Regulation of intracellular signalling by the terminal membrane proteins of members of the Gammaherpesvirinae

Melanie M. Brinkmann and Thomas F. Schulz
Institut für Virologie, Medizinische Hochschule Hannover, Carl-Neuberg Str. 1, D-30625 Hannover, Germany

The human \(\gamma_1\)-herpesvirus Epstein–Barr virus (EBV) and the \(\gamma_2\)-herpesviruses Kaposi’s sarcoma-associated herpesvirus (KSHV), rhesus rhadinovirus (RRV), herpesvirus saimiri (HVS) and herpesvirus ateles (HVA) all contain genes located adjacent to the terminal-repeat region of their genomes, encoding membrane proteins involved in signal transduction. Designated ‘terminal membrane proteins’ (TMPs) because of their localization in the viral genome, they interact with a variety of cellular signalling molecules, such as non-receptor protein tyrosine kinases, tumour-necrosis factor receptor-associated factors, Ras and Janus kinase (JAK), thereby initiating further downstream signalling cascades, such as the MAPK, PI3K/Akt, NF-\(\kappa\)B and JAK/STAT pathways. In the case of TMPs expressed during latent persistence of EBV and HVS (LMP1, LMP2A, Stp and Tip), their modulation of intracellular signalling pathways has been linked to the provision of survival signals to latently infected cells and, hence, a contribution to occasional cellular transformation. In contrast, activation of similar pathways by TMPs of KSHV (K1 and K15) and RRV (R1), expressed during lytic replication, may extend the lifespan of virus-producing cells, alter their migration and/or modulate antiviral immune responses. Whether R1 and K1 contribute to the oncogenic properties of KSHV and RRV has not been established satisfactorily, despite their transforming qualities in experimental settings.

Introduction

Members of the subfamily Gammaherpesvirinae are found in many species, probably including all Old and New World primates. They are subdivided into the \(\gamma_1\)-herpesviruses or lymphocryptoviruses and \(\gamma_2\)-herpesviruses or rhadinoviruses (reviewed by Greensill & Schulz, 2000; McGeoch et al., 2005). At present, lymphocryptoviruses appear to be confined to primate hosts, whereas rhadinoviruses are found in many more species. Among primate rhadinoviruses, the representatives of New World monkeys can be distinguished phylogenetically from those found in Old World primates. In addition, phylogenetic comparisons of mainly short sequence fragments, but also a few completely sequenced genomes, suggest strongly that there are two lineages of Old World rhadinoviruses, tentatively referred to as RV1 and RV2 (Rose et al., 1997; Greensill et al., 2000a, b; Lacoste et al., 2000b, c).

As judged mainly by the extensive research carried out on Epstein–Barr virus (EBV), the human lymphocryptovirus, lymphocryptoviruses establish latency in B lymphocytes (reviewed by Richardson & Kieff, 2001). EBV can also infect and establish a particular form of latency in epithelial cells in nasopharyngeal carcinoma (NPC; reviewed by Richardson & Kieff, 2001) and in some T cells, as deduced from its rare occurrence in T-cell lymphomas. The New World rhadinoviruses herpesvirus saimiri (HVS) and herpesvirus ateles (HVA) infect and transform T cells and can establish a latent pattern of infection in transformed T-cell lines; they can also be grown on fibroblast cultures (reviewed by Ensser & Fleckenstein, 2005). The human rhadinovirus Kaposi’s sarcoma-associated herpesvirus (KSHV) or Human herpesvirus 8 (HHV-8), the only representative of the RV1 lineage studied so far in this respect, infects B cells, epithelial cells, endothelial cells and cells of the monocyte/macrophage lineage; it establishes latency in B cells and endothelial cells in vivo (reviewed by Schulz, 2000, 2006). Rhesus rhadinovirus, the only RV2 representative to have been isolated in culture and studied in some detail, appears to infect B cells in vivo and can be grown on fibroblast cultures in vitro (Desrosiers et al., 1997; Bergquam et al., 1999; Wong et al., 1999).
One of the common features of primate \(\gamma\)-herpesviruses is the presence, at one or both ends of the coding region (‘long unique region’ or LUR) of the viral genome, of membrane-anchored proteins capable of triggering a variety of intracellular signalling pathways. As shown schematically in Figs 1, 2 and 3 and Tables 1 and 2, some of these share sequence elements or (predicted) structural features. Some, such as EBV LMP1 and HVS Stp and/or Tip, play a crucial role in the ability of these viruses to transform B cells (EBV) or T cells (HVS) \textit{in vitro}, and their transforming potential has been linked to the activation of particular signalling pathways. Stimulated by the link between transforming potential and the activation of certain signalling pathways, the terminal membrane proteins in other primate \(\gamma\)-herpesviruses have also been investigated more recently and the accumulated literature, reviewed here, may serve as a starting point to relate the biochemical properties of the different TMPs to the biological properties of the corresponding viruses.

**EBV latent membrane proteins LMP1 and LMP2A**

EBV is associated with infectious mononucleosis and several lymphoid malignancies, including African endemic Burkitt’s lymphoma, Hodgkin’s disease and lymphoproliferative disorders in immunodeficient individuals. EBV is also associated with two epithelial malignancies: NPC and oral hairy leukoplakia in AIDS patients (reviewed by Rickinson & Kieff, 2001). \textit{In vitro}, EBV can infect and efficiently transform and immortalize primary human B lymphocytes, leading to the outgrowth of transformed and immortalized lymphoblastoid cell lines (LCLs) that display elevated levels of several cellular activation antigens and adhesion molecules (reviewed by Rickinson & Kieff, 2001). In LCLs, viral gene expression is restricted to a small subset of latent genes that encode six Epstein–Barr nuclear antigens (EBNA1, 2, 3A, 3B, 3C and LP), three integral membrane proteins (LMP1, LMP2A and LMP2B) and two small nuclear RNAs (reviewed by Farrell, 1995). Five of these, namely EBNA1, 2, 3A, 3C and LMP1, have been shown to be essential for the process of B-cell immortalization (reviewed by Izumi, 2001).

**LMP1: biological properties.** LMP1 has transforming effects in rodent fibroblast cell lines, and Rat-1 cells expressing LMP1 are tumorigenic in nude mice (Wang \textit{et al.}, 1985, 1988b; Baichwal & Sugden, 1988; Moorthy & Thorley-Lawson, 1993a). LMP1 alters the growth of EBV-negative Burkitt’s lymphoma lymphoblasts and of primary B lymphocytes and induces many of the changes usually associated with EBV infection of primary B lymphocytes.

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**Fig. 1.** Genomic localization of the viral TMPs of HVS, HVA, RRV, KSHV and EBV. At the ‘left’ end of their genomes, monkey \(\gamma\)-herpesviruses HVS, HVA and RRV and human \(\gamma\)-herpesviruses KSHV and EBV encode membrane proteins with signalling activities and transforming potential. To facilitate the comparison with \(\gamma\)-herpesviruses, the EBV genome is depicted in the \(\gamma\)-herpesvirus convention, i.e. inverted with respect to the convention adopted by the EBV field. ORFs equivalent to R15, K15 or LMP2A/LMP2B, located at the ‘right’ end of the viral genomes of RRV, KSHV and EBV, respectively, have not been identified in the genomes of HVA or HVS. The length of the terminal repeats flanking the LUR varies between the different herpesviruses (not to scale). The LMP2 gene is transcribed across the terminal repeats from two spatially distinct promoter elements.
LMP1 increases plasma-membrane expression of adhesion molecules, induces higher levels of the lymphocyte function-associated antigen mRNA and functionally activates adhesion (Wang et al., 1988a, b). LMP1 expression can alter cell growth and gene expression in haematopoietic stem cells and epithelial cells (Dawson et al., 1990; Fahraeus et al., 1990; Fairbairn et al., 1993; Hu et al., 1993). Targeted expression of LMP1 in the skin or B-cell compartment of transgenic mice leads to the induction of epithelial hyperproliferation and lymphomagenesis, respectively (Wilson et al., 1990; Kulwichit et al., 1998). In monolayer cultures, LMP1 alters keratinocyte morphology and cytokeratin expression (Fahraeus et al., 1990). Cell differentiation is inhibited by LMP1 in raft cultures of immortalized human epithelial cell lines (Dawson et al., 1990). LMP1 is also critical for rendering LCLs tumorigenic in SCID (severe combined immunodeficient) mice (Dirmeier et al., 2003). LMP1 seems to play a role in virus production, as deletion of LMP1 from the EBV bacterial artificial chromosome derived from the Akata strain impaired virus release severely (Ahsan et al., 2005).

**LMP1 location and structure.** The LMP1 open reading frame (ORF) is located at the 'right' end of the EBV genome in the convention adopted by the EBV field – this corresponds to the 'left' end in the \( \gamma_2 \)-herpesvirus convention (Fig. 1). An N-terminal, 24 aa, arginine- and proline-rich, hydrophilic region of the LMP1 protein is followed by six transmembrane domains and a 200 aa cytoplasmic C terminus (Fig. 2). LMP1 is expressed in latently infected B cells in transplant lymphoma, NPC and Hodgkin's disease, as well as in B lymphocytes transformed by EBV in culture (reviewed by Rickinson & Kieff, 2001), and can...
be upregulated during the lytic cycle in epithelial and B cells (Boos et al., 1987; Contreras-Salazar et al., 1990; Chang et al., 2004). In addition to the full-length LMP1, a 'truncated' LMP1 protein, consisting of transmembrane domains 5 and 6 and the C-terminal domain, can be expressed in the lytic viral life cycle (Hudson et al., 1985); unlike full-length LMP1, this protein does not have transforming capabilities (Wang et al., 1988b; Baichwal & Sugden, 1989; Liebowitz et al., 1992).

