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REVIEW

Understanding HSV-1 entry glycoproteins

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SUMMARY

Herpes Simplex Virus-1 is a common infectious agent, but the precise detail of entry and infection of cells has only now begun to be clarified. Four viral surface glycoproteins (gB, gD, gH and gL) are required. This review summarises the known structure and function of each of these essential viral envelope glycoproteins, and explores what is known about their close cooperation with each other in mediating cellular membrane fusion. It is suggested that, following gD binding to one of its entry receptors, membrane fusion is mediated by gB and the heterodimer gH/gL. Significantly, these four entry glycoproteins also play a key role in the interaction between HSV and the host immune system. The glycoproteins serve an important role as targets of adaptive immunity. However, recent studies have demonstrated that the same proteins also play a key role in initiating the early innate immune response to HSV. Understanding the complex functions of these HSV proteins may be essential for successful development of vaccines for HSV. Copyright © 2007 John Wiley & Sons, Ltd.

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INTRODUCTION

Herpes Simplex Virus-1 (HSV-1) is one of the most common pathogenic agents, capable of infecting humans of all ages. The virus occurs worldwide. Infection results in a wide variety of clinical manifestations, ranging from asymptomatic infection, to the relatively minor but nonetheless significant common morbidity seen with the ulcerative and vesicular lesions of the skin and mucosae, up to sporadic encephalitis, which has both serious long term sequelae, and some mortality.

The Herpes Simplex Virus (HSV) virion consists of an electron dense core containing the double stranded linear DNA, enclosed by an icosahedral capsid. The capsid itself is surrounded by a proteinaceous layer—the tegument—and a lipid bilayer envelope on the surface. Of the 12 or more HSV-1 viral envelope proteins, infection in cell culture requires the coordinated action of at least four glycoproteins: D (gD), B (gB) and the heterodimer H (gH) and L (gL).

It was generally believed that HSV entry into cells occurs by direct fusion of virion envelope with the outer plasma membrane [1]. Recent reports have suggested, however, that at least three diverse pathways are implicated in HSV-1 entry into different cell types that are susceptible to infection: via direct fusion with the plasma membrane, via fusion within an acidic endosome, and via fusion within a neutral endosome. Fusion at the plasma membrane is known to occur in Vero cells in a pH-independent fashion [2]. In most cell types virions entry is by endocytosis, but this is also variable. For example, in HeLa cells, primary human keratinocytes and CHO-K1 cells transfected with a gD-receptor, fusion with endosomal membrane requires a low pH environment [3,4]. In contrast virus entry in mouse melanoma B78H1

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Abbreviations used

HSV, Herpes Simplex Virus; gD, glycoprotein D; gB, glycoprotein B; gH, glycoprotein H; gL, glycoprotein L; HS, heparan sulfate; HSPG, heparan sulfate proteoglycans; HVEM, Herpesvirus Entry Mediator; TNF, Tumour Necrosis Factor; 3-OS-HS, 3-O-sulfated heparan sulfate; PFD, pro-fusion domain; SU, surface subunit; TM, transmembrane subunit; HR, heptad repeat; VSV, Vesicular Stromatitis Virus; DC, dendritic cells; APC, antigen presenting cells; CTL, cytotoxic T-cells; NF-kB, nuclear factor-kB; IL-12, interleukin 12; ifn, type I interferon.

cells, expressing a human gD receptor, also involves virion endocytosis, but fusion does not require low pH [5]. Furthermore, this virus internalisation in B78H1 melanoma cells is completely dependent upon the expression of the gD receptor. In each of these pathways, fusion requires gD, gB, gHgL and a gD receptor [6]. It is still unclear however, whether these glycoproteins act in the same way, or form a similar fusion complex, or whether other cellular components are involved in these different entry pathways.

