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HERPESVIRUS GENES: MOLECULAR BASIS OF VIRAL REPLICATION AND PATHOGENICITY

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ABSTRACT

Herpesviruses possess large DNA genomes which contain from approximately 80 to 200 genes. These viral genes are divided into two groups based on whether they are essential or nonessential (dispensable) for virus growth in cell culture: the essential gene products include a set of replication proteins which accomplish the viral DNA replication, while the dispensable gene products include those important in influencing pathogenesis. This article briefly reviews the results of studies relating to the functions and roles of the gene products of human herpesviruses, particularly products associated with the herpes simplex virus.

Key Words: Human herpesviruses, Herpes simplex virus, Viral replication, Viral pathogenicity, Viral gene functions

INTRODUCTION

Herpesviruses are large DNA viruses whose genomes consist of a linear dsDNA molecule, 125–229 kbp. Their hosts range from lower vertebrates to humans. Virtually every vertebrate that has been carefully screened has been found to support at least one host-specific herpesvirus. In humans, five viruses (herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster virus (VZV), human cytomegalovirus (HCMV) and Epstein Barr virus (EBV)) have been identified as members of the herpesvirus family. In the past decade, however, three new human herpesviruses (human herpesviruses 6 (HHV-6), 7 (HHV-7) and 8 (HHV-8)) were discovered in AIDS patients. Together, these eight herpesviruses are associated with a variety of human diseases (Table 1), but their importance is increasing as a result of developments in medical technology and changing human behavior. They are frequently reactivated and sometimes cause severe diseases in immunocompromised hosts. Although the herpesvirus family, Herpesviridae, is divided into three subfamilies (Alpha-, Beta-, and Gamma-herpesvirinae), all herpesviruses share common features in their structure, gene organization, replication style and so forth. For example, while all of them have the ability to persist in their hosts throughout life, in the form of an episome in the nuclei of infected cells, still each herpesvirus occupies distinct ecological niches and causes characteristic diseases.

The features of each herpesvirus may be related to specific viral genes. The goal of our research at the Laboratory of Virology, Research Institute for Disease Mechanism and Control is to understand the role of herpesvirus genes and their involvement both in viral replication and in the disease process. In this paper, I briefly summarize the results of recent studies, including ours, on the functions and roles of the gene products of human herpesviruses, in particular HSV.

Table 1. Human herpesviruses

Subfamily	Virus	Genome size (kbp)	Disease
Alphaherpesvirinae	Herpes simplex virus 1 (HSV-1)	152	Gingivostomatitis, herpes labialis, encephalitis, genital herpes
	Herpes simplex virus 2 (HSV-2)	152	Genital herpes, neonatal herpes, herpetic paronychia
	Varicella-zoster virus (VZV)	125	Varicella, herpes zoster
Betaherpesvirinae	Human cytomegalovirus (HCMV)	229	Congenital cytomegalic inclusion disease, pneumonitis, retinitis, mononucleosis
	Human herpesvirus 6 (HHV-6)	162	Exanthem subitum (roseola)
	Human herpesvirus 7 (HHV-7)	160–170	Exanthem subitum?
Gammaherpesvirinae	Epstein-Barr virus (EBV)	186	Infectious mononucleosis, Bukitt's lymphoma, nasopharyngioma
	Human herpesvirus 8 (HHV-8)	?	Kaposi's sarcoma?

OVERVIEW OF HERPES SIMPLEX VIRUS

General features

Herpes simplex virus types 1 and 2 are prevalent human pathogens which cause a variety of diseases ranging from mild skin disorders to life-threatening encephalitis. HSV-1 typically causes recurent attacks of labial herpes, while HSV-2 is mainly responsible for recurent genital herpes. HSV-1 and HSV-2 share about 50% of base sequence homology and their genetic maps are very similar. They differ in restriction endonuclease cleavage sites, the apparent sizes of the viral proteins, antigenicity and some biological markers including plaque size, thermal stability and sensitivity to antiviral agents. Like other herpesviruses, HSV virions consist of four morphological elements: an inner core, an icosahedral capsid with 162 capsomers, a surrounding amorphous tegument, and an envelope containing a number of glycoproteins. The tegument contains about 20 distinct structural proteins with various functions, including the α-trans-inducing factor (α-TIF, UL48 product) and a virion-associated host shut-off protein (UL41 product).