Two regions in the C-terminal cytoplasmic domain of LMP1 have been defined as being critical for the contribution of LMP1 to the conversion of primary B lymphocytes to LCLs, and were therefore termed transformation effector sites (TES) 1 (aa 187–231) and 2 (aa 352–386) (Kaye et al., 1993, 1995, 1999; Izumi & Kieff, 1997; Izumi et al., 1997, 1999a) or C-terminal nuclear factor (NF)-κB-activating regions (CTAR) 1 (aa 194–232) and 2 (aa 351–386) (Fig. 2; Hammerskjöld & Simurda, 1992; Laherty et al., 1992; Huen et al., 1995; Mitchell & Sugden, 1995; Floettmann & Rowe, 1997).

**LMP1 activates NF-κB via its CTAR-1 and CTAR-2 regions by interacting with TRAFs and TRADD.** NF-κB plays a key role in most LMP1-stimulated gene expression (Devergne et al., 1998; He et al., 2000; Mehli et al., 2001; Zhang et al., 2001). Activation of the NF-κB pathway is linked to LMP1-induced immortalization of human primary B lymphocytes (Devergne et al., 1996; Izumi & Kieff, 1997; Cahir McFarland et al., 1999) and tumorigenic transformation of some rodent cell lines (He et al., 2000; Xin et al., 2001).

Both the canonical (reviewed by Ghosh & Karin, 2002) and the non-canonical (reviewed by Pomerantz & Baltimore, 2002) NF-κB pathways can be activated by LMP1. Activation of the non-canonical NF-κB pathway in fibroblast cell lines (Saito et al., 2003), epithelial and B cells (Atkinson et al.,...
associated factors (TRAFs) constitutively through its effect on NF-κB with TRAF-2, -3 and -5 have been reported to have little (Devergne et al., 1996, Franken et al., 1996; Eliopoulos et al., 1997). The binding affinity of the P\textsuperscript{284}QQAT motif of LMP1 is higher for TRAF-1 and -3 than for TRAF-2 and -5 (Mosialos et al., 1995; Devergne et al., 1996, 1998; Brodeur et al., 1997; Sandberg et al., 1997).

Although TRAF-3 was suggested earlier to act as a negative modulator by displacing TRAF-1 and TRAF-2 from CTAR-1 (Devergne et al., 1996; Kaye et al., 1996), recent experiments in murine TRAF-3-knockout B cells showed that it is crucial for LMP1-mediated JNK and NF-κB activation and upregulation of CD23 and CD80 (Xie et al., 2004). In mouse B cells, TRAF-3 was shown to mediate LMP1 signalling through direct interactions with CTAR-1 and indirect interactions with CTAR-2 (Xie & Bishop, 2004). TRAF-6 is recruited to the active LMP1 signalling complex by an indirect mechanism involving P\textsuperscript{284}QQAT of CTAR-1 and Y\textsuperscript{384} of CTAR-2 (Schultheiss et al., 2001).

However, mutations in CTAR-1 that disrupt association with TRAF-2, -3 and -5 have been reported to have little effect on NF-κB activation (Huen et al., 1995; Mitchell & Sugden, 1995; Brodeur et al., 1997; Sandberg et al., 1997; Devergne et al., 1998). Via its CTAR-2 region, LMP1 associates directly with two proteins involved in apoptosis, TNF-associated death domain (TRADD) and receptor-interacting protein (RIP) (Fig. 2; Izumi & Kieff, 1997). An LMP1 CTAR-2 double mutant (Y\textsuperscript{381},\textsuperscript{Y}\textsuperscript{382}) that fails to interact with TRADD is defective in NF-κB activation and in B-lymphocyte transformation (Izumi & Kieff, 1997), whereas RIP is not required for NF-κB activation (Izumi et al., 1999a). Although CTAR-1 is a weak NF-κB activator, it is sufficient for initial transformation, whereas CTAR-2, being the major NF-κB-activating region, is insufficient for transformation in the absence of CTAR-1 (Izumi et al., 1997).

Recent data generated in knockout murine embryo fibroblasts (or knockout 293 cells for IRAK-1) suggest that LMP1-mediated NF-κB activation does not depend on IKK\textsubscript{α}, IKK\textsubscript{β}, TRAF-2, TRAF-5, TAB2 or interleukin 1 (IL-1) receptor-associated kinase 4 (IRAK-4), but that IKK\textsubscript{β}, IRAK-1 and TRAF-6 are essential for LMP1 signalling (Luftig et al., 2003). Previous studies in which dominant-negative or wild-type TRAF-2 was overexpressed have led to controversial results concerning the role of TRAF-2 in LMP1-induced activation signals (Kaye et al., 1996; Eliopoulos et al., 1999b; Kieser et al., 1999). However, experiments with TRAF-2\textsubscript{-/-} B-cell lines have demonstrated that LMP1-mediated activation of NF-κB and JNK was not impaired in these cell lines, whereas CD40-mediated JNK activation was compromised by TRAF-2 deficiency (Xie et al., 2004). Filopodia formation promoted by LMP1 in fibroblasts and cells of epithelial- and B-cell origin (reviewed by Eliopoulos & Young, 2001) was shown to be mediated by the small GTPase Cdc42, independently of TRADD and TRAF-2 (Puls et al., 1999).

**LMP1 activates MAPK pathways.** LMP1 can activate the transcription factor AP-1 via mitogen-activated protein kinases (MAPKs) c-jun N-terminal kinase 1 (JNK1) and p38 in B cells and epithelial cells (Eliopoulos & Young, 1998; Eliopoulos et al., 1999a, b; Kieser et al., 1997). In 293 epithelial cells, JNK activation was dependent on aa 379–384, overlapping the CTAR-2 domain and TRADD-binding domain, but not on CTAR-1 (Kieser et al., 1997, 1999; Eliopoulos & Young, 1998; Eliopoulos et al., 1999b). Whereas CTAR-2-mediated NF-κB activation involves TRADD, TRAF-3 and TRAF-6, LMP1 signalling to JNK is independent of TRADD, TRAF-2, RIP and the p21 Rho-like GTPases Rac1 and Cdc42 (Kieser et al., 1999; Wan et al., 2004). LMP1 activates JNK via TRAF-6, the MAPK kinase TAK1, TAB-1 and the JNK kinases 1 and 2, whereas TRADD, TRAF-2, RIP, TAB-2, myeloid differentiation factor 80, IRAK-1 and IRAK-4 are not essential (Wan et al., 2004).

Furthermore, LMP1 activates the p38 MAPK through CTAR-1 and -2 via TRAF-6 (Eliopoulos et al., 1999a; Schultheiss et al., 2001). In epithelial cells, TRAF-6 is recruited to LMP1 complexes in the plasma membrane, but binding to LMP1 seems to be indirect (Schultheiss et al., 2001). p38 has been shown to mediate cytokine induction by LMP1 (Eliopoulos et al., 1999a; Vockerodt et al., 2001) and transcript stabilization of the chemokine IP-10 (Vockerodt et al., 2005).

**LMP1 and the JAK/STAT pathway.** The region between CTAR-1 and -2 (aa 233–350) includes four direct, imperfect repeats of a conserved PQDPDNTDG\textsubscript{N} sequence, a PPQLT sequence (aa 320–324) that resembles a PXQXT/S TRAF-binding motif, but does not function as one, a protease-cleavage site (between aa 241 and 242), 19 potential serine or threonine phosphorylation sites and sequences that vary in human isolates and have been reported to affect the ability of LMP1 to transform immortalized rodent fibroblasts (Fennewald et al., 1984; Hu et al., 1991; Li et al., 1996; Mehl et al., 1998). In addition, LMP1 has consensus sites that are characteristic for interaction with members of the Janus kinase (JAK) family. These consensus sites are classified as a box 1 motif, which is a proline-rich sequence (P\textsuperscript{P275}HDPL\textsuperscript{280}P and aa P\textsuperscript{P302}HDPL\textsuperscript{307}), and as box 2 motif, which may play a role in kinase activation (aa P\textsuperscript{P320}QQLTEVENK\textsuperscript{330}) (Gires et al., 1999). The region encompassing aa 233–350 is not critical for B-lymphocyte growth transformation (Izumi et al., 1999b).
**Table 1. Functional features of γ-herpesvirus TMPs**

