–macrophage colony-stimulating factor. The molecular mechanisms behind this increase have yet to be elucidated in further detail, but could involve either the selective inhibition of the degradation of ARE-containing transcripts or the selective stimulation of their translation; p38 MAPK may also be recruited to the kaposin B–MK2 complex. The effect was extended to the stabilization of a model ARE-containing transcript in KSHV-infected cells (McCormick & Ganem, 2005). Also of interest is the recent identification of micro-RNAs encoded within this region of the KSHV genome, including the kaposin locus (Cai et al., 2005; Pfeffer et al., 2005; Samols et al., 2005), which may be involved in the regulation of viral and cellular gene expression, perhaps including genes of the immune system.

Modulation of apoptosis as an effector component of the immune system

Apoptosis, an overview of which is provided in Fig. 3, is an important component of immune-system homeostasis, maintaining immunological unresponsiveness and providing a regulatory mechanism for innate and adaptive immune responses as apoptotic cells are internalized by phagocytes (Albert, 2004). It can be triggered by a variety of inducers. Ligands of the cell-surface death receptors cause them to form the death-inducing signalling complex (DISC) and activate the extrinsic apoptotic pathway. These receptors include the tumour necrosis factor receptor (TNFR) superfamily, one member of which is Fas (CD95). Alternatively, the intrinsic mitochondrial programme of cell death is activated by signals such as peroxides produced by macrophages or neutrophils, cell-cycle inhibitors, DNA damage, viruses and oncogenes.

In the context of virus infection, apoptosis undoubtedly results in the destruction of the viral genome and serves as an innate defence mechanism to limit virus replication and spread. Not surprisingly, many viruses have evolved multiple mechanisms for controlling host-cell apoptosis (reviewed by Benedict et al., 2002). Inevitably, these mechanisms contribute to cell transformation by oncogenic viruses. Indeed, some KSHV gene products, including LANA-1, LANA-2, vFLIP, vIL-6 and vCyclin, are immunomodulatory, as they influence apoptosis (or proliferation) either directly or indirectly. By this very function, they also promote cell transformation (Moore & Chang, 2003). Three KSHV proteins, vFLIP, vBcl-2 and vIAP, act directly on the apoptotic pathways.

vFLIP (K13/ORF71). ORF71, which is also called K13, encodes the KSHV FLIP protein (Thome et al., 1997). The vFLIP gene is expressed in KS and PEL cells from a polycistronic mRNA encompassing the latency locus (Talbot et al., 1999). As such, vFLIP may be an important component of KSHV pathogenesis. Indeed, this protein can inhibit the extrinsic apoptosis pathway by preventing the activation of caspases, including caspase 8 (Djerbi et al., 1999).

The potential role of vFLIP in KSHV pathogenesis is also reflected in its ability to drive cell transformation in vitro (Sun et al., 2003) and in vivo, as mice transgenic for the gene had a 6.5-fold increased incidence of lymphoma compared with their non-transgenic littermates (Chugh et al., 2005). In ectopic-expression studies, the mechanism appeared to involve constitutive NF-κB activation (Chaudhary et al., 1999). PEL cells (both primary and from established lines), naturally infected with KSHV, also have constitutive NF-κB activity that appears to be essential for cell proliferation (perhaps via IL-6 production) and apoptosis inhibition (Keller et al., 2000). Although other KSHV proteins, including K1, vGPCR and K15, can activate NF-κB, it is vFLIP that drives the constitutive NF-κB activation in PEL cells and is responsible for their survival in vitro and in vivo (Guasparri et al., 2004; Godfrey et al., 2005). vFLIP activates NF-κB through the IκB kinase (IKK) complex. It does so via members of the TNF receptor-associated factor (TRAF) family, which are adaptor molecules involved in transducing the signal from TNF receptors to the IKK complex that activates NF-κB. Specifically, TRAF2 and TRAF3 are required for vFLIP signalling, but only TRAF2 recruits vFLIP to the IKK complex (Guasparri et al., 2006). Thus, vFLIP is implicated in the pathogenesis of PEL and perhaps KS, and can be considered a tumour growth factor with dual roles of apoptosis prevention and tumour progression.