The HSV genome (approximately 100×10^6 Da) comprises two regions designated long (L) and short (S). Terminal repeat (TRL and TRS) and internal repeat (IRL and IRS) sequences bracket unique sequences (UL and US) of both L and S (Fig. 1). The complete sequence of the HSV-1 genome has been determined and the genome has been shown to contain at least 76 genes.[1] About half of the genes are not essential for viral replication in cultured cells (Table 2). However, it is likely that many of these dispensable genes play an important role in virus-host interactions in vivo.[2]

Viral Replication

The HSV virion possesses at least 11 glycoproteins, but only five, gB, gD, gH, gK and gL, are necessary to infect cells in cultures. A nonessential viral glycoprotein, gC, plays a major role in the initial association with a cellular receptor, the heparan sulfate moiety of cellular proteoglycans, while gB, gD and gH are absolutely required for entry into the cytoplasma. Fusion of the

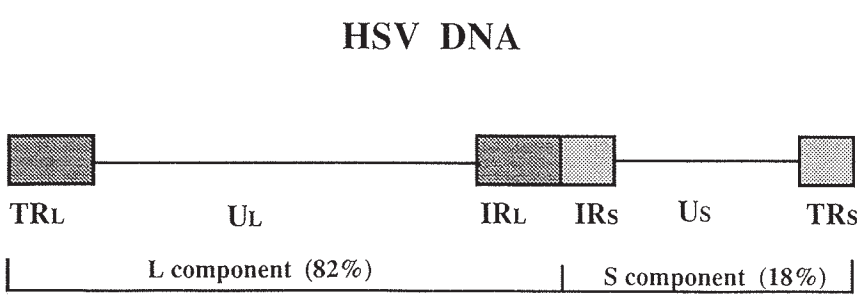

Fig. 1. Structure of Herpes Simplex Virus DNA

virion envelope with the plasma membrane occurs on the cell surface in a pH-independent manner. Viral capsids with a portion of tegument are transported to a nuclear pore, where viral DNA is released, circularized and transcription is initiated.

HSV gene expression is coordinately regulated and sequentially ordered in a cascade fashion.[2] Five genes (RL1, UL54, RS1, US1 and US12) are expressed as immediate early (IE or α) transcripts, and all except the US12 product (ICP47) have a role in the regulation of gene expression at the level of transcription or posttranscription. The RL1 (α0) and RS1 (α4) products are involved in the overall expression of both early (E or β) and late (L or γ) genes, and the UL54 product (α27) is essential for the expression of some late genes. The transcription of these IE genes is markedly stimulated by the tegument protein VP-16 (α-TIF).

The E genes comprise a much larger and more heterogeneous group. Many of the E gene products have enzymatic activities[3] and are involved in the synthesis of viral DNA and of dNTP precursors (Table 3). Seven E gene products are strictly required for HSV DNA replication. These include DNA polymerase (UL30), its accessory protein (UL42), the helicase-primase complex (UL5, 8 and 52), the single-stranded DNA binding protein (UL29) and the replication-origin binding protein (UL9). Type 1 and type 2 topoisomerases may also be required for viral DNA synthesis, but are of cellular origin.[4,5] Host cell DNA polymerases are not involved in the replication of viral DNA but in its repair.[6,7,8] The viral DNA replicates by a rolling circle mechanism in the presence of these proteins. The unit-length HSV DNA is cleaved from newly synthesized DNA concatemers and is packaged into preformed empty capsids. The nuclear matrix appears to be involved in the capsid formation.[9] Full capsids associate with tegument proteins near the nuclear membrane, and pass through the inner and outer membranes by budding and deenvelopment. Envelope glycoproteins are synthesized in the endoplasmic reticulum and interact with chaperons such as calnexin and Bip to fold properly.[10,55] Enveloped virions accumulate in the endoplasmic reticulum, and mature in the Golgi apparatus. The mature virions are released into the extracellular medium by exocytosis.

