REVIEW

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Gamma Herpesviruses: Pathogenesis of Infection and Cell Signaling

J. RAJČÁNI[a], M. KÚDELOVÁ[b]

[a]*Institute of Microbiology and Immunology, Jessenius Medical Faculty, Martin, Slovakia*
e-mail virural@savba.sk

[b]*Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia*

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ABSTRACT. Altered cell signaling is the molecular basis for cell proliferation occurring in association with several gamma herpesvirus infections. Three gamma herpesviruses, namely EBV/HHV-4, KSHV/HHV-8 and the MHV-68 (and/or MHV-72) and their unusual cell-pirated gene products are discussed in this respect. The EBV, KSHV as well as the MHV DNA may persist lifelong in an episomal form in the host carrier cells (mainly in lymphocytes but also in macrophages, in non-hornifying squamous epithelium and/or in blood vessel endothelial cells). Under conditions of extremely limited transcription, the EBV-infected cells express EBNA1 (EB nuclear antigen 1), the KSHV infected cells express LANA1 (latent nuclear antigen 1), while the MHV DNA carrier cells express the latency-associated protein M2. With the full set of latency-associated proteins expressed, EBV carrier cells synthesize additional EBNAs and at least one LMP (latent membrane protein 1). The latent KSHV carrier cells, in addition to LANA1, may express a viral cyclin, a viral Fas-DD-like ICE inhibitor protein (vFLIP) and a virus-specific transformation protein called kaposin (K12). In MHV latency with a wide expression of latency-associated proteins, the carrier cells express a LANA analogue (ORF73), the M3 protein, the K3/IE (immediate early) proteins and M11/*bcl-2* homologue proteins. During the period of limited gene expression, the latency-associated proteins serve mainly for the maintenance of the latent episomal DNA (a typical example is EBNA1). In contrast, during latency with a broader spectrum gene expression, the virus-encoded products activate transcription of otherwise silenced cellular genes, which leads to the synthesis of enzymes capable of promoting not only viral but also cellular DNA replication. Thus, the latency-associated proteins block apoptosis and drive host cells towards division and immortalization. Proliferation of hemopoetic cells, which had become gamma herpesvirus DNA carriers, can be initiated and strongly enhanced in the presence of inflammatory cytokines and by virus-encoded analogues of interleukins, chemokines and IFN regulator proteins. At early stages of tumor formation, many proliferating hemopoetic and/or endothelium cells, which had became transcriptionally active under the influence of chemokines and cytokines, may not yet be infected. In contrast, at later stages of oncogenesis, the virus-encoded proteins, inducing false signaling and activating the proliferation pathways, bring the previously infected cells into full transformation burst.

Abbreviations

AIDS	acquired immunodeficiency virus
AM	adherent mononuclear (cells)
Apo1	apoptosis receptor 1 (Fas receptor)
ART	activator of replication and transcription
BL	Burkitt's lymphoma
BRLF	*Bam*HI R leftward fragment (EBV DNA)
BZLF	*Bam*HI Z leftward fragment (EBV DNA)
CD	cluster of differentiation (leukocyte marker)
cdk	cyclin-dependent kinases
c-jun	cellular ju-nana (japanese expression for 17 sarcoma virus)
CMV	cytomegalovirus
CNS	central nervous system
CREB	(cAMP-response element)-binding protein
CTAR	C-terminal activator regions
DD	death domain
DED	death effector domain
DS	dyad symmetry (EBV DNA region)
EBER	Epstein–Barr encoded nonpolyadenylated RNA
EBNA	Epstein–Barr nuclear antigen
EBV	Epstein–Barr virus
FADD	Fas receptor-associated death domain
Fas	FS-7 associated cell surface (protein)
FGARAT	*N*-formylglycinamide ribotide aminotransferase
FLICE	FADD-like interleukin converting enzyme inhibitor protein (vFLIP)
FR	family of repeats
GAS	gamma-activated sequence
GPCR	G-protein coupled receptor
HAX-1	HS-1 associated protein X-1
HHV	human herpesvirus
HIV	human immunodeficiency virus
HL	Hodgkin's lymphoma
HLA	human leukocyte antigen
HS-1	hemopoetic specific protein 1
HSV-1	herpes simplex virus 1
HVS	herpesvirus saimiri
ICE	interleukin-1β converting enzyme
IE	immediate early proteins
IFN	interferon
IKK	inactivator kinases
IL	interleukin
IM	infectious mononucleosis

IRF	IFN regulating factor
IS	immunosuppression
ITAM	immunoreceptor tyrosine-based activator motif
K-bZIP	KSHV analogue of the EBV-specified Zta
KIP/CIP	kinase inhibitor protein/cyclin inhibitor protein
KS	Kaposi's sarcoma
KSHV	Kaposi's sarcoma (associated) herpesvirus
LANA	latent nuclear antigen
LCL	lymphoblastoid cell lines
LMP	latent membrane protein
LPD	lymphoproliferative disorders
LTP	large tegument protein
MAPK	mitogen activated protein kinase
MapK/MKK	mitogen activated kinase/kinase cascade
MCD	multicentric Castleman disease
MCP	monocyte chemoattractant proteins
MHV	murine herpesvirus
NFAT	nuclear factor activator of T cells
NF-κB	nuclear factor κB
NIK	NF-κB inducing kinase
NPC	nasopharyngeal carcinoma
OBP	*ori*-binding protein
PAN	polyadenylated nuclear RNA species
PEL	primary effusion lymphoma
Rb	retinoblastoma (proteins)
RR	ribonucleotide reductase (genes)
RS	Reed–Sternberg (cells)
Rta	R transactivator protein (R fragment encoded)
Sos	son of sevenless (protein)
STAT	signaling transduction and transcription
TK	thymidine kinase
TNF	tumor necrosis factor
TNFR	TNF receptor
TPA	4β,9α,12β,13α,20-pentahydroxytiglia-1,6-dien-3-one 12β-myristate 13α-acetate ('12-O-tetradecanoylphorbol 13-acetate')
TRADD	TNFR-associated death domain
TRAF	TNFR-associated factor
vCyclin	viral cyclin
VEGF	vascular endothelial growth factor
vFLIP	viral FLICE (caspase 1) inhibitor protein
vGPCR	viral G-protein coupled receptor
vMIP	viral macrophage inflammatory protein
VZV	varicella zoster virus
Zta	Z ('Zebra') transactivator protein (Z fragment encoded)

CONTENTS

1 BIOLOGICAL PROPERTIES OF GAMMA HERPESVIRUSES

Gamma herpesviruses comprise a herpesvirus subfamily, whose members, by tradition, have been regarded as lymphotropic. Their typical human representative, Epstein–Barr virus (EBV/HHV-4), however, replicates better in epithelial cells than in B cells. While EBV belongs to the Lymphocryptovirus genus (gamma-1 herpesvirus), the majority of gamma herpesvirus subfamily members belong to the Rhadinovirus genus (gamma-2 herpesviruses). Gamma herpesviruses persist lifelong in host lymphocytes. Their circularized plasmid-like DNA duplicates together with the host cell DNA. A special mechanism assures the distribution of viral DNA molecules into dividing host progeny cells. Due to the close relationship between viral and cell DNA replications, non-lytic infection of lymphocytes may cause lymphoproliferative disorders (Table I) in man as well as in mammals.

In contrast to EBV, which causes infectious mononucleosis at primoinfection, primary infection with rhadinoviruses (such as KSHV/HHV-8) is clinically silent. The latent KSHV reactivates in immunosuppressed subjects (often accompanying HIV infection or organ post-transplantation treatment).

Some other gamma herpesviruses (such as EBV and possibly MHV) promote the development of malignant disease also in natural (non-IS) hosts. The nonhuman primate and ungulate gamma herpesviruses are usually not recognized as pathogens for their natural hosts; on the other hand, they cause lymphoproliferative disease in heterologous hosts that are not too distantly related. As shown later, during latency, the gamma herpesviruses express non-structural proteins, which disregulate the host cell growth cycle. Several of their gene products (viral cyclins, LMP1, EBNA2, latent proteins), immortalize latent gamma-herpesvirus carrier cells in culture. When expressed in transfected cells, the latter become immortalized as well. In addition, gamma herpesviruses produce proteins interfering with cell signaling. These are either analogues

of cellular signal transduction pathway proteins or anti-apoptotic proteins (vFLIP, *bcl-2* product, GPCR). Finally, viral IL analogues (vIL-6, vIL-10) contribute to the proliferation of carrier lymphocytes. The aim of this paper is to describe the mechanisms of action of these unusual viral proteins, which participate in host cell transformation. Some latency-associated proteins help to maintain latency in dividing cells (a feature not found in alpha herpesviruses, which reside latent in nondividing cells). Others (vCyclins) activate the cellular *cdk*, which phosphorylate and inactivate the retinoblastoma protein(s) to drive cells into the next phase of the division cycle. Additional latent proteins constitutively produce false growth signals and activate their transmission along the intracellular signaling pathways. Our interest will be focused on the two human gamma herpesviruses (EBV and KSHV) and on the MHV. The latter, at least in laboratory mice, seems to mimick the cell transforming effects of KSHV and/or EBV, and therefore might be useful for studying the pathogenesis of Kaposi's sarcoma and/or the development of EBV-related lymphomas and sarcomas.

Table I. Overview of gamma herpesviruses causing lymphoproliferative disorders[a]

Virus[b]	Abbreviation	Natural host	Reference	Note[c]
Genus Lymphocryptovirus				
Epstein–Barr virus Human herpesvirus 4	EBV/HHV-4	man	Baer *et al*. 1984	DNA fully sequenced
Herpesvirus papio	HVP	baboon	Falk *et al*. 1976	
Genus Rhadinovirus				
Kaposi's sarcoma herpesvirus[d] Human herpesvirus 8	KSHV/HHV-8	man	Russo *et al.* 1996	DNA fully sequenced; tumors under immune deficiency conditions
Herpesvirus saimiri	HVS	squirrel monkey	Albrecht *et al.* 1992	DNA fully sequenced; tumors in owl and marmoset monkeys
Herpesvirus ateles	HVA	spider monkey	Albrecht 2000	DNA fully sequenced; tumors in marmosets
Murine herpesvirus Rhesus monkey rhadinovirus	MHV-68 RRV	bank vole *Macaccus* monkey	Virgin *et al*. 1997 Desrosiers *et al*. 1997	DNA fully sequenced;
Herpesvirus sylvilagus		cottontail rabbit	Medveczky *et al*. 1989	
Alcephaline herpesvirus 1	AHV-1	wilderbeest	Ensser *et al.* 1997	DNA fully sequenced; tumors in cattle and wild ruminants
Bovine herpesvirus 4	BHV-4	cattle	Bublot *et al*. 1992	association with lymphoproliferation not confirmed
Equine herpesvirus	EHV-2	horse	Telford *et al.* 1995	DNA fully sequenced; association with lymphoproliferation not confirmed

[a]Tumors developing in man under natural conditions are listed in Table V.
[b]*Italics* – viruses which are discussed in this paper.
[c]Only tumors developing in heterologous hosts are listed here.
[d]Originally referred to as Kaposi's sarcoma associated herpesvirus.

2 GENOME STRUCTURE OF GAMMA HERPESVIRUSES

It has become clear from the analysis of the genomes of at least eight gamma herpesviruses sequenced so far that they carry genes common for many (probably all) representatives of the alpha- and/or beta herpesvirus subfamilies. The family-common herpesvirus genes (Table II lists the gamma herpesvirus genes which are homologous also to HSV-1 genes) can be found among all the three kinetic classes (nonstructural immediate early genes, early genes as well as late structural genes). The total length of homologous sequences comprises at least 25 % of the total gamma herpesvirus DNA sequence. The family-common genes loca-

ted within the conserved regions of the gamma herpesvirus DNAs are arranged in blocks labeled either I–IV (according to Nicholas) or I–VII (van Regenmortel *et al.* 2000) (Fig. 1). As expected, the sequences of these individual genes, when compared to their homologous counterparts, are not fully identical; they usually reveal co-linear stretches, which may be longer among viral DNAs coming from the same subfamily than among the DNAs coming from members of different subfamilies (Nicholas 2000; Damania *et al.* 2000). For example, the family-common gB gene sequence variations within the gamma herpesvirus subfamily confirmed its further subdivision into two groups termed γ-1 and γ-2. The comparison of the DNA polymerase genes, on the other hand, has led to reclassification of the position of HHV-6 and HHV-7, now classified as beta herpesviruses. Both these relatively novel human herpesviruses were originally isolated from T lymphocytes, where they may reside in a latent form. Therefore, they were initially assumed to belong to the γ-subfamily. Recently, the genome comparisons showed that they belong to beta herpesviruses where they form the separate Roseolovirus genus (Gompels *et al.* 1995).