Besides structural similarities (Figs 2 and 3), the viral TMPs LMP1 and LMP2A of EBV, R1 of RRV, K1 and K15 of KSHV, Stp and Tip of HVS and Tio of HVA share functional features. The cellular signalling cascades initiated by these TMPs are summarized in Fig. 4.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>R1</th>
<th>K1</th>
<th>STP</th>
<th>LMP1</th>
<th>Tio</th>
<th>Tip</th>
<th>LMP2A</th>
<th>K15</th>
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<td>Direct interaction</td>
<td>Syk, but not Src</td>
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<td>Ras (StpC)</td>
<td>TRAF-1, -2, -3, and -5 via CTAR-1</td>
<td>Via SH3-B site with Lyn, Hck, Lck, Src, Fyn and Yes, but not Abl, PI3K or Grb2</td>
<td>Lck, TAP and p80</td>
<td>Csk (in epithelial cells)</td>
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<td>via cytoplasmic domain</td>
<td>TRAF-1, -2 and -3 (StpA and C)</td>
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<td>Via SH2-B site with Lck, Src and Fyn, but not PLC/5, Abl, Grb2 or Vav</td>
<td>TRAF-1 and -2 (StpB)</td>
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<td></td>
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<td>Syk, Vav1/3, PI3K,</td>
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<td>TRADD and RIP via CTAR-2</td>
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<td>STAT-1 and -3</td>
<td>Hax-1</td>
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<td>Lyn, PLC/2, Grb2,</td>
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<td>Phosphorylated by</td>
<td>Syk and Src (full-</td>
<td>Syk, Src at Y271,</td>
<td>Syk (StpA and B)</td>
<td>Not known, but is</td>
<td>In vivo by Lck, Src and Fyn</td>
<td>Lyn, Syk, Erk1</td>
<td>Phosphorylated at Y481</td>
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<td></td>
<td>length R1)</td>
<td>Y282, weakly by Lyn and ZAP70</td>
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<td>phosphorylated at T324 and S315</td>
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<td>Src, Lck, Fyn, Hck and Yes</td>
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<td>Lyn, Syk and Fyn</td>
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<td>Activated by</td>
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<td>Ligand-independent;</td>
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<td>LMP2A</td>
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<td>Induction of Ca²⁺ mobilization and cellular tyrosine phosphorylation</td>
<td>CD8–R1 chimera</td>
<td>K1 in B cells</td>
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<td>–</td>
<td>Tip-488 suppresses tyrosine phosphorylation</td>
<td>CD8–LMP2A chimera</td>
<td>No (CD8–K15 chimera)</td>
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<td>Induction of the activation of:</td>
<td></td>
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<td>NFAT</td>
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<td>STAT-1 and -3</td>
<td>Erk2 and JNK in epithelial cells, but not p38</td>
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<td>NF-κB, NF-κB via TRAFs (StpC)</td>
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<td>NF-κB via CTAR-1 and -2</td>
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<td>Lyn, Syk, PLC-γ/2, Cbl</td>
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<td>Erk2 (StpC)</td>
<td>p38 via CTAR-1 and -2</td>
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<td>PI3K/Akt</td>
<td>STAT-3 (StpA)</td>
<td>JNK1 via CTAR-2</td>
<td>Activation or suppression of Lck activity</td>
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<td>Inhibits PI3K and FKHR</td>
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<td>JAK-3 and STAT-1, -3 and -5</td>
<td>NF-κB (?) Inhibits GSK-3β, stabilizes β-catenin in epithelial cells</td>
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<td>Secretion of inflammatory cytokines (IL-6, IL-8, IL-1α/β, IL-10)</td>
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<td>VEGF, MMP-9</td>
<td>Induces cytokine secretion</td>
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<td>Induces expression of Fas, ICAM-1, CD40, LFA-3, A20, EGFR, CD23, CD80, Bcl2, VEGF and Cox</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes in HaCaT and HFK cells</td>
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<td>Effects on proliferation/apoptosis/cell morphology</td>
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<td>Can protect cells from apoptosis</td>
<td>–</td>
<td>Yes, via multiple routes</td>
<td>–</td>
<td>Promotes T-cell apoptosis</td>
<td>Promotes cell spreading and motility</td>
<td>–</td>
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<td>Localization in lipid rafts</td>
<td>–</td>
<td>Yes (unpublished results)</td>
<td>–</td>
<td>Yes</td>
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<td>Yes</td>
<td>Yes</td>
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</table>
Constitutively active signal transducers, activators of transcription (STATs) have been found in a variety of EBV-associated malignancies (Weber-Nordt et al., 1996; Chen et al., 1999, 2001; Kube et al., 2001). In epithelial cells, LMP1 expression was associated with an increase in activated STAT-3 and -5 (Chen et al., 2003). A reported direct interaction between LMP1 and JAK3 via aa 233–350 and activation of JAK3 and STAT-3 in EBV-transformed B cells remains controversial (Gires et al., 1999; Higuchi et al., 2002). Najjar et al. (2005) have recently reported that LMP1-induced phosphorylation/activation of STAT-1 in LCLs is almost exclusively due to the NF-κB-dependent secretion of alpha and gamma interferons.

LMP1 signalling leads to activation or repression of factors implicated in the control of cellular proliferation and apoptosis. The suppression of apoptotic death is a function of LMP1 that contributes to its oncogenicity. Activation of the NF-κB pathway by LMP1 appears to be essential for the inhibition of apoptosis, as a dominant-negative IκBα mutant and a chemical NF-κB inhibitor, Bay 11-7082, induced apoptosis in EBV-transformed LCL and LMP1-transfected cells (Cahir McFarland et al., 2000, 2004). LMP1 uses the NF-κB pathway to upregulate the expression of several antiapoptotic proteins, such as Bcl-2, A20 and Mcl-1 (Gregory et al., 1991; Henderson et al., 1991; Laherty et al., 1992; Milner et al., 1992; Martin et al., 1993; Rowe et al., 1994; Fries et al., 1996; Wang et al., 1996; D’Souza et al., 2004). Via the same pathway, transforming growth factor (TGF) β, a tumour-suppressor cytokine that inhibits cell proliferation due to cell-cycle arrest in the G1 phase, as well as Smad-mediated transcriptional responses (reviewed by Massagué & Wotton, 2000), are inhibited by LMP1 in epithelial cells and fibroblasts (Prokova et al., 2002). LMP1 represses the expression of E-cadherin, an invasion-suppressor gene, and triggers the invasive potential of cells (Fahraeus et al., 1992; Kim et al., 2000). The repression of E-cadherin gene expression seems to be mediated by activation of cellular DNA methyltransferases by LMP1 (Tsai et al., 2002).

Furthermore, LMP1 induces the antiapoptotic PI3K/Akt kinase pathway to promote cell survival and to induce actin remodelling via its p204-QQAT motif (Dawson et al., 2003). The PI3K inhibitor LY294002, but not the NF-κB inhibitor Bay 11-7085, could inhibit CTAR-1-induced focus formation and anchorage-independent growth in rodent fibroblasts (Mainou et al., 2005). LMP1 downregulates transcriptional expression of the metastasis-suppressor gene RECK via the MAPK Erk in an EBV-negative NPC cell line (Liu et al., 2003), prevents Ras-induced senescence in human fibroblasts and blocks expression of the p16INK4a tumour-suppressor gene (Yang et al., 2000). The p16INK4a-Retinoblastoma protein (Rb) pathway plays a critical role in preventing inappropriate cell proliferation and LMP1 inactivates the transcription factor Ets2, which is known to induce p16INK4a expression in Ras-induced senescence (Ohtani et al., 2001) by promoting the intracellular...
redistribution of Ets2 from the nucleus into the cytoplasm (Ohtani et al., 2003). In addition, LMP1 inactivates the downstream mediators of the p16INK4a–Rb growth-arrest pathway, E2F4 and E2F5 (Gaubatz et al., 2000; Ohtani et al., 2003). Manipulation of E2F4 seems to depend on both CTAR regions and involves the MAPK Erk pathway.

In epithelial cells, LMP1 may enhance proliferative signals and protect cells from apoptosis by inducing the expression of the epidermal growth factor receptor (EGFR) (Miller et al., 1995a, 1997). In an NPC cell line, LMP1 activated EGFR promoter activity in an NF-κB-dependent manner (Tao et al., 2004) and induced cyclooxygenase 2 and vascular endothelial growth factor (VEGF) (Murono et al., 2001). LMP1-induced nuclear accumulation of EGFR was found to accelerate G1/S transition through binding of EGFR to cyclin D1 and cyclin E (Tao et al., 2005). Activation of AP-1 may play a role in increasing the cyclin D1 promoter activity and downregulating p16 in epithelial cells (Song et al., 2004, 2005). In an NPC cell line, Faqing et al. (2005) observed LMP1-induced expression of survivin, enhanced levels of phosphorylated Rb and increased numbers of cells in the S phase. Everly et al. (2004) found downregulation of the cyclin-dependent kinase inhibitor (CDKI) p27 and increased levels of CDK2 and Rb in LMP1-expressing rodent fibroblasts.

In B cells infected with a derivative of EBV containing an NGF-R–LMP1 fusion protein, cross-linking with NGF antibody induced B-cell proliferation and supported G1/S transition, which was accompanied by upregulation of e.g. cmyc, pac1, junB, junD and irf-5 (Dirmeier et al., 2005). In human epithelial cells, LMP1 could repress p53-enhanced DNA repair and inhibited p53 transactivation (Liu et al., 2005).

### Table 2. Signalling motifs conserved between γ-herpesviral TMPs

<table>
<thead>
<tr>
<th>Motif/protein</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITAM (consensus)</td>
<td>(D/E)X7(D/E)X2YX7−10YX2L/I</td>
</tr>
<tr>
<td>LMP2A</td>
<td>DY74OPLGTGDSQSL85LGL</td>
</tr>
<tr>
<td>K1</td>
<td>DY77YSLHDCTEDY88TQP</td>
</tr>
<tr>
<td>TRAF-binding motif (consensus)</td>
<td>PXQXT/S</td>
</tr>
<tr>
<td>StpA-11</td>
<td>P66VQES</td>
</tr>
<tr>
<td>StpB SMH1</td>
<td>P41TQHT, P64VQET</td>
</tr>
<tr>
<td>StpC-488</td>
<td>P10IET</td>
</tr>
<tr>
<td>K15-M</td>
<td>INQ66SGLS, PPPF68PADE</td>
</tr>
<tr>
<td>K15-P</td>
<td>PDQ65SGMS, QATA70PTDD</td>
</tr>
<tr>
<td>LMP1</td>
<td>P20TQ28TADD</td>
</tr>
<tr>
<td>CD40</td>
<td>PVOET</td>
</tr>
<tr>
<td>SH2-B motif (consensus)</td>
<td>YYXL/I/V</td>
</tr>
<tr>
<td>K15-M</td>
<td>Y444ASI, Y490EEV</td>
</tr>
<tr>
<td>K15-P</td>
<td>Y431ASI, Y481EEV</td>
</tr>
<tr>
<td>LMP2A</td>
<td>Y112EEA</td>
</tr>
<tr>
<td>SH3-B motif (consensus)</td>
<td>PXXPFXR</td>
</tr>
<tr>
<td>Tio</td>
<td>PQLPPR</td>
</tr>
<tr>
<td>Tip-C488</td>
<td>PPLPPR</td>
</tr>
<tr>
<td>K15-M</td>
<td>P395PLPSR</td>
</tr>
<tr>
<td>K15-P</td>
<td>P387PLPF</td>
</tr>
</tbody>
</table>

LMP1 transmembrane domains are essential for signalling and localization to lipid rafts. The aminoterminal and transmembrane regions of LMP1 play a pivotal role in efficient signalling. LMP1 is able to form aggregates via its transmembrane domains (Hennessy et al., 1984; Liebowitz et al., 1986, 1987). LMP1 mutants lacking parts of the N-terminal transmembrane domains localize to the plasma membrane in BALB/c 3T3 cells or LCLs, but do not form aggregates, show no transforming activity and are not able to induce cellular gene expression (Wang et al., 1988a; Baichwal & Sugden, 1989; Martin & Sugden, 1991; Liebowitz et al., 1992; Kaye et al., 1993; Devergne et al., 1998). A chimeric protein composed of the N-terminal transmembrane domains of LMP1 and the C-terminal part of CD40 can signal in the absence of CD40 ligand (Floettmann & Rowe, 1997; Gires et al., 1997; Hatzivassiliou et al., 1998). Thus, the capacity of LMP1 to signal constitutively in the absence of any ligand is mediated by its transmembrane domains.