Virus-Cell Interaction

Shortly after infection with HSV, there occurs a marked decrease in host macromolecular synthesis. The early cessation in protein and DNA synthesis is caused by a structural component of HSV, and a more profound cessation of host cell metabolism occurs with the initiation of the expression of E and L genes.[2] When human embronic fibroblasts are infected with HSV-2, significant repair synthesis of cellular DNA is induced by 3–5 h postinfection (p.i.), and at 12 h p.i. more than 95% of cellular DNA synthesis is the repair type; infection of the cells with HSV-2 induces extensive strand breaks of cellular DNA.[11] Semiconservative replication of viral

Table 2. HSV genes and their functions

Gene	Essential (E) or dispensable (D)	Period of expression	MW	Function
RL1	D	IE	78K	Transcriptional regulator, IE110, ICP0, α0
RL2	D	L		ICP34.5, neurovirulence
RL3	D			LAT transcription unit
UL1	E	L	25K	Glycoprotein L, fusion
UL2	D	E	36K	Uracil DNA glycosylase
UL3	D		26K	? Nuclear phosphoprotein
UL4	D	L	22K	? Virion protein
UL5	E	E	99K	Helicase/primase complex, DNA-dependent ATPase
UL6	E		74K	Capsid protein, VP11, DNA cleavage-packaging
UL7	D		33K	?
UL8	E	E	80K	Helicase/primase complex,
UL9	E	E	94K	Replication origin-binding protein
UL10	D	L	51K	Glycoprotein M
UL11	D	L	10K	Myristylated virion protein, nucleocapsid envelopment
UL12	D	E	67K	Alkaline exonuclease
UL13	D	L	57K	Protein kinase, virion protein
UL14	E		23K	?
UL15	E	L	81K	Capsid formation, DNA packaging
UL16	D		40K	?
UL17	E	L	75K	?
UL18	E	L	34K	Capsid protein, VP23, Capsid formation
UL19	E	L	150K	Major capsid protein, VP5
UL20	D	L	24K	Virion transport, Intrinsic membrane protein
UL21	D		58K	? Nucleotidylylated phosphoprotein
UL22	E	L	90K	Glycoprotein H
UL23	D	E	41K	Thymidine kinase
UL24	D	L	30K	Membrane protein
UL25	E	L	63K	Capsid formation, DNA cleavage-packaging
UL26	E	L	62K	Proteinase
UL26.5	E	L		Substrate of UL26 protease, DNA packaging
UL27	E	L	100K	Glycoprotein B
UL28	E	L	86K	Capsid formation, DNA cleavage-packaging
UL29	E	E	128K	Major DNA-binding protein
UL30	E	E	136K	DNA polymerase
UL31	E	L	34K	? Nucleotidylylated phosphoprotein, Nuclear matrix
UL32	E	L	64K	Capsid formation, DNA packaging
UL33	E	L	14K	Capsid formation, DNA packaging
UL34	E	L	30K	Nonglycosylated, membrane-associated protein
UL35	E	L	12K	Capsid protein
UL36	E	L	336K	Tegument protein, DNA release?
UL37	E	L	121K	? Cytoplasmic phosphoprotein
UL38	E	L	50K	DNA-binding protein, Anchoring DNA in the capsid
UL39	D	E	124K	Ribonucleotide reductase (large subunit)
UL40	D	E	38K	Ribonucleotide reductase (small subunit)
UL41	D	L	55K	Host shut-off protein, virion protein
UL42	E	E	51K	DNA polymerase accessory protein
UL43	D	L	45K	? Membrane protein
UL44	D	L	55K	Glycoprotein C, C3b-binding activity
UL45	D	L	18K	? Membrane protein
UL46	D	L	78K	Tegument protein, α-TIF modifier
UL47	D	L	74K	Tegument protein, α-TIF modifier
UL48	E	L	54K	Major tegument protein, VP-16, α-TIF

