The Cycle of Human Herpes Simplex Virus Infection: Virus Transport and Immune Control

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After infection of skin or mucosa, herpes simplex virus enters the sensory nerve endings and is conveyed by retrograde axonal transport to the dorsal root ganglion, where the virus develops lifelong latency. Intermittent reactivation, which is spontaneous in humans, leads to anterograde transport of virus particles and proteins to the skin or mucosa, where the virus is shed and/or causes disease. Immune control of viral infection and replication occurs at the level of skin or mucosa during initial or recurrent infection and also within the dorsal root ganglion, where immune mechanisms control latency and reactivation. This article examines current views on the mechanisms of retrograde and anterograde transport of the virus in axons and the mechanisms of innate and adaptive immunity that control infection in the skin or mucosa and in the dorsal root ganglion—in particular, the role of interferons, myeloid and plasmacytoid dendritic cells, CD4+ and CD8+ T cells, and interferon-γ and other cytokines, including their significance in the development of vaccines for genital herpes.

Herpes simplex virus (HSV) type 1 infects 60%–80% of people throughout the world, whereas the prevalence of HSV-2 infection in adults varies markedly from country to country, from as low as 7% up to 80%, depending on sexual and, perhaps, contraceptive practices [1]. HSV-2 causes one of the most common sexually transmitted diseases, genital herpes, occasionally leading to neonatal herpes, which may result in severe morbidity or death. In addition, severe morbidity may result from recurrent genital herpes in immunocompromised patients. HSV-2 also appears to enhance the risk of acquisition of HIV by 2- to 3-fold [2]. HSV-1 causes ocular herpes, a major cause of blindness in the Western world, and is the most important cause of sporadic encephalitis, usually resulting in severe morbidity or mortality. HSV-1 is also causing an increasing proportion of genital herpes, particularly in adolescents [3]. Furthermore, HSV-1 is being engineered for use as a gene therapy vector to convey genes from the periphery to the central nervous system or for direct infection of cerebral and other tumors, resulting in infection and destruction of tumor cells but not of normal brain tissue [4].

HSV-1 and -2 usually infect via the oral or genital mucosa and replicate in stratified squamous epithelium; this is followed by uptake into ramifying unmyelinated sensory nerve fibers within the stratified squamous epithelium and then retrograde microtubule-associated transport to the cell body of the neuron in the dorsal root ganglion (DRG) adjacent to the spinal cord (or the trigeminal ganglion for HSV-1). Here, acute infection is followed by lifelong latent infection of these cells. Intermittent reactivation of the virus occurs spontaneously and results in anterograde microtubule-associated transport of the virus, usually back to the original infecting dermatome, where the virus crosses from the nerve terminal into the stratified squamous epithelium of skin or mucosa. Here, it replicates and is then shed into oral or genital secretions. This process may or may not result in clinical disease. Thus, the patient may discover the characteristic vesicles or ulcers of genital herpes, but, in most infected patients, small or atypical...
lesions are usually recognized only by expert clinicians or educated patients. In another 20% of patients, shedding may be entirely asymptomatic [5].

This cycle of infection is subject to immune control at several levels. Elucidation of these various mechanisms should help guide the development of therapeutic and better prophylactic vaccines. At entry, innate and adaptive immune mechanisms control the level of replication in the mucosa, thus determining the amount of virus that enters neurons, becomes latent, and reactivates from the DRG neurons. At the level of the DRG neuron, immune control determines control over latency and reactivation. After anterograde transport of virus to the axon terminals, adaptive immune mechanisms at the interface with mucosal epithelial cells may determine the level of replication and whether clinical disease or asymptomatic shedding occurs.

ENTRY OF HSV INTO THE SKIN/MUCOSA

HSV-1 and -2 have difficulty penetrating intact stratum corneum except when cracks or abrasions exist or this layer is very thin or nonexistent (labium minora and vagina in females, prepuce in males), allowing access to surface receptors on the epidermal keratinocytes and perhaps on Langerhans cells, resulting in infection. Both epidermal keratinocytes and dendritic cells (DCs) have been shown to express HSV receptors, nectin-1, and herpesvirus entry mediator (HVEM), on their surface [6, 7]. Recently, it has been shown that epidermal keratinocytes can also be infected via endocytosis (as well as via neutral fusion at the cell surface) [8]. HSV infection of keratinocytes results in the production of numerous cytokines, including interferon (IFN)-α, IFN-β, interleukin (IL)-1, IL-6, and β-chemokines, all present within vesicle fluid and shown to be liberated from infected keratinocytes in vitro [9]. Infection of Langerhans cells resident within the epidermis is also likely to liberate cytokines, as has been found in the supernatants of HSV-infected DCs in vitro [10].