Table II. Conservative herpesvirus genes common for all (alpha, beta, gamma) herpesvirus subfamilies and encoding the general herpesvirus proteins

Gamma HV gene block	EBV ORF	HHV-8 ORF	MHV-68 ORF	Protein/function[a]	HSV-1 ORF/gene block[c]
I	BALF2[b]	6	6	**ssDNA-binding protein**	UL29/II
I	BALF4	8	8	glycoprotein B	UL27/II
I	BALF5	9	9	**DNA polymerase**	UL30/II
II	BXLF2	22	22	**glycoprotein H**	UL22/IV
II	BcLF1	25	25	**major capsid protein**	UL19/V
III	BDRF1	29b	29b	terminase, DNA packaging	UL18/V
III	BGRF1	29a	29a	DNA packaging	UL6/VI
III	BGLF4	36	36	phosphotransferase	UL13/VI
III	BGLF5	37	37	alkaline exonuclease	UL12/VI
III	BBRF3	39	39	**glycoprotein M**	UL10/VI
III	BBLF2	40	40	**helicase**	UL5/VI
III	BBLF3	41	41	**helicase component**	UL8/VI
III	BKRF3	46	46	**uracil DNA glycosylase**	UL2/VII
III	BKRF2	47	47	**glycoprotein L**	UL1/VII
IV	BLLF3	54	54	dUTPase	UL50/III
IV	BSLF1	56	56	**primase**	UL52/III
IV	BMLF1	57	57	posttranscriptional regulator	UL54/III
IV	BMRF1	59	59	processivity factor	UL42/I
IV	BARF1	60	60	**ribonucleotide reductase**	UL40/I
IV	BORF2	61	61	**ribonucleotide reductase**	UL39/I
IV	BPLF1	64	64	**large tegument protein**	UL36/I

[a]**Bold** – common proteins typical for each gene block**.**
[b]*Bam*HI A leftward fragment 2 (*see* EBV genome convention; Farrel 1992).
[c]*See* Fig. 1.

As mentioned, the official classification of herpesviruses (van Regenmortel *et al.* 2000) has recognized 7 conserved gene blocks in which the family-common herpesvirus genes are arranged. Their order in the HSV-1 DNA is VII/VI/V/IV/II/I/III. All the conserved genes are located within the UL segment of HSV DNA (Fig. 1D), starting with the UL1 (gL) as first and ending with the UL54 (IE63/ICP27) as the last. The legend to Fig. 1D (and Table II) lists the marker genes representing individual blocks, namely, the gL and uracil DNA glycosylase genes (gL/UDG) for block VII, the gM and part of genes coding for the helicase/primase complex (gM, h/p) for block VI, the major capsid protein gene (MCP) for V, the gH gene for block IV, the DNA polymerase and ssDNA binding protein genes (pol/ssBP) for block II, the large tegument protein and ribonucleotide reductase genes (LTP/RR) for block I, and finally the rest of the primase/helicase helper protein genes (h/p) for block III – (bold type in the Table II). The individual blocks of homologous genes in the EBV genome are ordered from block II, IV, V, VI to block VII, followed by III and I and located between the terminal and the multiple internal repeats (Fig. 1C). The order of individual blocks in the EBV DNA is reversed (as compared to HHV-1/HSV-1 DNA) and, in addition, the position of blocks III and I is transposed (Figs 1A, B).

Out of the herpesvirus family-common genes, at least four encode envelope glycoproteins while another three encode capsid and/or tegument structural components (all belong to the kinetic class-γ pro-

teins). Another 11 proteins are either enzymes and/or proteins directly participating in DNA replication or involved in the synthesis of inevitable nucleotides (non-structural kinetic class-β proteins). One protein, the posttranscriptional viral mRNA regulator UL54 protein homologue is class-α IE (immediate early) protein (Phelan and Clements 1998).

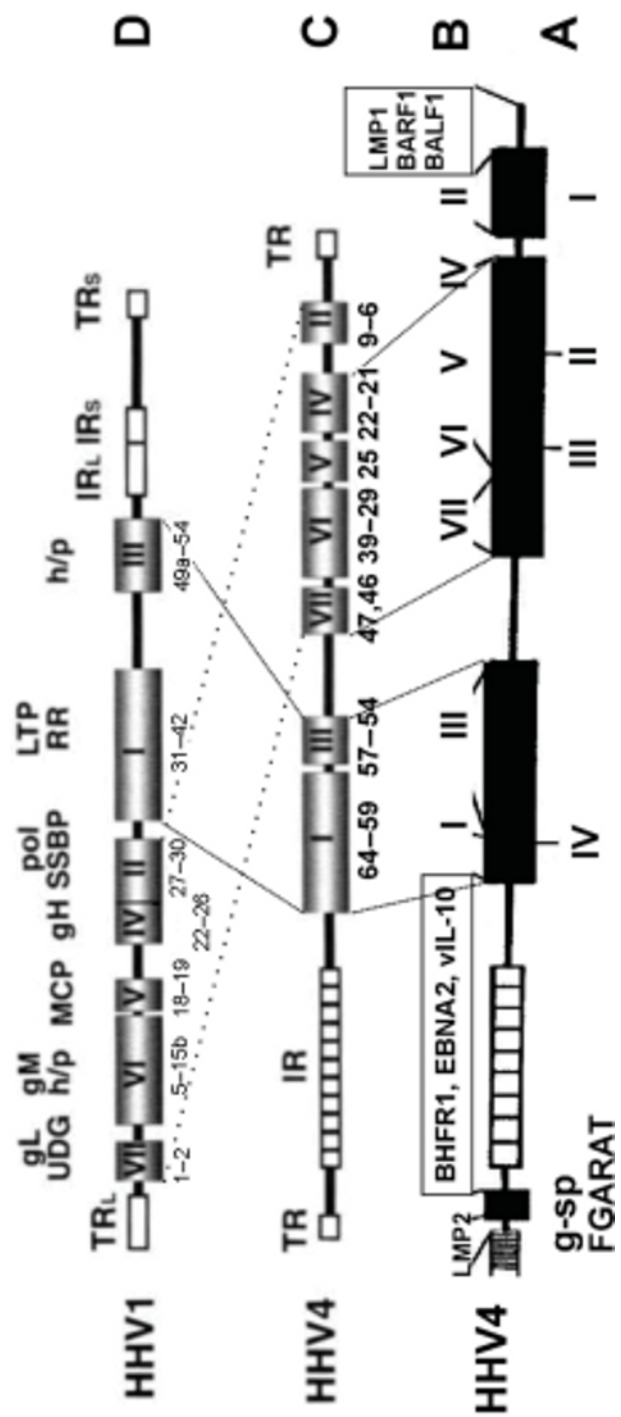

Fig. 1. Arrangement of the family-common herpesvirus genes in the herpes simplex virus 1 (HHV-1) and Epstein–Barr virus (HHV-4) genomes. **A**: Four conservative blocks of gamma herpesvirus genes (according to Nichols 2000) arranged in a reversed order (compare with Table II, *first column*)); the position of the γ-specific FGARAT gene is also shown (*see* Table III); **B**: seven conservative gene blocks according to the international classification (van Regenmortel *et al.* 2000); **C**: the conservative blocks as in B in a reversed order (from the *right* to the *left*), an opposite order is listed in Table II (*first column*); **D**: comparison of seven conservative gamma herpesvirus gene blocks with their position in the HHV-1 genome; note that the block complex III/I in the EBV DNA has been transposed as related to the position of the block complex VII/VI/V/IV/II.

In addition to the genes which are common for herpesviruses of all 3 subfamilies, several genes may be found among the members of at least two subfamilies. As an example, the thymidine kinase was found in alpha and gamma herpesviruses (protein II/21 in rhadinoviruses, BXLF1 in EBV and UL23 in HSV); such genes are called α/γ. Alternatively, the *ori*-binding protein encoded by the HSV-1 UL9 gene can be found in human CMV, being an example of the α/β subfamily-common genes. The gene coding for GPCR, probably an IL-8 receptor homologue, is present in many gamma herpesviruses as well as beta herpesviruses

(in human CMV its equivalent is UL78, while in HHV-6/HHV-7 the corresponding equivalents are UL51 and UL85, respectively). Thus, GPCR is an example of β/γ genes. The gene for Ox-2 (the N-CAM homologue) can be found in KSHV (K14 gene) and in RRV (R15 gene) as well as in human CMV. The latter gene is an example of a "cross-subfamily" gene, which is neither confined to a single subfamily, nor is common for each member of a single subfamily (Nicholas 2000).

Last but not least, a large group of genes comprises the gamma-specific ones, which fall into 2 categories: the common gamma-specific genes (present in each gamma herpesvirus) and the single gamma herpesvirus-specific genes, some of which might be present in several (but not in each) gamma herpesviruses. Among the general (widely shared) gamma-specific genes (Table III) one can find the gene coding for anti-apoptosis protein *bcl-2*, the gene coding for the cell cycle regulator vCyclin, and the nuclear latency genes (such as EBNA1 and LANA1) which are involved in the replication of the plasmid-like viral DNA when latent in cells which undergo occasional division. The group of virus-specific genes, which may have analogues present in several (but not in each) gamma herpesviruses, are, for example, genes coding for latent membrane proteins (LMP1, LMP2a, Tio/two in one) involved in pathological signaling inducing immortalization as well as genes coding for viral IL and/or chemokine analogues involved either in immune evasion or in stimulation of host cell proliferation (Table IV).

Table III. Genes common for the gamma herpesviruses and encoding γ1 and/or γ2 specific proteins

Protein	EBV ORF	KSHV ORF	MHV-68 ORF	HVS ORF	Gene/function
FGARAT	BNRF1	75	75a,b,c	3	*N*-formylglycinamide-ribotide aminotransferase
CCP		K4	4	4	complement control protein
Bcl-2	BALF1	16	M11	16	B cell leukemia-2 (anti-apoptotic) protein analogue, *Bax*-inhibitor
vFLIP		71		71	FLICE[a] inhibitory protein
vCyclin	BKRF1	72	72	72	an analogue of cellular cyclin D
EBNA1	EBNA1	73 (LANA)	73	73	EB (or latent) nuclear antigen
G-PCR	BLR1	74	74	74	G-protein coupled receptor (IL-8 receptor homologue)
Rta	BRLF1	50	50	50	R transactivator, IE protein
Zta	BZLF1	K8			Z transactivator, K-bZIP (leucine zipper)

[a]FLICE – FADD like ICE (IL-1β converting enzyme), FADD – Fas-associated death domain.

3 EBV PATHOGENESIS: MOLECULAR ASPECTS

The Epstein–Barr virus was discovered in lymphoid cells derived from Burkitt's lymphomas. It was soon recognized that Burkitt's lymphoma (BL) cell lines fall into several categories: cells which express structural viral proteins (capsid formation, lytic virus replication), those which express several immediate early and nonstructural antigens (latency III) but contain no virus particles, and cells which express a single latency associated nuclear antigen (EBNA1) only (Kerr *et al.* 1992). The latter stage of latency described in nonproducer BL cells (such as Raji) has been termed latency I (Magrath 1990; Magrath *et al.*1992). The latency I state is associated with silencing of the lytic Cp/Wp EBNA1 promoters and activation of a downstream positioned latency-associated promoter termed FQp. Therefore, EBNA1 (*see below*) is the only EBV-encoded protein found in latency I nonproducer BL cell lines. *In vivo,* latency I occurs in memory B lymphocytes of subjects who recovered from infectious mononucleosis and then carry the silenced circularized plasmid-like EBV DNA (Sample *et al.* 1991). Another form of latency, termed latency II, is characterized by expression of the LMP1. This protein is transcribed from a distinct promoter, termed LMP1p. *In vitro,* latency II occurs following EBV infection of T lymphoma cells expressing the CD21 receptor and in human epithelial cell lines expressing this receptor (Li *et al.* 1992). Experimental infection of primary B cells generates latency III (Fig. 2). The latter latency pattern involves the expression of a full set of EBNAs (EBNA1, 2, 3A, 3b, 3C) and LMP proteins (LMP1, LMP2a) and of the EBNA leader protein (Gregory *et al.* 1988). This type of latency is most frequent in B cells *in vivo*. It generates immortalized (permanently dividing) lymphoblastoid cell lines, which may occasionally undergo lytic virus replication (Pope *et al.* 1968). For this

reason, reactivation of infectious EBV production may occur in throat washings of most asymptomatic EBV carriers (Yao *et al.* 1985). However, in acute IM, productive replication of EBV occurs mainly in nasopharyngeal epithelium cells causing saliva to become infectious (Gerber *et al.* 1972). Latently infected B cells

Table IV. Unique gamma herpesvirus genes encoding specialized gamma herpesvirus proteins[a]

Protein	EBV ORF	KSHV ORF	MHV-68 ORF	HVS ORF	Gene/function
LMP1/ STP/Tio	BNLF1	K1		1	latent membrane protein 1/*saimiri* transformation (associated) protein/two in one (protein)
Serpin	BARF1		M1		chemokine receptor?
			M2		latency protein
DHFR		2		2	dihydrofolate reductase
vIL-6		K2			IL-6 analogue
IE1B		K3	K3		immediate early (protein)
					BHV-4 analogue/HLA class I
					inhibitor
Chemokine		K4 (MIP1)			macrophage inflammatory protein 1
		K5			immediate early (protein) BHV-4 analogue
Chemokine		K6(MIP3)			macrophage inflammatory protein 3
IE/SAg				14	superantigen (IE protein)
CD59			M6	15	cluster of differentiation marker (analogue)
	BCRF1				IL-10 homologue
v-IRFs		K9, K10, K11			IFN-regulating factors
Kaposin		K12			transformation protein, latency associated
Ox-2		K14			N-CAM homologue
Tip/LMP2	LMP2	K15			tyrosine kinase interacting protein/latent membrane protein 2

[a]Some of these proteins are analogues of cellular proteins and/or chemokines

also contain an abundant population of Epstein–Barr nonpolyadenylated RNAs (EBERs) accumulated mainly in the nucleus and complexed with the cellular La protein. Their function is obscure, but they may be involved in blocking the activity of the IFN-induced kinase, which inhibits translation by binding the initiation factor eIF2 (Glickman *et al.* 1988).