Gene	Essential (E) or dispensable (D)	Period of expression	MW	Function
UL49	E	L	32K	Nucleotidylylated tegument protein
UL49.5	E	L		? Membrane protein
UL50	D	E	39K	Deoxyuridine triphosphatase
UL51	D	L	25K	?
UL52	E	E	114K	Helicase/primase complex
UL53	E	L	38K	Glycoprotein K, Fusion, Viral exocytosis
UL54	E	IE	55K	Posttranscriptional gene regulator, IE63, ICP27, α27
UL55	D		20K	?
UL56	D		21K	? Virion-associated protein
RS1	E	IE	133K	Major transcriptional regulator, IE175, ICP4, α4
US1	D	IE	46K	Regulatory protein, IE68, ICP22, α22
US2	D		32K	?
US3	D	E	53K	Protein kinase
US4	D	L	25K	Glycoprotein G
US5	D		10K	Glycoprotein J
US6	E	L	43K	Glycoprotein D
US7	D	L	41K	Glycoprotein I
US8	D	L	59K	Glycoprotein E, Fc-binding activity
US9	D	L	10K	Tegument phosphoprotein
US10	D		34K	Virion protein
US11	D	L	18K	RNA-binding protein, Tegument protein
US12	D	IE	10K	Down-regulation of MHC class I, ICP47

1. The genes are divided into two groups based on whether they are essential (E) or dispensable (D) to virus replication in cell cultures.
2. The genes are expressed at the immediate early (IE), early (E) and late (L) phases of infection.
3. ICP = Infected cell-specific polypeptides
4. MW = Predicted molecular weight

Table 3. Herpesvirus-encoded enzymes

		HSV homologs			
	Enzymes	HSV	VZV	HCMV	EBV
Viral DNA replication	DNA polymerase	UL30	28	UL54	BALF5
	DNA helicase	UL5	55	UL105	BBLF4
	DNA primase	UL52	6	UL70	BSLF1
Nucleotide & DNA metabolism	Ribonuceotide reductase				
	large subunit	UL39	19	UL45	BORF2
	small subunit	UL40	18	–	BaRF1
	Deoxyuridine triphosphatase	UL50	8	UL72	BLLF2
	Thymidine kinase	UL23	36	–	BXLF1
	Thymidylate synthetase	–	13	–	–
	Alkaline nuclease	UL12	48	UL98	BGLF5
	Uracil DNA glycosylase	UL2	59	UL114	BKRF3
Protein modification	Protein kinase	UL13	47	UL97	BGLF4
		US3	66	–	–
		UL39[1)]	–	–	–
	Protease	UL26	33	UL80	–

1. The N-terminal portion of the large subunit of HSV ribonucleotide reductase has a protein kinase activity.

DNA predominates between 3 and 8 h p.i., but at 12 h p.i. about 50% of viral DNA synthesis is also the repair type. Although the induction mechanism of repair DNA synthesis remains unclear, the process of apoptosis, also called "programmed cell death", may be involved in this phenomenon. HCMV, unlike HSV, does not induce repair synthesis of cellular DNA even at late stages of infection.[12,13] Recent studies have shown that IE proteins 1 and 2 of HCMV can inhibit the induction of apoptosis.[14]

HSV is thus highly cytocidal and its productive infection results in rapid cell death. However, the virus can latently infect neurons in the sensory ganglia of humans and experimental animals. In the latent stage of infection, only one single transcription unit, the latency-associated transcription unit (LAT), is expressed, but there is no detectable synthesis of virus-encoded proteins in latently infected neurons. LAT appears to be involved in reactivation. The molecular mechanisms involved in latency have remained unclear partly because of a lack of appropriate in vitro latency models, although considerable efforts have been devoted to establishing such in vitro models.[15,16,17]

