Microreview

Herpes simplex virus: receptors and ligands for cell entry

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Summary
Entry of herpes simplex virus (HSV) into cells depends upon multiple cell surface receptors and multiple proteins on the surface of the virion. The cell surface receptors include heparan sulphate chains on cell surface proteoglycans, a member of the tumor necrosis factor (TNF) receptor family and two members of the immunoglobulin superfamily related to the poliovirus receptor. The HSV ligands for these receptors are the envelope glycoproteins gB and gC for heparan sulphate and gD for the protein receptors and specific sites in heparan sulphate generated by certain 3-O-sulfotransferases. HSV gC also binds to the C3b component of complement and can block complement-mediated neutralization of virus. The purposes of this review are to summarize available information about these cell surface receptors and the viral ligands, gC and gD, and to discuss roles of these viral glycoproteins in immune evasion and cellular responses as well as in viral entry.

HSV and disease
The most common manifestations of HSV infection are mucocutaneous lesions, commonly called cold sores or fever blisters if they occur on or near the lips. Such lesions can occur anywhere that the virus is inoculated, however. Lesions on the fingers (herpes whitlow) used to be an occupational hazard for dentists before the widespread use of surgical gloves. Herpes genitalis refers to the lesions on genitilia in sexually transmitted forms of disease. In addition, the cornea of the eye can be infected to cause keratitis. These lesions usually resolve without scarring and would not be a serious concern except that they can recur frequently. The virus enters sensory and autonomic neurons whose axons extend to the locale of the lesions and the virus sets up latent infections from which it can periodically be reactivated. Reactivated virus is transported back to the body surface to cause recurrent lesions. Occasionally the virus can also be transported to the central nervous system to cause encephalitis. Fortunately, this occurs rarely but, unfortunately, the factors that predispose to encephalitis are not known. Age must be one such factor because HSV infections in newborn infants can result in severe disseminated disease including neurological involvement. Two of the most important cellular targets in HSV disease are epithelial cells of skin and mucosa and neurons. Lymphocytes and other leucocytes can also be infected, a phenomenon of unknown significance with respect to the effectiveness of immune responses. Usually, spread of infection is by cell-to-cell contact, not via a hematogenous or lymphatic route. Some consideration will be given here to the requirements for viral entry into leucocytes, epithelial cells and neurons.

There are two serotypes of HSV, HSV-1 and HSV-2. Lesions caused by HSV-1 strains cannot be distinguished from those caused by HSV-2 but there are distinct genetic and biological differences between members of the two serotypes. For example, although both HSV-1 and HSV-2 can infect either oral or genital sites, HSV-1 is more likely to reactivate frequently from oral sites and HSV-2 is more likely to reactivate from genital sites (Lafferty et al., 1987).

Characteristics of HSV and the herpesvirus family
The family is large and diverse but all members share certain features in common. Each has a large double-stranded DNA genome encoding about 100–200 genes. In the virion, this genome is packed within an icosahedral capsid displaying 162 capsomers or morphological elements. The capsid is in turn coated with a layer of proteins called the tegument, all of which are enclosed within a membrane composed of lipids and more than a dozen viral proteins and glycoproteins. The virion is designed to protect the viral genome from adverse conditions in the extracellular environment and to permit cell invasion so that the viral genome can be released to the
cell nucleus for expression of its genes. The outer membrane or envelope of the virion is its infectivity organelle. Several of the glycoproteins in the envelope are essential for viral entry into cells and others may influence this process (Fig. 1).