LMP1 also localizes to intracellular membranes and the cytoskeleton and is associated with lipid rafts (Liebowitz et al., 1986, 1987; Mann & Thorley-Lawson, 1987; Moorthy & Thorley-Lawson, 1990, 1993b; Clauser et al., 1997; Ardila-Osorio et al., 1999; Higuchi et al., 2001; Kaykas et al., 2001). Studies with LMP1 transmembrane-deletion mutants in 293...
cells and human B lymphoblasts identified a motif present in the first transmembrane domain of LMP1 to be crucial for constitutive signalling and lipid-raft localization (Yasui et al., 2004). Lam & Sugden (2003) observed that the majority of LMP1 is localized to and signals from lipid rafts of intracellular membranes in the EBV-immortalized lymphoblastic cell lines 721 and 293, and is only detected at low levels at the plasma membrane. LMP1 protein expression was also observed in extracellular vesicles (Flanagan et al., 2003; Vazirabadi et al., 2003).

LMP2. The LMP2 gene is transcribed into two mRNAs across the circularized viral genome (i.e. across the terminal repeats) from two spatially distinct promoter elements (Fig. 1). Both transcripts are multiply spliced and exons 2–9 are shared by both mRNAs. Exon 1 of LMP2A encodes a 119 aa, hydrophilic, N-terminal cytoplasmic domain, whereas exon 1 of LMP2B is non-coding, with translation beginning in the common exon 2 before the first transmembrane sequence (Laux et al., 1988, 1989; Sample et al., 1989). The remaining exons encode 12 transmembrane domains and a 27 aa, hydrophilic C terminus.

Protein-interaction motifs in LMP2A. The LMP2A N-terminal cytoplasmic domain (aa 1–119) includes eight tyrosines (Fig. 2), some of which are phosphorylated constitutively (Longnecker et al., 1991; reviewed by Longnecker, 2000). The Y112EEA motif of LMP2A is homologous to the preferred Src-homology 2-binding (SH2-B) motif EEXXYEEV/I of Src family members of non-receptor protein tyrosine kinases (PTKs) (Songyang et al., 1993, 1994a, b) and is the binding site for the PTK Lyn (Fruehling et al., 1998). Tyrosine residues Y72QPL and Y101LGL of LMP2A form an immunoreceptor tyrosine-based activation motif (ITAM; Fig. 2). ITAMs are present in a variety of cellular immunoreceptors, such as the B-cell and T-cell antigen receptors (BCR, TCR), and play a central role in signal transduction of these receptors (reviewed by Benschop & Cambier, 1999). LMP2A is phosphorylated in vivo on serine and threonine residues (Longnecker et al., 1991) and is associated in vivo with the MAPK Erk1, which can phosphorylate LMP2A at residues S15 and S102 in vitro (Panousis & Rowe, 1997).

The N-terminal domain of LMP2A includes several motifs reminiscent of SH3-binding (SH3-B) motifs (reviewed by Pawson & Gish, 1992) and WW-domain interaction sites (reviewed by Sudol, 1996). SH3-B motifs are proline-rich regions recognized by proteins carrying non-catalytic SH3 domains. WW domains consist of paired tryptophan residues separated by 20–22 aa and bind specifically to the motif XPPPXY, of which LMP2A has two (PPPYY60 and PPPYY103). No SH3 domain-containing proteins have so far been reported to interact with LMP2A via its potential SH3-B motif, but LMP2A binds to several members of the Nedd4-like ubiquitin protein ligase family via its PPPPY motifs. This interaction results in ubiquitination and degradation of LMP2A and LMP2A-associated proteins, such as Lyn and Syk (Ikeda et al., 2000, 2001, 2002; Longnecker et al., 2000; Winberg et al., 2000; reviewed by Portis et al., 2004).

LMP2A blocks BCR signalling in LCLs and maintains viral latency. Normal BCR signal transduction following BCR cross-linking is blocked in LCLs generated with wild-type EBV, as measured by calcium mobilization, cellular kinase activation, induction of tyrosine phosphorylation and activation of transcription. In contrast, BCR signal transduction was found to be normal in LCLs infected with a recombinant EBV from which LMP2A had been deleted (Miller et al., 1993, 1995b), indicating that LMP2A can block BCR signalling in LCLs. In these LCLs, the N-terminal region of LMP2A was found to be constitutively tyrosine-phosphorylated and to be associated stably with Src family PTKs (Lyn and Fyn) and the Syk PTK (Burkhardt et al., 1992; Miller et al., 1995b). The Y112 of the SH2-B motif Y112EEA, essential for Lyn binding, and Y74 and Y80 of the ITAM, mediating Syk binding, were both required for blocking BCR signalling (Fruehling et al., 1996, 1998; Fruehling & Longnecker, 1997).

LMP2A/B do not affect EBV transformation of primary B lymphocytes into LCLs, LCL survival or virus replication (Longnecker et al., 1992, 1993a, b; Kim & Yates, 1993; Rochford et al., 1997; Speck et al., 1999). Studies with EBV recombinants revealed that BCR cross-linking on LCLs with null mutations in LMP2A results in activation of the lytic viral life cycle of EBV, whereas lytic infection is blocked in the presence of LMP2A. This block can be bypassed by raising intracellular free calcium levels by phorbol ester treatment (Miller et al., 1994a, b). In Burkitt’s lymphoma cell lines, LMP2A inhibited BCR-induced apoptosis, probably through inhibition of the activation of tyrosine kinases by BCR cross-linking (Fukuda & Longnecker, 2005). As LMP2A is expressed in most latently infected B lymphocytes in vivo, it is postulated to have a role in maintaining viral latency. However, a recent report described that, in the absence of other stimuli, LMP2A expression alone could lead to induction of the viral lytic life cycle in B cells (Schaadt et al., 2005). Short-term activation of LMP2A may therefore help to activate the lytic cycle, but long-term expression, as occurs during latency, is more likely to counteract lytic reactivation.

Given the role of LMP2A in bringing together Nedd4-like ubiquitin ligases with Lyn and Syk (see above), a model proposes that LMP2A exerts its dominant-negative effect on BCR signalling by withdrawing Src family PTKs and Syk from the BCR and targeting them for degradation. However, the PPPPY motifs of LMP2A do not seem to be essential for blocking BCR signalling in LCLs (Ikeda et al., 2001), which argues against this model. The dominant-negative effect of LMP2A on BCR signalling could be mediated by its localization to lipid rafts in LCLs: LMP2A was shown to exclude the BCR from entering lipid rafts, where the BCR would otherwise initiate normal B-cell signalling (Dykstra et al., 2001).
LMP2A drives B-cell development in vivo. LMP2A provides survival and development signals in an LMP2A-transgenic mouse line with B cell-specific expression of LMP2A (Caldwell et al., 1998). Contrary to expectations, even BCR-negative cells were capable of progressing out of the bone marrow and entering the peripheral immune system, where they subsequently persisted. Further, LMP2A is able to drive B-cell development when this LMP2A mouse line is crossed into a recombinase-activating gene (RAG)-null background (Caldwell et al., 2000). In wild-type mice, cells either lacking the BCR or B cells of RAG-knockout mice would not be able to proceed with normal B-cell development. The ITAM motif was shown to be critical for this function of LMP2A (Merchant et al., 2000). In B cells from LMP2A-transgenic mice, LMP2A activated the Ras/PI3K/Akt pathway to mediate B-cell survival (Portis & Longnecker, 2004b). Further, the adaptor protein SLPI65 (SH2 domain-containing leukocyte protein) was identified as a downstream effector of LMP2A (Engels et al., 2001) and Nedd4 ubiquitin ligases were shown to downregulate LMP2A in B cells from LMP2A-transgenic mice (Ikeda et al., 2003).

DNA microarrays of primary B cells from LMP2A-transgenic mice, LMP2A-expressing human B-cell lines and LCLs revealed that LMP2A has multiple effects on global gene expression. It increases expression of genes associated with cell-cycle induction and inhibition of apoptosis, alters the expression of genes involved in DNA and RNA metabolism and decreases expression of B cell-specific factors and genes associated with immunity (Portis & Longnecker, 2003, 2004a; Portis et al., 2003). Notably, these alterations mirror those described for Hodgkin/Reed-Sternberg cells present in Hodgkin lymphoma, in which LMP2A transcripts have been identified (reviewed by Thorley-Lawson, 2001; Portis et al., 2003).

Function of LMP2A in epithelial cells. In epithelial cells, LMP2A function might differ from that in B cells. Tyrosine phosphorylation of LMP2A in epithelial cells was triggered by cell adhesion to extracellular matrix proteins, but was not mediated by Src PTks. Overexpression of the C-terminal Src kinase (Csk, a negative regulator of Src) resulted in LMP2A phosphorylation in in vivo and in vitro assays, with the ITAM being the major site of in vitro phosphorylation (Scholle et al., 1999, 2001). Notably, LMP2A has transforming capabilities and inhibits cell differentiation in the human keratinocyte cell line HaCaT and human foreskin fibroblasts (HFKs) (Scholle et al., 2000; Morrison & Raab-Traub, 2005). In HaCaT cells and in telomerase-immortalized HFKs, LMP2A activated the Akt kinase (but not MAPks) (Scholle et al., 2000; Morrison et al., 2003). Activation of Akt seemed to be responsible for cellular transformation induced by LMP2A in HaCaT cells (Scholle et al., 2000).