vBcl-2 (ORF16). The KSHV vBcl-2 protein is encoded by ORF16 (Russo et al., 1996). It is expressed in late-stage KS lesions, but in PEL cells, only the transcript is detectable
Fig. 3. Apoptosis pathways. The extrinsic pathway of apoptosis (left side of illustration) is triggered by the binding of ligands to death-inducing membrane proteins. The two most well-described death domain-containing receptors are CD95 (Fas) and the type I tumour necrosis factor receptor (TNFR). The extrinsic pathway is initiated by the binding and trimerization of death receptors containing intracellular death domains, to form the death-inducing signalling complex (DISC). This event leads to the recruitment of Fas-associated death receptor (FADD). FADD in turn binds procaspase 8 and activates it, generating the ‘initiator’ caspase 8. Consequently, caspase 8 activates ‘effector’ caspases (caspases 3, 6 and 7). They then cleave proteins responsible for maintaining cellular and genome integrity, as well as repressors of pro-death enzymes, such as inhibitor of caspase-activated DNase (iCAD), thereby releasing CAD that fragments DNA. The result is cell death and phagocytic engulfment. The type I TNFR activates a similar mechanism of cell death. However, this receptor does not bind FADD directly, but rather via the TNFR-associated death domain (TRADD), which in turn recruits FADD to the receptor complex. The extrinsic pathway can be regulated by the intracellular FLICE inhibitory protein (FLIP), which binds to FADD and competes with DISC for caspase 8. FLICE is the acronym for FADD-like interleukin-1β-converting enzyme, the name by which caspase 8 is also known. KSHV vFLIP operates similarly. Cellular FLIP was identified through the recognition and study of viral FLIP proteins (Irmler et al., 1997). During conditions of cellular stress, such as DNA damage and growth-factor deprivation, the intrinsic apoptosis pathway (the pathway that is activated most frequently by the tumour-suppressor protein p53) is activated (right side of illustration). The intrinsic apoptotic pathway converges on the disruption of mitochondrial membranes. Sequestered within the intermembrane space of these organelles are pro-apoptotic proteins that, once released, signal the cell-death programme. The prototypical example is cytochrome c. When cytochrome c binds to apoptotic protease-activating factor 1 (APAF-1), pro-caspase 9 is recruited via caspase-recruitment domains (CARD), forming the apoptosome. In this complex, the initiator caspase 9 is auto-activated and, in turn, activates effector caspases, such as caspase 3, releasing CAD. The balance of the pro- and anti-apoptotic bcl-2 family proteins determines whether or not apoptosis proceeds. They are classified based on structural determinants: bcl-2 homology (BH) domains. These proteins include the pro-apoptotic BAX and BAK proteins, which, when activated, oligomerize and increase the permeability of the mitochondrial outer membrane. Anti-apoptotic members of the bcl-2 family, including bcl-2 itself and BCL-XL, regulate these proteins and therefore the intrinsic pathway. Granzyme B, one of the components of cytotoxic granules produced by CD8+ cytotoxic T lymphocytes and NK cells, can trigger apoptosis by activating effector caspase 3. However, it can also operate in a caspase-independent manner, for example by activating CAD directly. There is overlap between the intrinsic and extrinsic apoptotic pathways, as caspase 8 can cleave and activate the bcl-2 family protein BID, forming the pro-apoptotic truncated BID (tBID). tBID translocates to the mitochondria where it causes oligomerization of BAX and BAK. BID belongs to the 'BH3-only' subclass of bcl-2 proteins. Other BH3-only pro-apoptotic proteins include NOXA and PUMA. They are under the transcriptional control of p53 and invoke apoptosis through the intrinsic pathway in response to DNA damage. Regulation of both the intrinsic and extrinsic pathways can also occur through the activities of members of the caspase-inhibitor family, ‘inhibitors of apoptosis’ (IAPs), that can regulate both initiator and effector caspases, binding and inhibiting active caspases 3, 7 and 9. One of the most studied of the mammalian members of the IAP family is survivin (Altieri, 2003), not least because of its potential as a therapeutic target for certain malignancies (Schimmer & Dalili, 2005). The mechanism of KSHV vIAP inhibition of apoptosis is unclear, but it localizes to the mitochondria and ER. The mechanism behind the HAX-1 anti-apoptotic function is also still to be determined fully, but it is cleaved within the mitochondria in response to pro-apoptotic stimuli (Cilenti et al., 2004). K15 may augment this anti-apoptotic activity. This outline was compiled from recent reviews (Lieberman, 2003; Danial & Korsmeyer, 2004; Lowe et al., 2004).
and not the protein (Widmer et al., 2002). vBcl-2 shares 15–20% amino acid identity with human cellular homologues and inhibits apoptosis induced by virus infection and the pro-death protein BAX (Cheng et al., 1997; Sarid et al., 1997). Cellular members of the bcl-2 family often either homodimerize or heterodimerize with other family members, but there are mixed reports as to the capability of vBcl-2 in this regard (Cheng et al., 1997; Sarid et al., 1997; Huang et al., 2002). One possibility is that vBcl-2 does not heterodimerize with other bcl-2 family members, obviating negative regulation by such proteins (Cheng et al., 1997).

Therefore, as a lytic protein, vBcl-2 presumably inhibits apoptosis induced as a consequence of KSHV infection, ensuring that the cell survives long enough for the virus to assemble progeny. It may also counter any pro-apoptotic consequences of other KSHV proteins, such as the ORF72-encoded cyclin D homologue vCyclin (Ojala et al., 2000).

vIAP (K7). The product of KSHV K7 was identified as a survivin, or inhibitor of apoptosis (IAP), homologue by in silico analyses, followed by ectopic-expression studies that confirmed the apoptosis-inhibiting activity. For these reasons, it was named vIAP. Northern blot and RT-PCR studies of PEL cells revealed K7 to be a lytic gene. The protein localizes to the mitochondria and the ER, although the anti-apoptotic mechanism is unclear; it may operate as a molecular adaptor, recruiting activated caspases to anti-apoptotic regulatory proteins, like Bcl-2 (Wang et al., 2002).

p53 suppression. The p53 protein is one activator of the intrinsic apoptotic pathway and, not surprisingly, KSHV encodes several proteins that deregulate it. One of the most highly documented is LANA-1, which, in the context of KSHV replication, binds to the viral genome and an acidic region of the histone H2A–H2B dimer of the nucleosome, thereby tethering the episome to chromatin during mitosis and segregating it into daughter nuclei (Ballestas et al., 1999; Barbera et al., 2006). LANA-1 interacts with p53, repressing its transcriptional activity and its ability to induce apoptosis, thereby promoting the cell cycle (Friborg et al., 1999). Indeed, LANA-1 can promote S-phase entry (An et al., 2005), in part by driving the cell cycle through positive regulation of the retinoblastoma (RB) protein/E2F transcriptional pathway (Radkov et al., 2000).