The initial contact of HSV with a cell is believed to be binding of the virion to glycosaminoglycan (GAG) chains of cell surface proteoglycans. Heparan sulphate, one of several kinds of GAG, is preferred and is considered to be a binding receptor, as opposed to an entry receptor. Two of the virion glycoproteins, designated gB and gC, are capable of binding to heparan sulphate and either appears to be able to mediate the binding of virions to cell surface heparan sulphate. Although this binding significantly enhances the efficiency of HSV infection, it is not absolutely essential, at least not for the infection of cultured cells. If gC is absent from the virion, specific infectivity may be reduced as much as 10-fold as a result of reduced efficiency of virus binding to cells (Herold et al., 1991), but whether absence of gC has this effect is dependent on HSV serotype, perhaps on HSV strain and on cell type (Cheshenko and Herold, 2002). If both gB and gC are absent from the virion, binding to cells is severely reduced (Herold et al., 1994). Infectivity is abolished also, but this is in part because gB has an essential role in viral entry, as outlined below. Deletion of the heparan sulphate-binding domain from gB does not abrogate its essential role in entry (Laquerre et al., 1998). If cells are devoid of GAGs, susceptibility to infection is significantly reduced, but not abolished, unless entry receptors for HSV are absent (Shieh et al., 1992; Gruenheid et al., 1993; Banfield et al., 1995).

The interactions of HSV gB or gC with cell surface heparan sulphate are also not sufficient for viral entry. Following binding of the virus to the cell surface, cell entry requires that viral gD engage any one of several entry receptors. Binding of gD to one of these receptors triggers fusion of the viral envelope with a cell membrane, and thus entry of the viral nucleocapsid and tegument into the cell cytoplasm. This envelope-membrane fusion requires the action of other viral envelope glycoproteins (gB and a heterodimer of gH-gL) in addition to gD and the gD receptor (reviewed by Spear, 1993; Spear et al., 2000).

The entry receptors discovered to date fall into three classes (reviewed by Spear et al., 2000). They include HVEM (herpesvirus entry mediator), a member of the TNF receptor family; nectin-1 and nectin-2, members of the immunoglobulin superfamily; and specific sites in heparan sulphate generated by certain 3-O-sulfotransferases. Mice can be infected by HSV, and mouse and human forms of these receptors are nearly indistinguishable in their HSV entry activity. HSV-1 and HSV-2 differ somewhat in receptor preferences. Whereas both HVEM and nectin-1 are excellent entry receptors for both serotypes, nectin-2 is virtually inactive for HSV-1 entry but does have weak entry activity for HSV-2. The converse is true for 3-O-sulphate-modified heparan sulphate. Glycoproteins gB, gH and gL are structurally conserved among all herpesviruses and probably have conserved essential roles in viral entry. Glycoproteins gC and gD, on

![Fig. 1. Cell surface receptors and viral ligands that participate in HSV entry. The viral envelope contains more than a dozen viral glycoproteins but only five (gB, gC, gD, gH and gL) have been shown to participate in viral entry. Binding of virus to cells can be mediated by the binding of gB or gC to heparan sulphate (HS) chains on cell surface proteoglycans. This facilitates the binding of gD to one of its cell surface receptors. These include HVEM, a member of the TNF receptor family; nectin-1 and nectin-2, two members of the immunogoglobin superfamly; and specific sites in HS generated by certain 3-O-sulfotransferases. Binding of gD to any one of these receptors triggers fusion of the viral envelope with a cell membrane. This membrane fusion requires the action of gB and gH-gL heterodimers as well as gD and a gD receptor.](image-url)
the other hand, are conserved among most of the neurotropic alphaherpesviruses but have no recognizable structural homologues in members of the other two branches of the herpesvirus family.