This review will concentrate on the viral entry glycoprotein, their role in fusion with the cellular membrane and their influence on the host immune response. HSV entry occurs when extracellular virions attach to the cells surface via gC and gB, and then bind to a gD receptor activating the membrane fusion machinery comprised of gB, and the heterodimer gHgL [7,8] (Figure 1). In most cells virus endocytosis occurs between the attachment step and the membrane fusion. Concomitant virus binding on the cell surface and membrane fusion mediated by gD, gB and gHgL will then activate intracellular immunosignalling processes.

CELL SURFACE INTERACTION—GLYCOPROTEIN C (AND GLYCOPROTEIN B)

The initial step in HSV-1 entry into mammalian cells is the attachment of the virus to the cell surface. gC and gB interact independently with heparan sulfate (HS) proteoglycans to promote the initial attachment [9–11]. Yet these glycoprotein–HS interactions, although important, are not absolutely essential for viral entry, at least not in the infection of cultured cells. A virus mutant at the gB polylysine sequence responsible for the gB-HS interaction is still infectious, although virus binding is reduced; a virus lacking the gC glycoprotein is also still infectious [10,11]. If both gB and gC are absent, virus binding to cell surface is

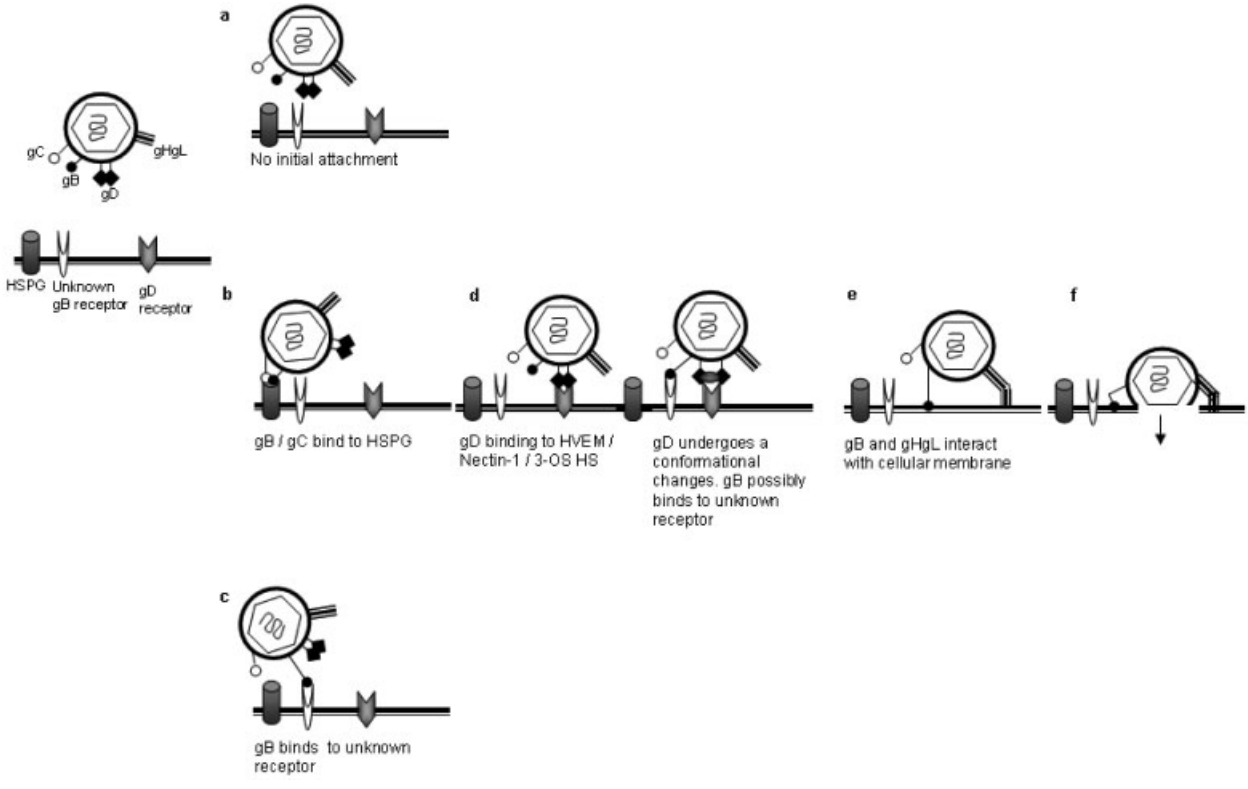

Figure 1. Model for direct HSV-1 entry by fusion with the cellular membrane. Initially, although not mandatory (a), gB (together with gC) binds with heparan sulfate proteoglycans (HSPG) (b) or an unknown gB receptor, independent of HS (c) allowing virus–cell attachment. This is followed by gD binding to a cell surface receptor: HVEM, nectin-1 or 3-O-sulfated heparan sulfate (d). gD undergoes conformational changes and transmits a signal gB and/or gH-gL. gB possibly interacts with an unknown cell surface component. gB and gH-gL form the fusion machinery that interact with the cellular membrane (e). gB and gH-gL mediate cell fusion and entry of the viral capsid (f). How gB, gH and gL work together to achieve fusion is unclear and steps 3 and 4 are drawn hypothetically

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severely reduced [10]. Infectivity is completely abolished in such virions, but this could be due to gB's critical role in membrane fusion, rather than to virion attachment at the cell surface. Recent data suggest however, that another receptor, independent of HS, may mediate interaction of gB with the cell surface, since a soluble form of gB was shown to interact with the surface of different cell types independent of HS [12]. The exact nature of this non-HS gB receptor is still unknown, and its role in attachment and/or fusion remains to be determined.

