TOPICAL REVIEW

Mechanisms of replication of alpha- and betaherpesviruses and their pathogenesis

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Abstract

The diseases caused by herpes simplex virus (HSV) and human cytomegalovirus (CMV) differ and distinct differences in biological properties of these viruses can be noticed at laboratory work. Despite of this, the structure of DNA and the replication cycle of both viruses shows remarkably common features. Analogous proteins encoded by both viruses, act at initiation of viral DNA transcription, at viral DNA synthesis, at nucleocapsid formation and envelopment. On other hand, considerable differences occur during maturation of virions and at their egress from infected cells. Both viruses in question developed strategies to escape immune recognition by cytotoxic T cells and/or to interfere with the antibody response. Both viruses are widespread in human population and are able to establish latency. Finally, their prevention and/or prophylaxis by effective vaccines has not been solved. Recently, the significance of both viruses has increased. HSV2 is an important pathogen acquired by sexual contact, while CMV reactivates under immunosuppression (post-transplantation, tumours, combined activation in the presence of human immune deficiency virus) and/or causes congenital infection. Chemotherapy of HSV mediated diseases seems more effective than that of CMV mediated infection, because the CMV inhibitor ganciclovir is much more toxic than the CMV inhibitor acyclovir and its derivatives. ***(Tab. 6, Fig. 5, Ref. 52.)***

Key words: herpes simplex virus, human cytomegalovirus, virus replication, analogous proteins, dissimilar biological properties.

The family *Herpesviridae* (vanRegenmortel et al, 2000), comprises 8 human intensively studied herpesviruses (Tab. 1), from which at least six are important pathogens for man. The majority of diseases related to herpesviruses develop due to reactivation of covert (latent) infection. Latency is a form of chronic virus persistence, when the virus does not undergo replication, thus it persists in a nonproductive form. Herpesviruses, especially those belonging to subfamilies *Alpha* and *Beta* are ubiquitous, i.e. they infect the majority of population. Among healthy blood donors in Bratislava we found that 80 % had antibodies against herpes simplex virus 1 (HSV1) in virus neutralization and ELISA tests. In various populations, the seropositive rate of human cytomegalovirus (CMV) antibodies ranged from 45—80 % (Britt and Alford, 1996).

Alphaherpesviruses are characterised by a relatively shorter replication cycle (not longer than 16—18 hrs according to the various susceptible cells); they spread well from cell to cell but they also are easily released from infected cells, in which they multiply causing clear-cut cythopatic effect and formation of eosinophilic intranuclear inclusion bodies (of type B according to Cowdry). *In vitro* they infect cells originating from various animal species. In nature, various species (not only humans) host many Alphaherpesviruses, but for each virus a species can be identified to which it had been at best adopted. In such host species the particular virus undergoes latency most frequently

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Tab. 1. Classification of human herpesviruses, related diseases and clinical syndromes.

Subfamily	Virus species	Disease
Alphaherpesvirinae	Herpes simplex virus 1 (HSV1) (Human herpesvirus 1)	Musculocutaneus herpes (mainly labial) keratitis, oesophagitis. Gingivostomatitis (at primoinfection), encephalitis, hepatitis. *Eczema herpeticum.*
	Herpes simplex virus 2 (HSV2) (Human herpesvirus 2)	Genital herpes. Herpetic disease of neonates (skin manifestations, pneumonia, hepatitis, encephalitis).
	Varicella - zoster virus (Human herpesvirus 3)	Chickenpox, herpes zoster.
Betaherpesvirinae	Human cytomegalovirus (Human herpesvirus 5) (Human CMV)	Congenital malformations (microcephaly, hepatosplenomegaly) Infectious mononucleosis-like syndrome, posttransfusion syndrome, colitis, sialadenitis, pneumonia,
	Human herpesvirus 6 (HHV6)	(post-transplantation disease, HIV complications)
	Human herpesvirus 7 (HHV7)	*Exanthema subitum* (HHV6 type B)
Gammaherpesvirinae	Epstein Barr virus (EBV) (Human herpesvirus 4)	Infectious mononucleosis, tonsillitis hepatitis. Late effects: Burkitt's lymphoma, nasopharyngeal carcinoma, Non-Hodgkin lymfomas. (possibly Hodgkin's lymphoma).
	Human herpesvirus 8 (HHV8) Kaposi sarcoma associated herpesvirus	Kaposi's sarcoma

showing in it the lowest degree of pathogenicity. Within the host body, the Alphaherpesviruses prefer to spread along nerves, while intraaxonal transmission predominates.