Expression and properties of the HSV binding and entry receptors

Heparan sulphate is thought to be ubiquitously expressed, at least on cells that stay put in tissues, as opposed to circulating cells of the immune system. This does not necessarily mean that binding sites in heparan sulphate for gB, gC or gD have the same distribution as heparan sulphate. Heparan sulphate chains are synthesized as repeating disaccharide units of N-acetyl-glucosamine and glucuronic acid and then modified, in some regions of the chain but not others, by a sequence of enzymatic reactions including de-acetylation of the glucosamine, sulphation of the amino group, epimerization of the glucuronic acid to iduronic acid, and O-sulphations at the 2-OH position in the iduronic acid and the 6-OH and 3-OH positions in the amino sugar (reviewed by Lindahl et al., 1998). These reactions generate regions in heparan sulphate (approximately 6–12 residues) that differ with respect to positions of epimerized uronic acid and sulphate groups and that can bind proteins with great specificity. The site in heparan sulphate to which gD can bind is generated by the action of enzymes that can yield an octasaccharide of the following structure: UA-GlcNS-IdoUA2S-GlcNAc-UA2S-GlcNS-IdoUA2S-GlcNH3S6S (Liu et al., 2002). The demonstration that gD could bind to this sequence in heparan sulphate built on findings that an expression plasmid encoding mouse 3-O-sulfotransferase-3α could convert resistant Chinese hamster ovary (CHO) cells to susceptibility to HSV-1 entry (Shukla et al., 1999). Some isoforms of 3-O-sulfotransferases can generate gD-binding sites (provided the other relevant enzymatic activities are expressed) whereas others generate antithrombin-binding sites (Liu et al., 1999; Shukla et al., 1999; Xia et al., 2002). The structures in heparan sulphate to which gB and gC bind have not yet been determined. However, it is clear from competition studies that gB and gC bind to different structures and that HSV-1 and HSV-2 forms of gC also bind to different structures (Gerber et al., 1995; Trybala et al., 2000). Determination of the distribution of binding sites for gB, gC and gD in heparan sulphate will require use of specific viral probes for these sites. It cannot be done just by determining where various enzymes are expressed, because it is not yet clear what influences the overall coordination of their activities to generate specific sites.

HVEM (also known as HveA, ATAR, TR2, TNFRSF-14) is expressed in a variety of tissues and cell types, including T and B lymphocytes, other leucocytes, epithelial cells and fibroblasts, but probably not in neurons (Montgomery et al., 1996; Hsu et al., 1997; Kwon et al., 1997; Marsters et al., 1997). HVEM is the principal receptor for HSV entry into activated human T lymphocytes, but not for a number of other human cell types (Montgomery et al., 1996). The viral ligand for HVEM is gD (Whitbeck et al., 1997) whereas the natural cellular ligands for HVEM are LIGHT and lymphotoxin-α (Mauri et al., 1998). LIGHT is also a ligand for the lymphotoxin-β receptor (LTβR). Studies of the normal physiological roles of HVEM have focused on immune responses and on LIGHT as the functional ligand (lymphotoxin-α binding is of lower affinity and of unknown significance). These studies (reviewed by Croft, 2003; Granger and Rickert, 2003) have been aided by the fact that patterns of expression for HVEM and LTβR are largely non-overlapping. For both mice and humans, HVEM is expressed in a variety of tissues and cell types, as mentioned above, whereas LTβR expression is more restricted. With regard to immune responses, it is relevant that HVEM, but not LTβR, is abundantly expressed on NK-T cells and naive CD8+ T cells and is expressed at variable levels on some CD4+ T cells and dendritic cells. On the other hand, LTβR is expressed on the stromal cells of lymphoid organs and is critical for lymphoid organ development. LIGHT expression is tightly regulated and is detected on activated lymphocytes, NK cells and immature dendritic cells. In vitro studies have shown that binding of LIGHT to HVEM can provide a second signal for T cell activation. These signals are transmitted via cytoplasmic TNF receptor-associated factors (TRAFs) and lead to activation of NFκB or JNK/AP-1. Several in vivo studies, including some with LIGHT gene K/O mice and LIGHT gene transgenic mice, have demonstrated that LIGHT–HVEM interactions contribute to CTL-mediated immune responses, allograft rejection and graft-versus-host disease.