Several other KSHV proteins have been reported to repress the transcriptional activity of p53. They include vIRF-1 and LANA-2 (vIRF-3) (see above), K-bZIP (the product of K8) (Park et al., 2000) and the lytic-switch protein RTA (Gwack et al., 2001).

Taken together in the context of tumour formation, there may be functional cooperation between these proteins that regulate p53 activity and other KSHV proteins. For example, whilst vCyclin is oncogenic, it is so only in the absence of functional p53 (Verschuren et al., 2002).

Regulation of complement

KSHV complement-control protein (KCP). Complement bridges innate and adaptive immune responses as well as humoral and cell-mediated immunity, and is antiviral. Complement effects on virus infection include (i) lysis of infected cells and enveloped virus via formation of a pore or ‘membrane-attack complex’ in the cell or viral surface, (ii) coating of infected cells and virions with component C3b to enhance phagocytosis or block viral infection and (iii) production of potent anaphylatoxins, which exert a variety of effects on the immune system, including recruitment of inflammatory cells to the site of infection.

Complement activation occurs through the cleavage of proenzymes to enable the formation of the C3 and C5 convertase enzymic complexes, with the release of smaller chemotactic and anaphylatoxin fragments. The covalent attachment of C4b and C3b to pathogen and infected-cell surfaces also enhances recognition by phagocytes and increases the humoral response to those pathogens (Watanabe et al., 2003; Bower et al., 2004). For detailed diagrams of the complement-activation pathways and their regulation, the reader is referred to the review by Blue et al. (2004).

To protect host cells from autologous complement attack, soluble and membrane-bound complement regulators have evolved to limit inflammation to the infected site. An important group of these regulators is encoded in the regulators of complement activation (RCA) gene cluster at chromosome 1 (locus 1q32). All of these proteins, including membrane cofactor protein (MCP; CD46), complement receptor 1 (CR1; CD35), decay-accelerating factor (DAF; CD55), factor H (FH) and C4b-binding protein (C4BP), contain between four and 35 short-consensus-repeat (SCR) domains and share significant homology, as well as complement-inhibition mechanisms (Kirkitatdje & Barlow, 2001).

Complement does not require previous antigen exposure to be fully effective and accelerates and enhances the generation of an adaptive immune response to pathogens. Therefore, it represents a potentially important antiviral immune response. This possibility is borne out by the diversity of complement-evasion strategies adopted by viruses that we are now beginning to understand (reviewed by Blue et al., 2004).

The ORF4 gene of KSHV encodes a lytic-cycle protein called KSHV complement-control protein (KCP) that inhibits activation of the complement cascade. KCP consists of four N-terminal SCR domains, a dicysteine motif, a long serine (S)- and threonine (T)-rich domain and a C-terminal hydrophobic element sufficient to act as a transmembrane anchor (Spiller et al., 2003a, b). KCP has also been named kaposica (Mullick et al., 2003). The full protein is 550 residues in length, including a 19-residue signal peptide, but PEL cells naturally infected with KSHV also express two isoforms of KCP in which either the S/T region or the S/T

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region plus the dicysteine motif is removed through alternative splicing of the ORF4 transcript. All three isoforms retain the four SCR domains and the transmembrane region and all can regulate complement through two separate mechanisms (Spiller et al., 2003a): firstly, by directly accelerating the decay of the classical pathway C3 convertase enzyme complex, and secondly, by acting as a cofactor for factor I-mediated inactivation of C3b and C4b, two components of the C3 and C5 convertases (Blue et al., 2004). Decay of the classical pathway C3 convertase and cofactor activity for C4b inactivation depend on three N-terminal SCR domains (SCR1–3; Spiller et al., 2006) and a positively charged linker region between SCR1 and 2. There is no evidence for an abundant soluble form of KCP, unlike, for example, that encoded by the related rhadinovirus herpesvirus saimiri (Albrecht & Fleckenstein, 1992).

Structurally and functionally, KCP belongs to the RCA protein family. However, KCP may be multifunctional. In addition to being expressed on the surface of naturally and experimentally infected cells, KCP is associated with the envelope of purified KSHV virions, where it potentially protects them from complement-mediated immunity. Furthermore, recombinant KCP binds heparin, an analogue of the known KSHV cell-attachment receptor heparan sulphate, via the SCR1–2 region where it can facilitate KSHV binding to target cells (Spiller et al., 2006).