ENTRY RECEPTOR INTERACTION—GLYCOPROTEIN D

Following gC and/or gB interaction with the cell surface, the next stage in viral entry requires the interaction of gD with one of several potential entry receptors: (a) Herpesvirus Entry Mediator (HVEM), a member of the TNF receptor family; (b) nectin-1, a member of the immunoglobulin superfamily; and (c) 3-O-sulfated heparan sulphate [8,13–16]. The differential use of these receptors is important, and may help to account for the entry of HSV into such a wide range of different cell types [17–20].

HSV-1 glycoprotein D is a type I membrane glycoprotein consisting of 369 amino acids with an N-terminal ectodomain of 316 amino acids and three N-linked oligosaccharide attachment sites [21]. The protein's ectodomain core is of a V-like domain of the immunoglobulin fold (residues 56–184) [22]. Within the N-terminal extension to the core, a 37 residue hairpin structure (bending at residue 21) forms the entire site which contacts the receptor—HVEM [22,23]. In the absence of HVEM, the N-terminus adopts an extended and flexible conformation. Since the nectin-1 receptor binds to a different area and does not require the N-terminal hairpin of gD, it has been suggested that it interacts with gD when the N-terminus is in its extended unbent conformation [22,24].

The C-terminal extension (residues 260–316) can also play a vital role in viral entry at this stage. Similar to the N-terminal extension, the C-terminal is flexible, and this flexibility is necessary for correct gD function [25]. The C-terminus of the gD ectodomain, past residue 260, does not participate in a direct interaction with either receptor [24,26], but rather wraps around the gD core and blocks the formation of the HVEM binding hairpin, at the same time concealing the residues involved in nectin-1 binding [24,27]. Mutagenesis of gD at the C-terminus has shown that residue 294 (Trp294) is the necessary anchor in bringing the C-terminus in close proximity to the N-terminus, hence interfering with receptor binding [25]. The structure of gD in its pre-receptor binding form suggests, that in order for the receptors to bind, the C-terminus must move. Furthermore, the movement of the C-terminus upon binding of a receptor is important for the function of gD in activating the mechanism leading to membrane fusion [25].