In HFK cells, Akt activation by LMP2A (ITAM-dependent) leads to phosphorylation and inhibition of the pro-apoptotic forkhead transcription factor FKHR and of the glycogen synthase kinase-3β (GSK-3β). Inhibition of GSK-3β by LMP2A results in the stabilization of the proto-oncogene β-catenin and depends on the ITAM and PY motifs of LMP2A (Morrison et al., 2003; Morrison & Raab-Traub, 2005). In B cells, LMP2A expression also mediated constitutive activation of Akt via PI3K, depending on Syk and Lyn, but in the absence of a survival phenotype (Miller et al., 1995b; Swart et al., 2000). In the Burkitt’s lymphoma cell line Ramos and the gastric carcinoma cell line Hsc-39, LMP2A may inhibit TGF-β1-mediated apoptosis through activation of the PI3K/Akt pathway (Fukuda & Longnecker, 2004). However, transgenic expression of LMP2A in mouse epithelium showed no effect on epithelial differentiation or survival (Longan & Longnecker, 2000).

In 293 cells transfected stably with LMP2A, MAPks Erk2 and JNK, but not p38, were activated, and activation of the Erk pathway was implicated in LMP2A-mediated cell migration (Chen et al., 2002). In squamous epithelial cells, LMP2A and LMP2B promoted cell spreading and motility, and studies with pharmacological inhibitors indicated that tyrosine kinases are involved (Allen et al., 2005). In NPC cells, LMP2A activated mTOR (mammalian target of rapamycin), a mediator of growth signals and proliferation, probably via the PI3K/Akt pathway (Moody et al., 2005).

Summary of EBV LMP1 and LMP2A. The signalling pathways engaged by EBV LMP1 are reminiscent of those triggered by CD40 or TNFR-1 following interaction with their cognate ligands (Eliopoulos et al., 1997; Gires et al., 1997; Sandberg et al., 1997; Floettmann et al., 1998; Hatzivassiliou et al., 1998; Kilger et al., 1998; Kieser et al., 1999; Uchida et al., 1999). The interaction between CD40 and CD40 ligand is important for multiple steps in T cell-dependent B-cell responses, including B-cell survival and proliferation, germinal centre and memory B-cell formation and antibody isotype switching, as well as affinity maturation (Durie et al., 1994; Dadgostar et al., 2002). LMP1 may therefore represent a constitutively active functional homologue of CD40, which ensures the survival of latently EBV-infected B cells. LMP2A mimics signalling patterns induced by the B-cell antigen receptor and is therefore thought to provide signals for survival and maturation that are normally triggered by the contact of a B cell with its antigen [see references in the paper by Mancao et al. (2005)], in addition to helping to maintain latency.

KSHV TMPs K1 and K15

KSHV was originally identified in Kaposi’s sarcoma (KS) tissues in 1994 by Chang et al. (1994) and is also associated with primary effusion lymphoma (PEL) and the plasma-cell variant of multicentric Castleman’s disease (MCD) (reviewed by Schulz, 2000, 2006). The TMPs of KSHV are the highly variable K1/VIP and the multiply spliced K15, encoded, respectively, at the ‘left’ and ‘right’ ends of the 138 kbp coding region of the viral genome. Unlike LMP1 and LMP2A, however, both are mainly expressed during the...
lytic-replication cycle (Lagunoff & Ganem, 1997; Glenn et al., 1999; Choi et al., 2000a; Jenner et al., 2001; Nakamura et al., 2003).

**K1/VIP.** Encoded by ORF K1, K1/VIP (variable ITAM-containing protein) is a 46 kDa, type I transmembrane glycoprotein (289 aa) (Fig. 1; Russo et al., 1996; Lagunoff & Ganem, 1997; Lee et al., 1998a). Its extracellular N-terminal domain contains several N-glycosylation sites and displays a high degree of genetic variability between different KSHV isolates. This led to the definition of five major subtypes of K1 (A–E), each containing several distinct variants (Nicholas et al., 1998; Cook et al., 1999, 2002; Hayward, 1999; Zong et al., 1999; Lacoste et al., 2000a). K1 has been shown to oligomerize via its extracellular domain (Fig. 4; Lee et al., 1998a; Lagunoff et al., 1999). The cytoplasmic domain of K1 of 38 aa contains an ITAM, which is highly conserved between different K1 subtypes and is similar to the one found in LMP2A (Fig. 3; Table 2; Lee et al., 1998b).

The K1 protein has transforming capabilities: K1 can induce morphological changes and focus formation in rodent fibroblasts (Lee et al., 1998a) and can substitute functionally for the StpC gene in the context of the HVS genome with regard to immortalization of common marmoset T lymphocytes to IL-2-independent growth and the induction of lymphomas in primates (Lee et al., 1998a). The in vivo transforming potential of K1 was also demonstrated in the background of murine γ-herpesvirus 73 (Douglas et al., 2004). By using monoclonal antibodies (mAbs) to the N-terminal domain of K1, K1/VIP expression has been demonstrated by immunohistochemistry in MCD tissue, but not in KS lesions (Lee et al., 2003).

**Signal-transduction pathways activated by K1.** A chimeric protein consisting of the extracellular and...
transmembrane domain of CD8 fused to the cytoplasmic domain of K1 induced cellular tyrosine phosphorylation and calcium mobilization upon stimulation with a CD8 antibody when expressed stably in KSHV-negative B cells (BJABs) (Lee et al., 1998b). In this system, a single motif of the ITAM (Y²⁷¹YSL or Y²⁸²³TOP) was shown to be sufficient for induction of cellular tyrosine phosphorylation, but both were important for mobilization of intracellular calcium. Further, this CD8–K1 chimera is phosphorylated at Y²⁷¹ and Y²⁸² of its ITAM motif upon α-CD8 stimulation (Fig. 3). Co-transfection studies in Cos1 cells indicate that Syk could be a kinase responsible for this phosphorylation (Lee et al., 1998b) and that this is followed by subsequent recruitment of the SH2 proteins Syk, Vav and PI3K. In glutathione S-transferase (GST)-pulldown assays, GST–K1 interacted with SH2 domain-containing proteins Lyn, Syk, p85ζ of PI3K, phospholipase C-γ2 (PLCζ2), RasGAPζ12, Vav 1/3 and protein tyrosine phosphatases 1/2 and Grb2 upon tyrosine phosphorylation, with each tyrosine of the ITAM motif contributing to the interactions in distinct ways (Lee et al., 2005). Phosphorylated forms of Syk, Cbl and PI3K, but not of Vav and Blk, were detected in antibody-stimulated CD8–K1-expressing BJAB cells (Lee et al., 1998b). Phosphorylated Syk kinase and phosphorylated PLCζ2 were also detected in B cells transfected with full-length K1, depending on an intact ITAM (Lagunoff et al., 1999). Syk interaction was also observed with a K1 ITAM peptide phosphorylated on both tyrosines (Lagunoff et al., 1999). In line with these data, signal transduction of full-length K1 is impaired in a Syk-deficient cell line, pointing to a role of this PTK in downstream signalling events of K1 (Lagunoff et al., 1999).

K1/VIP constitutively activates the transcription factor nuclear factor of activated T-cells (NFAT) in KSHV-negative B cells as a consequence of homodimer formation via the extracellular domain (Lagunoff et al., 1999). K1-mediated NFAT activation was shown to be dependent on PI3K, Syk and PLCζ2 (Lee et al., 2005). K1 mAbs recognizing aa 92–125 (encompassing the C2 and Ig-like region, Fig. 3) could efficiently induce an increase in intracellular calcium concentration and cellular tyrosine phosphorylation in B cells transfected stably with K1 (Lee et al., 2003, 2005). However, in PEL cells, the transfected CD8–K1 chimera was not able to induce intracellular calcium mobilization or activation of NFAT upon antibody stimulation, and was impaired in its ability to induce cellular tyrosine phosphorylation (Lee et al., 2002).

In B cells, K1 was found to activate PI3K and Akt and to inactivate the negative regulator of the PI3K/Akt pathway PTEN, depending on the ITAM (Lee et al., 1998b; Tomlinson & Damania, 2004). Activated Akt phosphorylates, and thereby inactivates, pro-apoptotic factors such as caspase 9, GSK-3β, Bad and members of the FKHR family of transcription factors, which results in cell survival. K1-expressing cells showed increased phosphorylation of FKHR, but not of Bad, caspase 9 or GSK-3β, and K1 was shown to be able to protect cells from FKHR- and Fas-mediated apoptosis (Tomlinson & Damania, 2004).

Induction of the expression and secretion of VEGF by K1 has been shown in epithelial and endothelial cell lines (Wang et al., 2004). K1 can induce the expression of matrix metalloproteinase 9 in endothelial cells (Wang et al., 2004). Inhibition of Lyn kinase activity in KVL-1 cells, a cell line derived from a K1 lymphoma, resulted in decreased VEGF induction and NF-κB activity (Prakash et al., 2005). When K1 was expressed in BJAB cells, Lyn kinase activity was found to be increased, with concomitant VEGF induction and NF-κB activation (Prakash et al., 2005). The same group demonstrated that systemic administration of the NF-κB inhibitor Bay 11-7085 or an anti-VEGF antibody reduced K1 lymphoma growth significantly in nude mice.