Betaherpesviruses have a limited range of host organisms. This property is reflected *in vitro* when these viruses replicated only in cells derived from the host species. Their replication cycle is slow (lasts several days) and their release from infected cells is inefficient. Also betaherpesviruses develop latency. Because they do not show preferential neural spread, they usually persist in leukocytes, in cells of reticuloendothelial system and also in epithelium cells of renal tubuli and salivary gland ducts. At productive replication, infected cells become enlarged and contain intranuclear inclusion bodies. The Gammaherpesviruses replicate slowly and reveal lymphotropic properties. They code for lymphokines and or other proteins influencing intracelullar signalisation. Therefore, these viruses may be related to several malignant diseases. Their properties will be reviewed elsewhere (Rajčáni and Kúdelová, 2002). Here we shall consider the analogous molecular biologic traits of Alpha- and Betaherpesvirus replication.

Structure of herpesvirus particles

The HSV1 virion has a diameter of 150—200 nm; it consists of four structures which can be identified by electron microscopy (Fig. 1): 1) The electron dense (dark) core, containing the viral DNA and at least one basic core protein. 2) The capsid, which has a shape of dodecaedron, and which consists of 150 hexamers and 12 pentamers (altogether 162 subunits); 3) The homogeneous electron translucent tegument and 4) The outer envelope, formed by a lipid bilayer derived from infected cell membrane and containing up to 11 virus coded glycoproteins (Roizman et al, 1969). The size of Alpha- and/or Betaherpesvirus virions may vary; however, the diameter of herpesvirus capsids (110 nm) remains fairly constant.

Both, HSV as well as CMV contain double stranded (ds) DNA organised as follows: TR_L—U_L—$IR_L IR_S$—U_S—TR_S (Fig. 2), where the indexes L and S mean the long and short portions of the DNA stretch. The internal (I) and terminal (T) repetitive (R) sequences show a more complex structure when analysed in

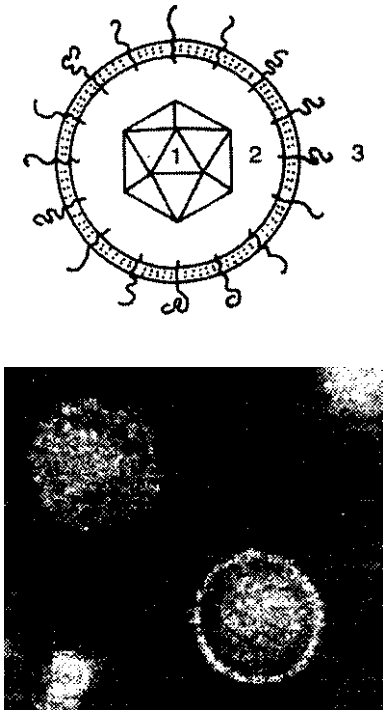

Fig. 1. Above: the basic structure of herpesvirus particle. 1. - Capsid. 2 - Tegument. 3. - The lipid envelope containing viral glycoproteins. Below: the electron microscopic picture of naked herpes simplex virus capsids after removal of the envelope. The particles were purified on sucrose gradient (Matis et al., Acta virol. 15, 1971, p. 521).

detail; in HSV1 and/or 2 DNA it can be written as follows: $\underline{a}_L\underline{a}_n\underline{b}—U_L—\underline{b}'\underline{a}'_m\underline{c}'—U_S—\underline{ca}_S$ (Roizman a Sears, 1996). The a_n a a'_m sequences symbolise homologous regions repeated *n* or *m* times. The *a* sequence, which has about 700 nucleotides (nts) and is present at both ends of the genome, contains the packaging signal enabling recognition of the end of each DNA molecule which should be packed into each virion (Tab. 2). The HSV-1 DNA consists of $152x10^6$ base pairs (bp), while the CMV DNA has about $240x10^6$ bp. The Unique Long (UL) HSV1 DNA segment harbours 56 genes, while the Unique Short (US) segment of this virus has at least 12 genes. The number of genes encoded by the relevant CMV segments is nearly twofold. Each repetitive region flanks the unique region at both sites, thus the number of genes they encode is doubled (Tab. 2). A few genes are encoded by the non-coding (3'—5') complementary DNA strand. Most important in this respect for HSV1 and 2 is the region in part complementary to the ICP0 (RL2) gene located between the UL56 gene and partially overlapping the RL2 gene. This area codes for RNAs transcribed during latency is designated LAT (latency associated transcripts). Taken together, the HSV genome has at least 80 genes, from which 5 are doubled. The real number or genes may not be final, since the identification of genes believed to be encoded by the complementary sequence is still not settled. The number of proteins synthesized in susceptible cells infected with HSV (infected cell protein, ICP) surpasses 70, the estimated number with CMV is over 150 proteins. Less than the half of HSV ICPs becomes structural part of virions (virion protein, VP). Purified HSV1 virions may show 24—33 proteins by electrophoresis (Spear and Roizman, 1972; Heine and Roizman, 1974). We found that about the half of VPs are highly immunogenic in man, as human sera react with up most 16 structural HSV1 polypeptides (Murányiová et al, 1991).