Mutated gD with insertions/deletions in the C-terminus, between residues 275 and 300, were found to be impaired in cell entry [28,29]. Furthermore, soluble forms of gD with residues 1 to 285, but not shorter forms, were found to be sufficient in rescuing the infectivity of non-infectious gD-null HSV mutants [30]. It has been suggested that, following gD binding to one of the cellular entry receptors, gD may transmit a signal to gB and/or gH-gL which interact with the cellular membrane to mediate cell fusion. This signalling was found to be triggered by a proline rich region in the C-terminus of gD (residues 262–285) located in proximity to the viral membrane, named the pro-fusion domain (PFD) [25,30] (Figure 2).

Several studies have also shown a role for gD in inhibiting cell apoptosis (ref).

FUSION—GLYCOPROTEIN B AND THE GLYCOPROTEIN H/GLCOPROTEIN L COMPLEX

The most complex and least understood stage in HSV-1 entry into mammalian cells is the fusion of the virion envelope with the cellular membrane. In the absence of gB, gH or gL, HSV cannot enter target cells [25,28–32]. Since gD does not have the characteristics of a fusion protein, it is assumed that the central fusion machinery involves gB and the heterodimer gH/gL.

In many viruses the fusion process is preformed by specific proteins in the viral membrane. To date, two completely unrelated structural classes have been identified, designated type I and type II fusion proteins. In the type I model (identified in orthomyxoviruses [33,34], paramyxoviruses [35], retroviruses [36], filoviruses [37] and coronaviruses [38]), the fusion proteins form homotrimers that are cleaved proteolytically into a surface (SU) subunit and a transmembrane (TM)

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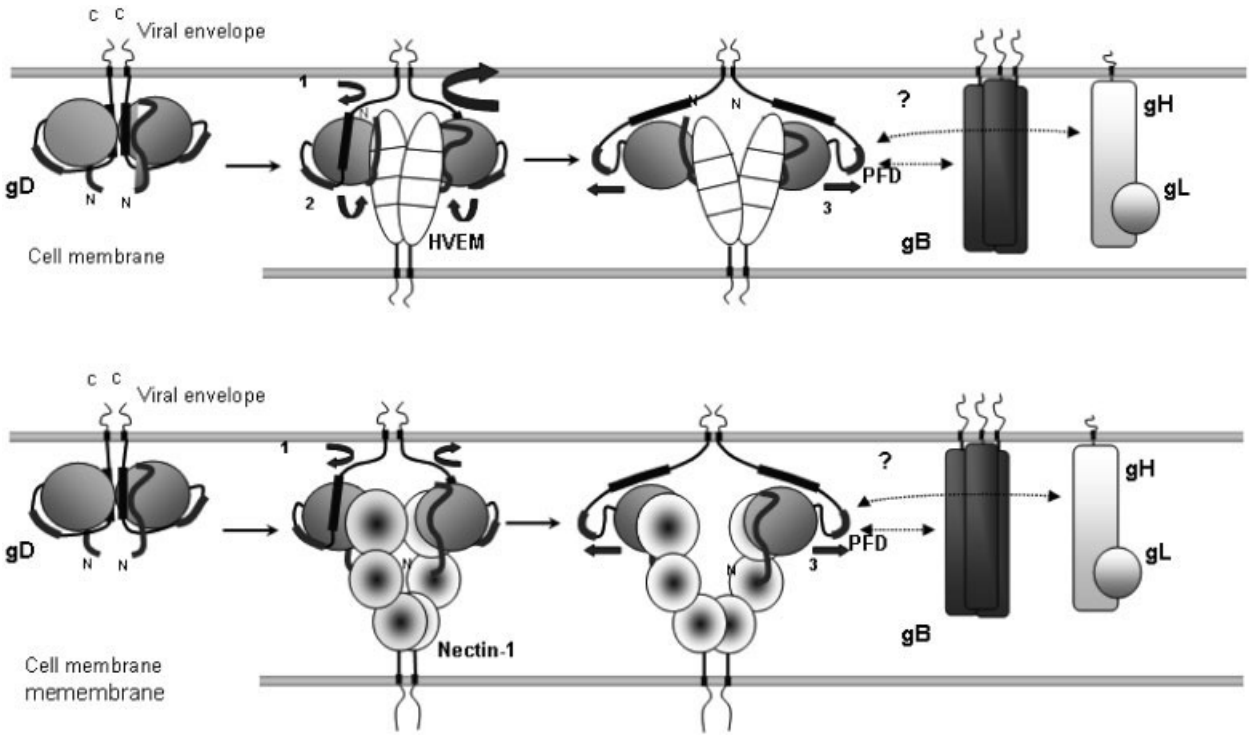