RETROGRADE TRANSPORT OF HSV-1 IN NEURONS

HSV is rapidly taken up by neurons in the mucosa or skin in guinea pig models, although amplification in keratinocytes probably provides the bulk of virus entering sensory nerve terminals. After entry into cells, including neurons, the surface glycoproteins of the virus are left behind in the cell membrane as a consequence of virus–cell membrane fusion. Many of the outer tegument proteins are phosphorylated and dissociate from the virion [11]. Indeed, most of the tegument proteins appear to be lost from the internal core or nucleocapsid. The exact tegument proteins remaining on the capsid have yet to be defined, but this process allows the capsids with a complement of only a few or even 1 tegument protein to bind to molecular motors associated with microtubules and to be transported rapidly in a retrograde direction to the cell body [12]. There is evidence that the principal retrograde molecular motor dynein, in association with dynactin, is involved in this transport [13]. The viral protein(s) binding to this molecular motor have yet to be fully defined. In the related alphaherpesvirus pseudorabies virus, at least 3 inner tegument proteins appear to remain on the capsid during retrograde transport: US3, UL36, and UL37 [14, 15]. However, this may not necessarily be the case with HSVs (authors’ unpublished data). It is likely that the viral proteins binding to dynein are such inner tegument proteins or outer capsid proteins. In fact, we have shown that VP26, a small 12-kDa protein perched externally on the capsid that binds to the major capsid protein VP5 in its hexon configuration, also binds to dynein as a recombinant protein [16]. Furthermore, when HSV capsids are assembled in vitro from their constituent proteins with or without VP26, only the capsid with VP26 on its surface is transported to the nuclear membrane after deposition of both types of capsids into the cytoplasm by microinjection [16]. It is possible that the external position of VP26 may allow it to bind to dynein during retrograde transport in vivo if not covered by any of the other inner tegument proteins. Nevertheless, there must be another (possibly inner tegument) protein binding to dynein, because VP26 can be deleted from the virus without completely impairing retrograde transport in mice [17]. Herpesviruses often show redundancy of important functions, and it seems likely that this will also be the case with retrograde and anterograde transport.

ROLE OF IFN AND THE INNATE IMMUNE RESPONSE IN INITIAL INFECTION BY HSV

IFNs, macrophages, NK cells, and γδ T cells have all been shown to play a role in innate immunity [18–20] (figure 1A). In murine models, the role of IFN-α and -β in protection against HSV-1 infection has long been known. However, recently, Toll-like receptors (TLRs) have also been shown to be important mediators of innate immune responses in HSV infection in the genital tract and, perhaps, systemically. HSVs interact with both TLR-2 and -9 [21]. The interaction of HSV-1 and -2 with TLR-2 appears to be at the surface [21], whereas the interaction of HSV-2 with TLR-9 appears to be via viral DNA within the endosomes, particularly of plasmacytoid DCs [22]. This latter interaction is a potent stimulus to the production of IFN-α. Indeed, plasmacytoid DCs are the main effectors of the previously well-described stimulation of IFN-α production from human blood mononuclear cells by HSV antigen [23]. Whether plasmacytoid DCs are present within human lesions is currently under investigation.
immune control of latency or reactivation from neurons cannot be studied in humans, so accurate animal models of latency and reactivation are needed. In humans, both spontaneous and induced (e.g., by UV light) reactivation from latency followed by recurrent disease occurs, but spontaneous reactivation does not occur in the mouse [24]. In guinea pigs and rabbits, in which such spontaneous reactivation occurring in disease does occur, a full range of reagents to characterize immune cells is not available. Nevertheless, careful examination of murine DRG neurons during the phase of HSV latency shows that latency is somewhat “leaky,” as shown by the presence of HSV RNA and proteins in occasional neurons [25]. Latency needs to be maintained by the immune system, particularly by noncytolytic CD8+ T cells (specific for HSV structural proteins), which lie in apposition to neurons and secrete IFN-γ [24]. Experiments with IFN-γ (or receptor) knockout mice and/or subsequent addition of IFN-γ within 24 h of ex vivo culture support its importance in preventing reactivation, possibly through inhibition of the function of the key immediate early viral protein ICP0. CD4+ T cells are also present and may help maintain latency [24].