Virus pathogenicity

The outcome of a virus infection is greatly influenced by both the host and the virus strain. Various animals, especially mice, have been used to study the mechanism by which HSV causes diseases. Studies using inbred strains of mice have shown that mouse strains vary in their resistance to HSV following intraperitoneal infection, and have later pointed to natural defence mechanisms as being responsible for this genetically determined difference in resistance.[18] There is good evidence to suggest that the macrophage restriction of virus replication, natural killer cell activity and early production of interferon α/β participate in determining the outcome of HSV infection, although their relative roles remain indeterminate. It is also known that various HSV strains and mutants demonstrate great differences in virulence after infection of an individual mouse strain. Table 4 shows the neurovirulence phenotype of the viruses tested in our laboratory.[19-23] Some strains can cause fatal encephalitis at low doses of infection (less than 10^2 PFU)

Table 4. Neurovirulence of herpes simplex virus in mice

Virus	Characteristics	Neurovirulence phenotype
HSV-1 KOS	Laboratory strain, wild-type	class I
KOS/hrR3	RR (UL39)-deficient mutant	class III
KOS/TK$^-$	TK (UL23)-deficient mutant	class III
HSV-1 SP23	Laboratory strain, wild-type	class I
SP23/N38	US9,10,11,12-deletion variant	class I
HSV-1 HF	4kb-deletion in the Bam HI B fragment	class II
HSV-1 SKa	Laboratory strain, small plaque	class III
HSV-1 TN-1	gC-negative isolate	class I
RTN-1	gC-rescued virus	class I
HSV-2 186	Laboratory strain, wild-type	class I
186/L1BR1	US3 PK-deficient mutant	class II
186/L1B-11	US3 PK-rescued virus	class I
HSV-2 YN	fresh isolate	class I

Class I strains induce lethal encephalitis both by the intracerebral and the peripheral routes of infection. Class II strains are highly virulent only by the intracerebral route. Class III strains are highly attenuated by both routes of infection.

following intraperitoneal and footpad inoculation of mice, while other strains cannot induce any detectable sign of neurological disease after infection with as much as 10^8 PFU even if the growth rates of these viruses in most cell types are not significantly different from those of the virulent strains. The investigation of the mechanisms by which individual HSV strains show different pathogenicity is of fundamental importance since it facilitates our understanding of the pathogenesis of HSV infections.

To understand how specific HSV genes are involved in the development of diseases, various approaches have been employed. One useful approach is to make genetically engineered mutants which fail to express a specific gene and then to compare their biological properties with those of the parental wild-type virus in cell cultures and in animal models.[20,21] By application of such an approach, investigators have identified pathogenically relevant genes and their products.

HERPES SIMPLEX VIRUS GENES AND THEIR FUNCTIONS

UL30 DNA polymerase and fidelity

When cultured host cells are infected with a herpesvirus, a novel DNA polymerase which is immunologically and biologically distinct from host cell DNA polymerases is induced and easily detected in the nuclear extracts of infected cells. Because of their central role in viral DNA replication, herpesvirus polymerases have been purified and extensively characterized with respect to their substrate specificity, reaction optima and kinetic behavior.[24,25] The purified preparation of HSV DNA polymerase consists of two polypeptides having molecular masses of 140 kDa and 65 kDa. The former polypeptide is the DNA polymerase and the latter is the UL42-encoded DNA binding protein, which has the ability to increase the processivity of the polymerization. The polymerase-UL42 protein interaction is very tight and essential for viral DNA replication.