In the context of KSHV tumorigenicity, it has been postulated that lytic viral proteins may contribute to tumorigenicity by exerting paracrine effects. In reporter assays, K1 activated AP-1 in BJAB cells (Lagunoff et al., 2001; Lee et al., 2005) and NF-κB and AP-1 when expressed transiently in Cos1 cells (Samaniego et al., 2001). In KS endothelial cells and B cells transfected with K1, NF-κB-dependent promoter activity was induced. In KS cells expressing K1, the induction of secretion of inflammatory cytokines implicated in KS lesion formation, such as IL-6, IL-12 and granulocyte–macrophage colony-stimulating factor, was observed (Samaniego et al., 2001; Prakash et al., 2002). In B cells, transfected K1 induced the expression of IL-1β, IL-8, IL-10, monocyte-derived chemokine and Rantes (Lee et al., 2005).

In K1 transgenic mice, serum IL-12 levels were impaired severely and basic fibroblast growth factor (bFGF) expression was upregulated in lymphocytes and tumours (two of 13 mice developed tumours) of K1 mice (Prakash et al., 2002). bFGF is an autocrine growth factor for endothelial cells that promotes growth and angiogenesis of AIDS-KS cells (Samaniego et al., 1995). In B lymphocytes of K1 transgenic mice, NF-κB and Oct-2 were constitutively active and tyrosine phosphorylation and the activity of the PTK Lyn were increased (Prakash et al., 2002).

**K1 can downregulate BCR surface expression and has an effect on the viral life cycle.** Similar to LMP2A, K1/VIP downregulates the expression of the BCR at the cell surface of BJAB cells (Lee et al., 2000). The underlying mechanism seems to involve the extracellular domain of K1, which interacts with the μ chains of the BCR complex, thereby inducing the retention of BCR subunits in the endoplasmic reticulum (Lee et al., 2000). K1 expression has been reported to both augment (Lagunoff et al., 2001) and repress (Lee et al., 2002) lytic replication; the latter effect requires a functional K1 ITAM motif. A KSHV microarray showed that the majority of viral genes were downregulated in TPA-treated BCBL-1 CD8–K1 cells (Lee et al., 2002).
KSHV K15: predicted structure, evolution and conserved motifs. Like LMP2A, K15 is encoded by a multiply spliced gene of eight exons, which is located between the viral terminal-repeat region and ORF75 and features up to 12 transmembrane domains (Fig. 1). However, there is little protein sequence similarity between the two and the cytoplasmic domain is at the C-terminal end of K15, whereas it is at the N-terminal end of LMP2A (Fig. 2; Glenn et al., 1999; Poole et al., 1999; Choi et al., 2000a).

Expression of K15 transcripts was identified in unstimulated KSHV-positive PEL cells and found to be upregulated upon lytic-cycle induction (Glenn et al., 1999; Poole et al., 1999; Choi et al., 2000a). Multiple, alternatively spliced transcripts are generated from the K15 gene, with the most prominent transcript containing eight exons (Fig. 1). The sequences of all K15 cDNA clones isolated so far are predicted to contain a common C-terminal cytoplasmic region (encoded by exon 8) linked to a variable number of transmembrane domains. Several splice variants identified use an alternative splice donor and start codon in exon 1, which would be predicted to join a part of exon 1 out of frame with the other exons, resulting in an alternative 6 aa at the N terminus of the resulting protein (Glenn et al., 1999; Choi et al., 2000a). In transfection experiments, the eight-exon K15 isoform gives rise to a protein with an apparent mass of approximately 45 kDa that associates with lipid rafts (Choi et al., 2000a; Brinkmann et al., 2003).

In uninduced PEL cell lines, a 23 kDa protein was detected by Western blotting with a mAb raised to the C-terminal domain of K15 (Sharp et al., 2002). However, in epithelial cells infected with recombinant KSHV, an approximately 45 kDa protein was detected with a polyclonal K15 antibody upon lytic-cycle induction (M. M. Brinkmann & T. F. Schulz, unpublished results), in keeping with the increased mRNA expression upon induction of the lytic cycle (Glenn et al., 1999; Choi et al., 2000a; Jenner et al., 2001; Nakamura et al., 2003) and the protein observed in transient-transfection assays (Choi et al., 2000a; Brinkmann et al., 2003).

Two different forms of ORF K15, K15-P (predominant, found in the majority of KSHV genomes tested) and K15-M (minor), have been identified (Glenn et al., 1999; Poole et al., 1999), which are almost identical concerning splicing pattern and protein structure, but show as little as 33 % amino acid identity (Glenn et al., 1999; Poole et al., 1999; Choi et al., 2000a). As most K15 studies published so far have been performed with the P type of K15, only signalling motifs of K15-P are depicted in Fig. 2.

The putative signalling motifs in the cytoplasmic C-terminal domain of the two K15 variants are highly conserved (Table 2), suggesting the conservation of associated functional properties. As depicted in Fig. 2 and Table 2, the cytoplasmic domain of K15-P contains a proline-rich motif that could potentially serve as an SH3-B motif (PP^{287}PLPP) and a motif that is reminiscent of a TRAF-binding site (ATQ^{475}PTDD). Furthermore, two potential highly conserved SH2-B sites are present in the cytoplasmic domain of K15-P (VFGY^{431}ASIL and DDLY^{481}EEVL). However, the YASI motif is not preceded by a negatively charged amino acid in either K15 type and may not serve as an SH2-B motif, but rather as an internalization motif.

Signal-transduction pathways activated by K15. The cytoplasmic domain of K15-P and K15-M can interact with TRAFs 1, 2 and 3 (Glenn et al., 1999; Brinkmann et al., 2003). The cytoplasmic domain of K15-P was further shown to interact with Hax-1, partly through the Y^{431}ASI motif of K15-P (Fig. 2; Sharp et al., 2002). Hax-1 can inhibit Bax-induced apoptosis (Sharp et al., 2002), but the relevance of its interaction with K15-P is not clear. Via its C-terminal domain, K15-P also interacts with members of the Src family of PTKs (Src, Hck, Lck, Fyn and Yes) and is phosphorylated in vitro at Y^{481} of its putative SH2-B motif (Y^{481}EEV) by these kinases (Brinkmann et al., 2003). The same tyrosine residue was shown to be phosphorylated constitutively in BJAB cells transfected with a CD8–K15-P chimera (Choi et al., 2000a). Following cross-linking, the CD8–K15-P chimera inhibited BCR signalling through the putative SH3-B (PP^{387}PLPP) and SH2-B (Y^{481}EEV) motifs (Choi et al., 2000a). When expressed in 293 epithelial cells, the K15-P eight-exon isoform activates the NF-κB and AP-1 transcription factors and the MAPK Erk2 via the classical Ras/Raf/MEK pathway and JNK1, and this depends on an intact Y^{481} residue (Brinkmann et al., 2003).

RRV R1

Two viral isolates of RRV (Desrosiers et al., 1997) have been sequenced completely: RRV 17577 (Searles et al., 1999; GenBank accession no. AF083501) and RRV 26-95 (Alexander et al., 2000; GenBank accession no. AF210726). RRV belongs to the Old World RV2 lineage of γ2-herpesviruses and has been detected predominantly in B lymphocytes (Rose et al., 1997; Mansfield et al., 1999; Greensill & Schulz, 2000). The ORF at the left-hand side of the LUR was termed R1, due to its resemblance to KSHV K1 (Figs 1 and 3; Searles et al., 1999; Alexander et al., 2000).

R1 structure and conserved motifs. Similar to K1/VIP, R1 is a glycosylated, type I transmembrane protein of 423 aa (Fig. 3) with an apparent molecular mass of 70 kDa by SDS-PAGE. Its N-terminal extracellular domain (aa 1–224) is 27 % identical (40 % similar) to KSHV K1 and shows homology to CD16 (Damanía et al., 1999). The extracellular domains of K1 and R1 contain cysteine residues that may form disulfide linkages. The single transmembrane domain of R1 is succeeded by a long, cytoplasmic, C-terminal domain of 170 aa (aa 235–423), which contains a number of potential SH2-B motifs (Fig. 3). The five distal SH2-B motifs are of the consensus sequence YXXL and, of these motifs, Y^{394}HGL and Y^{407}NHL or Y^{407}NHL and Y^{419}DWL could potentially
resemble an ITAM motif. The five membrane-proximal motifs, of the consensus YXXA/P/T/V, also have the potential to bind to SH2 domain-containing proteins (Damania et al., 1999). The R1 protein is localized on cytoplasmic, possibly endosomal, membranes (Damania et al., 1999).

**Signal-transduction pathways activated by R1.** Full-length R1 interacts directly with the PTK Syk, but not Src, and is phosphorylated by Syk in vitro and in vivo (Damania et al., 2000). A chimeric protein consisting of the extracellular and transmembrane domains of the CD8 receptor fused to the cytoplasmic tail of R1 elicits intracellular calcium mobilization, cellular tyrosine phosphorylation and NFAT activation in B cells upon stimulation with an x-CD8 antibody (Damania et al., 2000). Full-length R1 also activates NFAT constitutively (Damania et al., 2000), indicating that R1 is capable of inducing events leading to B-lymphocyte activation.

Like K1, R1 has oncogenic properties: expression of R1 in Rat-1 cells induces morphological changes and focus formation, and injection of R1-expressing Rat-1 cells into nude mice results in formation of multifocal tumours (Damania et al., 1999). When the Stp gene in the HVS genome was replaced with either the R1 or K1 gene under control of the Stp promoter, these recombinant viruses could immortalize T lymphocytes from common marmosets to IL-2-independent growth, similar to wild-type HVS (Damania et al., 1999).

**RRV sequences resembling K15.** The right-hand side of the LUR of RRV, between ORF75 and the terminal-repeat region, has the potential to encode an ORF reminiscent of EBV LMP2A and KSHV K15 (Fig. 1). This ORF was therefore termed R15 for RRV isolate 26-95 by Alexander et al. (2000), but unfortunately the Ox-2 homologue of RRV isolate 17577 was also termed R15 by Searles et al. (1999). Computational analysis of the region between ORF75 and the terminal repeats revealed an ORF with a potential splicing pattern similar to that of K15 and LMP2A, and this splicing could be verified by RT-PCR (unpublished observations). Similar to K15, this ORF has the potential to encode a transmembrane protein composed of up to 12 transmembrane domains joined to a cytoplasmic C-terminal domain, which features several potential SH3-B motifs and motifs suitable for phosphorylation by cellular kinases, but not a YEEV-like motif noted to be crucial for K15-mediated signal transduction (unpublished observations).