Herpesvirus replication cycle

The HSV1 replication cycle lasts 16—18 hrs, while the human CMV growth cycle extends for 48—72 hrs. Already within a few minutes after virion attachment, they interact firmly with the cell surface. This process is mediated by glycoproteins gC, gB and gD (Tab. 3). Two kinds of cellular receptors interact with the virion glycoproteins mediating attachment (reviewed by Rajčáni and Vojvodová, 1998): 1) The outer surface glycosoaminoglycans (GAGs), former mucopolysaccharides, which cover

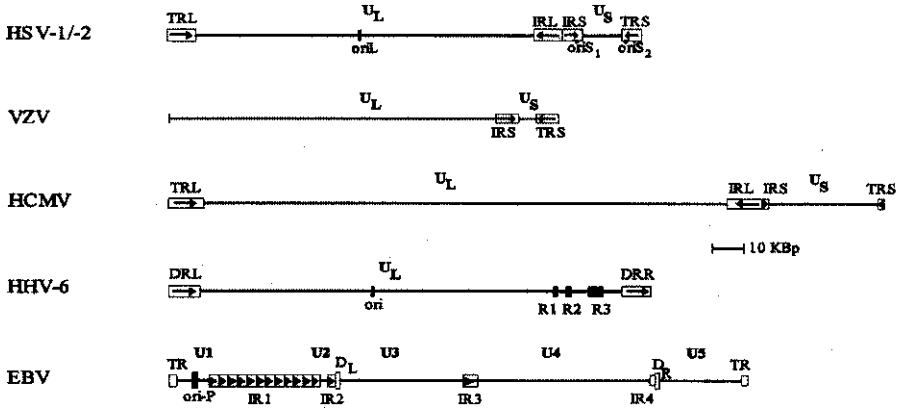

Fig. 2. Comparison of the DNA structure of most important human herpesviruses. The structure of HSV1, HSV2 and CMV DNAs is similar. The DNAs of both HSV types differ from CMV DNA in the length of their unic segments, but not in the position of their repetitive sequences. VZV = varicella zoster virus, HCMV = human CMV, EBV = Epstein-Barr virus, HHV6 = human herpesvirus 6.

Tab. 2. Comparison of genes and open reading frames (ORFs) of Alpha- and Betaherpesviruses.

DNA region	HSV1 (ORF number)	Human CMV (ORF number)
a (identical terminal sequence)	*pac 1* and *pac 2* signals	*pac1* and *pac2* signals
TR_L	2 (+2 in complementary strand)	14
U_L	56 (+1 in complementary strand)	134
IR_L	2 (+2 in complementary strand)	14
IR_S	1	1
U_S	12 (+1 in HSV2)	36
TR_S	1	1
a (identical terminal sequence)	*pac1* and *pac2* signals	*pac1* and *pac2* signals
Genes (ORFs) together	71 (3 + 2 doubled, additional 1 in the complementary U_L strand; 2+2 putative LAT related ORFs)	185 (+ 15 doubled)
kbp	152	230
Homologous DNA strands	Blocks A,B,C,D,E,F,G = 43 kbp	Blocks A,B,C,D,E,F,G = 44 kbp

Tab. 3. Functional similarities between HSV and human CMV (HCMV) glycoproteins.

Gene		Glycoprotein		Function
HSV1	HCMV	HSV1	HCMV*	
UL27	UL55	gB	gB	Adsorption to GAG receptors, virion entry, virus neutralizing epitopes, *syn*3 locus (in HSV1 gB).
UL22	UL75	gH	gH	Forms complex with gL, initiation of penetration, virus neutralising epitopes.
UL1	UL115	gL	gL	Forms complex with gH, participates in penetration.
UL44	US1-5	gC	gC/II	Adsorption to GAG receptors, interaction with complement C3 (inhibited by heparin).
UL10	UL100	gM	gM	Interaction with desmosomes, cell to cell spread.
US6	?	gD	not found	Adsorption to protein receptor(s), penetration domain. transsynaptic spread.
US8	not found	gE	not found	Binding to IgG Fc fragment, complexing with gI, penetration into polarized cells (or nerve) endings, initiation of neural spread.
US7	?	gI	?	Complexing with gE, modulates interaction with IgG.
UL53	?	gK	?	Participation in egress of enveloped virions when crossing the cytoplasm; *syn* 1 mutations (in HSV only).
US4	?	gG	?	Penetration and cell to cell spread, specific for HSV2, truncated in HSV1.
?	US6-11	?	gpUS6 family	Function unclear (not a gD analogue)