HSV DNA polymerase, like other herpesvirus polymerases, contains an associated 3′-to-5′ exonuclease activity and shows a clear ability to preferentially excise a terminal nucleotide mispair, the classical criterion by which proofreading was demonstrated for procaryotic polymease.[26,27] On a ϕX174 natural DNA template, the herpes polymerase is more accurate than purified eucaryotic DNA polymerases α and β; the error rate is similar to E. coli polymerase I. However, the high accuracy is maintained under conditions which are used to inhibit proofreading in procaryotic DNA polymerases, suggesting that the HSV DNA polymerase may be relatively accurate even in the absence of proofreading.

The herpes DNA polymerases are very susceptible to genetic analysis, because they are the targets for various inhibitors including aphidicolin (Aph), phosphonoacetic acid (PAA) and antiviral nucleoside analogs. We have isolated HSV mutants resistant to Aph and PAA, and have analyzed these mutants, which carry drug-resistant polymerases.[6,27,28] The mutants can replicate in cultured cells as efficiently as the wild-type virus, but have different rates of spontaneous mutation; Aph^r isolates have mutator phenotypes, while PAA^r isolates have antimutator phenotypes. Investigation of the mutant polymerases showed that these DNA polymerases have similar exonuclease/polymerase ratios, suggesting that altered basepairing selectivity of the mutant enzymes is involved in the different mutation frequencies. We have also determined the nucleotide sequence of the DNA polymerase gene of HSV type 2,[29,30] and have identified the mutation site responsible for the Aph-resistance phenotype. The Aph^r mutant polymerase has an aminoacid substitution from a tyrosine to a histidine in the well-conserved region of the DNA polymerase.[31] Our results indicate that the single amino acid change can confer resistance to Aph, hypersensitivity to PAA, an increased affinity to dCTP and dTTP, and is also responsible for the mutator phenotype.

Recent application of the baculovirus-expression system greatly facilitated the identification of the functional roles of individual proteins, herpesvirus DNA polymerase catalytic subunits and their accessory proteins.[32,33] We have overexpessed and purified the individual components of EBV DNA polymerase by using a baculovirus expression system.[34] The accessory subunit exhibits a greater binding affinity for double-stranded DNA but has neither polymerase activity nor exonuclease activity. The accessory protein stimulates the polymerizing activity more than 10-fold compared with that of the catalytic subunit alone. Moreover, we found that although the processivity of the catalytic subunit is low on singly primed M13 single-stranded DNA circles, the addition of the accessory subunit results in completely processive replication and the generation of full-length products.[35,36] A similar interaction is observed with other herpesvirus DNA polymerases.

UL23 thymidine kinase, UL39 ribonucleotide reductase and neurovirulence

Herpesvirus thymidine kinase (TK) was first reported in 1963, and has been one of the most discussed viral proteins in recent years because the TK gene has proven to be a useful vehicle to introduce viral nucleic acid in studies of molecular and cellular biology[37,38] and also because the antiviral nucleoside analogs such as acyclovir and BVdU have been shown to be selectively phosphorylated by the enzyme.[39] HSV TK is composed of two identical subunits each containing 376 amino acids, and the native protein has binding sites for both the natural nucleoside substrates thymidine and deoxycytidine, and also for the phosphate donor ATP. The enzyme, unlike cellular TK, also possesses thymidylate kinase activity. Although TK is not essential for viral replication in cell cultures, it is important for viral pathogenesis in animal models and in humans. TK-negative mutants exhibit restricted replication in neural tissues, and fail to reactivate from latent infections of mouse sensory ganglia.[40]