Nectin-1 and nectin-2 are also expressed in a variety of tissues and cell types, including epithelial cells, fibroblasts and neurons. The cDNAs for nectin-1 and nectin-2 were originally described in the literature as encoding poliovirus receptor-related proteins 1 and 2 (Prr1 and Prr2) (Eberlé et al., 1995; Lopez et al., 1995). Because they were not poliovirus receptors, they were renamed HveC and HveB respectively (Geragthy et al., 1998; Warner et al., 1998). They were then renamed nectin-1 and nectin-2, because Y. Takai and colleagues had discovered their roles in cell adhesion. By now, at least four nectins and other nectin-like molecules have been identified (reviewed by Takai and Nakanishi, 2003). These cell surface glycoproteins are members of the immunoglobulin superfamily and are closely related to each other and to the poliovirus receptor. The nectins are cell adhesion molecules that can co-
localize with cadherins in adherens junctions and can also engage in cell adhesions independent of cadherins. The nectins dimerize in the plane of the membrane and nectin homodimers engage in trans interactions with the same member or other members of the nectin family on adjacent cells, or with HSV gD. These trans interactions probably signal various intracellular events. A variety of extracellular and intracellular signaling molecules, such as scatter factor/hepatocyte growth factor, Ras, Cdc42 and Rac small G proteins, regulate the formation and disruption of cell junctional complexes in dynamic fashion. Nectin-1, -2 and -3 are broadly expressed in a variety of tissues and cells. Nectin-4 appears to be localized to the placenta in human and mouse peripheral and central nervous systems, and exhibit the phenotypes expected with respect to neurological defects. Nearly every neuron of the nectin-1(–/–) genotype is not more pronounced, especially with regard to developmental defects of the hands and, in some cases, mental retardation. Unfortunately, it has not been possible to obtain sera from such individuals to test for evidence of HSV infection. Fibroblasts and lymphoblastoid cells are available from the Coriell Mutant Repository and exhibit the phenotypes expected with respect to HSV entry. The fibroblasts expressed HVEM and nectin-2, but not nectin-1, and were more resistant to HSV infection than wild-type control cells. At high doses of virus, the cells could be infected with appropriate virus strains, however, presumably via HVEM or nectin-2 (F. Struyf and P.G. Spear, unpublished studies). It is somewhat surprising that the phenotype of the nectin-1(–/–) genotype is not more pronounced, especially with respect to neurological defects. Nearly every neuron of the mouse peripheral and central nervous systems expresses nectin-1 mRNA (Haarr et al., 2001) and Takai and colleagues have defined junctions in the brain that involve nectin-1/nectin–3 trans interactions (Mizoguchi et al., 2002). The only phenotype associated with knock-out of nectin-2 in the mouse is male sterility (Bouchard et al., 2000). The cause is absence of appropriate nectin-2/nectin-3 trans interactions between Sertoli cells and spermatids (Mueller et al., 2003). The absence of more severe phenotypes when nectin-1 or nectin-2 are not expressed, coupled with co-expression of these proteins in many cell types, suggests that there may be some redundancies of function in cells that normally express both.

**Interactions of gC with heparan sulphate and the C3b component of complement**

All alphaherpesviruses studied to date express a member of the gC family that has both heparan sulphate-binding and C3b-binding activities. As mentioned above, this glycoprotein is dispensable for the replication of virus in cultured cells, despite its role in virus binding to heparan sulphate. HSV strains obtained from patients almost invariably express gC, however, and it is clear that gC can have a role in viral virulence, as outlined below.

**Protection from complement neutralization**

The C3b-binding activity of gC is associated with protection of virus from antibody-independent neutralization by complement components (reviewed by Friedman, 2003). Both HSV-1 and HSV-2 retain full infectivity in the presence of complement whereas mutants unable to express gC are neutralized by complement, even in the absence of anti-HSV antibodies (McNearney et al., 1987; Hidaka et al., 1991; Gerber, et al., 1995; Friedman et al., 1996). Neutralization of gC-negative virus requires complement activation and presence of C5 but does not require C6, C8 or factor D, indicating that the alternative complement pathway and complement components beyond C5 are not required (Friedman et al., 2000). Both HSV-1 gC and HSV-2 gC (gC-1 and gC-2) have been shown to bind to C3, C3b, iC3b, C3c, but not to C3d (Tal-Singer et al., 1991; Kostavasili et al., 1997). HSV-1, but not HSV-2, gC can block the binding of C5 and properdin to C3b (Fries et al., 1986; Hung et al., 1994; Kostavasili et al., 1997). This gC-1-specific blocking cannot provide a full explanation for the ability of both serotypes to resist complement-mediated neutralization. Some species specificity for interactions of complement with alphaherpesvirus gCs has been reported (Huemer et al., 1993). Also, it was reported that human and guinea pig sera, but not mouse sera, could neutralize HSV-1 infectivity in the absence of anti-HSV antibodies (Hidaka et al., 1991; Huemer et al., 1993). However, compelling evidence was presented that HSV-1 mutants, unable to express gC or altered in ability to bind to C3, were attenuated in wild-type mice but exhibited near wild-type virulence in C3-deficient mice (Lubinski et al., 1998; 1999).