Figure 2. Model for receptor mediated activation of HSV gD. In its unbound state, gD is shown as a putative dimer in an autoinhibited conformation (left). Upon binding to HVEM (top) or nectin-1 (bottom) gD undergoes a series of conformational changes numbered chronologically: (1) displacement of the C-terminus; (2) formation of a N-terminal hairpin loop in the case of HVEM binding; (3) extension of the C-terminus and exposure of the profusion domain (PFD). Hypothetical molecular interactions with the fusion machinery (gH/gL, gB) are indicated with dotted arrows. This figure is adapted from Krummenacher *et al*., 2005. EMBO J. 24:4144–4153, Figure 6

subunit anchored to the viral membrane. These fusion proteins are extended to a rodlike structure in response to an activating trigger. A hydrophobic α-helix fusion peptide at the N-terminus of the TM subunit is exposed and able to penetrate the target cell membrane. A further conformational change in the TM subunit brings together two heptad repeats (HR-1 and HR-2) located downstream of the fusion peptides, to form a coiled coil structure resulting a stable hairpin shaped conformation. This folding back of the fusion protein upon itself brings the viral and cellular membranes into close contact allowing the lipids in the outer membranes to mix [39–41]. In the type II model (applicable to flaviviruses [42,43] and alphaviruses [44]), the fusion protein (which bears an internal fusion peptide) is folded flat on the viral surface, in tight association with a second protein as a heterodimer. The activating cleavage occurs in the second protein. Then receptor binding leads to an irreversible rearrangement of the fusion protein into a trimer protruding from the viral envelope. This allows the penetration of the exposed hydrophobic hairpin loop into the target cell membrane [39,40,45]. A fold-back movement of the fusion protein then brings the viral and cellular membranes together.

In contrast, HSV and other members of the herpesviruses are peculiar in that they rely on three distinct glycoproteins (gB, gH and gL) in addition to the receptor binding gD for fusion. The key role in HSV-1 fusion is thought to be played by the glycoproteins B and H, both conserved glycoproteins in the Herpesviridae family [46–48]. Which one of these is actually performing membrane fusion is still debated. However, recent advances on the structures of the HSV fusion glycoproteins indicate that herpesviruses may use some kind of intermediate mechanism with components similar to both type I and type II.

In its organisation, gH resembles viral fusion glycoproteins—a possible α-helix contained within the ectodomain of gH (at residues 377–397) has the characteristics of an internal fusion peptide

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and two downstream heptad repeats (HR-1 and HR-2) could potentially interact and adopt a coiled coil conformation [49]. Mutation or site deletion of the gH α-helix results in HSV's inability to enter cells; its replacement with heterologous fusion peptide of HIV gp41 or of VSV-G partially restores virus infectivity and gH fusion activity [50]. Furthermore, disrupting the HR's capability in forming coiled coils, also affected the ability of gH to function in the infectivity and cell fusion of cultured cells [49,50]. Although gH/gL do not form trimers, these mutagenesis data suggests a function for gH as a fusion protein in viral entry.

This being so, gH's function is dependent on forming a heterodimer complex with gL, which acts as a gH chaperone for proper processing and gH trafficking to the viral envelope. HSV-1 gL is a short (224 amino acids) glycoprotein lacking a transmembrane domain [51–53]. Upon expression in a complex with gH, gL is associated with the viral envelope; and, in the absence of gH, gL is secreted from cells [52]. However, HSV-1 virions lacking gL also lack gH, and although they bind to the cell surface, they do not penetrate the cellular membrane [53,54].