**Deregulating cell–cell contact: the central event of T cell-dependent immune responses**

Modulators of the immunological synapse. Unlike B cells, T cells are not able to recognize soluble antigens. Instead, they recognize antigenic determinants associated with self MHC molecules on the surface of antigen-presenting cells (APCs). When T cells recognize antigenic peptides on APCs, the TCR complex and accessory molecules often colocalize at the site of cell–cell contact. The accessory molecules include those involved in adhesion and coactivation, an intracellular signal-transduction complex and elements of the cytoskeleton. This cluster of molecules at the site of T cell–APC contact has been called either a supramolecular activation cluster (SMAC) (Monks et al., 1998) or an immunological synapse (IS) (Grakoui et al., 1999) and is considered a dynamic structure at which information exchange occurs between the participating cells (Trautmann & Valitutti, 2003; Friedl et al., 2005). Whilst the concept was originally applied to the CD4+ T helper-cell synapse, it can also be considered in the context of cytotoxic T lymphocytes (CTLs) (Stinchcombe et al., 2001), B-cell acquisition of antigen (Batista et al., 2001), NK-cell surveillance (Davis et al., 1999) and myeloid lineage-cell interactions (e.g. monocytes/macrophages) (Barclay et al., 2002).

Given the nature of the components, the IS is probably responsible for initiating and sustaining TCR signalling to orchestrate the appropriate immune response, as suggested by Trautmann & Valitutti (2003). The IS may also ensure polarized T-cell cytokine secretion, directing either a Th1 or Th2 response. As the IS is the site of cell–cell contact and the main signalling event that sets thresholds for T-cell activation, these thresholds must be met to ensure either suitable immune responses against pathogens and toxins, including differentiation into effector and memory cells or maintenance of immunological tolerance. If thresholds are set incorrectly, the host is either susceptible to pathogen escape or, conversely, autoimmune diseases. Full T-cell activation, necessary for antigen elimination, occurs once the TCR-induced signals reach a critical threshold, which is dependent on the antigenic-peptide dose, its agonistic activity and the T-cell microenvironment (reviewed by Friedl et al., 2005). Therefore, the T-cell response is also influenced by the nature of the APCs that display peptide–MHC complexes and costimulators to the T cells. For example, mature dendritic cells express high levels of the MHC molecules and the costimulators that provide second signals to T cells. Some APCs may even engage in active processes to manipulate IS formation and stabilization. Nevertheless, following cognate receptor–ligand interaction at the IS, physiological T-cell activation can occur and the process can be divided into a series of temporal stages: T-cell polarization, initial adhesion, IS formation (initial signalling) and IS maturation (sustained signalling).

Thus, IS formation in general, and the signal-transduction process more specifically, are potential targets for intervention to modulate immune responses by modifying either the microenvironment or gene-expression profiles in inducer or responder cells. Concomitantly, the IS could represent a target for viruses to modulate the first stages of immune activation and provide immunological unresponsiveness as an adaptive evasion strategy (Table 2). One aspect of differences in biology between different types of APC concerns the site of the KSHV reservoir. Although not incontrovertible, as other sites are possible, current evidence supports the concept that the major reservoir is the B cell, not least because this is the predominant cell type in the peripheral blood infected with KSHV (Ambroziak et al., 1995). It is conceivable that evolutionary pressure has selected B cells as the site of KSHV latency due to their relative inability to recruit patrolling leukocytes, including T cells (Friedl et al., 2005), another manifestation of KSHV immune evasion.

A description of modulation of the IS by KSHV (summarized in Table 2) follows.

**KSHV modulators of MHC and accessory molecules.**

CTLs are the main protagonists of virus elimination, killing virus-infected cells before virions are produced (see Fig. 4). Thus, in the context of virology, their principal function is surveillance against virus infection. Most virus-specific CTLs are CD8+ T cells that recognize cytotoxic, usually endogenously synthesized, viral antigens in association with class I MHC on any nucleated cell. Full activation and differentiation of CTLs as effector cells, like other lymphocytes, requires at least two signals. The first is the antigenic peptide–MHC complex and the second is either
produced by Th cytokines or costimulators expressed on infected cells. Therefore, if a virus inhibits the MHC class I-restricted antigen-presentation pathway, that virus-infected cell would become invisible to CTL surveillance. Even reduced antigen presentation might restrict CTL-activation thresholds from being reached, although as few as three to ten peptide–MHC complexes within the IS can induce target-cell elimination (Purbhoo et al., 2004). However, NK cells are activated by, and can eliminate, those cells displaying reduced MHC class I density (see below). In addition, IFN upregulation of MHC class I protein expression may abrogate the effect of virus inhibition (Brander et al., 2000). The outcome of this dynamic process determines whether the virus or the host benefits.

There are many steps in the processes of cytosolic antigen processing and presentation that can be targeted to reduce antigenic-peptide presentation in the context of MHC class I (Fig. 4). Inhibition of the endogenous antigen-presentation pathway by viruses that establish persistent or latent infections, such as herpesviruses, is especially prevalent (reviewed by Lilley & Ploegh, 2005). Indeed, herpesviruses reactivate from latency in the face of fully primed host immunity. Therefore, it is important for their host cells to evade CTLs, at least for the period immediately following reactivation and before virion production.