The initial attachment of EBV to susceptible cells is mediated by the major envelope glycoprotein, gp350/220, which interacts with the CD21/CR2 complement receptor (Nemerow *et al.* 1985). Post-attachement events involve the gL/gH/gp42 glycoprotein complex formation (Speck *et al.* 2000). Clearly, gH requires gL in order to mediate membrane fusion and penetration. This complex, lacking gp42, is efficient for EBV entry into epithelial cells occurring in the absence of the CD21 receptor (Wang *et al.* 1998; Molesworth *et al.* 2000). Thus, adsorption to epithelial cells is mediated by a distinct attachment mechanism. The virion gp42 is needed for B cell penetration, in which latency III (a still nonlytic stage of the genome) is frequently established. The latter glycoprotein interacts with the HLA class II molecule, especially with HLA-DP, -DR or -DQ isotypes, explaining why persons carrying the above mentiones HLA isotype preferentially develop IM (Haan *et al.* 2000). As already mentioned, during primoinfection, B cells develop the third type of latency (latency III) and not lytic replication, because they are less permissive for EBV replication than the nasopharyngeal epithelium cells. The Cp/Wp directed transcription of EBNA mRNA and the LMP1 mRNA are regularly detected in the B cells coming from patients at the onset of IM (Tierney *et al.* 1994). Such tonsils also contain many B cells expressing EBERs, as well as EBNA2 and LMP1 antigens (Rickinson and Kieff 1996). The atypical mononuclear cells, occurring in large numbers in blood during acute IM, are predominantly lymphoblasts of the CD8 subset, which proliferate due to the expression of the above mentioned EBV-specific antigens as well as due to the expression of nonviral (cellular) proteins

(receptors) activated in B cells during EBV infection (for example, CD23, CD21, CD39, CD40, CD44 and others). Because CD8 T cells are essential for recovery from IM, in X chromosome-linked immunodeficiency, which is related to impaired T cell receptor differentiation, a fatal form of IM develops called X-linked lymphoproliferative syndrome. Alternatively, T-cell deficient AIDS patients having EBV infection develop oral hairy leukoplakia within their mouth cavity, characterized by thickening of squamous epithelium cells. This process based on impaired squamous epithelium differentiation program due to EBV infection.

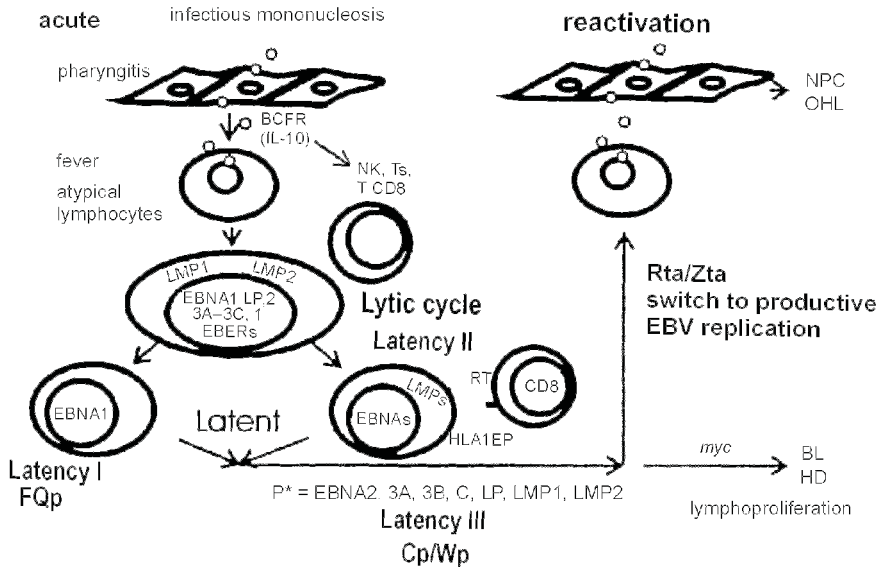

Fig. 2. Molecular pathogenesis of EBV infection (modified according to Kieff 1996). During acute stages post-infection, the virus replicates in the pharyngeal epithelial cells and nonproductively infects B cells (these then express EBERs, EBNAs, LMPs). The CD8 T cells react to the appearance of viral antigens in B cells. T cells proliferate, forming atypical blastic elements in the peripheral blood. During latency, the virus persists in B cells, where various forms of latency may develop (latency I – expression of EBNA1 only, latency II – EBNA1 and LMP1 expression; latency III – expression of a full set of latency-associated proteins). The latter form of latency may result in virus reactivation or lymphoproliferation occurs due to constant expression of LMP1 and other viral proteins exerting cell growth signaling. Additional intercurrent infections (malaria) in combination with EBV latency cause chromosomal translocations and *c-myc* activation (for details *see* text).

The key step towards lytic replication of EBV resides in the transcription of two transactivator proteins, termed BZLF1 and BRLF1 (Speck 1997; Zalani *et al.* 1996; Ragoczy *et al.* 1999). Both transactivator proteins are needed for the expression of structural EBV proteins as well as for replication of viral DNA from the lytic Ori_L initiation site (Feederle *et al.* 2000). The *Zta* (zipper transactivator encoded by the BZLF1 gene) is a basic leucine-zipper protein that binds to specific motifs in the promoter sequences of many early and late EBV genes. Similarly as the HSV ICP4 transactivator protein, it has an activation domain which acts in accord with cellular transcription factors such as TBP (TATA-binding protein) and TfIIID (Flint *et al.* 2000). The availability (expression) of *Zta* is regulated by several cellular transcription activator proteins, namely through at least two (or three) signaling cascades. *In vitro* (in non-producer BL cells), *Zta* expression can be activated by phorbol esters such as TPA. The cross-linking of B-cell surface IgM receptors due to antigen interactions was recognized as natural activator signal (it activates the tyrosine kinase-phospholipase/C-calcineurin pathway or acts *via* LMP1 expression). These stimuli recruit the cellular enhancer transcription proteins SP1, Atf1 and Mef2d, which positively regulate the initiation of *Zta* transcription from the EBV genome when latent in B cells. In contrast to reactivation of latency III, in latency II the LMP2a expression blocks B-cell receptor mediated antigen stimulated signaling (*see below*).

Several malignancies were found related to EBV infection (Table V). In a smaller portion of patients with X-linked lymphoproliferation, the anaplastic growth of B cells becomes dominant resulting in uncontrolled immunoblastic lymphoma. Such lymphomas may develop also in EBV-infected AIDS patients (Pedersen *et al.* 1991). With the introduction of a profound immunosuppressive therapy directed against the T-cell-mediated immune response, post-transplant lymphoproliferative disorders (LPD) of varying degree and severity were recognized following organ transplantation; their association with EBV has been sugges-

ted and in part confirmed (Klein and Purtillo 1981). However, the majority of non-Hodgkin lymphomas, which develop in non-immunocompromised patients, do not show EBNA and/or EBER positivity; this fact raises doubt on EBV etiology of LPDs and/or non-Hokgin lymphomas in other than immunocompromised subjects (Craighead 2000a).

Table V. EBV-associated lymphoproliferative diseases[a]

Tumor	Subtype	EBV positivity, %	Antigen expression	Latency type
Burkitt's lymphoma	endemic sporadic	85–100	EBNA1	I
Nasopharyngeal carcinoma		100	EBNA1, LMP1/2	II
Hodgkin's disease	mc/ld[b]	<90	EBNA1, LMP1/2	II
T-cell lymphoma		100	EBNA1, LMP1/2	II
Immunoblastic (B cell) lymphoma	transplantation or AIDS associated	100	EBNA1, 2, 3A, 3B, 3C, LMP1/2	III

[a]According to Rickinson and Kieff (1996).
[b]Mixed cellularity or lymphocyte depleted forms.

A lymphoproliferative condition most frequently associated with EBV is BL, which is endemic mostly in Africa and in South-East Asia. The disease apparently develops due to local circumstances such as malaria infection (Burkitt 1962). Rarely, a sporadic lymphoma unrelated to malaria can be recognized in developed countries (O'Connor *et al.* 1960). Both the sporadic and the endemic BL revealed a similar histological appearance (large lymphoid cells a occasional "starry sky" macrophages) and common typical histochemical markers (CD10 and the CD77BL glycolipid moiety). The BL patients display reciprocal translocations of the *c-myc* gene locus between chromosomes 8 and 14 (the heavy chain immunoglobulin locus) or 8 and 22 (the light chain immunoglobulin locus) (Bernheim *et al.* 1981; Dalla-Favera *et al.* 1982). The translocations in question liberate the *c-myc* locus from its usual transcription control (Nishikura *et al.* 1983). As mentioned above, the cell lines established from BL tumors frequently show latency III phenotype, rarely latency I phenotype. Stable group I latency cells show heavy methylation of the resident EBV DNA, especially in the Cp/Wp promoter region (Emberg *et al.* 1989; Jansson *et al.* 1992). Switching from the Qp promoter transcription to the Cp/Wp promoter driven transcription is the first step and also a prerequisite for activation of the latent genome, which then proceeds into activated B cells with the help of *Zta* and *Rta* proteins. The expression of these transactivation proteins is regulated by numerous mechanisms that are related to signal transduction cascades, which activate EBV replication in B cells. The *Zta* transcripts are spliced from the primary EBNA transcripts. The availability of the *Zta* mRNA is cell-regulated by means of at least 2 enhancer sites (promoter site ZI binds the cellular leucine-zipper proteins Mef2d and SP1, while the ZII promoter site binds the cellular Atf transcription factor). An excess of *Zta* downregulates its own transcription *via* binding to the ZIII promoter site (Chi and Carey 1993). The latency III BL cells express many B cell markers such as CD23 and CD21 (Kieff 1996). How exactly the EBV contributes to pathogenesis of BL is still poorly understood, since neither LMP1 nor EBNA2 are continuously expressed in noncultured BL cells found in the lymphoid tumor (*i.e.* noncultured BL cells show latency I). On the other hand, when EBV infects the nonpermissive B cells *in vitro,* these become latency III EBV carriers, with new antigens induced and immortalized. Subsequently they may undergo recombination events and chromosome translocations. Thus latency III may occur at an early stage of any lymphoproliferative process. The stable retention of EBV DNA and consecutive EBNA1 expression in BL cells argues for some role of this nuclear protein in maintaining and segregation of the viral episomal genome and in B cell growth achieved by binding to *oriP* (the latency promoter) and to appropriate viral and cellular gene promoters (Rickinson and Kieff 1996; Leight and Sugden 2000).

The link between nasopharyngeal carcinoma (NPC) and EBV infection was suggested by seroepidemiological studies. NPC cells are EBV DNA positive and express EBERs, which are markers of latent EBV infection of both the epithelium and lymphoid cells (Gillighan *et al.* 1990). Furthermore, NPC cells express EBNA1 translated from the long transcripts initiated at FQp and possess the *Bam*HI QUK splice structure, while the Cp/Wp promoter remains downregulated (Smith and Griffin 1992). In contrast to BL cells, LMP1 and LMP2a transcripts are present in NPC cells (Niedobitek *et al.* 1992). Nevertheless, the contribution of EBV to NPC development is still not fully elucidated. The tumor is clearly confined to the

South-Eastern Asian population and to particular HLA haplotypes such as HLA-A11 and B13 (Lu *et al.* 1990). Cytogenetic studies showed deletions on the short arm of chromosome 3 at sites postulated for tumor suppressor (anti-onc) genes other than p53 (Choi *et al.* 1993).