* according to Mocarski et al., 1996

surface of many cells; 2) The protein receptors previously designated herpesvirus entry mediators (HVEM). More recently, HveA was recognised belonging to the tumour necrosis factor (TNF) receptor family, while HveB and HveC were found belonging to the nectin family. The interaction with GAG is mediated by gC and gB molecules, which initiate adsorption (Herold et al, 1991; Spear et al, 1993). The late phase of adsorption is mediated by gD, which interacts with the protein receptors (Whitbeck et al, 1997). Early penetration starts due to the activity of the gH/gL complex believed to initiate membrane fusion (Dessai et al, 1988; Forrester et al, 1992). During late adsorption and early penetration phase, which is pH independent, fusion bridges are formed between the virion envelop and the cell membrane (Fuller and Lee, 1992; Roizman and Spear, 1996). Fusion may be related to

Tab. 4. Analogous capsid (gamma)-proteins in HSV1 and human CMV.

HSV		HCMV		Properties and function
UL19	VP5	UL86	MaCP*	The main capsid component (hexons).
UL38	VP19C	UL46	MiCP**	Attachment of DNA inside the capsid.
UL18	VP23	?	?	Type A (basic) capsid component.
UL35	VP26			Type A (basic) capsid component.
UL26.5	VP22a	UL80a		Scaffolding protein in B type capsid,
	VP21			removed from the type C capsid after DNA packaging.
UL26	VP24	UL80		Serine protease, N-terminal cleavage

* major capsid protein; ** minor capsid protein

a confirmation change in gD and probably also in gB molecules (Handler et al, 1996). Virus neutralising antibodies react with specific domains of gB, gD, gC and gH which are neither identical with the domains related to membrane fusion nor with those interacting with cellular receptors. Antibodies just interfere with the above mentioned functional domains for steric reasons. In addition, the cytoplasmic domain of gB, which is the site of *syn3* mutations (Walev et al, 1994; Rajčáni et al, 1996; Košovský et al, 2000), has a control function. The *syn3* mutations impair this function allowing non-controlled membrane fusion.

The replication cycle starts with penetration of the viral genome (DNA) into cytoplasm followed by its transport across nuclear pores into cell nucleus. This event is especially tedious in sensory neurons, the nuclei of which are located in distant sensory ganglia, usually several hundred or even up to thousand milimeters away from the penetration site. In these, the capsids are transported along the neurotubules by means of the transporter protein dynein by the so called quick axonal transport. The dynein complex interacts with the capsid protein UL19 and also with the basic core protein UL31 (Ye et al, 2000). Furthermore, the uncoated capsids may be transported along the actin filaments in any non-neural susceptible cell. Within the nucleus, the viral DNA undergoes circularisation. Immediate early transcription is initiated by cellular transcription factors such as Oct-1, AP1, SP1, ATF and NFB. In HSV-infected cells, Oct-1 interacts with a virion tegument protein called α-TIF/VP16 (transcription initiation factor) (reviewed by Hayward, 1993). A similar protein, designated pp71, was recognized in CMV virions (Liu and Stinski, 1992). Contemporarily with the immediate early (IE) transcription, which starts at the promoters of IE virus genes (Mackem and Roizman, 1980) by the help of the cellular RNA polymerase II, an another independent early event begins in the cytoplasm. Here, an another viral tegument protein, called *vhs* (virion host shutoff), gradually decreases the biosynthesis of cellular proteins (Fenwick and McMenamin, 1984). Later on, the early host shutoff is followed with a more efective inhibition of cellular proteosynthesis mediated by several other early virus-coded proteins (Roizman et al, 1965).

Inititation of lytic replication

At least two immediate early HSV polypeptides, namely ICP0 and ICP4 act as transactivator proteins for transcription of the early (ß) and late (γ) HSV genes. The alternative immediate early proteins encoded by CMV are IE1 (several transcripts) and IE2; they transactivate the transcription of all other CMV genes, the promoters of which do not contain sequence motifs recognised by the immediate early transcription complex. The ICP4 IE protein (encoded by the RS1 gene) is an HSV specific transactivator, which associates with the cellular transcription factor SP1 (and not with Oct-1) to transcribe the early (ß) genes recognising their promoter motifs. ICP4 synthesis ceases at late intervals p.i., because this polypeptide limits its own transcription by a feedback mechanism. A similar effect was observed with IE2 CMV polypeptide, coded by the UL122 gene; it functions as cofactor for transcription of many early and late CMV genes and is also able to down regulate its own transcription. The IE HSV protein ICP47 (encoded by US12 gene) inhibits the transport of class I HLA glycoproteins from the site of their synthesis at rough ER to the Golgi vesicles; thus, it interferes with the mechanism of antigenic peptide recognition by cytotoxic T cells (Hill et al, 1995).