**KSHV and RRV summary.** The functional properties of the K1 and R1 transmembrane proteins suggest an involvement in signalling circuits linked to the B-cell receptor. However, both K1 and R1 are expressed during the lytic-replication cycle and it is therefore difficult to envisage a role similar to that proposed for LMP2A, i.e. the provision of survival signals for latently infected B cells that ensure their escape from the apoptotic fate met by the vast majority of B cells that do not encounter the appropriate antigen. A contribution to prolonged cell survival during the lytic-replication phase and a role in regulating latency in B cells appear possible, however, on the basis of the currently known properties of K1 and R1.

K15 combines aspects of signalling of LMP2A (e.g. the recruitment of Src kinases; inhibition of BCR-induced signalling) with those of LMP1 (e.g. the recruitment of TRAFs and the activation of NF-κB and JNK). The currently available data are compatible with a role of K15 in protecting virus-producing cells against apoptosis.

**HVS: saimiri transforming protein (Stp) and tyrosine kinase-interacting protein (Tip)**

The simian herpesviruses HVS and HVA induce T-cell lymphomas and leukaemias in several primate species other than their natural host (Melendez et al., 1969a, b; Daniel et al., 1974; reviewed by Fickenscher & Fleckenstein, 2001). HVS, a T-cell-specific virus, naturally infects squirrel monkeys without causing disease (Melendez et al., 1968; Falk et al., 1972). The left end of the HVS genome was found to be highly variable and HVS strains were therefore classified into the three subgroups A, B and C, which differ with respect to their oncogenic potential (Desrosiers & Falk, 1982; Medveczky et al., 1984, 1989). HVS strains A and C immortalize common marmosets to IL-2-independent growth and the most highly oncogenic HVS strain, C, can also immortalize human, rabbit and rhesus monkey lymphocytes and cause fulminant lymphoma in rhesus monkeys and some New World primates (Desrosiers et al., 1986; Szomolanyi et al., 1987; Biesinger et al., 1990, 1992; Bröker et al., 1993). Among HVS C strains, isolate HVS C-488 is more transforming than isolates HVS C-484 and C-139 (Fickenscher et al., 1997). These biological differences have been assigned to the genes encoded at the left end of the LUR of HVS (Fig. 1).

**Oncogenic potential of StpA and StpC-Tip.** ORF1 of HVS strain A11 encodes the oncogenic protein saimiri transforming protein (Stp) A (164 aa) (Murthy et al., 1989) and ORF1 of strain B (SMHI) encodes the StpB protein (171 aa) (Fig. 1; Choi et al., 2000b; Hö r et al., 2001). Two proteins, termed StpC (ORF2) and tyrosine kinase interacting protein (Tip), are derived from a bicistronic transcript of HVS strain C-488 (Fig. 1) and are responsible for the oncogenic potential of HVS strain C (Biesinger et al., 1990, 1995; Jung et al., 1991; Medveczky et al., 1993a, b; Lund et al., 1995, 1996; Fickenscher et al., 1997; Duboise et al., 1998a). StpA has been shown to be required for in vitro T-cell transformation and in vivo leukaemogenesis (Desrosiers et al., 1985, 1986; Murthy et al., 1989). Recombinant HVS lacking either StpC or Tip is unable to immortalize T lymphocytes in vitro to IL-2-independent growth or to produce fatal lymphomas in infected common marmosets, as induced by wild-type HVS. However, StpC and Tip are dispensable for replication and persistence (Duboise et al., 1998a). Tip
expression alone is not sufficient for oncogenic transformation in rodent fibroblasts (Jung et al., 1991), but induces T-cell lymphomas in transgenic mice (Wehner et al., 2001) and rabbits infected with an HVS Tip-484 deletion mutant survive (Lund et al., 1997a). In contrast to StpB, StpA and StpC transform rodent fibroblasts (Jung et al., 1991), and transgenic mice expressing StpC-488 or StpA-11 develop tumours (Murphy et al., 1994; Kretschmer et al., 1996).

**StpA and StpC are structurally similar.** Although StpA, B and C are only weakly homologous (StpB is 28% identical to StpA and 22% identical to StpC), they show structural similarities. In both StpA and C, a highly acidic N-terminal end is followed by collagen-like repeats (Gly–X–Y, with X or Y being a proline or glutamine residue) and a hydrophobic membrane anchor. Whilst StpC has 18 direct uninterrupted repeats of a collagen motif (G–P–P or G–P–Q, encompassing 54 aa) in its N terminus (Fig. 3), StpA has only nine that are not repeated directly (Biesinger et al., 1990; Geck et al., 1990; Lee et al., 1997). StpB lacks the collagen-like cluster (Jung & Desrosiers, 1991, 1994; Lee et al., 1997). Several lines of evidence point to an important role of the collagen repeats of StpA and StpC in transformation. First, StpB, which is not capable of transforming rodent fibroblasts, lacks collagen repeats, but when 18 collagen-repeat sequences are introduced into its N terminus, it is able to oligomerize, activate NF-κB and transform rodent fibroblasts (Choi et al., 2000b). Second, a mutation disrupting the collagen repeats has been shown to abolish the transforming activity of StpC-488 (Jung & Desrosiers, 1994). StpC-488 is membrane-bound and localizes primarily to perinuclear compartments in rodent fibroblasts (Jung & Desrosiers, 1991, 1994). Stpc (102 aa) has apparent masses of 20 and 22 kDa, with the 22 kDa form being phosphorylated in vivo at serine residue S

**StpA, StpB and StpC: cellular ligands and signal transduction.** StpA and B both contain SH2-B motifs of the consensus YAEV/I and all three Stp proteins have TRAF-binding sites (Table 2). Y

\[ \text{Y}^{115} \]

of the StpA Y

\[ \text{Y}^{115}\text{AEV} \]

motif is crucial for binding to Src and is phosphorylated by Src in vitro (Lee et al., 1997). Tyrosine phosphorylation of StpB by Src (in vivo) leads to subsequent binding of Lck and Fyn in vitro (Lee et al., 1997). Activation of Src kinases by StpB in 293-T cells was not observed by Choi et al. (2000b). Another group reported that StpB was, like StpA, phosphorylated in vitro and in vivo in the presence of Src in Cos cells (Hör et al., 2001). Hör et al. (2001) further showed that Src binding to StpB was abolished when Y

\[ \text{Y}^{118} \]

of the potential StpB SH2-B motif Y

\[ \text{Y}^{118}\text{AEI} \]

was mutated, and that Src binding to StpA and StpB seems to occur via the SH2 domain of Src. Furthermore, StpA binds STAT-3 (via StpA aa P

\[ \text{P}^{34}\text{TPYL}^{38} \]

and mediates phosphorylation of STAT-3 by binding Src, which results in the activation of STAT-3 transcriptional activity (Chung et al., 2000; Park et al., 2004). StpB was also shown to bind STAT-3, but to activate STAT-3 only weakly (Park et al., 2004).

Although StpA-11 and StpC-488 bind to TRAF-1, -2 and -3 via their TRAF-binding sites (Table 2) and StpB interacts with TRAF-1 and -2, StpA and StpB are not able to activate NF-κB (Lee et al., 1999; Choi et al., 2000b). Activation of NF-κB by StpC in epithelial cells is dependent on an intact TRAF-binding motif (residues P

\[ \text{P}^{10} \]

or I

\[ \text{I}^{11} \]

; Lee et al., 1999) and the presence of functional TRAF-2 and NIK (Sorokina et al., 2004). Interestingly, studies with recombinant HVS carrying the StpC gene with a P

\[ \text{P}^{10} \]

→ R

\[ \text{R}^{10} \]

point mutation showed that TRAF interaction and NF-κB activation by StpC are not essential for transformation of common marmoset T lymphocytes in vitro and in vivo, but are crucial for immortalization of primary human T lymphocytes (Lee et al., 1999). Stable expression of StpC-488 and a dominant-negative TRAF-2 mutant in Rat-1 cells dramatically suppressed NF-κB activity and the transformation of Rat-1 cells by StpC (Lee et al., 1999). These data suggest that binding to TRAFs seems to be a major component of the oncogenic potential of StpC, reminiscent of the LMP1 protein of EBV, but TRAFs do not seem to be the only effectors for StpC-associated oncogenicity.

Cellular Ras has been identified as an additional interaction partner of StpC. StpC is able to activate the Ras signalling pathway, as indicated by a two- to fourfold increase in the ratio of Ras-GTP (active form) to Ras-GDP and constitutive activation of the MAPK Erk2 (Jung & Desrosiers, 1995). Ras binding to StpC was shown to be abolished with StpC mutants showing no or reduced transforming potential (Jung & Desrosiers, 1994, 1995). Replacement of StpC with Ras in the context of the HVS genome showed that Ras could substitute for the StpC-488 protein in lymphocyte transformation, but with lower efficiency (Guo et al., 1998).

**Intracellular ligands of Tip.** Two different variants of the Tip protein have been described: Tip-484 of HVS strain 484 (ORF2, 214 aa; Geck et al., 1990; Lund et al., 1995) and Tip-488 of HVS strain 488 (ORF1, 256 aa; Biesinger et al., 1990, 1995). They show 71% amino acid identity, with Tip-484 lacking 42 aa (aa 37–79 of Tip-488) in its N-terminal domain.