Epidemiological studies also raised the possibility that EBV is associated with Hodgkin's lymphoma (HL) and that IM increases the risk of HL development (Rosdal *et al.* 1974). Histological criteria for HL are the disrupted architecture of affected lymph node and the presence of Reed–Sternberg (RS) cells. The HL tumor tissue consists mainly of either lymphocytes (lymphocyte predominant form) or fibroblasts (nodular sclerosing form) or reveals a mixed cellularity. A lymphocyte depleted form has also been described. The RS cells express lymphocyte activation markers (CD30, CD70 and the IL-2 receptor) but rarely the B- or T-cell specific markers. EBERs and LMP1 antigens are regularly present, strongly suggesting latency II pattern (Herbst *et al.* 1991; Pallesen *et al.* 1991). However, the association between EBV and HL has been challenged, because there is no correlation between LMP1 and *bcl-2* protein expression in RS cells (Armstrong *et al.* 1992).

4 KAPOSI'S SARCOMA HERPESVIRUS (KSHV/HHV-8): A RECENTLY EMERGED HUMAN PATHOGEN

About 130 years ago, the Hungarian dermatologist Kaposi described a case of rare idiopathic pigmented skin sarcoma in an elderly Jewish male of Mediterranean origin. During the last two decades Kaposi's sarcoma (KS) became very frequent in AIDS patients (Friedman-Kien 1981). The disease may also occur in allotransplant recipients who underwent IS therapy (Akhtar *et al.* 1984). The DNA of the new gamma herpesvirus, termed KS associated (KSHV), has been identified in nondifferentiated spindle cells within skin lesions (Chang *et al.* 1994) and, before the onset of the disease, in mononuclear peripheral blood cells of HIV-positive KSHV carriers (Whitby *et al.* 1995). The virus spreads by sexual contact mainly among homosexual HIV-positive men, but in Central African countries where KSHV is endemic, also among HIV-negative subjects, where the virus can be acquired in childhood due to close family contacts (Schultz 2000). Rarely, HIV-negative Jewish males acquire the classical disease in the region of the Mediterranean Basin. Early stages of the disease show formation of aberrant vessels (lymphatic–venous shunts) lined by discontinuous endothelial cells. The sarcomatous lesions consist of fibroblastoid spindle cells (or KS cells), believed to represent proliferating elements derived from endothelium cells (Craighead 2000*b*). In early vascular lesions a small proportion of KS cells and endothelium cells show positivity for KSHV DNA, while in advanced nodular (sarcomatous) lesions the majority of spindle cells contain this DNA (Staskus *et al.* 1997). Furthermore, an EBNA1 protein analogue, called LANA (latent nuclear antigen), encoded by KSHV (Table III), was found to be expressed in the KS spindle cells (Dupin *et al.* 1999). In addition to KS, cells bearing the episomal KSHV DNA were found in other lymphoproliferative conditions, namely in angiofollicular lymph node hyperplasia, termed Castleman disease (CDe) and in body cavity-based non-Hodgkin lymphoma, the so-called primary effusion lymphoma (PEL). Castleman originally described a relatively benign lymphadenopathy, located in the mediastinum, in which the lymph node structure resembled to that of thymus (Castleman 1956). Histologically, the aberrant B cells ($CD5^+$, $KiB3^-$) along with plasmacytoid monocytes and light chain producing plasma cells represent a hallmark of CDe (Palestro *et al.* 1999). A key event in the pathogenesis of CDe is overproduction of B-cell growth factor(s), such as IL-6, leading to B-cell proliferation and plasma cell differentiation. A systemic multicentric variant at this condition (multicentric Castleman disease, MCDe), in which the Hassel's body-like central follicular structure is surrounded by abundant proliferating plasmablasts of B-cell origin, has been associated with HIV-1 infection and AIDS (Frizzera *et al.* 1983; Dupin *et al.* 2000). KSHV DNA as well as LANA was detected in the plasmablasts surrounding the hyalinized germinal centers containing rare capillaries (Dupin *et al.* 1999; Sharp and Boshop 2000). The lymphomatous effusions in PEL occur in pleural and peritoneal cavities but in the absence of a tumor mass. They are usually comprised by large pleomorphic cells expressing LANA1 (ORF73) and several other KSHV-encoded proteins (listed in Tables II–IV) such as the viral IL-6 (vIL-6/K2), the viral DNA polymerase processivity factor (ORF59), the capsid proteins encoded by ORF26 and ORF65 and the vIRFs (Katano *et al.* 2000).

The proliferation of KS cells and of the plasmablasts in PEL is maintained by a series of gene products such as the vCyclin, the viral interleukins, the KSHV K1-encoded LMP1 analogue and others (*see later*). In HIV-positive patients, the KS cells usually do not express enhanced cellular cyclin D activity. However, vCyclin (ORF72) expression is higher especially in the early lesions, supporting the "hit and run" hypothesis (Kennedy *et al.* 1999). The vCyclin/ORF72 and the ORF71 (encoding the anti-apoptotic protein vFLIP) are transcribed into a common bicistronic transcript. While the bicistronic transcript is translated to

vCyclin by the usual cap-dependent ribosomal machinery, the vFLIP protein is translated with the help of a ribosomal entry site, located upstream of the vFLIP gene within the vCyclin coding sequence (Bieleski and Talbot 2001; Grundhoff and Ganem 2001). The translation product vFLIP is a FLICE (Fas-associated death domain-like IL-1β converting enzyme) inhibitor and has been proposed to block apoptosis mediated by the Fas-receptor or the TNFRα ligand (Table III). In MCD, plasmablasts proliferate due to an overexpression of vIL-6, which is produced within the altered lymph nodes. Namely, vIL-6 induces a growth factor, termed vascular endothelial growth factor (VEGF), which is present in MCD lymph nodes, but not in normal ones (Nishi and Maruyama 2000). The details of cytokine-mediated paracrine growth will be discussed later.

As described above, both EBV and KSHV DNAs reside in B lymphocytes possibly in episomal (circularized) form expressing at least a single latency associated protein (EBNA1 or LANA1) or several latency-related proteins. To initiate the cascade transcription of nonstructural (early) and late structural virus genes, immediate early transactivator protein(s) must be produced that initiate lytic virus replication. The EBV IE transcription is regulated by several cellular transcription cofactors which modify the transcription and splicing of EBNA1 mRNA in order to prepare the messenger RNA for transactivator proteins *Zta* and *Rta* (encoded by BZLF1 and BRLF1 genes); both transactivator proteins are required for full expression of EBV proteins during lytic replication (Feederle *et al.* 2000). The KSHV gene K8 codes for leucine zipper protein K-bZIP (Table III) which is an analogue of EBV-specified *Zta* (Polson *et al.* 2001). The nearby located ORF50 of KSHV DNA encodes the Rta analogue called activator of replication and transcription (ART) (Wang *et al.* 2001). The latter protein seems to play an important role in the transition from latency to productive infection, since its C-terminal domain (amino acids 527–634) binds to a 16 bp consensus sequence within the IE K8 gene promoter (K-bZIP protein expression) and within the promoter of an additional IE gene, the ORF57. The latter encodes the HSV-1 IE63/ICP27 protein analogue, which regulates the nucleocytoplasmic transport of viral mRNA (Bello *et al.* 2000). The ART protein also binds to the promoter, which directs the transcription of an abundant non-coding polyadenylated nuclear (PAN) RNA species analogous to EBER (Song *et al.* 2001). The functional K-bZIP protein is expressed in KSHV virion producing cell lines derived from PEL lesions. It has a DNA-binding leucine-zipper domain in combination with a basic domain (bZip); it can be phosphorylated by cellular cyclin dependent kinases (*cdk*s) indicating some link between the host B cell proliferation and productive KSHV replication. In IE transcription, once activated, the expression of delayed early (non-structural) and late (structural) KSHV genes follows in a cascade resembling more HSV-1 lytic replication than the highly sophisticated EBV lytic reactivation. Once the lytic origin of KSVH DNA replication is provided in *cis*, the minimal late promoter regions are sufficient for transcription and expression of late genes (Chang and Ganem 2000). The requirement of viral DNA synthesis for efficient γ-gene transcription can be explained by altering the inhibited state of the originally latent genome during viral DNA copying (absence of any methylation in critical promoter regions, removal of negative inhibitory proteins, *etc*.).

5 MURINE HERPESVIRUS (MHV): ANIMAL MODEL FOR HUMAN GAMMA HERPESVIRUS

Blaškovič *et al.* (1980) reported the isolation of a new herpesvirus from free living rodents *Apodemus flavicollis* and *Clethrionomys glareolus*. The novel MHV, in contrast to the mouse cytomegalovirus, replicated in cell cultures of various species such as chick, rabbit, hamster, mink, swine, monkey as well as in cells of human origin (Svobodová *et al.* 1982). When newborn mice were inoculated with MHV-68 by oral or intranasal (i.n.) routes, the virus spread quickly to lungs (necrotising pneumonia), liver, spleen, kidneys, heart muscle, striated muscles and spinal ganglia (Blaškovič *et al.* 1984). In juvenile and adult mice, which were given various doses of MHV-68/MHV-72, hematogenous dissemination from lungs to heart muscle, spleen, liver, thymus, kidneys and mammary glands has been demonstrated by immunofluorescence (Rajčáni *et al.* 1985), by plaque assay and Southern blot analysis (Rašlová *et al.* 2001). Electron microscopy confirmed the replication of MHV in capillary endothelium cells of damaged alveolar septa. In survivors, especially in infected adults, persistent infection of spleen, lungs and kidneys has developed, but also trigeminal ganglia became involved. Additional studies (Rajčáni *et al.* 1986) confirmed the absence of neural spread of MHV-68 to Gasserian ganglia. On the other hand, explantation increased the rate of virus recovery from neural as well as non-neural tissues indicating the presence of non-productive latency without lytic virus replication. Summing up, the latency established in outbred mice seemed more dynamic than it is the case in HSV-1/2 latency, so that the term persistent infection (implicating continuous production of small amounts of infectious virus at least in lungs or spleen) was used in the above mentioned pilot studies.

MHV-68 has received worldwide attention when a Cambridge group showed that this virus is genetically related to EBV and to HVS (Efstathiou *et al.* 1990). They found that at least 9 MHV-68 DNA genes had a relatively high homology with EBV genes coding for proteins which contained 49–87 amino acids long stretches completely homologous with the corresponding EBV genes. Only the ribonucleotide reductase large subunit gene was found to be homologous also to the corresponding VZV and HSV-1 genes (cf. Table II) while the DNA polymerase gene was more related to EBV than to HSV-1. Therefore, the authors suggested that the new virus should be classified as a member of the gamma herpesvirus subfamily. These findings confirmed the statement that MHV was a new murine herpesvirus distinct from mouse CMV. However, it also became clear that the virus in question does not belong to the alpha herpesvirus subfamily as suggested in some pilot studies (Svobodová *et al.* 1982). Due to these results, and due to some new biological properties, which became more obvious in Balb/c than in conventional mice (spleen atrophy in acute disease as well as splenomegaly in healthy survivors at late intervals post-infection), Sunil-Chandra *et al.* (1992*a*,*b*) postulated that the virus is lymphotropic and infects predominantly B cells. Nevertheless, the above authors confirmed the crucial role of lungs in the pathogenesis of acute and chronic MHV disease stating that both the lungs and spleen are major sites of latency. In contrast to the dynamic latency observed in conventional MHV-infected mice, Balb/c mice developed a more typical non-productive form of latency including spleen, where viral DNA was present in the absence of acute virus replication. When μMT transgenic mice (which do not produce heavy μ chains and therefore do not possess mature B cells) were used to establish latency, the MHV DNA persisted in lungs, namely in the pulmonary epithelium cells (Stewart *et al.* 1998). In such mice, the IgM deficient but CD21 positive B cells may also be used for establishment of latency but, more frequently, macrophages and NK cells represent the main cell population, in which the viral DNA survives. This has been already shown by Mistríková *et al.* (1994), who claimed that adherent peritoneal and/or peripheral blood mononuclear cells (mainly macrophages) participate in virus dissemination during acute infection and may represent an important reservoir of latent virus in healthy adults which did not undergo acute disease. The population of peritoneal and bone marrow cells most frequently harboring the latent MHV DNA was identified as T-cell depleted adherent mononuclear cells (AMC) enriched by means of the anti-macrophage F4/80 monoclonal antibody (Weck *et al.* 1996, 1999). In addition, acute virus reproduction was not needed for establishment of MHV latency, a scenario already known with HSV. Last but not least, it was demonstrated that clearance of MHV producing B cells from blood can be achieved by cytotoxic T cells since microglobulin β-2 deficient mice showed elevated titers of the virus in the blood and spleen and developed a long lasting viremia in comparison with MHC I non-deficient mice. Furthermore, mice depleted of CD8 T cells failed to resolve the pulmonary disease and died. In contrast, the μ-chain deficient mice showed lower virus titers in the spleen confirming the importance of mature (IgM plus) B cells for acute (lytic) virus replication at early intervals post-inoculation. Summing up, mature B cells and macrophages replicate MHV at early intervals, while CD8 T cells participate in the clearance of infected B cells. Macrophages and possibly NK cells (in addition to lung epithelium) are the main site of MHV latency in lungs, spleen and bone marrow. In consequence of the trigeminal ganglion involvement after corneal inoculation (Rajčáni *et al.* 1986), the neurotropism of MHV was further investigated. In adult mice deficient in IFN-α/β receptor gene the virus replication appeared to be prominent at a peripheral inoculation site (similarly as in newborn and juvenile conventional mice) followed by perivascular CNS involvement (Terry *et al.* 2000). As expected, there was no spread of the virus along fila olphactoria after i.n. inoculation.