Viral DNA reproduction

A key position among the non-structural early proteins belongs to polypeptides participating in the viral DNA synthesis (Roizman a Sears, 1990; 1996; Mocarski, 1996; Rajčáni and Durmanová, 2000). The HSV DNA synthesis starts with binding of the UL9 polypeptide to the oriL (or OriS) sites. This is followed by the binding of ICP8 (UL29) polypeptide, which has been known since a long time to stabilise the „lagging" DNA single strand for adding the nucleosides in an opposite 3'—5' direction. The new „lagging" strand DNA of HSV is formed by the help of helicase/primase complex, consisting of at least 3 proteins, produced by genes UL5, UL8 and UL52. A similar function in CMV DNA synthesis is accomplished from polypeptides encoded by genes UL70, UL102, and UL105. The primase produces a short RNA (primer) sequence needed for initia-

Tab. 5. Clinical syndromes caused by HSV1 and HSV2.

Acute disease (primoinfection)	Recurrent disease (after reactivation)
Stomatitis, pharyngitis, tonsillitis	*Herpes labialis* (predominantly type 1), unusual forms (*herpes facialis*)
Eczema herpeticum (mainly type 1, on inflamed skin)	
Herpes progenitalis (type 2)	*Herpes progenitalis* (type 2)
Ocular herpes (keratoconjunctivitis, chorioretinitis); in newborn frequently type 2	*Ulcus cornae* (preferentially type 1)
Herpetic disease of newborn (generalized skin infection, interstitial pneumonia, hepatoadrenal necrosis, meningoencephalitis, predominantly type 2).	
Either primoinfection or recurrent disease: encephalitis, acute necrotising hepatitis, pneumonia. In immunocompromised patients: oesophagitis, severe skin involvement, pneumonia.	

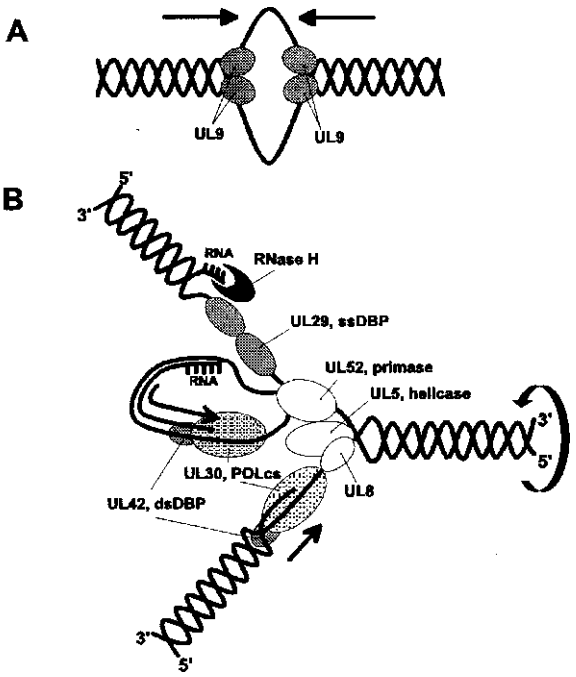

Fig. 3. Cartoon showing the herpesvirus DNA replication (according to Knopf, 2000). A. Unwinding of both DNA strands and binding of HSV UL9 protein (helicase activity) at the site of future replication fork (OriS). The CMV analogue for UL9 has not been identified. B. Synthesis of both nascent DNA strands. The HSV DNA polymerase activity (adding of nucleosides in 5‘ to 3‘, direction) is exerted by protein UL30 (in case of CMV by protein UL54). Their cofactors are UL42 (for HSV) and UL29 (for CMV). The lagging DNA strand is fixed by proteins UL29 (HSV) and UL57 (CMV), respectively. The proteins forming the helicase/primase complex for initiation of DNA synthesis in the opposite direction: for HSV they are products of genes UL5, UL8 and UL52; for CMV they are products of genes UL70, UL105 and UL107.