KSHV encodes two proteins, K3 and K5 (Russo et al., 1996), that share 40 % amino acid identity (Nicholas et al., 1997a) and are now called modulator of immune recognition 1 and 2 (MIR1 and MIR2), respectively (Coscoy et al., 2001). When they are expressed during lytic replication, they are localized predominantly at the endoplasmic reticulum (ER) and increase endocytosis of surface MHC class I molecules for their degradation by the endolysosome (Coscoy & Ganem, 2000; Ishido et al., 2000a; Stevenson et al., 2000). This mechanism differs from that of other known viral inhibitors of MHC class I expression, which interfere for example with the synthesis or assembly of MHC class I chains or retain them in the ER (reviewed by Lilley & Ploegh, 2005). The function of MIR2 is specific for human leukocyte antigen (HLA)-A and HLA-B, which are responsible for presenting antigen to CTLs, and, to a lesser extent, HLA-E, but not HLA-C. The latter two molecules provide a

Table 2. Potential mechanisms of KSHV modulation of the immunological synapse (IS)

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<th>IS target</th>
<th>KSHV mechanism</th>
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<tr>
<td>Effector-cell activation threshold</td>
<td>Latency: viral gene expression is minimized, limiting the diversity and dose of viral epitopes presented and thereby reducing the likelihood of effector-cell activation thresholds being reached</td>
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<tr>
<td></td>
<td>Blocking viral antigen presentation: MIR1 and MIR2</td>
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<td>Inhibiting T- and NK-cell costimulatory-molecule expression or activity: MIR2, perhaps vOX2</td>
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<tr>
<td>Effector-cell function</td>
<td>Incorrect growth- and differentiation-factor levels in the extracellular microenvironment: cellular IL-8 modulation, vIL-6 and vCCLs</td>
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<td>Interfering in cell polarization in the site of immune response, in the case of chemokines and chemokine receptors and cytoskeletal molecules: vCCLs, especially vCCL2</td>
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<td></td>
<td>Modulation of IS environment indirectly through corrupting cytokine networks: e.g. vIRFs</td>
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<td>Delivery of negative-regulatory signal: vOX-2</td>
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<td>Deregulation of T-cell signalling: e.g. K1</td>
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<td>Deregulation of B-cell signalling: e.g. K1 and K15</td>
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<td>Inhibition of antigen presentation by infected cells: e.g. ORF 45, vIRFs</td>
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Fig. 4. The endogenous pathway of antigen presentation. In the virus-infected cell, viral proteins translated in the cytosol (1) are degraded in the proteasome into peptides containing antigenic determinants (2). The antigenic peptides are transferred into the ER by TAP proteins embedded in the ER membrane in an energy-dependent mechanism (3). In the ER, the peptides are loaded onto the antigen groove of newly synthesized chains of MHC class I molecules (4). A trimolecular complex, consisting of MHC I a chain, b2-microglobulin and antigenic peptide, then traverses the Golgi apparatus (5) and is presented on the surface of the plasma membrane. Here, the complex of the MHC class I molecule and antigenic peptide is displayed at the cell surface, where it may be recognized by the cognate T-cell antigen receptor of a CD8+ cytotoxic T lymphocyte (6). CD8 binds an invariant site in the a3 domain of the MHC class I molecule. KSHV MIR1 and MIR2 proteins, located predominantly in the ER, downregulate MHC class I surface expression by increasing endocytosis of the antigen-presenting complex.
negative-regulatory signal to NK cells (see below) and, in contrast to MIR2, MIR1 downregulates HLA-A, -B, -C and -E significantly (Ishido et al., 2000a).

The molecular details behind MIR1 and 2 activities are now understood in some detail. The proteins belong to the family of enzyme type 3 (E3) ubiquitin ligases and can regulate the last step of ubiquitination: when lysine residues in the intracytoplasmic tail of MHC I molecules are ubiquitinated, the molecules are endocytosed and degraded by the lysosome (Coscoy et al., 2001; Hewitt et al., 2002). Importantly, MIR1, but not MIR2, has now been shown to have E3 ligase activity in a lysine-independent manner, ubiquitinating cysteine residues of target molecules and possibly serine residues. Perhaps the evolution of this previously unknown mechanism of ubiquitination broadens the specificity of KSHV for modulating the cell-surface expression of other components of the IS (Cadwell & Coscoy, 2005).

PEL cell lines infected with latent KSHV also express reduced levels of MHC class I and transporter associ- ated with antigen processing-1 (TAP-1) molecules compared with B-lymphoblastoid cell lines derived by Epstein–Barr virus (EBV) transformation. Consequently, they are deficient in antigen processing and presentation, and are therefore less susceptible to CTL killing (Brander et al., 2000). However, as MIR1 and MIR2 are lytic proteins and are not expressed during latency, it is unknown which latent protein (or proteins) is responsible for this activity. It may be that this antigen-presentation deficiency of PEL cells is dictated by the cell phenotype rather than by virus infection. Alternatively, K3 might be expressed during latency (Taylor et al., 2005).

Because KSHV predominantly infects the B lymphocyte and perhaps the monocyte (Ambroziak et al., 1995), the possible effects of this virus on the MHC class II antigen-presentation pathway could be significant, although to date there is no evidence in this regard, even with the MIRs (Coscoy & Ganem, 2000; Ishido et al., 2000a).

T-cell activation. Activation of Th cells is the central event in the development of adaptive immune responses. Initiation of this activation requires APC and CD4+ T-cell contact and specific antigen recognition by the TCR, stable adhesion of the T cells to the APCs and transduction of signals to the CD4+ T cells. The TCR recognizes the foreign peptide associated with MHC II and provides the first signal for T-cell activation. T cells also express other molecules, such as CD28, CD40, CD2 and LFA-1, which do not recognize antigen, but bind to specific ligands on the APC and participate in responses to antigens. These T-cell molecules are collectively called accessory molecules. Their physiological role within the IS is to deliver signals (the ‘second signal’) to the T cells or to function as adhesion molecules.