An important feature during either acute or chronic infections with strains MHV-68 (Usherwood *et al.* 1994) and MHV-72 (Mistríková and Mrmusová 1998; Rašlová *et al.* 2000) is the lymphoproliferative changes of T and B cells found in lungs, spleen and peripheral blood of relatively resistant adult Balb/c mice in association with limited virus replication. In splenomegaly, cytofluorimetric analysis revealed increased numbers of B lymphocytes and CD4 as well as CD8 T cells. After i.n. inoculation, a substantial population of infected B cells appears in mediastinal lymph nodes, where reactive CD8 T cells proliferate as first. Then, within several months, their numbers fall back to onset levels (Stevenson and Doherty 1998). A continued proliferation of CD8 as well as CD4 T cells (the later ones produce IFN-γ) could be demonstrated in the spleen of affected mice. Recently, increased amounts of both T cells and of blastic CD19 B cells were found in the blood of Balb/c mice at early and late intervals p.i. with MHV-72 (Mrmusová *et al.* 2002). Lymphoproliferative infiltrates developing in the spleen and lung consisted of a mixed population of B and T cells (Sunil-Chandra *et al.* 1994). The lymphoblastic elements present in lymphomas expressed the pan T CD45 marker (Mistríková *et al.* 1999). The frequency of lymphoproliferative disorders in MHV-infected mice varied between 9–13 %; their rate increased after IS either with cyclosporin or FK506 (Sunil-Chandra *et al.* 1994; Mistríková *et al.* 1999, 2000). Due to the high age of some chronically infected mice (the animals were kept under observation for up to 2 years), tumors other than non-Hodgkin lymphomas were also found

(spinocellular carcinomas of skin, anaplastic carcinomas, non-differentiated hemoblastomas and sarcomas). MHV was recovered by explantation (as a sign of positive latency) from nearly all lymphomas and sarcomas and from a proportion of anaplastic epiteloid cell tumors (*unpublished results*). Summing up, it seems that the pathogenesis of MHV infection in Balb/c mice shows similarity to EBV induced IM (atypical lymphocyte proliferation in the blood, spleen and lymph nodes). Furthermore, the lymphoproliferative changes in the spleen of latent MHV carriers histologically resembled either EBV-related non-Hodgkin lymphoma or KSHV-related multicentric lymph node hyperplasia (multicentric Castleman disease).

Recently, considerable efforts were made to identify the latency associated genes from those transcribed during individual phases (immediate early, early and late) of virus growth cycle (Fig. 3). The transcription of MHV-68 mRNAs during latency has been compared in peritoneal macrophages and splenocytes (a population of macrophages and CD19 B cells) harvested from IgM deficient mice (Virgin *et al.* 1999). In the absence of lytic gene transcription (no IE transcripts such as K3/IE1, ORF50/*Rta* and M8; no E transcripts such as ORF6/ssDBP and ORF9/DNApol, no ORF8/gB, no ORF25/MCP, and no M7/gp structural gene transcription) at least 4–9 latency candidate genes were transcribed in a differential manner. In peritoneal macrophages mainly M2 (latency associated protein) and M11 (*bcl-2* analogue) genes, the ORF73/LANA1 analogue (in comparison to KSHV) and the ORF74/GPCR gene were transcribed. In cells derived from the spleen, besides the M2 gene, transcription of M3 and M9 genes was prominent. It is noteworthy, that these latter genes were not found to be transcribed in peritoneal macrophages. Thus, while the latently infected peritoneal cells did not express the M3 and M9 genes, the latent spleen cells expressed not only the M2 but also the M3 and M9 genes.

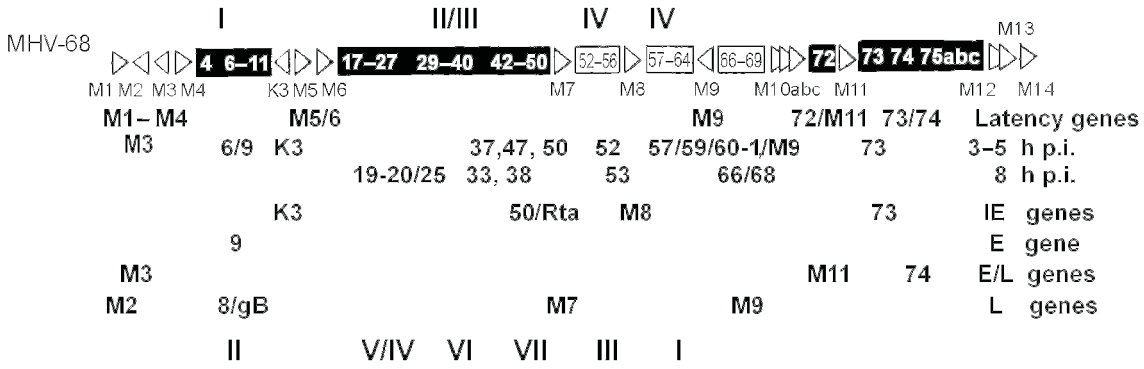

Fig. 3. The MHV-68 genome (modified according to the *in vivo* data of Virgin *et al.* 1999 and according to the *in vitro* data of Rochford *et al.* 2001 and Ahn *et al.* 2002). Four conservative gene blocks as shown in Table II in comparison with seven conservative gene blocks (*see* Fig. 1); the latency gene candidates according to Virgin *et al*. (1999): **M1** – serpin; **M2** – latency associated protein (expressed in macrophages and spleen B cells); **M3** – (expressed in spleen cells); **M4** – complement control protein (CCP); **M5** – IE superantigen analogue (HVS gene homologue); **M6** – ?; **M9** – capsid protein (expressed since 5 h p.i., not a true latency protein; cf. Ahn *et al*. 2002); ORF72 – vCyclin (homologue); **M11** – *bcl-2* homologue, herpesvirus specific (cf. Table III); ORF73 – LANA (latency nuclear antigen); ORF74 – GPCR (G-protein coupled receptor; IL-8 receptor homologue); IE/E non-structural gene expressed since 3–5 h p.i. (Ahn *et al*. 2002): ORF73 – immediate early protein; early nonstructural genes expressed since 3–5 h p.i. (Ahn *et al.* 2002): M3 – chemokine binding protein; ORF6 – ssDNA binding protein; ORF9 – DNA polymerase; K3 – IE1 protein, BHV-4 – gene homologue; ORF37 – alkaline exonuclease; ORF50 – *Rta* homologue (early transcriptional transactivator); ORF52 – unknown function, highly conserved among herpesviruses; ORF57 – IE transcript regulator (analogue of ICP27 from HSV-1); ORF59 – DNA polymerase processivity factor, an HSV-1/UL42 analogue; ORF60/61 – both RR subunits, HSV-1 UL40/39 analogues; cf. Table II); further early non-structural genes expressed since 8 h p.i.: ORF37 – alkaline exonuclease; structural genes expressed since 8 h p.i.: ORF19 – tegument protein; ORF20 – fusion protein; ORF25 – major capsid protein; ORF38 – early membrane protein; **M7** – an MHV-specific glycoprotein, gp150; ORF68 – glycoprotein (?) ORF33 – tegument protein, unclear function; ORF53 – (?); the immediate early (IE), the early (E), the early late (EL) and late (L) genes were listed according to Rochford *et al*. (2001). IE genes: K3, ORF50, **M8** – recently classified as ORF57 and ORF73; E genes: M3, M11, ORF74; L genes: M2, ORF8 (gB) and M9.

When using the cDNA array technique to study the early stages of lytic MHV replication *in vitro* (Ahn *et al.* 2002) the ORF73 (LANA analogue in comparison with KSHV) was found to be expressed with an α-gene kinetics from 5 h p.i. The early non-structural genes expressed at 5 h p.i. at the onset of lytic virus replication are the putative transactivator protein ORF50/*Rta* (not transcribed in latent cells) and the several genes involved in initiation and continuation of viral DNA replication (Fig. 3). The first structural gene was the M9 (capsid protein) expressed in overwhelming amounts from 5 or 8 h p.i., respectively. Thus, the ORF73/LANA1, the ORF74/GPCR and even the M11/*bcl-2* latency candidate genes cannot be regarded as

truly expressed throughout the latent infection defined as nonproductive MHV DNA persistence with highly limited gene expression.

Rochford *et al.* (2001) quantified the expression from selected lytic (K3, ORF50/*Rta*, M8, ORF9/DNApo1 and ORF8/gB) and the above candidate latency genes by multiprobe RNAase protection assay based on RT-PCR generated riboprobe templates. Following lytic infection of mouse 3T3 cells, the MHV genes could be classified according to their expression as immediate early (K3, ORF50/*Rta*), M8, M9 and ORF73/LANA), early (ORF72/vCyclin), early/late (M3, M11/bcl-2 and ORF74/vGPCR) and late (M2, M9/capsid and ORF8/gB). *In vivo*, viral gene expression could be detected in the lung as early as 1 d p.i. All latent transcript candidates were expressed in lungs (days 3–6) during the acute (virus-productive) phase of infection. From them, only two (M3 and M9) were detected in the spleen (on days 10–16) and three (M3, M9 and M11) were found in mediastinal lymph nodes (MLN) (on days 2–16) at both latency sites. Interestingly, the kinetics of expression was strikingly different between lungs, spleen and MLN. No expression of candidate latency transcripts ORF73, ORF74 and M2 was found in the spleen or in the MLN. Moreover, two lytic transcripts K3 and M8 were found to be also expressed. Levels of K3 transcripts become almost equivalent to those of M3 and M9 both in the spleen and MLN. Thus, the authors suggested the existence of further latency associated candidates indicating the establishment of latency-like DNA persistence in the absence of real nonproductive replication – K3 and M8. The function of the M8 protein is still unknown but may be similarly important as M9 for immune evasion and the establishment of latency. These results give rise to some uncertainty as to which genes are really latency-associated in the strict meaning of this term, and point to the possibility that different forms of MHV latency exist (in parallel to EBV). In the case of most strictly defined latency probably only M2 is expressed. It is possible, however, that additional genes may be expressed still in the absence of virus replication. Under such conditions the expressed genes may help to maintain the non-productive DNA persistence. These findings, furthermore, reflect the findings described in conventional mice, in which virus replication was seen more frequently than in Balb/c mice due to spontaneous reactivation.

The K3 gene product, similarly as the bovine herpesvirus 4 IE1 protein homologue, downregulates the HLA class I glycoprotein expression on the surface of cells (Stevenson *et al.* 2001), *i.e.* it has a function comparable with that of HSV1/2 IE protein ICP47. M3 is a broad-spectrum chemokine-binding secreted protein transcribed during acute infection and at early stages of latency (van Berkel *et al.* 1999). M9 is a structural (capsid) protein extensively expressed from the earliest stages of virus replication which is thought to show essentially a late expression kinetics. The M11, a *bcl-2* homologue, inhibits TNF-α induced apoptosis (Roy *et al.* 2000). It is an early/late gene continuously expressed in lungs and spleen of mice with persistent MHV infection. The expression of M9 and M11 in spleen and lungs points at a dynamic form of latency, while macrophages show a static form of latency. Possibly, various forms of MHV latency can spontaneously reactivate less or more frequently, depending on the pattern of limited expression of IE lytic genes such as K3, M9, M11. More recently, the M2 gene was suggested to represent a typical latency-associated protein, having been detected in nonproductively infected splenocytes (Husain *et al.* 1999), in nonproductively infected peritoneal exudate cells (Virgin *et al.* 1999) as well as in B cells coming from MHV-related lymphomas (Husain *et al.* 1999). After i.n. infection, the M2 mutant virus exhibited decreased establishment of latency and inefficient reactivation from latency. Noteworthy, the M2 mutant virus had no significant effect on the establishment of latency in macrophages (Jacoby *et al.* 2002).