tion of the opposite strand DNA synthesis (reviewed by Knopf, 2000). The DNA polymerase (UL30 and UL42) complex is know able to add the nucleosides in both directions at the replication fork (theta-replication). Because the maternal double strand becomes circularised before replication, the nascent strand unwinds from the maternal strand by a „rolling circle“ mechanism while the complementary strand is synthesised by means of the above described mechanism from shorter fragments, which are finally ligated to form an „endless“ double strand (DNA concatemers). At packaging into the capsids, the „packaging signals“ at both *a* termini ($\mathbf{a}_L$ and $\mathbf{a}_S$) must be recognised and cut by the terminase complex (UL15 and UL28 proteins, see below). The herpesvirus DNA replication is a good target for several efficient virus replication inhibitors, from which acyclovir (HSV and varicella-zoster virus DNA polymerase inhibitor) and ganciclovir (CMV DNA polymerase inhibitor) emerged as most prominent (rewieved by Rajčáni, 1999). Both act in a phosphorylated form (as triphosphates). Addition of the first phosphate group is provided by a specific virus coded protein, HSV thymidine kinase (an UL23 gene product) which phosphorylates acyclovir to ACVP, while the UL97 CMV protein phosphorylates ganciclovir. ACVP cannot arise in CMV infected cells, since CMV lacks thymidine kinase.

Capsid formation

The packaging of HSV DNA into capsids is preceded by the formation of precursor capsids, consisting of following polypeptides: the major capsid protein VP5 (UL19), protein VP19C (UL38) and VP23 (UL18). All these are transported into nucleus and aggregate into procapsids of type A by the help of polypeptides UL17 and UL32. Then further proteins are added, namely VP26 (UL35) and the scaffolding protein precursors, products of genes UL26 and UL26.5. The cleavage of latter is a basis for maturation of procapsids into type B capsids, which become ready for DNA packaging. The original product of genes UL26 and UL26.5 is the ICP35 polypeptide, which is self cleaved into capsid protein VP24 (the N-terminus serine protease) and the C-

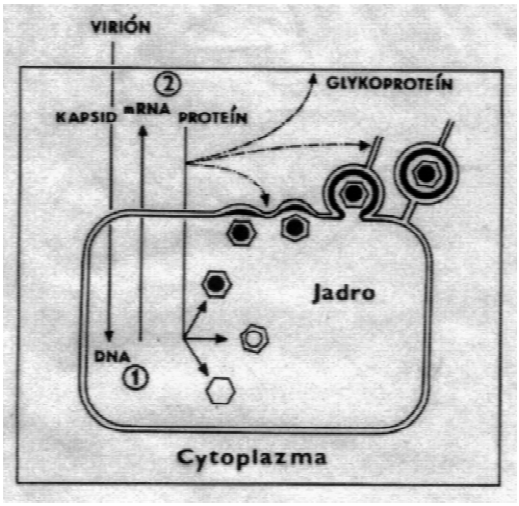

Fig. 4. The herpesvirus replication in infected cells starts at adsorption and penetration; then the capsids are transported towards the nucleus and the viral DNA moves into nucleus. 1 = initiation of DNA transcription, IE mRNAs are transported into cytoplasm and translated. 2 = the IE proteins return into nucleus, initiate further transcription followed by a second wave of translation. The generated early proteins assist at DNA replication. The structural capsid proteins are also transported into nucleus, where they aggregate into type A and B capsids. The newly synthesised viral DNA is packaged into type C capsids. 3 = the viral glycoproteins are produced from precursor polypeptides and incorporated into the cell membrane system. The mature capsids are enveloped at crossing the nuclear membrane. Finally, the particles leave the infected cells (egress).

terminus precursor polypeptide. The latter is further cleaved into a short peptide (VP21) and the scaffolding protein VP22a (Robertson et al, 1996). VP21 is the C-terminus of the original UL26 polypeptide; it has only 29 amino acids and remains associated with the VP5 capsid protein. The described cleavage is essential for the capsid type B maturation before DNA packaging (Kennard et al, 1995; Thomsen et al, 1995). The type B capsid contains 7 polypeptides, while the scaffolding protein VP22a is finally removed from the fully matured type C capsid. The mechanism of CMV capsid formation follows similar principles (Tab. 4).

The packaging of herpesvirus DNA into type B capsids is similar to events, which occur during DNA packaging into the bacteriophage head. In the beginning, the HSV coded UL15 protein (one terminase subunit) binds to the „packaging" motif in the terminal *a* DNA sequence. After binding a helper protein (UL6), the whole complex is transported to the capsid wall. After interaction with the second terminase subunit (UL28), the active terminase cleaves the concatemeric HSV DNA always at the same site between the two packaging signals located within the $\mathbf{a}_L$ and $\mathbf{a}_S$ terminal repeats. The helper UL6 protein now interacts with the scaffolding protein VP22a and with the internal capsid bound peptide VP21 in order to get packed and hooked inside the mature type C capsid. The completed capsids undergo envelopment at the internal lamella of nuclear membrane, where they gain all viral glycoproteins which in the meantime became inserted into the cellular membrane system (Tab. 3).