KSHV MIR2, but not MIR1, reduces surface expression of at least two molecules involved in the IS, B7-2 (CD86) and ICAM-1 (CD54), inducing their endocytosis and degradation (Ishido et al., 2000b; Coscoy & Ganem, 2001). B7-2 is one ligand that binds to the CD28 co-stimulatory molecule and ICAM-1 is a well-known adhesion molecule that interacts with LFA-1. Both ligands are important in IS formation and T-cell activation and the functional consequence of their downregulation by KSHV is reduced T-cell stimulation by antigen-presenting B cells (Coscoy & Ganem, 2001). Hence, MIR2 is an example of a viral modulator of IS formation and T-cell costimulation, reducing the visibility of KSHV-infected B cells to T-helper cells, thereby decreasing T-cell responses, cytokine release and the production of co-stimulatory signals for CTL generation (Coscoy & Ganem, 2001).

Another class of T cells and NK cells includes those that are restricted by CD1 proteins, a family of MHC class I molecules. The five CD1 proteins present lipids to T cells through their TCRs and are encoded by the genes CD1a–e. Evidence supporting the involvement of CD1d-restricted T cells in controlling viral infections was largely circumstantial until recently (Hegde & Johnson, 2005), when MIR2 was shown to remove CD1d from the surface of cells infected with lytic KSHV and to reduce CD1d-restricted T-cell activation (Sanchez et al., 2005). MIR1 may function similarly (Sanchez et al., 2005). MIR2-induced ubiquitination of the unique lysine on the cytosolic tail of CD1d was required for the endocytosis, although degradation of CD1d was not expedited (Sanchez et al., 2005). In addition to assigning a role for CD1d-restricted T cells in antiviral immunity, these findings suggest a mechanism by which KSHV also evades NK-cell activity; CD1d-restricted T cells regulate NK-cell activity and include a subset of ‘NK T’ cells.

Downregulation of NK-cell function. NK cells are a population of lymphocytes whose major function is in innate immunity, operating while the adaptive immune response to a pathogen evolves. Unlike the receptors of T and B cells, NK cell-receptor genes do not undergo rearrangement and the cells do not participate in clonal expansion. They are therefore poised for immediate response, killing virus-infected cells through the exocytosis of perforin- and granzyme-containing granules (Fig. 3) and secreting cytokines, mainly IFN-γ, to activate CTLs and macrophages.

The role of NK cells in virus diseases is perhaps best illustrated by human and animal herpesvirus infections; there is abundant literature in this regard (reviewed by Lodoen & Lanier, 2005). It is becoming increasingly apparent that the scenario in which NK cells are activated in the face of virus-mediated MHC class I downregulation is more complex and that herpesviruses, in particular, have evolved NK-modulating activities when MHC class I expression is downregulated (Lodoen & Lanier, 2005; Tomasec et al., 2005).

KSHV MIR1 and MIR2 downregulate MHC class I expression (see above), providing the opportunity for NK-cell
activation. MIR2 downregulates ICAM-1 and B7-2 expression, potentially protecting infected cells from NK cell-mediated cytotoxicity by destabilizing the IS (Ishido et al., 2000b), although the extent of this protection in vivo is debatable (Coscoy & Ganem, 2001). Given the significance of NK cells in innate immune defence against some virus infections, it is possible that KSHV has evolved mechanisms to circumvent their activation, which perhaps remain to be understood completely. However, in vivo studies with MHV-68 in NK-deficient mice suggest that these cells do not control this virus infection (Usherwood et al., 2005). Therefore, MHV-68 is unlikely to have evolved NK-evasion strategies. Whether the lack of NK-cell control is true of other gammaherpesvirus infections, including KSHV, remains to be determined.

B-cell activation. KSHV K1 encodes a type 1 membrane glycoprotein that is induced significantly during lytic replication (Lagunoff & Ganem, 1997). This gene is at a position in the KSHV genome equivalent to that of the EBV gene encoding the LMP-1 protein (Young & Rickinson, 2004). The K1 extracellular domain is highly variable, other than 12 conserved cysteine residues (Zong et al., 1999; McGeoch, 2001). The conserved cytoplasmic domain contains a functional immunoreceptor tyrosine-based activation motif (ITAM), similar to that involved in signalling by immune receptors, including the B-cell antigen receptor (BCR) and TCR complexes (Lee et al., 1998a, b; Lagunoff et al., 1999). Constitutive, ligand-independent, ITAM-dependent signalling is thought to result from homomultimerization of the extracellular domain of K1, phosphorylation of tyrosine residues in the ITAM by Src family kinases, followed by recruitment of various Src homology 2 (SH2)-containing signalling proteins, including the B-cell-specific Syk kinase. These events, as well as ITAM-independent signalling, activate AP-1 and NFAT in a cell type-specific manner (Lee et al., 1998a, 2005; Lagunoff et al., 1999, 2001). A K1-specific ligand may also be involved in regulating these processes, but has yet to be identified (Lagunoff et al., 2001; Lee et al., 2002). Thus, K1 appears to activate B-cell signalling, inducing inflammatory-cytokine production by B cells (Lee et al., 2005). It can also modestly augment RTA-induced KSHV lytic reactivation in this cell type, but is itself unable to trigger reactivation (Lagunoff et al., 2001); others suggest that K1 contributes to maintaining viral latency (Lee et al., 2002).