**ROLE OF THE IMMUNE SYSTEM IN MAINTENANCE OF LATENCY AND PREVENTION OF VIRUS REACTIVATION**

Immune control of latency or reactivation from neurons cannot be studied in humans, so accurate animal models of latency and reactivation are needed. In humans, both spontaneous and induced (e.g., by UV light) reactivation from latency followed by recurrent disease occurs, but spontaneous reactivation does not occur in the mouse [24]. In guinea pigs and rabbits, in which such spontaneous reactivation occurring in disease does occur, a full range of reagents to characterize immune cells is not available. Nevertheless, careful examination of murine DRG neurons during the phase of HSV latency shows that latency is somewhat “leaky,” as shown by the presence of HSV RNA and proteins in occasional neurons [25]. Latency needs to be maintained by the immune system, particularly by noncytolytic CD8+ T cells (specific for HSV structural proteins), which lie in apposition to neurons and secrete IFN-γ [24]. Experiments with IFN-γ (or receptor) knockout mice and/or subsequent addition of IFN-γ within 24 h of ex vivo culture support its importance in preventing reactivation, possibly through inhibition of the function of the key immediate early viral protein ICP0. CD4+ T cells are also present and may help maintain latency [24].

**ANTEROGRADE TRANSPORT OF HSV FROM DRG NEURONS TO PERIPHERY**

The process of alphaherpesvirus assembly and egress from the cell body of DRG neurons appears to be similar to that in cultured cell lines [26, 27]. The study of anterograde transport of HSV from the cell bodies of neurons in the DRG to the periphery is difficult. Early studies relied on empirical sampling of HSV-infected mice or rabbits or of neurons cultured in vitro at different time points and the identification of a small number of enveloped or unenveloped capsids in the DRG and peripheral nerves. The region of the axon in which these enveloped capsids were observed was either proximal (close to the cell body) or uncertain [28].

To study the process of anterograde transport more exactly, we adapted the 2-chamber systems of Lycke and colleagues [28, 29], as shown in figure 2. Intact DRGs were placed in the central chamber, while autologous epidermal explants were placed in the external chamber. The addition of nerve growth factor resulted in the outgrowth of axons from the DRG, which then penetrated an agarose plug in a barrier between the 2 chambers, extended into the exterior chamber, and interacted with epidermal cells. Inoculation of virus into the inner chamber resulted in infection of the DRG and anterograde transport of the virus within axons. Indeed, the only route of virus particles from the inner to the outer chamber was via these axons. Virus crossed from the axons into the epidermal explants, as observed by serial fixation and confocal microscopy for viral antigen. Furthermore, somewhat surprisingly and controversially, ultrathin sections taken from behind the advancing front of viral antigen were examined by transmission electron microscopy and showed the presence of unenveloped nucleocapsids in these axons [30].

Follow-up observations of infected axons in this system by transmission immunoelectron microscopy showed unenveloped capsids adjacent to microtubules immunolabeled for capsid (VP5) and surrounded by tegument proteins (VP16), whereas glycoproteins and other tegument proteins were observed within axonal vesicles in distal axons [31, 32]. Unenveloped capsids were demonstrated in infected axons during anterograde transport but not in uninfected axons by rigorously controlled and blinded electron microscopic observations and by
Both transmission immunoelectron microscopy and scanning immunoelectron microscopy. In our laboratory, previous studies have observed, usually in cross-sections, >100 unenveloped capsids but no enveloped capsids in the mid- or distal axon [26, 30–32] (authors’ unpublished data). These studies were further supported by the examination of capsid, tegument, and envelope protein localization by serial fixation and immunofluorescence, demonstrating differences in the kinetics of the appearance of glycoprotein and capsid protein in axons. Furthermore, the tegument protein US11 was found to directly interact with the heavy chain of the anterograde motor kinesin [33]. The above observations suggested separate capsid and glycoprotein transport and assembly at the axon terminus (although the latter has not been visualized). Other laboratories have also provided further evidence for such anterograde axonal transport of unenveloped HSV-1 capsids [34].

Our most recent studies of anterograde transport of HSV-1 in human fetal DRG axons showed, by confocal microscopy, antigenic colocalization of all 3 classes of HSV-1 components (capsid, tegument, and envelope proteins), mainly in axonal varicosities (axonal swellings usually at branch points) and growth cones at the axon terminus. These findings correlated with the presence of enveloped capsids in vesicles, which were observed in clusters at these sites by transmission electron microscopy. In these growth cones, tegument (VP22) and envelope (gD) proteins, alone or together, were detected on vesicles by transmission immunoelectron microscopy, consistent with the suggestion that some tegument and envelope proteins may be transported separately or together in association with axonal vesicles [35]. This is consistent with real-time fluorescence studies of pseudorabies virus in chick DRG neurons [14, 26]. Early availability of these proteins on vesicles in the distal axon could allow later assembly and envelopment of virions in the varicosities and growth cones, by a process similar to that in the trans-Golgi network.