Tab. 6. Clinical syndromes in human CMV infection.

- Infectious mononucleosis syndrome (mainly at primoinfection of immunocompetent individuals).
- Anicteric hepatopathy (hepatosplenomegaly, lymphocytosis).
- Posttransfusion syndrome in seronegative recipients (severe infectious mononucleosis syndrome, pneumonia; predominantly due to reactivation of the latent virus from donor white blood cells).
- Reactivation of "endogenous" CMV in immunocompromised patients (AIDS, leukemia) and/or in immunosuppressed patients (i.e. after transplantation): infectious mononucleosis syndrome (active viraemia), interstitial pneumonitis, colitis, encephalitis (in AIDS two viruses multiply in the brain - HIV and HCMV). (Note: HIV *tat* protein reactivates HCMV IE transcription; HCMV replication is facilitated due to decreased cytotoxic T cells). CMV also potentiates HHV8 activation.
- Congenital infection: A. Generalised cytomegalic disease of newborn (hepatitis, jaundice, atypical lymphocytes, thrombocytopenia, splenomegaly). B. Chronic encephalitis - periventricular ependymitis, calcifications, microcephaly, hydrocephaly, deathness. C. Pneumonia. D. Other congenital malformations: heart malformations, palatoschisis. E. Abortive forms: viruria (kidney tubuli), shedding in saliva (salivary glands).

Envelopment of virions

The viral glycoproteins are synthesised at the rough endoplasmic reticulum, further glycosylated at trans-Golgi and finally transported to the cell membrane, including the nuclear membrane (Fig. 4). The egress of HSV particles is dependent on the presence of gK (reviewed by Rajčáni and Kúdelová, 1998). According to most recent views, the enveloped particles, present in the perinuclear space, may become deenveloped when entering cytoplasm and finally reenveloped within the transporter vesicles before expelled from cells. The viral gM which interacts with the desmosomes (for example in the squamous epithelium) helps in cell to cell spread without final envelopment. Though the above described events in HSV1 and 2 replication cycles on one hand and in the replication of CMV on other hand, showed many similarities, distinct biological features exist distinguishing them, which should be stressed here. 1) Alphaherpesviruses replicate in cells originating from various species, cytomegaloviruses replicate only in cells derived from the natural host species. This might be explained in part that way, that the HSV CP0 IE polypeptide is a non-specific (more universal) transactivator, which acts as cofactor for corresponding activator proteins in a wide variety of cell lines. In contrast, the ability of CMV IE1 and IE2 polypeptides to transactivate early transcription is confined to specific cellular cofactors from the homologous species. 2) The replication cycle of CMV lasts much longer than that of HSV. This difference comes from the longer maturation of CMV capsids, from longer formation of tegument and a prolonged envelopment process.

The tegument of CMV virions contains two potent and characteristical immunogens, namely phosphoproteins pp150 (UL32)

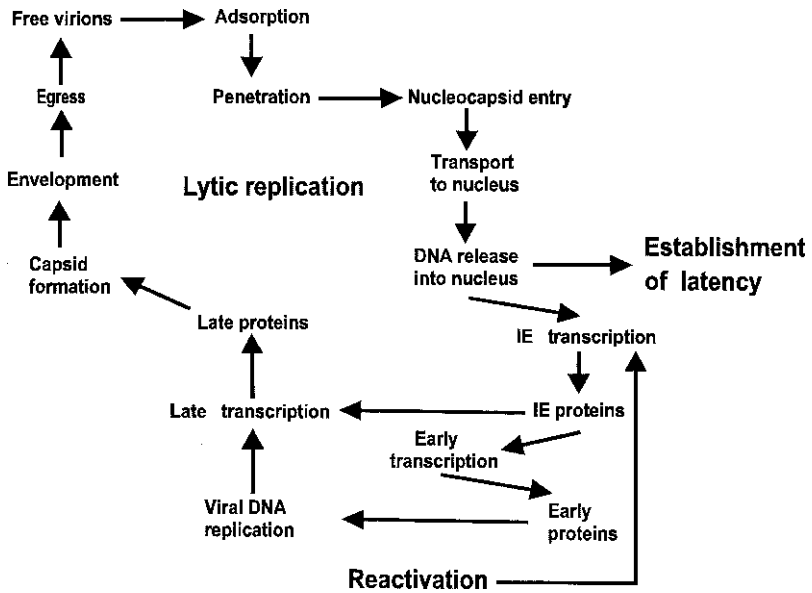

Fig. 5. The lytic replication cycle and HSV latency (modified from Subak-Sharpe and Dargan 1998). Upon penetration the virions loose their envelope. Tegument proteins and the capsid are transported towards the nucleus. In the nucleus, the cascade expression of virus coded proteins begins (α, β and γ). The viral mRNA leave the nucleus and become translated within cytoplasm. The viral proteins are transported either to the nucleus (capsid proteins) or to the endoplasmic reticulum (envelope glycoproteins). The virions mature at two steps: first they are enveloped at nuclear membrane, next the mature particles are transported to the cell surface. Latency can established from the very beginning avoiding lytic replication.