Ribonucleotide reductase (RR) is a key enzyme in the de novo synthesis of DNA precursors, catalyzing the reduction of all four ribonucleoside diphosphates to the corresponding 2′-deoxyribonucleoside diphosphates. HSV encodes its own RR which is distinct from cellular RRs; HSV RR consists of two nonidentical subunits, termed RR1 (140 kDa) and RR2 (38 kDa).[41] The large subunit is encoded by the UL39 gene, and the small subunit is encoded by the UL40 gene. The eukaryotic RRs are highly regulated by triphosphate nucleosides and have an absolute requirement for ATP and Mg^{2+}, while HSV RR is free of allosteric control by dATP and dTTP and is fully active in the absence of ATP and Mg^{2+}. These properties of the viral enzyme are responsible for the buildup of large deoxyribonucleoside triphosphate pools for viral DNA synthesis. For example, infection of Vero cells with HSV causes a marked increase (approximately 10-fold) in the dTTP pool size of infected cells; this increase is not dependent on the induction of viral TK, but that of viral RR, indicating that HSV RR is of primary importance even in the increase of dTTP pool size.[42]

To determine how HSV RR is involved in the pathogenicity of HSV, the replication of RR-deficient mutants were investigated after intracerebral and corneal inoculation in newborn and adult mice.[20] The mutants failed to replicate in the brains of mice > 8 days old but exhibited significant virulence in newborn mice as a result of viral replication in the brains. Our studies indicate that the induction of viral RR is essential for growth in the central nervous system in adult mice but is not essential for the establishment and maintenance of latency in vivo.

US3 protein kinase and intraperitoneal pathogenicity

HSV also encodes enzymes catalyzing protein modification, such as protein kinase and protease (Table 3). At present, three open reading frames, US3, UL13 and UL 39 are identified as protein kinase-related genes. The US3 gene was originally found to be homologous to the

protein kinase gene family of eukaryotes and retroviruses by DNA sequencing analysis. We have purified the US3-encoded protein kinase from post-ribosomal cytoplasmic fractions of Vero cells infected with HSV-2.[43] The enzyme has a molecular mass of 66 kDa and is relatively resistant to high concentrations of salts. When the substrate specificity was investigated using synthetic oligopeptides, the peptides containing arginyl residues on the amino-terminal side of the target seryl residue were found to be the best substrates for the US3 protein kinase.

To study the roles of the enzyme in replication and pathogenicity, we have constructed a US3 lacZ insertion mutant of HSV-2 which cannot induce the US3 protein kinase activity, and have characterized it in vitro and in vivo.[21,44] Although the US3-deficient mutant replicates in Vero cells as efficiently as the parental wild-type HSV-2, the virulence of the mutant for mice is remarkably reduced, depending on the routes of infection; after inoculation by footpad and intraperitoneal routes, the mutant is more than 10,000-fold less virulent than the parental virus, but it exhibits only about a 10-fold decrease in virulence following the ocular and intracerebral infection. In the intraperitoneal inoculation into adult mice, the replication of the mutant in the liver and spleen is severely restricted, but in newborn mice the mutant can grow as well as the parental virus in these organs. The adoptive transfer of peritoneal macrophages from adult mice results in marked inhibition of mutant replication. Moreover, the mutant, unlike the parental virus, cannot replicate in peritoneal macrophages from adult mice. When the transneuronal spread of these viruses was immunohistochemically studied in immunocompetent and immunosuppressed mice, it was found that the mutant principally retains the capacity to spread in the central nervous system.[45] These results suggest that the US3 protein kinase-deficient mutant loses the ability to overcome the mononuclear-phagocytic defense system and thereby loses its pathogenicity by intraperitoneal and footpad routes.

To learn more about the biological role of the US3 protein kinase, we sought to identify the target proteins of the enzyme. Studies of in vitro phosphorylation with extracts of infected cells suggest that the protein kinase is involved in phosphorylation of the UL12 alkaline nuclease and the tegument protein encoded by US9.[46,47] The deletion of the US9 gene does not much affect the virulence of HSV in mice,[22] but the deficiency of the alkaline nuclease strongly inhibits the replication of HSV even in cultured cells. It thus seems that the phosphorylation of the alkaline nuclease by the US3 protein kinase has critical importance for the HSV replication in macrophages. However, the precise mechanism remains to be elucidated.