In contrast, a recent report has challenged this “separate transport” or “subassembly” hypothesis. In a 3-chamber system, enveloped particles within vesicles were observed in the proximal and mid-axon [36]. How can these different observations be reconciled? At present, there are no clear answers. Different alphaherpesviruses, such as HSV, pseudorabies virus, and varicella-zoster virus, may vary markedly in the proteins used for various functions, but it seems unlikely that they would use completely different processes for axonal transport [37]. Quantitative differences in the relative importance of the unenveloped versus enveloped capsid transport pathways are more likely.

**Figure 2.** Two-chamber system for examining anterograde axonal transport of herpes simplex virus in human or rat dorsal root ganglion (DRG) neurons.

**IMMUNE RESPONSE TO HUMAN RECURRENT HERPES IN THE SKIN**

Several laboratories, including our own, have concentrated on studying immune control of HSV infection in the human skin, especially in recurrent oral or genital herpes, demonstrating the importance of the cell-mediated immune response to HSV in control and clearance of recurrent infection. The increased severity and persistence of recurrent herpes as a presenting syndrome of AIDS reflects the key role of T cells, especially CD4+ T cells [38]. In the Merigan laboratory in 1983, we used biopsy samples of recurrent herpetic lesions and immunohistochemical methods to demonstrate the importance of CD4+ T cells, both as a source of IFN-γ and as cytotoxic effectors early in the course of infection, followed by a later influx of CD8+ T cells [39, 40]. This was later confirmed by Koelle et al. [40] in studies of T cell clones derived from such biopsy samples. We also demonstrated that the IFN-γ secreted by CD4+ T cells restored major histocompatibility complex (MHC) class I expression on infected epithelial cells, thus overcoming the HSV ICP47–induced blockade of MHC class I expression [41] and allowing recognition by CD8+ cytotoxic T cells [42]. IFN-γ also stimulated MHC class II expression on keratinocytes throughout the lesion, allowing recognition by CD4+ T cells. Therefore, it is not surprising that the frequency of recurrent herpes was found to correlate with levels of IFN-γ produced by blood CD4+ T cells [43]. β-Chemokines, IL-12, and IFN-α, -β, and -γ produced by both epithelial cells and the infiltrating immune cells within herpetic lesions are critical for the control of this highly cytopathic virus [44, 45]. β-Chemokines attract monocytes and T cells into the lesions, and IL-12 entrains CD4+ T cell secretion to a Th1 pattern (especially IFN-γ), which activates cytotoxic CD8+ T cells, through IL-12 and IFN-α (figure 1B). The later infiltration with CD8+ cytotoxic T cells corresponds with clearance of virus from the lesions [40]. Study of the immunology of human recurrent herpetic lesions has helped guide the development of prophylactic as well as therapeutic vaccine candidates, suggesting the need to induce Th1 rather than Th2 patterns of response [46].
Homing and infiltration of monocytes and T cells.
Although it now seems clear that earlier infiltration of CD4⁺ T cells and monocytes than of CD8⁺ T cells into herpetic lesions is an important sequence, the mechanisms responsible for the differential homing of the T cell subsets are unclear. Recent investigations have demonstrated an up-regulation of E-selectin on cutaneous venule endothelial cells in recurrent herpetic lesions, as expected in any inflammatory lesions [47]. This could be a result of secretion of IL-1β by keratinocytes and, as the lesion progresses, by IFN-γ from CD4⁺ T cells. In blood, HSV-2–specific CD4⁺ and CD8⁺ T cells express the E-selectin ligand and/or the similar cutaneous T cell–associated antigen and E-selectin ligand after in vitro stimulation with HSV-2. Cutaneous lymphocyte-associated antigen and/or E-selectin ligand may be up-regulated by cytokines such as IFN-α, IL-12, and transforming growth factor–β secreted in blood or lesions by immune cells and keratinocytes [48]. Further work is needed to fully understand these mechanisms of differential T cell homing and entry into lesions and their relative importance.