6 GAMMA HERPESVIRUS GENES PROMOTING DNA REPLICATION, CELL DIVISION AND TRANSFORMATION

6.1 Nuclear gamma herpesvirus proteins and latency maintenance

EBNA1 and the ORF73/LANA are gamma herpesvirus proteins expressed during latency in cells carrying the circularized (episomal) virus genome. Latency, as defined here, means the presence of gamma herpesvirus DNA in the absence of proteins needed for virus reproduction. The production of EBNA1 (latency I) does not serve for the initiation of virus replication cycle, but rather to maintain latent episomal DNA as an episomal plasmid, which must be distributed into the daughter cells during division. The protein may also function as a key transcriptional regulator of the latent gene expression (Leight and Sugden 2000). The viral DNA component required for duplication and for maintenance of the EBV genome is the latent origin of replication, *oriP*, which acts in *cis* (Yates *et al.* 1985). The *oriP* sequence is composed of a family of repeats (FR) and a region of dyad symmetry (DS), separated by an approximately 1-kbp non-specific sequence (Rawlings *et al.* 1985). The FR sequence contains 20 copies of a 30-bp element, each of which is equipped

with a 16-bp palindrome showing EBNA1 binding properties (Ambinder *et al.* 1990). The dyad symmetry sequence region consists of two 65-bp palindromes in an opposite orientation, at which the EBV DNA plasmid synthesis starts and, finally, close to which the DNA synthesis terminates (Gahn *et al.* 1989). In addition, the dyad symmetry region contains four EBNA1 binding sites which compete for binding with the kinesin-like DNA binding (*kid*) cellular protein linking the EBV plasmid to the spindle apparatus (Tokai *et al.* 1996).

EBNA1 has at least three important domains (Ambinder *et al.* 1991). A signal peptide (at amino acids 379–386) was identified as being responsible for the nuclear localization, which binds this protein to an import receptor (importin) at the nuclear pore (Kim *et al.* 1997). The N-terminal peptide domain containing a GGA motif inhibits the ubiquitin/proteasome mediated degradation of EBNA1 to prevent it from being presented as a foreign viral immunogenic peptide by MHC I glycoproteins. Thus, EBNA1 is not recognized by the receptors of cytotoxic (CD8) T cells. The third domain located at the C-terminus (amino acids 451–608) allows a site-specific DNA binding and dimerization of the EBNA1 molecule. Besides binding to *oriP*, this site has the ability to form dimers bound to DNA and thereby to link or loop out the intervening DNA sequence. EBNA1 is also able to bind RNA *in vitro,* including the EBER molecules. For better understanding of EBNA1 function, its DNA binding property should be discussed separately for DNA copying, for maintenance of the EBV plasmid and for segregation of the newly copied viral DNAs into daughter cells during host cell division. The DS region of the EBV plasmid is needed for replication of the EBV genome; this occurs even with viral DNA lacking the DS sequence from *oriP* (Mackey *et al.* 1999). Since EBNA1 does not possess any helicase activity (in contrast to the *ori*-binding protein/UL9 of HSV1/2), it must associate with a cellular DNA replication protein, such as replication protein A (RPA). The exact mechanism of EBV plasmid replication is not known (Zhang *et al.* 1994). In contrast to the dyad symmetry region of *oriP,* the FR region provides the maintenance plus segregation functions. According to a hypothesis, the EBNA1 plasmid not only associates with *oriP* by means of the C-terminal dimerization domain at the DS region, but in addition, the chromosome attachment protein(s) interact with the linking regions of the EBNA1 molecule, which flank the GGA motif-rich N-terminal domain. Most probably, the cellular EBP2 associates with LR assuring the "homing" of the episome at the metaphase chromosome(s). Then the EBV plasmid can be duplicated during the S phase of cell division. When the DNA replication becomes completed, the linkage between the dyad symmetry region and *kid* protein is exploited for parting of the duplicated episomal DNA. In addition to the above mentioned roles of EBNA1, it should be noted that any origin of DNA replication (*oriP* not excluding) may contain sequences enhancing and/or silencing the DNA synthesis. The binding of EBNA1 to *oriP* indeed enhances transcription from the *Bam*HI Q promoter. It seems that this viral nuclear protein positively upregulates its own expression. In summary, the latency I pattern does not promote immortalization of carrier cells by itself, since transition from latency I to latency II requires additional genome transcription from an independent promoter. Burkitt's lymphoma develops from the latency I status due to chromosome translocations. In EBV latency II (Fig. 2), when the membrane signaling protein LMP1 becomes expressed in addition to EBNA1, the former acts as a potent NF-κB activator (*see below*); this is the case in NPC, in a proportion of non-Hodgkin T-cell lymphomas and Hodgkin lymphomas (cf. Table V).

The KSHV ORF73/LANA1 protein is expressed in KS spindle cells carrying the latent genome (Kedes *et al.* 1997). LANA1 is a highly acidic protein equipped with a leucine-zipper domain and with a cytoplasmic C-terminus with overall basic charge composed of 190 amino acids. It was found to inhibit efficiently the p53 protein so that it eliminates its activity at promoting p21 transcription (removal of cyclin/*ckd* inhibition) and diminishes the apoptotic response generated by p53 (Friborg *et al.* 1999). Unlike EBV, which codes for a cell transforming LMP1 expressed independently in DNA virus carrier cells, the transcription of KSHV ORF73 (LANA1) is closely associated with the expression of another two cell growth promoting genes, namely ORF72 (codes for vCyclin) and ORF71 (codes for the anti-apoptotic vFLIP). The transcripts governed by the same promoter are either a tricistronic mRNA (unspliced as well as spliced), or a bicistronic transcript presumed to represent ORF72 mRNA (Dittmer *et al.* 1998). The cluster of latent genes in the KSHV genome predicts some functional link between LANA and vCyclin expression in KS spindle cells carrying the latent KSHV episome. No single ORF71/vFLIP gene transcripts have so far been identified, but it is possible that the vFLIP anti-apoptotic protein is translated from the same bicistronic message with the help of an internal ribosomal entry site. These findings strongly suggest that both LANA1 and vCyclin are expressed in KS and PEL tissues. Interestingly, deregulated cellular cyclin D is increased and activated in many human tumors (Motokura *et al.* 1991) and it can be assumed that vCyclin would exert a similar effect (*see later*). The whole mechanism how KSHV induces proliferation of endothelium cells into nondifferentiated KS spindle cells is not quite clear, *i.e.* the genesis of tumor formation seems very complex. An abundant 700-bp transcript, T0.7 (transcribed from the K12 gene), coding for the protein called kaposin

was found in KS spindle cells (Staskus *et al.* 1997). When the K12 was transfected into Rat-3 cells, sarcoma formation was observed (Muralidhar *et al.* 1998). The transformed cells caused tumors in *nu*/*nu* mice. The exact mechanism of kaposin action has not been elucidated but its strongly hydrophobic terminus suggests a possible analogy with the papillomavirus E5 protein action. The latter polypeptide interacts with the subunit of cellular vacuolar ATPase hindering the acidification of vacuolar interior and degradation of cell surface receptors such as the epidermal and/or the platelet derived growth factor receptors (EGFR, PDGFR). It is possible that kaposin prolongs the presence of growth factor receptors, making cells more sensitive to growth factor signaling (Cohen *et al.* 1993). Furthermore, KSHV encodes additional proteins linked to proliferation, such as the viral G-protein coupled receptor (Cesarman *et al.* 1996; Arvanitakis *et al.* 1997; Bais *et al.* 1998), and at least two above mentioned apoptosis antagonists: the viral FLICE- (FADD-like IL-1β converting enzyme) inhibitor protein (vFLIP), which is a proteinase attacking caspase 1, and a *bcl-2* like protein (ORF16). Finally, it encodes vIL-6 and several IFN regulating factor homologues (vIRFs). All these proteins may contribute to cell transformation in accord with the K1 and K15 gene products (LMP analogues), which will be discussed next (Table VI).

Table VI. Gamma herpesvirus gene expression during latency

Virus	Gene expression			Genes expressed[a]
	latency	highly restricted	moderately restricted	
EBV	I	yes		EBNA1
	II		yes	EBNA1, **EBNA2**, **EBNA3a**, **3b**, **3c**;
	III		yes	**LMP1** and **LMP2**
KSHV	I[b]	yes		**ORF73/LANA**
	II[b]		yes	**ORF73/LANA**, **ORF74/v-FLIP**, **ORF72/vCyclin**; **K1/LMP1**; **K15/LMP2** and **K12** (kaposin)
MHV-68	I[b]	yes		M2
	II[b]		yes	M2, M3, **K3/IE1**; **M11/bcl-2**, **ORF73/LANA**

[a]**Bold** – genes whose products may contribute to cell transformation. [b]Proposed by authors.

6.2 Latent membrane proteins and cell signaling

EBV was the first human gamma herpesvirus in which a transforming protein, interfering with cell signaling, had been characterized (Baichwal *et al.* 1988). The protein in question, called latent membrane protein 1, has 6 transmembrane domains and a cytoplasmic domain of 199 amino acids. The LMP1 molecules form oligomers by a process which itself leads to activation of cellular signaling protein pathways. To activate transcription in non-infected B cells (by cofactors such as NF-κB or *c-jun*), helper proteins associate with the C-terminal activator regions (CTARs) of receptor proteins such as the CD40 B-cell receptor and the TNF receptor (TNFR). Like CD40, the LMP1 cytoplasmic domain (C-terminus associated receptor 1, CTAR1) interacts with the TNFR-associated factor (TRAF2), an adapter protein capable of activating NF-κB, *i.e*. its transcription activator complex p50/p65 (Fig. 4). This complex is then translocated to the nucleus to initiate the transcription of several surface B cell marker proteins (CD23, VLA) and to induce transcription of enzymes needed for B cell proliferation. The p50/p65 mediated activation of cellular transcription is itself a complex process, mediated by a phosphorylation cascade (for review *see* Chabot-Fletcher 2000). The target of this cascade (represented by several IKKs, inactivator kinases) is an inactivator protein I-κB, which binds to the p50/p65 complex in order to retain the inactive transcription activator (inactive NF-κB) within the cytoplasm of nonstimulated B cells (Bauerle and Baltimore 1988). However, in stimulated cells, the phosphorylated I-κB is released from the p50/p65 complex and degraded by ubiquitination. Before this happens, another protein, the NF-κB inducing kinase (NIK), must be activated. NIK represents a pivotal molecule, which becomes activated upon stimulation through the cytoplasmic domain of various surface receptors, such as TNFR-associated death domain (TRADD). TRADD can activate (*via* TRAF) also another transcription pathway, the effectors of which are the *c-jun*/AP1 transcription factors (Song *et al.* 1997). The AP1 (activator protein 1) pathway itself is activated by additional series of kinases such as, *e.g.*,

mitogen activated protein kinase (MAPK). Summing up, in non-infected cells, when the CD40 receptor and/or TNFR (both surface molecules) interact with appropriate ligands, they activate a series of phospho-