**gC and HSV virulence**

Although gC is dispensable for the replication of HSV in cultured cells, it can have a role in HSV virulence in the intact animal. Reports on the effects of gC deletions on HSV virulence are mixed. For example, deletion of gC was found to have no effects on HSV-1 virulence in the mouse after corneal, intraperitoneal or intracerebral inoculation (Minagawa et al., 1997). On the other hand, deletion of gC abrogated HSV-1 replication in human skin implanted.
into SCID-hu mice (Moffat et al., 1998), attenuated virulence in a rabbit seizure model (Stroop and Schaefer, 1989) and attenuated virulence in mice inoculated intradermally (zosteriform model), provided the mice expressed the C3 component of complement (Lubinski et al., 1998; 1999). HSV-2 mutants have not been as extensively studied for pathogenesis. An HSV-2 mutant with a deletion of 130 bp in the gC open reading frame was reported to cause disease similar to that of wild-type virus after intra-vaginal or intracerebral inoculation (Johnson et al., 1986).

**Structural features of HSV-1 and HSV-2 gC and mapping of binding sites**

Members of the gC family are type I membrane glycoproteins with both N-linked and O-linked glycans. gC-1 and gC-2 exhibit 65% identity in amino acid sequence with most of the divergence in the N-terminal region. This region in both gC-1 and gC-2 is mucin-like, with numerous O-linked glycans, but relatively low conservation of amino acid sequence. Part of this region is missing in gC-2. For gC-1, amino acid substitutions in basic and hydrophobic residues between amino acids 129 and 160 and at position 247 (Fig. 2) significantly impaired binding to heparan sulphate and also impaired binding of virus to cells (Mardberg et al., 2001). Mutations that can reduce binding of gC-2 to heparan sulphate have not been identified. Linker-insertion mutations and amino acid substitutions in several regions of gC-1 and gC-2 (Fig. 2) impaired binding to C3b (Seidel-Dugan et al., 1990; Hung et al., 1992). It is evident from Fig. 2 that there is some overlap in regions of gC-1 that are critical for binding to both heparan sulphate and C3b. In fact, heparan sulphate can inhibit the binding of gC-1 to C3b (Rux et al., 2002).

Deletion of amino acids 275–367 in gC-1 abrogated C3b binding whereas deletion of amino acids 33–123 did not (Hung et al., 1992). However, the latter deletion reduced somewhat the affinity of gC-1 for heparan sulphate (Rux et al., 2002) and also prevented gC from blocking the binding of C5 or properdin to C3b (Hung et al., 1994; Kostavasili et al., 1997). Interestingly, HSV-1 mutants with deletion 275–367 in gC-1 were able to induce the secondary lesions occurring by zosteriform spread in inoculated mice, but only in C3-deficient mice, not in wild-type mice. The other deletion (33–123) had less effect and the double deletion caused no more impairment of viral virulence in wild-type mice than that observed with the 275–367 deletion alone. Thus, the authors concluded that the C3b-binding activity of gC-1, not its ability to block the binding of C5 or properdin to C3b, was most important for protecting virus from complement-mediated inactivation *in vivo* (Lubinski et al., 1999).

Although attention was first focused on gC for its ability to bind to heparan sulphate and mediate the binding of virus to cells, experimental evidence available to date suggests that the C3b-binding activity of gC, conferring protection against complement inactivation, may be more important for the contribution of gC to HSV virulence. There are models of HSV disease in which gC has a critical role, as described above, and in which the involvement of complement has not yet been assessed. Clearly, more must be done to characterize these models and also to investigate a possible relationship between heparan sulphate binding and protection from complement.