Viral antigens recognized by T cells. The next important question is which of the key immunogenic proteins within the HSV virion. Using blood lymphocytes from HSV-2–infected patients that were restimulated with whole HSV in vitro and incubated with IFN-γ–stimulated epidermal cells infected with recombinant vaccinia virus containing a repertoire of HSV proteins, we showed that the surface glycoproteins gD and gB (produced late in the replication cycle) are key targets for CD4⁺ T cells in most patients, and ICP27 (a protein present only at early stages in infected cells) is the target for CD8⁺ T cells in most patients [49] (both gB and ICP27 are also recognized by CD8⁺ T cells in mice [50, 51]). However, the immunogenicity of individual proteins in mice and humans does not always correlate. Koelle et al. [52] cloned T cells out of recurrent herpes simplex lesions and reacted them against B cells infected with HSV-1, HSV-2, and recombinants of the two. In this system, several proteins from the tegument of the virus have been found to be important targets for type-specific immunity (HSV-2 but not HSV-1). The differing results may be complementary (i.e., HSV-1 and HSV-2 may have been cross-reactive and type-specific epitopes), but testing of these 2 systems in vivo is required. Although these studies were conducted in humans with recurrent herpes simplex, glycoprotein D seems to be a good candidate for prophylactic vaccines, and here CD4⁺ T cell responses are still likely to be important [53].

Role of DCs. Human herpes simplex, whether initial or recurrent, is an epidermal disease. Therefore, the primary DC likely to be involved in HSV antigen uptake is the Langerhans cell, and, indeed, recently we have demonstrated HSV structural antigens within these cells during recurrent herpes simplex (authors’ unpublished data). Involvement of dermal DCs in the upper region of the dermis has not been excluded. Older and more recent studies of Langerhans cells and other DCs presenting antigen in lymph nodes initially appear to be contradictory. Earlier studies suggested that depletion of Langerhans cells from skin, after HSV-1 infection of mice via the footpad, led to increased HSV virulence [54]. However, 2 recent studies have demonstrated that Langerhans cells are not the cells presenting HSV antigen to CD8⁺ T cells in lymph nodes. After epidermal scarification with the virus, it was observed that CD8⁺ DCs present HSV antigens to CD8⁺ T cells in draining lymph nodes 2 h after infection [55], and after vaginal inoculation of HSV, it was found that dermal DCs present HSV antigen to CD4⁺ T cells [56]. The apparent paradox of initial HSV antigen uptake by Langerhans cells but presentation by another DC subtype may be explained by transfer of the antigens from one DC subtype to another.

In view of the difficulty in obtaining sufficient numbers of immature human Langerhans cells, monocyte-derived DCs have been used as a model. Both immature and mature monocyte-derived DCs express the HSV receptors nectin-1, nectin-2, and HVEM and can be infected with HSV-1 and HSV-2, but only immature monocyte-derived DCs (which more closely resemble the immature sessile Langerhans cells in epidermis) produce virus at low levels. HSV infection results in asynchronous down-regulation of the key costimulatory molecules CD40, CD80, CD83, and CD86, preventing proper maturation of the DCs [10, 57]. Interestingly, MHC class I is not down-regulated on infected monocyte-derived DCs, unlike most other infected cells. Similarly, in most other cell types, HSV-1 produces antiapoptotic effects, whereas in DCs, both HSV-1 and HSV-2 induce apoptosis progressively throughout the cell sheet over 24 h [57, 58]. However, these apoptotic cells are preferentially taken up by uninfected bystander cells, and the HSV antigens contained within these apoptotic cells are then cross-presented (on MHC class I) to CD8⁺ T cell clones [58]. This suggests a mechanism for the antigen transfer between DC subtypes observed in murine models and suggests that the immunoevasive mechanisms of costimulatory molecule down-regulation and apoptosis of DCs by HSV can be counteracted by uptake by bystander DCs. Preliminary experiments with immature Langerhans cells in vitro show a similar down-regulation of costimulatory molecules, but the other effects need to be verified in this cell type (authors’ unpublished data). Whether bystander Langerhans cells or subjacent dermal DCs respond in human infection in a fashion similar to that in the murine models remains to be elucidated. In addition, uninfected immature monocyte-derived DCs undergo partial maturation after uptake of inactivated HSV. Inactivated HSV is also a potent stimulator of IFN release by blood plasmacytoid DCs.
and is responsible for the majority of IFN-α released by peripheral blood mononuclear cells stimulated with HSV antigen, a long-standing observation (figure 1A) [23]. Whether such plasmacytoid DCs infiltrate herpetic lesions as they do cutaneous psoriasis lesions remains an open question.