Like gH, gB is conserved among the Herpesviridae family [48]. HSV-1 gB is a 904-amino acid type I membrane glycoprotein composed of a 696-amino acid ectodomain, a 69-amino acid transmembrane domain, and a 109-amino acid carboxy-terminal domain. The cytoplasmic domain of gB is the longest among HSV-1 glycoproteins [55]. The carboxy-terminal hydrophobic region was proposed to have three segments containing 20–21 amino acids corresponding to residues 727–746 (segment 1), 752–772 (segment 2) and 775–795 (segment 3) which can traverse the membrane three times [56]. Studies using mutants with deletions in the predicted membrane-anchoring sequences suggest segment 3 as the membrane-anchoring domain and segments 1 and 2 may be peripherally associated with the membrane [57].

The recently solved crystal structure of HSV-1 gB [58] reveals a trimeric ectodomain made up of five distinct domains in each protomer (identified as I–V). The rod-shaped trimer is organised around a central helical core reminiscent of type I fusion proteins. On the other hand HSV gB is not proteolytically cleaved and might contain an internal fusion peptide similar to type II fusion proteins. Studies involving virus-neutralising monoclonal antibodies directed towards specific sites on the gB ectodomain found that antibodies that affected fusion reacted with residues in the mid-region of the ectodomain, suggesting that domain IV of the gB ectodomain is exposed on the surface of the virion and may be required for viral penetration and cell fusion [58,59].

Interestingly, the HSV gB ectodomain structure is homologous to the fusion protein G of Vesicular Stomatitis Virus (VSV)—a rhabdovirus with single-stranded RNA genome [60]. As protein G of VSV is known to be the viral fusion protein, this structural similarity strongly suggests that gB might be the effector of membrane fusion during HSV entry.

Regardless of which of gB or gH is the central fusion effector, these glycoproteins must act in concert to promote virus entry. How they are activated by gD binding to cell receptors, and how this machinery functions, remains to be elucidated.

IMMUNE RESPONSE

These detailed studies of HSV-1 glycoproteins have highlighted several important aspects of HSV-1 infection. In particular, while other enveloped viruses rely on only one or two glycoproteins for fusion, this virus, in common with other members of the alpha-herpesvirus subfamily, requires the complex interaction of four glycoproteins—gB, gD, gH and gL. It appears that these same four surface glycoproteins, as well as acting in viral entry into cells, also play a key role in the immune response to HSV-1, both as well-established targets of effector T- and B-cells, and also (as has been documented in recent studies from our own and other laboratories) in the initial induction of innate immunity and in modulation of dendritic cell (DC) function.

There have been several studies showing that these HSV-1 surface glycoproteins are targets for adaptive responses. Animal experiments suggest that viral glycoprotein gD induces neutralising antibodies [61]. Furthermore, mice immunised passively with monoclonal antibodies to gB, gC, gD or immunised actively with either recombinant or synthetic viral glycoproteins were protected from lethal virus challenge [62–64]. HSV-1 specific neutralising antibodies were also obtained in response to the gH-gL heterodimer, although their protective efficiency against viral challenge varied considerably in published studies [65,66]. It is likely that these antigen specific antibody

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responses are themselves controlled via $CD4^+$ T-cell help, and this in turn is activated by MHC class II presentation of T-cell epitopes on antigen presenting cells (APC), such as DC.

Probably the most important component of the adaptive host immune response is due to $CD8^+$ cytotoxic T-cells (CTL) responding to specific viral antigens. Various reports showed that gD, gB and to a lesser extent gH serve as a target antigens for CTLs during primary infection [63,67–71]. There are also HLA-DR $CD4^+$ T-cells in the HSV-1 lesions that have cytotoxic activity, and these T-cells have been shown to recognise the gB, gH and gD glycoproteins [71]. Presumably, peptides derived from these glycoproteins are initially presented on MHC class I and/or class II on APC, and hence this pathway also implicates DC.