The kinetics of K1 expression, at least in vitro, are inconsistent with a straightforward tumorigenic role; whether they differ in vivo is unclear. Nevertheless, the protein is oncogenic and implicated in KSHV pathogenesis because it mediated rodent fibroblast transformation in vitro upon ectopic expression and was lymphomagenic in marmosets when substituted for the STP oncogene in a recombinant herpesvirus saimiri (Lee et al., 1998b). Moreover, in transgenic mice, K1 induced lymphoproliferative disease through ITAM-driven constitutive activation of the Src kinase Lyn and concomitant NF-κB activation and VEGF production (Prakash et al., 2005).

In conclusion, K1 has multiple roles in signal transduction, presumably to confer virus-infected cell survival, which may also have immunomodulatory activity. In the context of immune modulation, K1 protein interacts with the heavy (\( \mu \)) chains of the BCR, retaining this complex in the ER and preventing it from reaching the cell surface (Lee et al., 2000). K1 is also anti-apoptotic (Tomlinson & Damania, 2004).

K15 is manifested in the KSHV genome as one of two divergent alleles, each of eight coding exons, located at the right end of the KSHV genome. Each allele encodes a protein with up to 12 membrane-spanning domains and a C-terminal cytoplasmic tail containing intracellular-signalling motifs (Glenn et al., 1999; Poole et al., 1999; Choi et al., 2000; Brinkmann et al., 2003) and can be alternatively spliced (Glenn et al., 1999). The two alleles are designated K15-P (predominant) and K15-M (minor) (Poole et al., 1999; McGeoch, 2001). Although the proteins that they encode are structurally similar, they share only 33 % amino acid sequence identity; most published functional studies have been performed with K15-P. Whether K15 is a lytic-cycle protein or is expressed during latency is equivocal: in PEL cells, mRNA analyses indicate that the gene is expressed most abundantly upon reactivation (Glenn et al., 1999; Choi et al., 2000), whereas Western blotting studies suggest that the protein is latency-associated (Sharp et al., 2002).

As with K1, the functions of K15 remain to be fully elucidated, but already resemble those of EBV LMP-2A and LMP-1. Indeed, K15 has a similar location in the KSHV genome to the gene encoding LMP-2A in the EBV genome (Young & Rickinson, 2004). Moreover, immunomodulatory activities are emerging: the C-terminal region contains conserved motifs, such as SH2- and SH3-binding domains (Glenn et al., 1999; Choi et al., 2000), and binds TRAF1, TRAF2 and TRAF3 (Glenn et al., 1999). In addition, a chimaera in which the cytoplasmic tail of K15 was located in the membrane by the CD8\( \alpha \) chain extracytoplasmic domain inhibited BCR signalling. In contrast to K1, the K15-specific effect was to reduce tyrosine phosphorylation and intracellular calcium influx (Choi et al., 2000). However, Brinkmann et al. (2003) found that the nature of the transmembrane anchor affected K15 signalling: full-length K15, but not isoforms resulting from alternative splicing, activated the NF-κB pathway, so other K15 sequences are also important for modulating cell signalling by the cytoplasmic tail. These authors also showed that TRAF2 interaction with full-length K15, at an SH2-binding motif (YEEVL) phosphorylated by several Src tyrosine kinase family members, was necessary for K15-mediated activation of the Ras/MAPK and AP-1. Through its interaction with HAX-1, K15 may also inhibit apoptosis (Sharp et al., 2002).

Although difficult to reconcile the lytic-expression kinetics of K1 and perhaps K15 with a survival strategy for KSHV
latently infected cells, a conciliatory perspective could argue for leaky expression of K1 and K15, during either latency or abortive lytic replication (see above). Thus, KSHV has evolved at least two strategies to subvert BCR signalling during lytic replication and perhaps also during latency in some cell types: (i) K1-mediated retention of the BCR complex in the ER (Lee et al., 2000) and (ii) inhibition of BCR signal transduction by K15 (Choi et al., 2000). As B cells are the likely reservoir of latent KSHV, this strategy may then usurp control of infected-cell signalling to prevent erroneous activation and concomitant KSHV reactivation. Alternatively, lytic expression of K1 and K15 may simply extend the life of the infected cell to maximize progeny virion production.

Other immunomodulatory activities

K14 (vOX2). The vOX2 protein shares 36% identity with human OX2, a member of the immunoglobulin (Ig) superfamily of proteins. Cellular OX2, referred to as CD200, is expressed on the surface of many cell types, including endothelial cells, B cells, T cells, neuronal cells and tonsil follicles (Morris & Beech, 1987; Wright et al., 2001).