and pp65 (UL83). Their presence is related to the regulation of gene expression and to nuclear matrix of host cells. The pp65 appears relatively early (ß-polypeptide), a property useful for the quick identification of cells replicating CMV in culture (before cytopathic changes develop) by means of monoclonal anti-pp65 antibodies. The slow and inefficient egress of CMV particles from host cells, may be, at least in part, explained by the absence of gK (reviewed by Rajčáni a Kúdelová, 1998).

The continuing problem of latency

A striking property of herpespeviruses in general is their ability to undergo latency (Fig. 5). For herpesviruses, latency can be defined as a state of continuous DNA persistence in the absence of virus replication (Rajčáni and Szántó, 1979). This may but need not be accompanied by the synthesis of virus coded proteins. Several herpesviruses synthesise at least a few immediate early or early (non-structural) proteins during latency. However, HSV DNA is believed to remain inactive in this respect, though an RNA species, designated LAT (latency associated) is being transribed (reviewed by Ho, 1992). The silenced HSV DNA remains circularised in the nuclei of pseudounipolar sensory neurons (Rock and Fraser, 1985), after being transported from the mucous membranes and/or skin along neurotubules by quick intraaxonal transport (Paine, 1964). During latency, the number of HSV genome copies remains stabile in neurons of Gasserian ganglion similarly as the number of LAT copies transcribed (Hill et al, 1996). The number of neurons harbouring the latent genome varies (0.1—5 %) similarly as the number of LATs per DNA molecule (ranges from 10^1 to 10^3) depends of the amount of infectious virus which had penetrated the corresponding nerves endings (Sawtell et al, 1998). The LATs are not inevitable for the establishment of latency, but are related to the frequency and efficiency of reactivation (Spivack et al, 1995; Perng et al, 1996). It can be assumed that in the absence of α-TIF/VP16, the onset of effective viral DNA transcription is related to the expression of IE and E proteins such as ICP0, ICP4 and thymidine kinase (reviewed by Preston 2000; Rajčáni and Ďurmanová, 2000). There is neither clear how the essential regions of IE HSV gene promoters are blocked (methylation?) in order to suppress transcription, nor has been elucidated how is the blockade lifted at reactivation. The CMV DNA persists in a unknown conformation and under conditions of limited expression of IE1 protein (Jordan, 1983) or in the absence of any protein expression (Taylor-Wiedman et al, 1994). In various models of experimental CMV latency, semipermissive cells expressed certain IE genes and a limited number of ß-genes without replication of viral DNA and without infectious virus production (deMarchi et al, 1983; LaFemina and Hayward, 1988). Latency with limited transcription of IE genes appears in monocytes, macrophages and lymphocytes (Rice et al, 1984). CMV does not multiply in non-differentiated pluripotent bone marrow cells, but these can harbour the latent DNA. CMV latency has been described in acinar epithelium cells of salivary glands, in the epithelium of renal ducts and in various lymphatic elements within sinuses of spleen and lymph nodes.

The dissimilar pathogenesis and clinical sings

The pathogenesis of infection and clinical signs elicited by HSV (Tab. 5) and CMV (Tab. 6) differ profoundly. However, a common feature is the infection of CNS (encephalitis) caused by both viruses. HSV preferentially infects skin and mucous membranes and spreads along nerves to establish latency in regional sensory ganglia. CMV preferentially infects nasopharyngeal tissue and salivary glands causing viraemia. Latency established in lymphatic tissues and macrophages reactivates during immunosuppression (after transplantation, during AIDS). HIV virus codes for a regulatory protein (*tat*), which may contribute to expression of CMV IE genes leading to reactivation. After repeated transfusions of white blood cells (from seropositive donors) latent infection reactivates causing the posttransfusion syndrome (especially in seronegative recipients) Newborn acquire HSV during delivery and closely after birth (from ward personnel or from other contacts with infectious labial herpes). In contrast, viraemia (even when latent) may infect the offspring causing congenital infection. Various malformations develop when the virus replicates in embryonic tissues. In addition to hepatitis B, CMV is the most frequent cause of congenital virus infection. The third important virus in this respect is HIV; rubella virus follows on forth place after being eliminated, at least in developed countries, due to successful vaccination.

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