**Interactions of gD with its receptors**

All alphaherpesviruses studied to date, except varicella-
zoster virus (the cause of chicken pox and shingles), express a member of the gD family. It seems likely that members of the gD family encoded by different herpesviruses have conserved the ability to bind to members of the nectin family. Nectin-1 is highly conserved among mammalian species (Shukla et al., 2000; Milne et al., 2001). HSV and bovine and porcine herpesviruses can all use human, mouse and porcine forms of nectin-1 for cell entry (Geraghty et al., 1998; Shukla et al., 2000; Milne et al., 2001), in part explaining the broad host ranges of these viruses, at least with respect to cultured cells. In the case of HSV, the other known gD receptors are nectin-2, HVEM and specific sites in heparan sulphate generated by 3-O-sulphotransferases.

**Structure of gD and interface with HVEM**

X-ray structures of a large portion of the HSV-1 gD ectodomain, crystallized alone and in complex with a portion of HVEM (Carfi et al., 2001), have revealed the following: (i) the core of the gD ectodomain is an Ig-fold with unconventional disulphide bonding; (ii) an N-terminal extension from the Ig-fold assumes a hairpin shape in the complex with HVEM and has all the residues that make contact with HVEM; (iii) this N-terminal extension is disordered in the crystals of gD alone; (iv) a domain of gD downstream of the Ig-fold forms an α-helix which is sandwiched between the N-terminal hairpin (or disordered region) and the Ig-fold. Residues in HVEM (Connolly et al., 2002) and in the N-terminal hairpin of HSV-1 gD that are critical for HVEM–gD interactions have been determined by mutational analyses, as will be discussed more fully below for gD. Although the structure of HSV-2 gD has not yet been determined, mutational analyses indicate that HSV-1 and HSV-2 gD are very similar in their interactions with HVEM and nectin-1.

**Effects of gD mutations on physical and functional interactions with entry receptors**

The approach has been to introduce the desired mutations into the gD open reading frame and then to test ability of the mutant gDs (i) to bind to the various receptors and (ii) to participate with gB and gH-gL in inducing membrane fusion. The former assay uses soluble forms of gD, in some cases hybrids of the gD ectodomain fused to the Fc of rabbit IgG, for quantification of their ability to bind to cells transfected to express each of the relevant receptors (Geraghty et al., 2000; 2001). For the latter assay, cells expressing gB, gH-gL, various forms of wild-type or mutant gD (effector cells) are mixed with target cell populations expressing each of the entry/fusion receptors to quantify cell fusion activity (Pertel et al., 2001). It is clear from these studies that the structural features of gD critical for functional interactions with the various entry receptors are distinct, as summarized below:

(i) Deletion of amino acids 7–32, comprising all the HVEM contact residues revealed in the X-ray structure (Fig. 3), eliminated physical and functional interactions of gD-1 or gD-2 with all receptors (HVEM, nectin-2 and 3-O-sulphated heparan sulphate) except nectin-1 (Yoon et al., 2003). This applies to both the mouse and human forms of the receptors. Binding of these mutant forms of gD-1 and gD-2 to mouse or human nectin-1 and cell fusion activities with nectin-1 were indistinguishable from those of wild-type gD-1 and gD-2, indicating that the major contact site for nectin-1 in gD is downstream of amino acid 32.

(ii) Certain insertions or deletions in gD-1 eliminated binding and activity with HVEM alone or with both HVEM and nectin-1, without affecting expression of the gD-1 mutants on the cell surface (Jogger et al., 2003). The former insertions are in the N-terminal region and their effects are predictable from the X-ray structure of the gD-HVEM complex. The latter insertions and deletion are in the major alpha-helix mentioned above or in the E beta strand of the Ig fold (Fig. 3). It seems likely that the latter mutations indirectly affect the conformation of the HVEM contact sites in the N-terminal hairpin and also affect the conformation of the nectin-1 contact sites, which are undoubtedly different but have not yet been precisely defined.