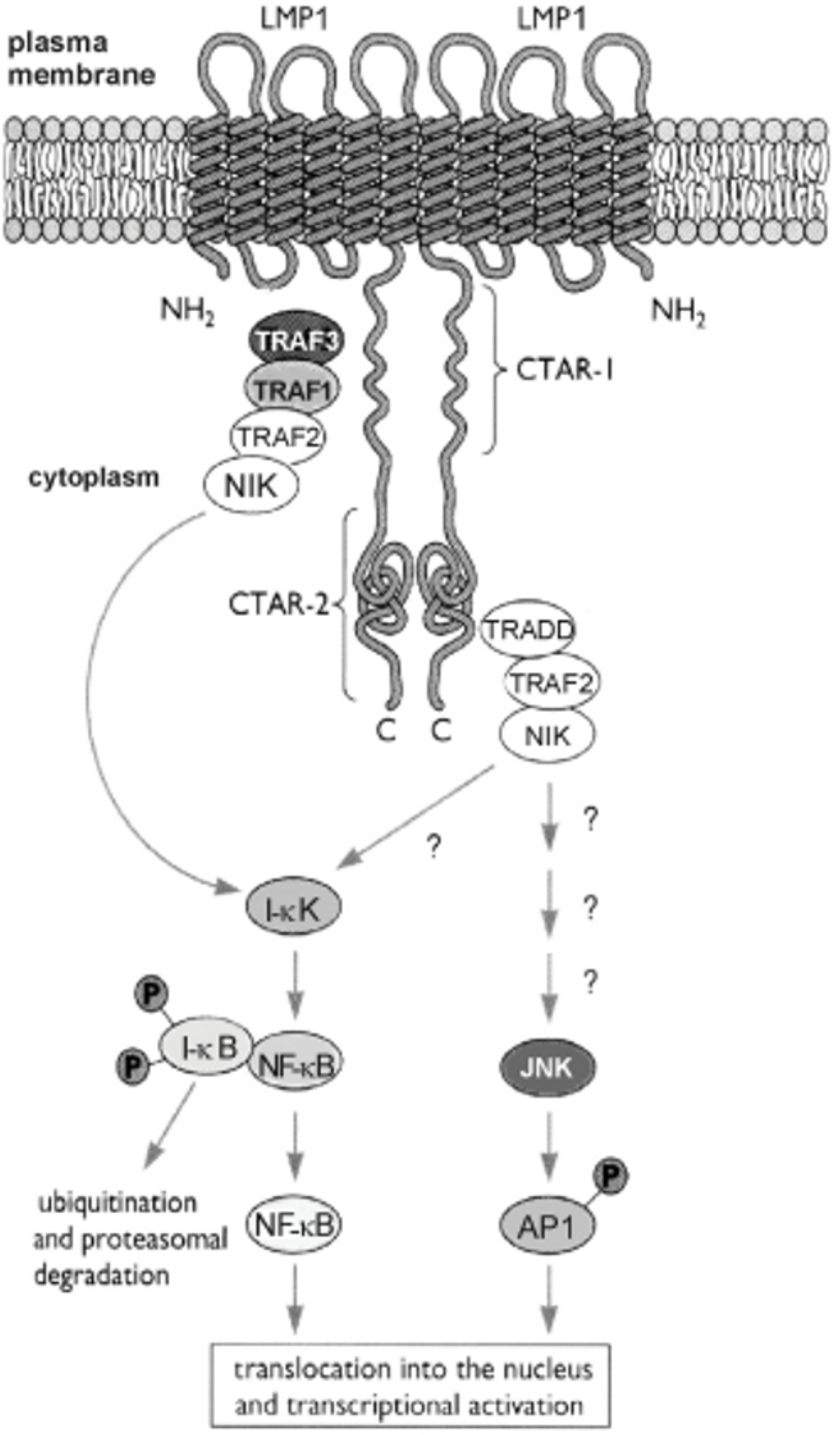

Fig. 4. The latent membrane protein (LMP1) dimer (according to Flint *et al.* 2000) and interactions of its CTAR (C-terminal associated receptor) domains with the cellular signaling pathway activators TRAF, NIK and IKK; TRAF – TNF receptor associated factor, TRADD – TNF receptor associated death domain, NIK – NF-κB inducing kinase, IKK – inactivator kinase (kinase), I-κB – inactivator κB, NF-κB – nuclear factor κB (a *Rel* family protein), AP-1 (activator protein 1) includes members such as *c-jun* characterized by a basic region-leucin zipper DNA-binding domain; M(AP)K(K)-mitogen (activator protein) kinase (kinase); JNK – c-jun N-terminal kinase; MapK can be phosphorylated by activated *Ras*/GTP (cf. Fig. 5).

rylation events which lead to nuclear translocation of transcription factors p50/p65 (active NF-κB) and/or *c-jun*/AP1 (Fig. 5). On the other hand, TNFR stimulation may lead to apoptosis, by either direct or indirect activation of various effector caspases, such as caspase 8 (Hsu *et al.* 1995). Nevertheless, apoptosis is more frequently launched by receptors such as Fas/Apo1/CD95 or the decay receptors DCR and DR (Ahskenazi and Dixit 1998).

The LMP1 expressed in EBV carrier B cells (in the latency types II and/or latency type III), is a constitutively active signaling molecule which mimics the effects of (absent) receptor/ligand interactions and thus becomes the source of false positive signaling (Gires *et al.* 1997; Izumi *et al.* 1997). An interesting property of LMP1 expressing B cells is their ability to aggregate due to self-oligomerization. This also contributes to mimicking of a ligand-induced activation at the CD40 receptor. In this way, an immortalization of EBV DNA carrier B cells, rather than their apoptosis, occurs. This appears to happen also due to activation of additional cellular chemokine receptors (*see later*) as well as due to the expression of virus-encoded regulator anti-apoptotic proteins such as *bcl-2* and *bclx* (Adams and Cory 1998; Eliopoulos *et al.* 1999; Damania *et al.* 2000).

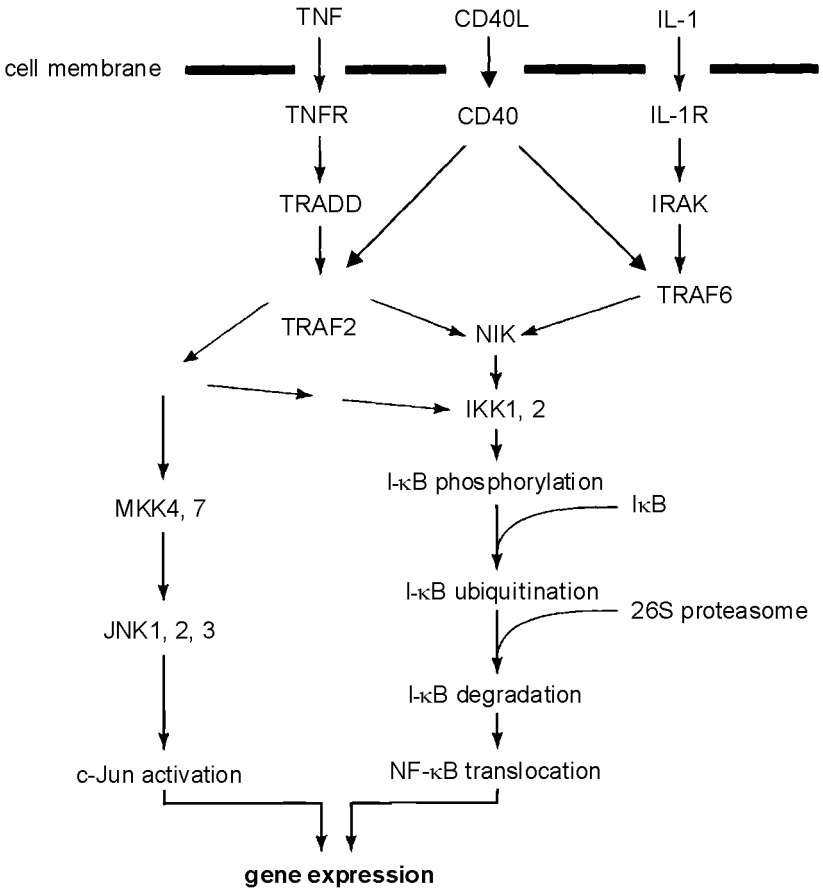

Fig. 5. Bifurcation of intracellular signal transducing pathways *via* the TNF, CD40 (cluster of differentiation 40) and IL-1 receptors (TNFR, IL-1R, respectively; according to Manning 2000, modified); for further explanation *see* legend to Fig. 4.

The K1 gene of KSHV (an EBV LMP1 gene analogue) encodes a 46-kDa transmembrane glycoprotein (Lee *et al.* 1998*a*), whose sequence has turned out to be extremely variable (Kasolo *et al.* 1998). However, its C-terminal cytoplasmic tail is more conserved containing an immunoreceptor tyrosine-based activator motif (ITAM) (Lagunoff *et al.* 1999). The ITAM motif of the K1 transmembrane protein may function similarly as the ITAM attached to the cytoplasmic tail of the T or B cell receptors, but unlike the latter, its activation does not require a ligand/receptor interaction. Taken together, it again functions constitutively. In KSHV infected endothelium and spindle cells, the K1/ITAM domain phosphorylates several signal pathway transduction proteins such as Syk and p85 (Lee *et al.* 1998*b*) and, finally, induces expression of

a transactivator protein termed nuclear factor of T cells (NFAT). The rightmost end of the KSHV genome situated opposite to the K1 gene encodes another signaling transmembrane protein called K15, which is an EBV LMP2a analogue (Choi *et al.* 1999). The cytoplasmic tail of K15 (as well as of LMP2a) contains SH2 and/or SH3 binding sites (*src* homology domains) capable of combining with proteins exerting tyrosine kinase activity, such as *Lyn*, *Fyn*, *Syk* and *Csk* kinases and other members of the *src*-tyrosine kinase family (Burkhardt *et al.* 1992). These kinases participate in proliferative signal transduction in response to various growth factors (ligands) interacting with receptors on T cells, endothelial cells, B cells, platelets, bone marrow cells, brain glial cells and fibroblasts. Unlike noninfected cells, in which tyrosine kinase activation is cAMP dependent and requires ligand–receptor interactions, in KSHV or EBV infected cells the K15 or LMP2a cytoplasmic tails are constitutively tyrosine phosphorylated by the above cellular enzymes. In EBV latency III with LMP2a expression, the B-cell receptor-associated signal transduction can be downregulated, so that the latency carrier cells would not become activated upon antigen stimulation. A similar effect has been described in KSHV infected cells (Choi *et al.* 1999). K15 is expressed especially in PEL and Castleman disease. This protein has the ability to associate with HAX-1, the HS-1 associated protein X-1. HAX-1 shows anti-apoptotic activity, but K15 protein does not influence apoptosis. Because HAX-1 also affects contacting, which promotes actin polymerization *via* phosphorylation, it can be assumed that K15 may influence endothelial cell motility (Sharp *et al.* 2002).

6.3 Gamma herpesvirus-encoded cyclins

vCyclins expressed during infection of cells by cyclin encoding viruses may mimic cellular cyclins and functionally substitute them even if they are down-regulated by p53 and the cyclin inhibitors KIP/CIP (kinase or *cdk* inhibitor proteins) (Mittnacht and Boshoff 2000). In non-infected cells, the cyclins are cofactors which complex with cyclin-dependent kinases (*cdk*). To activate transcription, *cdk* phosphorylates the retinoblastoma (Rb) protein. After this, the phosphorylated *Rb* protein is released from the promoter region of silenced genes, especially those participating in cellular DNA replication. The *Rb* anti-onc protein blocks transcription of enzymes involved in cellular DNA synthesis and repair. In association with these different functions, there are several cyclin/*cdk* complexes regulating either the transition from G_1 to S phase (duplication of cellular DNA) or the transition from G_2 to M phase (DNA control and repair). The cellular cyclins are controlled *via* inhibitors such as the p21 protein, which belongs to the KIP/CIP family. The expression and synthesis of the latter is regulated by the p53 anti-*onc* protein. When activated, p53 upregulates the transcription of KIP, which in turn inhibits the cyclin/*cdk* complexes.

KSHV encodes a vCyclin resembling cellular type D cyclins (Chang *et al*. 1996), while MHV-68 encodes a type A/E vCyclin (van Dyck *et al.* 1999). KSHV similarly as the HVS-encoded vCyclin binds and activates the cellular *cdk6*, which is usually activated by cellular type D cyclin molecules (Swanton *et al.* 1997). The resulting phosphokinase activity phosphorylates the *Rb* protein, which in turn becomes inactivated allowing the expression of DNA polymerase and other related cellular enzymes to proceed. In addition, KSHV vCyclin modulates the p27/KIP levels inducing p27 degradation (Ellis *et al.* 1999). The latter effect can never be achieved by cellular type D cyclins, but is known to occur in the presence of type A/E cyclins (Mann *et al.* 1999). As mentioned above, the KSHV vCyclin ORF72 is flanked by ORF71 encoding the viral FLICE inhibitor protein (vFLIP) – which is in fact a proteinase/caspase-1 inhibitor – and by ORF73 encoding the LANA1 protein. Common translation of vFLIP and vCyclin arises from a bicistronic transcript with an internal ribosomal entry site indicating that their synthesis is highly linked. Thus, expression of the anti-apoptotic protein vFLIP correlates with the expression of vCyclin when cells are being driven to the synthetic phase and when cellular as well as viral DNA synthesis is promoted.

6.4 Gamma herpesvirus-encoded virokines and chemokines

Chemokines are a superfamily of small structurally related cytokines which selectively promote adhesion, chemotaxis and activation of leukocyte populations (Baggiolini *et al.* 1997; Nelson and Krensky 1998). All biological effects of chemokines are mediated by a large group of receptors, whose transmembrane domain crosses the cell membrane seven times (seven-span, serpentine) and whose cytoplasmic domain generates GTP as a second messenger (GPCR). A typical feature of chemokines is the presence of four conserved cystein residues (*i.e.* the four-cystein motifs). The most important are the C-C subgroup peptides (with no intervening amino acids) and the CXC subgroup peptides (with a single intervening amino acids). KSHV encodes at least three chemokines termed viral macrophage inflammatory proteins (vMIP1/K4, vMIP2/ORF4.1 and vMIP3/K6). The vMIP1 acts as an antagonist of the C-C receptor 8 (Dairaghi *et al.* 1999), vMIP2 binds to C-C receptor 3 to inhibit the chemotaxis of monocytes (Boshoff *et al*. 1997), while vMIP3 interacts with C-C receptor 4 (Stine *et al.* 2000). In addition, these virus-encoded chemokines can

bind to some C-X-C receptors (Kledal *et al.* 1997). All of them are chemoattractant receptors for T_H2 lymphocytes.

