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Antiviral Research 63S1 (2004) S19–S26
Antiviral Research
www.elsevier.com/locate/antiviral

Review

HSV shedding

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Abstract

Viral shedding of HSV occurs frequently in infected individuals. HSV is shed asymptomatically from multiple anatomical sites and shedding, like exposure, is a significant risk for transmission. However, the relationship between shedding frequency, viral titer and transmission is unknown. HSV-2 shedding is affected by the site and time since acquisition of infection. The advent of sensitive PCR techniques has shown that the magnitude and frequency of viral shedding is higher than shown previously with viral culture techniques. It has also clearly demonstrated that suppressive (daily) antiviral therapy reduces clinical and subclinical reactivation rates, and has been successfully used in the prevention of recurrent oral and genital HSV infections. A recent study has demonstrated that daily antiviral therapy with valaciclovir can significantly reduce transmission of HSV-2 between discordant heterosexual couples in monogamous relationships.

Keywords: HSV; Shedding; Transmission; PCR; Antiviral

1. Introduction

Viral shedding of HSV occurs frequently in infected individuals. For HSV-2 genital infections, clinical and subclinical reactivation is very common. While shedding in the oro-labial area is less well studied, several studies indicate that asymptomatic salivary shedding also occurs frequently. It is clear such asymptomatic shedding can lead to transmission of virus to others. Shedding, like exposure, is a significant risk for transmission between sexual partners and mother to infant. However, the relationship between shedding frequency, viral titer and transmission is unknown. The advent of sensitive PCR detection techniques has advanced the understanding of both the magnitude and frequency of subclinical shedding as well as the impact of antiviral therapy.

Antiviral drugs reduce clinical and subclinical rates of HSV excretion and have been successfully used in the prevention of recurrent oral and genital HSV infections. A recent study has demonstrated that daily antiviral therapy with valaciclovir can significantly reduce transmission of HSV-2 between discordant heterosexual couples in monogamous relationships (Corey et al., 2004). These results have raised questions as to whether these clinical effects would be similar for other antiviral agents in the same class and for different patient populations.

2. HSV-1 infection

Most HSV-1 infections result from contact with infectious salivary secretions and result in symptomatic mucocutaneous HSV-1 in the mouth or subclinical infection that results in trigeminal ganglion latency. Subsequent to the development of latency, reactivation of HSV-1 in the oral cavity occurs. In the last decade, genital HSV-1 infection is becoming reported more frequently in the US and Europe;

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doi:10.1016/j.antiviral.2004.06.004

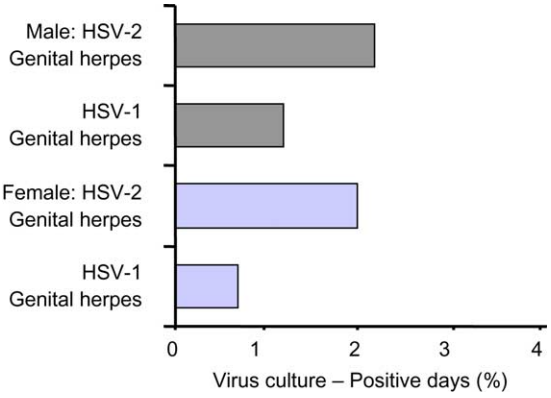

Fig. 1. Comparison of asymptomatic shedding between HSV-1 and HSV-2 genital herpes (Wald et al., 1995).