(iii) The N-terminal region of gD-1 or gD-2 is required for functional interactions with nectin-2, but not nectin-1, and the actual amino acid sequence within the first 53 amino acids determines whether nectin-2 can be recognized as a cell fusion or entry receptor. Nectin-2 is a very poor entry/fusion receptor for gD-1 but more active for gD-2. Hybrids of gD-1 and gD-2 containing the first 53 amino acids from gD-2 (only 6 amino acid differences from the gD-1 sequence) resembled gD-2 in cell fusion activity with nectin-2 (Zago and Spear, 2003). Single or double substitutions into the gD-1 sequence were not as effective as conferring activity with nectin-2 as all 6 substitutions. Alternatively, certain single amino acid substitutions in gD-1, at positions conserved between gD-1 and gD-2 (L25P, Q27P, Q27R, L28A), can enable gD-1 to use nectin-2 as a receptor for entry and cell fusion (Warner et al., 1998; Lopez et al., 2000; Connolly et al., 2003; Yoon et al., 2003). The L25P, Q27P and Q27R mutations in gD-2 have no effect on the ability of gD-2 to mediate nectin-2-dependent cell fusion (Yoon et al., 2003). Various lines of evidence indicate that nectin-2 interacts with the wild-type form of gD-2 at much lower affinity than does nectin-1. It seems likely that the major contact site on gD for nectin-2 is...
downstream of amino acid 32, as is true for nectin-1, but that there may be secondary contact sites in the N-terminal region that are critical for interaction with nectin-2 but not nectin-1. Alternatively, the N-terminus may affect the conformation of other domains of gD-2 in a way that is critical for interactions with nectin-2 but not with nectin-1.

Preliminary results indicate that multiple amino acid substitutions downstream of amino acid 32 in gD-1 or gD-2 can abrogate functional and physical interactions with nectin-1 and nectin-2 but not with HVEM (C. Jogger, S. Manoj, D. Mysofsky and P.G. Spear, unpublished data). By introducing various gD mutations into the viral genome, it should be possible to obtain viral mutants capable of using only nectin-1 or only HVEM as an entry receptor. These mutants will be invaluable for assessing the role of each receptor in infection of certain target cells in cultured cells and in experimental animals and for studies designed to determine whether interactions of gD with each receptor, either during viral entry or viral replication, influences the cell response to virus infection.

Potential significance of alternative usage of receptors by HSV

The ability of HSV-1 and HSV-2 to use multiple receptors for entry into cells could in part reflect simple redundancy but there may be greater significance to the existence of multiple receptors. First, these viruses may use different receptors to enter different cell types, governed by the natural patterns of receptor expression. For example, primary human T lymphocytes express HVEM, but not nectin-1, and HVEM can be used for HSV entry into these cells (Montgomery et al., 1996; Geraghty et al., 1998). Cells of neuronal origin express nectin-1, but not HVEM (Montgomery et al., 1996; Geraghty et al., 1998; Mauri et al., 1998), and it was reported that nectin-1 could mediate HSV entry into rat neurons (Richart et al., 2003). Second, different receptors could be used for entry into the...
same cell type under different conditions. Epithelial cells express nectin-1, nectin-2 and HVEM (Geraghty et al., 1998; Warner et al., 1998) but the nectins can be localized to junctions and remain inaccessible to virus until the junctions are disrupted (Yoon and Spear, 2002). Therefore, entry of virus into cells of an intact epithelium may require either damage to the epithelium or use of a receptor such as HVEM, instead of nectin-1. On the other hand, cell-to-cell spread of HSV infection in an intact epithelium could depend on use of one of the nectins because exit of virus from polarized cells is directed to lateral surfaces (Johnson et al., 2001) where nectins would be localized. In fact, it has been shown that nectins can enhance the cell-to-cell spread of HSV infection in cell monolayers, as assessed by plaque size (Sakisaka et al., 2001). Third, single amino acid substitutions in HSV-1 or HSV-2 gD could alter receptor usage, as described above, suggesting that natural polymorphisms could influence the cell types infected and therefore pathogenesis. Fourth, interaction of gD with one of its receptors (either in the same cell or on an adjacent cell) may transduce signals or interfere with signal transduction via the natural ligand. For example, cell junctions could be modified or appropriated by virus, through interactions of gD with one of the nectins (Krummenacher et al., 2003). Also, the normal responses of leucocytes to foreign antigen or to inflammatory cytokines could be affected by interactions of gD with HVEM. It has been reported that cells expressing gD can inhibit T cell responses (La et al., 2002). The viral mutants altered in gD may permit investigation of some of these possibilities.

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