**Future studies.** Longitudinal studies of recurrent genital herpes and asymptomatic genital shedding of HSV in humans indicate a decrease in frequency initially over 3 months and then again over years, suggesting a maturation of the immune response. The reasons for this are unclear and may reflect an effect on memory cells or even on maturation of homing responses. In addition, studies of innate and adaptive immune responses in initial human HSV infection are difficult and therefore infrequently undertaken. More work needs to be done on this topic to compare with the abundant studies in murine models.

**VACCINES AND IMMUNITY**

Until recently, a search for a vaccine candidate to prevent genital herpes had been unsuccessful, partly because of low antigen concentrations and a focus solely on neutralizing antibodies. The use of a vaccine candidate that incorporated high concentrations of recombinant soluble glycoprotein D, which is widely recognized in human populations [49], and the adjuvant deacylated monophosphoryl lipid A derived from the cell walls of bacteria showed partial efficacy in the prevention of genital herpes and a trend toward prevention of infection with HSV-2. Although these results remain to be confirmed by the ongoing HERPEVAC trial, it is of particular interest that the vaccine showed an efficacy of 73%–74% only in women (not men) and only in those who were seronegative for both HSV-1 and HSV-2 [53]. The reasons for this sex bias and apparent evidence of cross-protection of HSV-1 against disease are of considerable interest.

The success of this vaccine candidate has been attributed to the role of the adjuvant in inducing Th1 patterns of immune and cytokine response, especially induction of IFN-γ, in both guinea pig models and human phase 1 trials [59] as well as during the trial itself (L. R. Stanberry, A. L. Cunningham, S. L. Spruance, M. Denis, G. Dubin, and D. I. Bernstein, unpublished data). No induction of T cell cytotoxicity was demonstrated. Neutralizing antibody was induced, but not to the very high levels induced by another vaccine candidate (from Chiron), containing HSV-2 glycoprotein D and glycoprotein B with MF59 adjuvant, in clinical trials [60] (L. R. Stanberry, A. L. Cunningham, S. L. Spruance, M. Denis, G. Dubin, and D. I. Bernstein, unpublished data). The sex bias in the immune response may be due to local effects of this Th1 response in enhancing genital mucosal T cell responses, which therefore enhances resistance of the female genital mucosa so that it is more similar to that of male genital mucosa. The latter has greater intrinsic resistance because of the presence of a thick intact stratum corneum in penile skin (with the exception of the prepuce). However, women also display greater systemic Th1 responses than do men, which could partly explain the sex difference. Recently, murine studies showing gender differences in immune responses to HSV have been published [61].

The trial results with the GlaxoSmithKline candidate vaccine Simplirix seem to demonstrate several key principles for a vaccine against HSV. First, it is possible to obtain substantial protection against disease with a single recombinant viral protein combined with an adjuvant that induces the correct form of immune response. If these correlates of protective immunity can be confirmed as CD4+ T cell Th1 responses (especially producing IFN-γ) in the current HERPEVAC trial, they will be helpful in the design of surrogate markers for evaluating other vaccine candidates, such as DNA vaccines and replication-defective mutants [62]. Nevertheless, the current vaccine candidate eventually needs to be improved to achieve ≥95% efficacy in both males and females.

For example, the design of future vaccine candidates must also be aimed at inducing innate immune responses and at controlling viral infection at the level of both the genital mucosa and the DRG. The former is probably more important to the prevention or reduction of the viral inoculum entering cutaneous sensory nerve endings and, thence, the DRG, thus reducing the number of latently infected DRG neurons, which determines subsequent reactivation rates and the frequency of recurrent herpes. Failure to stimulate innate immunity by the current vaccine candidate might be partly responsible for the discrepancy between prevention of genital herpes and genital HSV-2 infection; that is, stimulation of the adaptive immune response may reduce levels of virus in the DRG and subsequent disease but may not prevent symptomatic infection and shedding. Alternatively, induction of CD8+ T cell immunity and/or neutralizing antibody with different HSV-2 antigens and adjuvants may synergize with the induced CD4+ T cell Th1 response, as in natural infection [39, 40, 42]. Mucosal immunization is also a worthwhile strategy and could be aimed at stimulating adaptive and/or innate immune mechanisms after oral or nasal delivery [63]. Finally, the effects of all such vaccine candidates on asymptomatic genital shedding and subsequent transmission are of great importance epidemiologically.

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