Viral proteins and immune evasion

Viruses have evolved a variety of mechanisms by which they circumvent the host's antiviral response. Recent studies have shown that large DNA viruses possess many genes which interfere with specific parts of the host immune system such as interferons, complements, cytokines, neutralizing antibodies and cyotoxic T lymphocyte (CTL) recognition.[48,49]

HSV-encoded glycoprotein C (gC) is one of the major glycoproteins on the virion envelope, and it plays an important role in virus attachment onto cell membrane proteoglycans.[49] It contains a heparan sulfate-binding domain. In addition, gC has the ability to bind both a component of complement, C3b, and factor X. Studies with mutant HSVs have demonstrated that gC provides protection against complement-mediated neutralization either in the presence or absence of antibodies. Since the protection afforded by gC against complement is small in the presence of antibody, it appears that gC primarily influences the alternative complement pathways; namely, gC may be most effective in protecting HSV from complement during primary infection, before antibodies are present. These studies suggest important roles for this glycoprotein in vivo. Nevertheless, many investigators have reported that gC-negative mutants produce diseases paralleling those caused by the wild-type in mice. Our recent studies, however, have

Table 5. Herpesvirus proteins that modulate host immune responses

Virus	Viral proteins	Host response
HSV	gC (UL44)	Complement (C3b-binding activity)
	gE-gI complex (US7, US8)	Antibody (Fc receptor)
	IP47 (US12)	CTL (MHC class I down-regulation)
HCMV	US11 product	CTL (MHC class I down-regulation)
	US2 – US5 products?	CTL (MHC class I down-regulation)
	US28 product	Cytokine (C-C chemokine receptor)
	UL18 product	CTL? (MHC class I heavy chain homolog)
EBV	BCRF1 product	Cytokine (IL-10 activity)

demonstrated that gC is important for the induction of some specific lesions in a murine pneumonia model (unpublished observations).

$CD8^+$ CTLs recognize intracellular viral or other foreign protein antigens only in association with class I major histocompatibility complexes (MHC) on the cell surface. Class I MHC molecules consist of a polymorphic membrane-spanning 45 kDa heavy chain noncovalently associated with a 12 kDa light chain, β2 microglobulin (β2m). Peptides generated from degradation of endogeneous proteins within the cytoplasma associate with the heavy chain and β2m at the time of their assembly in the endoplasmic reticulum. After assembly, the complex is transported to the cell surface, where it may be recognized by $CD8^+$ CTLs. Herpesviruses have evolved to evade $CD8^+$ CTLs by the down-regulation of MHC class I complexes (Table 5). In cells infected with wild-type HSV, the reduction may be partly a result of the effect of the UL41 product in shutting down host protein synthesis. However, a mutant lacking the UL41 gene can also down-regulate the surface expression of MHC class I antigens. Class I molecules are synthesized in normal amounts in cells infected with the mutant, but the heavy chains are retained in the endoplasmic reticulum and rapidly degraded.[49] Recent studies have shown that the US12 product, ICP47, is necessary and sufficient to cause MHC class I retention in the endoplasmic reticulum, and that ICP47 inhibits peptide transport across the ER membrane so that nascent class I molecules fail to acquire antigenic peptides.[50] In the case of HCMV, infection with the virus causes the increased synthesis of MHC class I molecules,[51,52] but results in the reduction of its cell surface expression.[52,53] Studies using deletion mutants have demonstrated that two loci within the HCMV US region are independently involved in down-regulation,[54] although the molecular mechanisms remain unknown.

CONCLUSION

The study of human herpesviruses is rapidly progressing, and the functions of many herpesvirus genes have been elucidated. Since HSV has been most actively investigated among them, we can now explain its replication cycle in considerable detail and outline the virus-host interaction at the molecular level. In recent studies, HSV has even been promoted as a therapeutic gene-transfer vector for the introduction of DNA into the central nervous system. Further understanding of the functions and roles of herpesvirus genes will shed light on new strategies to prevent and cure human diseases caused by these viruses.

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