Both Tip proteins can form stable complexes with the T-cell kinase Lck (Biesinger et al., 1995; Lund et al., 1996). The binding domain in Tip for the Lck kinase has been mapped to (i) a region encompassing the potential Tip SH3-B motif (10 aa) that has been shown to bind to the SH3 domain of Lck, (ii) a CSKH (C-terminal Src-related kinase homology) domain (10 aa) that mediates binding to the C-terminal half of Lck and (iii) the 18 aa region between these two motifs (Fig. 3; Jung et al., 1995b; Lund et al., 1996; Hartley et al., 2000; Schweimer et al., 2002; Bauer et al., 2004). The Lck-binding domain of Tip-484 is sufficient for activation of Lck in vitro and in vivo and for in vitro STAT-3 binding and activation if expressed in T cells (Lund et al., 1999). Furthermore, Tip can be phosphorylated in vitro by Lck (Biesinger...
et al., 1995; Jung et al., 1995a, b). The SH3 domains of Src, Lck, Hck, Lyn, Fyn and Yes were shown to bind to Tip peptides (Schweimer et al., 2002).

Tip-488 interacts with a WD repeat domain-containing endosomal protein termed p80. This interaction was reported to lead to lysosomal-vesicle formation and subsequent targeting of the Lck kinase into lysosomes for degradation. Furthermore, Tip interaction with p80 and Lck resulted in the downregulation of TCR and CD4 surface expression, respectively (Park et al., 2002). Tip localizes to lipid rafts in transfected T cells and HVS-C488-transformed common marmoset T cells (Park et al., 2003). Whilst lipid-raft association of p80 depended on co-expression of Tip in transfected 293 cells, interaction of Tip and Lck was required for the recruitment of the TCR to lipid rafts (Park et al., 2003). Cho et al. (2004) described that Tip can block TCR signalling and immunological-synapse formation by sequestering Lck.

Effect of Tip on Lck kinase activity. It is a contentious issue whether Tip binding to Lck results in an increase or decrease of Lck kinase activity (reviewed by Isakov & Biesinger, 2000). In T cells and 293 cells transfected with Tip-484 (Lund et al., 1997a; Hartley et al., 1999, 2000) and in HVS-484-infected human peripheral blood T lymphocytes (Lund et al., 1997a), Lck kinase activity was found to be elevated. Expression of Tip-488 was also reported to enhance Lck kinase activity in T cells (Wiese et al., 1996). Others observed downregulation of cellular tyrosine phosphorylation and suppression of Lck and Zap-70 activity in stably Tip-488-transfected T cells (Jung et al., 1995a). In addition, the transformed phenotype of NIH 3T3 fibroblasts expressing activated Lck kinase was suppressed by coexpression of Tip-488 (Jung et al., 1995a).

However, in one study (Kjellen et al., 2002), Tip-484 and -488 were analysed in parallel and both were able to stimulate Lck kinase activity in vivo and in vitro. Mutation of Y114 in Tip-488 was found to enhance the suppression of cellular tyrosine phosphorylation and to increase Lck-binding activity compared with wild-type Tip (Guo et al., 1997). In contrast, Kjellen et al. (2002) found that mutant Tip-488 Y114F could stimulate Lck activity as well as wild-type Tip.

Studies with Tip-488 in the context of the complete HVS genome showed that a Tip protein in which the prolines in the SH3-B motif have been substituted by alanines (HVS-TipΔSH3-B) was unable to bind Lck, but was still able to immortalize common marmoset T lymphocytes in vitro and in vivo (Yoon et al., 1997; Duboise et al., 1998b), suggesting that Lck binding to Tip is not essential for immortalization. However, abrogation of Lck binding to Tip resulted in altered characteristics of the transformed cells/lymphomas (Duboise et al., 1998b).

Tip activates NFAT, NF-κB, STAT-1 and -3. Tip-484 induces the binding of STAT-1 and -3 to DNA in T cells in an Lck-dependent manner (Lund et al., 1997b, 1999). Tip-484 is phosphorylated by Lck at Y72 (Y114 in Tip-488) of the Y72XPQ motif and, subsequently, STAT-1 and -3 are phosphorylated and STAT-dependent transcription is induced in 293-T cells (Hartley & Cooper, 2000; Kjellen et al., 2002). STAT-3 can be phosphorylated in vitro by a complex of GST–Tip and Lck, and T cells infected with recombinant HVS or expressing recombinant Tip showed a significant increase of DNA-binding activity of STAT-1 and -3 and increased in vivo phosphorylation of STAT-3 in the presence of Lck (Lund et al., 1997b). Tip-484 and -488 were shown to stimulate NFAT- and STAT-3-dependent transcription in T cells (Hartley et al., 2000; Kjellen et al., 2002). A recombinant HVS strain C-488 virus containing a mutated Tip (Y114XPQ to F114XPQ) lost the capability to activate STAT-1 or STAT-3, but was still able to transform human T lymphocytes, implying that growth transformation by HVS is independent of STAT activation (Heck et al., 2005).

Furthermore, Tip-488 interacts with the nuclear RNA-export factor Tap (Tip-associated factor) and co-expression of Tip and Tap in T cells results in upregulated surface expression of adhesion molecules and activation of NF-κB (Yoon et al., 1997). However, the role of Tap in Tip signalling is not clear. When expressed in T lymphocytes, Tip was shown to promote T-cell apoptosis by Fas in the presence of active Lck (Hasham & Tsygankov, 2004). Tip and StpC were shown to act synergistically in the induction of NF-κB activity and IL-2 gene expression in T cells (Merlo & Tsygankov, 2001).

HVA two-in-one (Tio) protein

HVA, an HVS-related virus of spider monkeys (Melendez et al., 1972c), causes fulminant lymphomas in various New World primates (Melendez et al., 1972a, b). HVA isolates from two strains (810 and 73) have been described to transform monkey T cells to permanent growth in vitro and in vivo (Hunt et al., 1972a, b; Laufs & Melendez, 1973; Melendez et al., 1973).

Tio shares sequence similarity with StpC and Tip of HVS. In transformed monkey T cells, a single, spliced mRNA derived from the highly variable ‘left’ region of the HVA genome (strain 73) is transcribed and gives rise to a protein of 269 aa with a predicted mass of 29 kDa (Albrecht et al., 1999; Albrecht, 2000). The term ‘Two-in-one’ (Tio) for this protein is derived from its sequence similarity to HVS strain C oncogenic proteins StpC and Tip (Fig. 3). Tio shares 36% amino acid identity in its N-terminal one-third with StpC, whereas its C-terminal two-thirds are 33% identical to Tip (Fig. 3; Albrecht et al., 1999). However, the collagen repeats noted in StpA and C (see above) are fewer in number (four) and interrupted by proline-rich regions in the Tio protein (Fig. 3). Tio and Tip share a CSKH motif, a conserved SH3-B motif (187PQLPRR) and a serine-rich motif of unknown function (Fig. 3; Albrecht et al., 1999). Tyrosine residues
of motifs Y^{136}IPW (Y^{127}TF in Tip) and Y^{167}PKN (Y^{155}PPD in Tip) are conserved between Tip and Tio, whereas the tyrosine residue of the putative SH2-B motif of Tio, Y^{171}KKL, is not conserved (Fig. 3).

**Tio interacts with and is phosphorylated by PTKs.** In lysates of transformed monkey T cells and in 293-T cells transfected with recombinant Tio, protein bands of 43 and 46 kDa are detected. Additionally, a homodimeric form of Tio is observed in 293-T cells (Albrecht et al., 1999). When co-transfected in 293-T cells with Src family members of PTKs Lck, Src and Fyn, a tagged Tio protein is in vivo-phosphorylated on tyrosine and binds directly to Lck, Src and Fyn. Fluorescence-spectrometry assays performed with a Tio peptide encompassing its SH3-B motif revealed that this binds to GST–SH3-domain fusion proteins of Lyn, Hck, Lck, Fyn, Src and Yes, but not of Abl, the p85 

subunit of PI3K or Grb2 (Albrecht et al., 1999).

Direct binding of Tio to the SH2 domains of Lck, Src and Fyn is only observed when Tio is tyrosine-phosphorylated (Albrecht et al., 1999). Interaction of Tio with SH2 domains seems to be specific for members of the Src family of PTKs, as binding of Tio to SH2 domains of Abl, Vav, Grb2 and PLCg was not observed. Thus, Tio combines functions of Tip regarding its interaction with PTKs of the Src family via its SH3-B motif and with StpA/B with respect to binding to Lck, Src and Fyn via its SH2-B motif in a phosphotyrosine-dependent manner.

Recombinant HVS-C488 in which the StpC and Tip genes were replaced with the Tio sequence of HVA strain 73 is transformation-competent in cultured monkey and human T cells, implying that Tio is an oncoprotein (Albrecht et al., 2004). By using this Tio-recombinant HVS, Albrecht et al. (2005) showed that mutation of the SH3-B site of Tio abolished binding of Lck and subsequent phosphorylation of Tio at Y^{136} by Lck, leading to a loss of transforming activity.

**Summary of HVS and HVA.** StpA, StpB, StpC, Tip and Tio augment survival signals for latently infected T cells. As for LMP1, the NF-kB pathway appears to contribute to the transforming potential of some Stp proteins, but others, e.g. the Ras/ERK pathway, are involved as well. Recruitment of Lck is mediated by Tip and Tio, and may contribute to the transforming properties of the latter.

**Outlook**

Our present knowledge of the signal-transducing properties of γ-herpesviral TMPs illustrates interesting similarities between proteins with apparently divergent functions in the viral life cycle. In spite of some quite detailed understanding of signalling pathways engaged by these different viral proteins, it is still not possible to predict why the same signalling pathway may, for example, be linked to transformation in the case of some, but not other, viral proteins. Expression of these proteins during latency appears to be associated with an essential (LMP1, StpA, StpC, Tip, Tio) or at least contributing (LMP2A) role in tumorigenesis, probably by mimicking physiological signals required for the survival of B or T cells. K1, K15, R1 and R15, expressed during lytic replication, may provide similar survival signals to virus-producing cells and thereby extend their lifespan. Whether this translates into a contributing role in the oncogenic qualities of KSHV and RRV is currently unclear.

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