Signal delivery of CD200 occurs through binding CD200 ligand (i.e. receptor: CD200R) (Gorczynski et al., 2000; Wright et al., 2000, 2003). CD200R consists of a family of subtypes, all of which may recognize CD200, implicating further complexity in immunomodulation by CD200 (Wright et al., 2003; Gorczynski et al., 2004a, b). Mammalian CD200R subtypes, including a human one, have an intracytoplasmic tail of at least 60 residues that may transduce signals upon receptor ligation (Barclay et al., 2002; Wright et al., 2003; Gorczynski et al., 2004a). The distribution of CD200R family members in humans is more restricted – to myeloid and T cells (Wright et al., 2003) – than that of CD200, suggesting the CD200–CD200R interaction acts locally to modulate inflammatory-cell activity at sites of infection. Thus, CD200 delivers immunosuppressive signals to myeloid cells (and perhaps T cells) by ligating its cognate receptors; the three MAPKs (ERK, JNK and p38 MAPK) are inhibited after CD200R ligation, possibly explaining the negative activity of CD200 (Zhang et al., 2004).

The KSHV vOX2 protein, encoded by lytic gene K14 (Jenner et al., 2001), may imitate some immunomodulatory functions of CD200 to provide a microenvironment favouring either virus replication and dissemination or persistence in vivo. However, the function of KSHV vOX2 (and, indeed, other viral OX2 proteins) has been characterized incompletely. Two published vOX2 reports contradict each other. Chung et al. (2002) suggested that vOX2 stimulates myeloid-lineage cells to produce proinflammatory cytokines. Foster-Cuevas et al. (2004) suggested that the vOX2 protein does the reverse. Our own studies support the hypothesis that vOX2 is immunosuppressive. Recombinant extracellular vOX2 was expressed as a fusion protein with the C-terminal domain of human IgG1 Fc, and functional studies with the purified protein in vitro revealed that it reduced the oxidative burst of primary human neutrophils and the production of IL-8 by rIFN-γ-treated myeloid cells to basal levels (Rezaee et al., 2005). IL-8 production by rIFN-γ-treated and untreated peripheral primary monocytes was also inhibited significantly by recombinant vOX2 (S. A. R. Rezaee & D. J. Blackbourn, unpublished observations). In vivo, vOX2–Fc suppressed inflammation significantly, which correlated with reduced cell infiltration in the carrageenan-mouse model of acute inflammation (Rezaee et al., 2005), suggesting that vOX2 could modulate chemotaxis and indicating a profound anti-inflammatory activity for vOX2.

ORF37 (SOX). Against the backdrop of certain KSHV lytic proteins, such as K1, K15 and vGPCR, potentially manipulating host-cell survival either through paracrine effects or subverting cell-signalling pathways by inducing gene expression (see above) is the confounding activity of the ORF37 protein, SOX (shutoff and exonuclease). ORF37 encodes a DNase putatively involved in resolution of replicative intermediates produced during DNA synthesis, and is conserved throughout the Alpha-, Beta- and Gammaherpesvirinae. The SOX protein participates in RNA degradation to accelerate mRNA decay and inhibit host-cell gene expression, effecting ‘host shutoff’ within 12 h of lytic reactivation (Glaunsinger & Ganem, 2004b); the DNase activity of the KSHV protein still exists, but can be separated from that of host shutoff (Glaunsinger et al., 2005). Certain cellular genes, notably IL-6, escape shutoff and are upregulated during lytic growth, but the majority are apparently downregulated (Glaunsinger & Ganem, 2004a). Thus, another potential immunomodulatory capacity of KSHV can be inferred, as presumably genes involved in antigen processing and other antiviral activities are shut off by SOX. It is difficult to reconcile host protein-synthesis shutoff, itself a rather blunt immune-evasion strategy, with the sophistication and complexity of other KSHV immunomodulatory activities discussed above, and perhaps the two activities should be considered separately. Nevertheless, SOX is included in the present review for completeness.

Concluding remarks

Investigating virus modulation of immune responses can lead to a deeper understanding of molecular mechanisms, the revealing of new immune-system responses or components and the discovery of new classes of immune modulators with therapeutic potential for inflammatory diseases. With regard to the discovery of new immune-system responses or components through virus research, several recent examples set a precedent, including at least two emanating from KSHV research. First, the study of the HIV Vif protein identified a new innate immune response to exogenous retroviruses (Sheehy et al., 2002), mediated by the cellular protein CEM15 or APOBEC3G, a DNA deaminase, which destroys or mutates the virus genome (Harris et al., 2003).
Second, it was recognized through its binding to paramyxovirus V proteins that mda5 is a central player in the signal-transduction cascade that can activate IFN-β expression (Andrejeva et al., 2004). Third, it was recognized that a novel ubiquitin E3 ligase, KSHV MIR1, can operate on cytosine residues, where previously only lysine residues were thought to be ubiquitinated (Cadwell & Coscoy, 2005). Fourth, understanding KSHV kaposin B stabilization of ARE-containing transcripts identified a mechanism of virus regulation of cellular mRNA stability (McCormick & Ganem, 2005).

The therapeutic opportunities for human diseases presented by understanding viral immune modulators are immense, as these activities have evolved under selective pressure to become highly specific. An important example is viral inhibition of the inflammatory response. Uncontrolled inflammatory reactions are the main causes of almost all types of hypersensitivity, resulting in autoimmune diseases (such as rheumatoid arthritis, systemic lupus erythematosus and glomerulonephritis), inflammatory diseases (such as chronic liver disease following hepatitis virus infections), and also post-infection diseases (such as Crohn’s disease) and also post-infection diseases (such as viral mimicry of cytokines, chemokines and their receptors.

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