It is noteworthy, however, that HSV has evolved several methods of evading these CTLs, facilitating persistence in the host. Relevant to the present review, gD binding to HVEM has been implicated in interfering with the nuclear factor-κB (NF-κB) signalling pathway and hence with T-cell activation [72]. Most of the other forms of evasion that have been described are not discussed in detail here, since they do not directly involve the surface glycoproteins (73–77).

Past efforts to develop an HSV vaccine in humans have used a range of constructs from live attenuated virus to the use of recombinant viral subunits [78,79]. However, to date, all have failed to reach the endpoint of protection from infection in clinical trials. Although successful in protecting against wild-type challenge in animal models, a live modified HSV-1/HSV-2 construct was a poor immunogen in phase I clinical trials [80,81]. Recombinant glycoprotein vaccines have given promising results in animal models [65,82,83]. Recombinant HSV-1 gD induced an antibody response in Japanese macaques, which could be increased following intracutaneous boosting [84]. A variable T-cell response was also detected, and it was suggested that this vaccine might be valuable not only in HSV-1 infection, but also in herpes B virus infection, which expresses a homologous gD protein, and which can cause a lethal encephalomyelitis when transmitted to humans. An interesting novel approach, thus far also only reported in mice, has been the development of synthetic lipopeptides, where the lipid moiety acts as an adjuvant via the DC, and the gD peptide is the specific antigen to which the T-cell response in induced [85]. This may well act via an interleukin-12 (IL-12) pathway, since an IL-12p35 expressing virus also induces a degree of viral clearance in an ocular herpes model [86].

Recombinant gB and gD proteins have also been used as candidate subunit vaccine in human subjects. Thus far, a combined HSV-2 recombinant gDgB subunit vaccine with adjuvant, despite inducing high titres of specific antibodies, provided only a transient protection to infection [87]. Another recombinant gD vaccine given with a more effective adjuvant, also failed to produce full protection to infection although resistance to HSV-2 was demonstrated in women who were seronegative for HSV at the time of vaccination [88].

INTERACTION WITH APC

HSV-1 readily infects macrophages [89–91] and DC [92–94], leading to a series of functional changes in which antigen presenting capabilities are impaired. Despite this, as outlined above, the host is usually able to overcome the acute phase of infection by mounting a strong immune response. This is linked to the production of a range of immediate cytokines, which can both precede and control the evolution of the antibody and cytotoxic T-cell responses. Interestingly, at least one phase of this early response of cytokine production is dependent on viral glycoprotein interaction with the cell surface. The very same initial molecular interactions that are required for viral entry appear also to activate cellular signalling pathways responsible for an early burst of type I interferon (ifn) secretion [95], which may be key to the capacity of the host to overcome infection [96].

Infection of immature DC by HSV-1 leads to a series of both morphological and functional changes. There is partial DC maturation (as judged by surface phenotype), at the same time there is concomitant impairment of antigen presenting function and eventual apoptosis [92–94]. The exact pathway by which HSV-1 enters DC has not been characterised, although it likely occurs in a similar fashion to other cell types via the binding of gD to one of two entry receptors: HVEM and nectin-1, followed by gB and/or gHgL mediated membrane fusion. It is interesting that gD binding to HVEM triggers a direct signalling response, leading to NF-κB activation [72], in a similar way to which LIGHT, a known member of the tumour necrosis