The ORF74 of MHV-68 encodes a CXCR2 chemokine receptor homologue (Virgin *et al.* 1997) (termed oncogenic G protein-coupled receptor) (Wakeling *et al.* 2001) and a M3 gene-encoded soluble chemokine-binding protein that could potentially interfere with chemokine–receptor interactions. Recently, M3 protein has been shown capable of binding a broad variety of chemokines neutralizing cellular responses to chemokines *in vitro* (Parry *et al.* 2000). *In vivo* it probably attenuates the immune response to virus infection and assists the mechanisms regulating leukocyte recruitment that could lead to enhanced viral replication (Sarawar *et al.* 2002).

In addition to the secreted chemokines, EBV, KSHV as well as MHV code for an IL-8 receptor homologue. The corresponding gene is BLR1 and/or ORF74, respectively (Table III). The viral GPCR after interaction with IL-8 activates the secretion of VEGF. The viral GPCR could be induced *in vitro* in PEL derived lymphoma cell line (Arvinatakis *et al.* 1997). The VEGF production in KS tissues is limited to a few KS cells which underwent expression of multiple latency genes (Table VI) and of additional IE and E proteins including trans-activating ones (ORF50 and K8). Secretion of VEGF may promote proliferation of conventional endothelium cells and their transition to the transformed KS endothelium cell phenotype. This occurs due to the presence of additional stimulation factors (cytokines) such as vIL-6 (Molden *et al.* 1997). In CD and PEL, vIL-6 appears to be responsible for proliferation of many uninfected B cells that make up these tumors. In turn, vIL-6 enhances VEGF secretion (Ensoli *et al.* 2000). Furthermore, the basic fibroblast growth factor (bFGF) produced by the spindle cells within the KS lesions induces vascular proliferation and promotes angiogenesis (Samaniego *et al.* 1995). Both growth factors act in accord with HIV-1 transactivator protein *tat.* Inflammatory cytokines (IFN-γ and monocyte chemoattractant proteins, MCPs) contribute to the accumulation of circulating B cells carrying the latent KSHV DNA and stimulate lytic virus activation. As already mentioned, in general, following receptor–lingand interaction any GPCR molecule (either cellular or virus-encoded) , undergoes dimer formation which activates the tyrosine kinase activity of its cytoplasmic tail. An adapter protein such as *Grb2*, containing SH2 and SH3 domains, interacts with a receptor (*Sos)* protein. The *Grb2*/*Sos* complex exchanges GTP with *Ras* so that *Ras*/GTP is formed. The latter binds to a cytoplasmic serine-threonine kinase such as *Raf* in order to induce the phosphorylation signal, which is further transferred *via* the MapK/MKK (mitogen activated kinase/kinase) cascade. Finally, *c-jun*/AP1 or other transcription factors are translocated into the nucleus in order to activate transcription.

It seems that vIL-6 is the most important virokine produced by KSHV gene K2 (Neipel *et al.* 1997; Nikolas *et al.* 1997) and secreted from KSHV-infected lymphocytes, fibroblasts, monocytes and/or endothelium cells. The IL-6 receptor signal transducing moiety gp130 can be activated by vIL-6 directly, *i.e.* without the need of any coreceptor to phosphorylate and translocate the corresponding transcription factors STAT1, 3 or 5 (Molden *et al.* 1997). In addition, the above mentioned *Ras*/MKK signaling pathway may become activated (Moore and Chang 2002). It is likely that these activated signaling pathways may induce anti-apoptotic proteins as well. Neither vIL-6 nor vMIPs are expressed during restricted latency in KSHV DNA carrier cells (Table VI) so that they cannot act at initial stages of KS development (Canon *et al.* 1999). This is in contrast with the large amounts of this virokine produced by early infiltrating cells. Furthermore, HIV-encoded *tat* is released from HIV-infected T cells, and is also an efficient transcription activator of KSHV transcription (Ensoli *et al.* 1993). The synergistic effect of HIV-*tat* and bFGF (basic fibroblast growth factor) has been already mentioned. Summing up, early stages of KS are characterized by a few spindle shaped cells expressing KSHV latency genes and many proliferating non-infected cells reacting to the presence of cellular and viral inflammatory chemokines (Cannon *et al.* 1999).

The IRF (IFN regulating factor) family of transcription factors is involved in regulating gene expression in response to IFN signaling. KSHV possesses a mechanism allowing it to prevent the induction of cellular IFN genes that are important components of natural (non-specific) immune response. The virus induced IRF molecules have an N-terminal DNA binding motif and a C-terminal repressor/transactivator region. Though the first characterized vIRF1/K9 (Table IV) does not directly bind to DNA, it inhibits the IFN-α-gene promoter expression, down-regulates p21 expression and prevents IFN-α/β-induced growth arrest (Gao *et al.* 1997). The vIFN-2 encodes 163 amino acids protein and shares the N-terminal region with IRF1 which binds to the NF-κB binding site (Burysek *et al.* 1999). The vIFN-3 interferes with the transcription of IFN-α in infected cells (Lubyová and Pitha 2000). IFN regulators are involved also in the IFN-γ response inhibition, which is of special interest, since this class two IFN is a cytokine rather than a classical virus inhibitor (Vilcek and Sen 1996). In non-infected cells, interaction of IFN-γ with corresponding receptor causes Janus-kinase (JAK) phosphorylation which activates the signaling transduction and transcription (STAT) protein homodimer formation, an alternative pathway similar to that activated upon IL stimulation. The activated STAT-1 homodimers migrate to the nucleus, where they act as transcription

cofactors by recruiting the γ-activated sequence (GAS) element and its co-adapters such as HAT (the histone acetyltransferase) and CBP/p300 (the CREB-binding protein) necessary for initiation of transcription at the IFN-responsive promoters (Goodman and Smolik 2000). The vIRFs inhibit this signaling mechanism by binding to STAT co-adapter proteins and interfering with their transcription promoting function.

Because IFNs up regulate the *cdk* promoter, the expression of which is regulated by p21 (KIP), vIRFs interfere also with the function leading to cell arrest. The p53- and p21-induced cell cycle arrest can be lifted *via* different KSHV-encoded products: the vIRFs, LANA1/K1 and LANA2/K15 proteins. In this way, KS cells expressing early (nonstructural) KSHV products are shifted from apoptosis to a state of upregulation of cell division promoting regulatory proteins. Apoptosis, in addition, is prevented by the virus-encoded *bcl-2* analogue (ORF16) and by the early expression of ORF71/K13/vFLIP. Especially the latter is an efficient anti-apoptotic protein, since it acts as an inhibitor of caspase 8 activation. The vFLIP has a similar death effector domain (DED) as the Fas receptor-associated death domain (FADD) adapter protein, which is an efficient transducer of the apoptosis activation signaling to inactive caspases. Since the DED of caspase 8 associates with vFLIP as well as with the death domain of FADD, in fact the viral FLICE (*i.e*. the virus-encoded FADD-like ICE) inhibitor protein efficiently interferes with the induction of apoptosis. vFLIP can also inhibit the TNFR-mediated cellular apoptosis induced signaling. Finally, the viral *bcl-2* analogues (encoded by many gamma herpesviruses; Table III) can prevent apoptosis either due to vCyclin/*cdk6* over-expression or by *BAX* binding (Ojala *et al.* 1999). In KS sarcoma, however, vFLICE expression may be more frequent than the *bcl-2* analogue expression.

7 FUNCTIONAL SIGNIFICANCE OF THE GAMMA HERPESVIRUS-SPECIFIC GENES

The steps of KSHV oncogenesis and transformation by gamma herpesviruses discussed here include an initial non-specific phase of cell proliferation mediated by inflammatory cykines and chemokines which is followed by an activation of latency with an expression of a wide spectrum of latency-associated viral genes (Table VI). The expression of genes such as LMP1 (transition of EBV latency to latency II and/or III) or the latency-associated KSHV genes (such as ORFs71–73 and the K1, K12 and K15 genes) activates host cell DNA transcription. This can result in driving host cells towards division. Latency II/latency III in EBV carrier B cells, for example, causes expression of CD markers and cytokine receptors, a state which renders them sensitive to the action of corresponding cytokines and growth factors. Furthermore, heterologous infections such as HIV and the presence of their transactivation proteins (HIV-tat) or other stimuli activate the expression of EBV or KSHV-encoded transactivator proteins (*Zta*/*Rta*), which turn on the transcription of additional virus-encoded regulatory proteins, viral virokines, viral chemokines and growth receptors (Table VII). Expression of structural proteins and virion formation may be important for virus spread, but it is not relevant for oncogenesis. The block of lytic virus replication might be even of advantage for cell transformation and tumor formation, since the above effects are launched by nonstructural viral proteins.

8 GAMMA HERPESVIRUS GENOMICS: CELL GENE PIRATING AND EVOLUTION

The majority of herpesvirus genes contain no introns and their mRNAs need no splicing. This indicates that herpesviruses might have acquired the cellular genes through mRNA intermediates. Conversion from mRNA into DNA could occur with the help of reverse transcriptase supplied by a co-infecting retrovirus. The idea of possibility of direct virus-to-virus gene transposition is supported by identification of gene inserts of virus origin such as the adeno associated virus type 2 *rep* gene sequence in the HHV-6 genome (Thomson *et al.* 1991). In addition to the acquisition of sequences from heterologous viruses and/or of cellular gene sequences, there is also some evidence of gene loss and/or of reduplication of gene sequences in herpesvirus DNAs. Gamma herpesvirus genes such as FGARAT, GCPR, IRF or MIP might be present in multiple copies. The presence of transcripts transcribed by the RNA polymerase III, from tandem repeat sequences encoding the EBERs, tRNAs or mRNAs, respectively, points at the fact that these are non-coding repetitive elements. Whether such sequences are just compensations for sequence deletions or whether they are of functional significance should be further investigated. As an evidence of gene loss can be understood the absence of ori-binding protein gene in the gamma herpesvirus DNA (Inoue *et al.* 1994). Similarly, the presence of GCPR IL-8 receptor gene in KSHV but not in MHV-68 DNA indicates its loss from certain gamma herpesvirus genomes (Nicholas 2000). Coincidence of genetic divergence at particular genomic locations suggests that these loci allow more readily the loss or acquisition of foreign sequences from an

hypothetical ancient progenitor virus genome. It is possible that more variations occur at the DNA termini due to nicks and cuts during concatemer cleavage and packaging events. The divergent loci flank both DNA

Table VII. A survey of gamma herpesvirus genes involved in cell transformation: from latency to oncogenesis[a]

KSHV	EBV	MHV	Responsible protein
Cell proliferation due to cytokine and virokine action			
vIL-6			viral IL-6; growth factor with a wide spectrum of target cells
		M1, serpin	immune regulator receptor (?)
	ORF4/CCP	ORF4/CCP	complement control protein
K4/MIP1			macrophage inflammatory proteins
K4.1/MIP2			
K6/MIP3			
K9/IRF			IFN regulatory proteins interfering with the JAK/STAT signaling pathway
K10/IRF			
K10.l/IRF			
K11.1/IRF			
	BCFR1		viral IL-10 homologue, inhibitor of inflammatory cytokines
ORF74/vGPCR		ORF74/vGPCR	viral G-protein coupled receptor (activates *Ras*/*c-jun*/AP-1 signaling)
Anti-apoptosis proteins			
ORF16	BALF1	M11	functional *bcl-2* homologue
ORF71/vFLIP			*Fas*-DD-like (ICE) inhibitor protein; interferes with death domain signaling
Proteins directly affecting intracellular signaling pathways			
ORF72/vCyclin		ORF72/vCyclin	vCyclin D analogue; cdk6 activator
K1	BNLF1/LMP1		latent membrane protein 1; the EBV transformation protein activates NF-κB; K1 interacts with ITAM adapter protein
K12			kaposin, an E5 transformation protein analogue
K15	LMP2		SH2/SH3 binding and tyrosine kinase activation
ORF73/LANA1		ORF73/LANA	latent nuclear antigen, interacts with Rb protein, activates E2F
K10.1/LANA2	BYRF1/EBNA2		inhibits p53-dependent transcription

[a]Cf. with Tables III, IV and VI.

termini and are located between the conserved gene blocks I to II, and III to IV as depicted in the MHV-68 genome (Fig. 3). Both terminal regions of MHV-68 as well as of KSHV DNA contain the latency associated and acquired virus specific or subgroup-specific genes confirming the notion that these diverging regions undergo more frequent recombination events. In contrast to the pirated genes, the core structural and the essential non-structural proteins are encoded by conservative genes (Table II) organized in homologous blocks (Fig. 1). Acquisition of new genes could confer on the virus new and specific properties altering its biological behavior on the basis of natural selection. Whatever the mechanism, the phenomenon itself provides a powerful tool for rapid adaptation of herpesviruses giving the family members considerable diversity.

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