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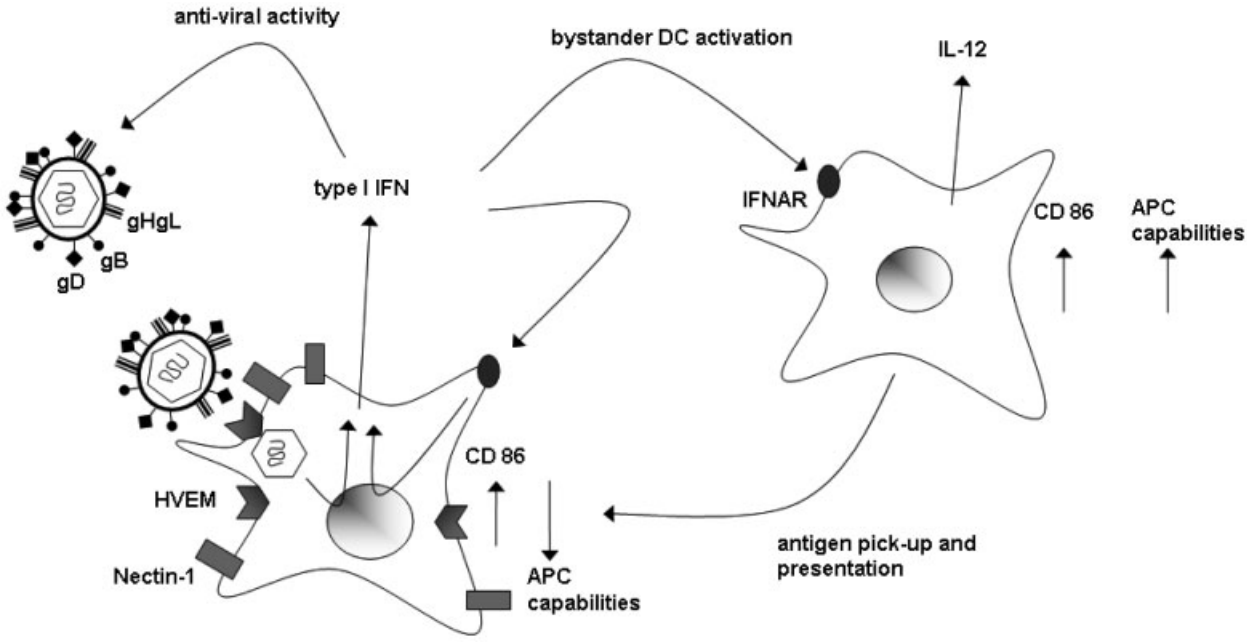

Figure 3. HSV, possibly through the binding of the envelope glycoproteins, triggers DC maturation (demonstrated by cell surface expression of CD86) and the secretion of type I IFN in immature infected DCs. As a result, bystander immature DCs are activated to pick up viral antigen and/or dying/dead cells for the presentation to T-cells in order to trigger an effective cellular adaptive immune response

factor (TNF) superfamily, binds to HVEM and triggers the same NF-κB pathway [97–100]. In the monoblast-like cell line, U937 this is linked with the apoptotic pathway with inhibition of apoptosis [72]; in primary cells, this is the process that is thought to lead to the secretion of type I IFN, responsible for the first wave of viral control [94,101–103]. Studies have shown that type I IFN released by HSV infected DC, in addition to its anti-viral activity, also acts as a danger signal to bystander uninfected DC, inducing maturation, release of IL-12 and migration of DC cross-presenting antigen [96,104]. This would promote a more effective Th1 immune response, and provide the link between the immediate and adaptive phases (Figure 3). Thus interaction between viral glycoproteins and the DC surface may be critical for this process to occur [101].

CONCLUSIONS

Because of their essential part in the early step of virus infection, the entry glycoproteins of HSV are targets for antiviral drug development and also candidate vaccine components. Recent advances in our understanding of their structure and function has increased our knowledge of the early interaction of the virus with the target cells, including cells from the immune system. The role that these glycoproteins play in the early immune response by binding to APCs is becoming more apparent. However, additional knowledge on the HSV glycoproteins, their structure and function in cellular entry, and their specific role in initiating the immune response, will contribute to the rational design of an efficient vaccine against HSV.

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