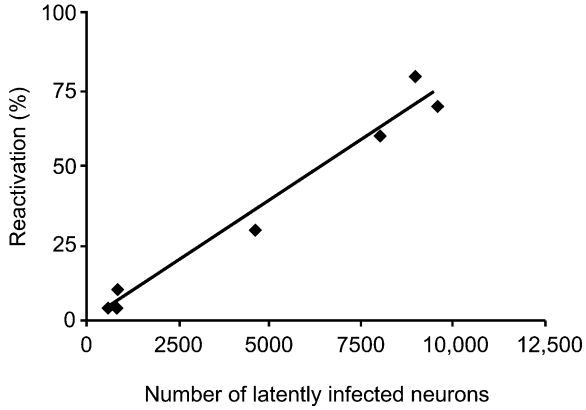

Fig. 2. Reactivation of HSV-1 is directly related to the number of neurons infected. Reprinted from Sawtell (1998) with permission from the American Society for Microbiology.

nearly half of all first episodes of genital herpes in Canada and the UK result from HSV-1 infection. Early studies using viral culture found that HSV-1 was shed asymptomatically in the oral cavity 6% of the time (Spruance, 1984). In a more recent study in oral/maxillofacial surgery outpatients, the rate of asymptomatic shedding was found to be slightly lower at 2.7% by culture and 4.7% by PCR (Tateishi et al., 1994). The rate of asymptomatic shedding increases with immunosuppression. There are few data on the transmission of HSV-1 from patients who are seropositive for HSV-1 but have a negative history of herpes labialis.

The greatest frequency of asymptomatic shedding of HSV-1 occurred during false prodromal symptoms, presumably when there was viral reactivation which did not lead to lesions. In cases of genital HSV-1 infection, the rates of asymptomatic shedding are considerably lower than those associated with asymptomatic genital shedding of HSV-2, in both men and women (Wald et al., 1995; Fig. 1).

Ocular HSV-1 infections are the most common cause of blindness secondary to infection in the developed world and offer some interesting issues with regard to transmission. Around 70% of people are exposed to HSV-1, but ocular HSV disease only occurs in a small proportion of these individuals (approximately 1 in 2000; Verdier and Krachmer, 1984). Nonetheless, this equals nearly 150 000 cases in the US alone. The majority of people with ocular HSV disease have a history of herpes labialis. One explanation is that inoculation of the eye via the conjunctivae occurs during primary oral infection. Alternatively, the virus could spread through the ganglia. In a murine study of ocular or oral HSV-1 infection, the incidence of latent infection of the trigeminal ganglion was related to the severity of the peripheral infection (Tullo et al., 1982). Following ocular inoculation, latent infection could be demonstrated not only in ophthalmic branches of the ganglion but also in those ganglionic segments innervating the face. Similarly, severe primary infection of the lip resulted not only in latent infection of the mandibular branch of the trigeminal ganglion, but also of maxillary and ophthalmic branches. Thus, in mice virus can spread through the peripheral nervous system. This may explain the observation that HSV disease can suddenly appear in the eye, even if the eye has not previously been affected by HSV-1 infection.

The rate of virus reactivation in mice correlates directly with the number of latently infected neurons in the ganglia. In a study using groups of mice whereby latently infected neurons ranged from 1.9 to 24%, reactivation of HSV (induced by hyperthermic stress) correlated with the number of infected neurons ($r = 0.9852$, $P < 0.0001$) (Sawtell, 1998; Fig. 2). Clinically, it is not known how many neurons are infected within a patient, but it is likely that the more neurons that are infected, the greater the rates of reactivation and more shedding that occurs.

3. HSV-2

3.1. Shedding and reactivation

Factors that affect viral shedding in HSV-infected individuals include: (1) site (Table 1), (2) time since the acquisition (Table 2), (3) natural history, including the state of the immune system, as immunosuppressed patients excrete virus more frequently, and (4) virus type. For HSV-2, symptomatic genital lesions (Fig. 3) are associated with high genome copies of mucosal HSV DNA; shedding in the absence of lesions (asymptomatic or subclinical shedding) is also common, occurring most frequently from rectal sites (Wald et al., 2000).

The risk of shedding decreases with time after the primary infection, such that after 10 years it is approximately 70% lower than during the first 6 months of infection.

Having a history of symptomatic genital herpes does not influence the rate of subclinical shedding of HSV (Table 3). People who are HSV seropositive, but did not have symptomatic genital herpes, were equally likely to shed HSV as those people who had symptomatic genital herpes.

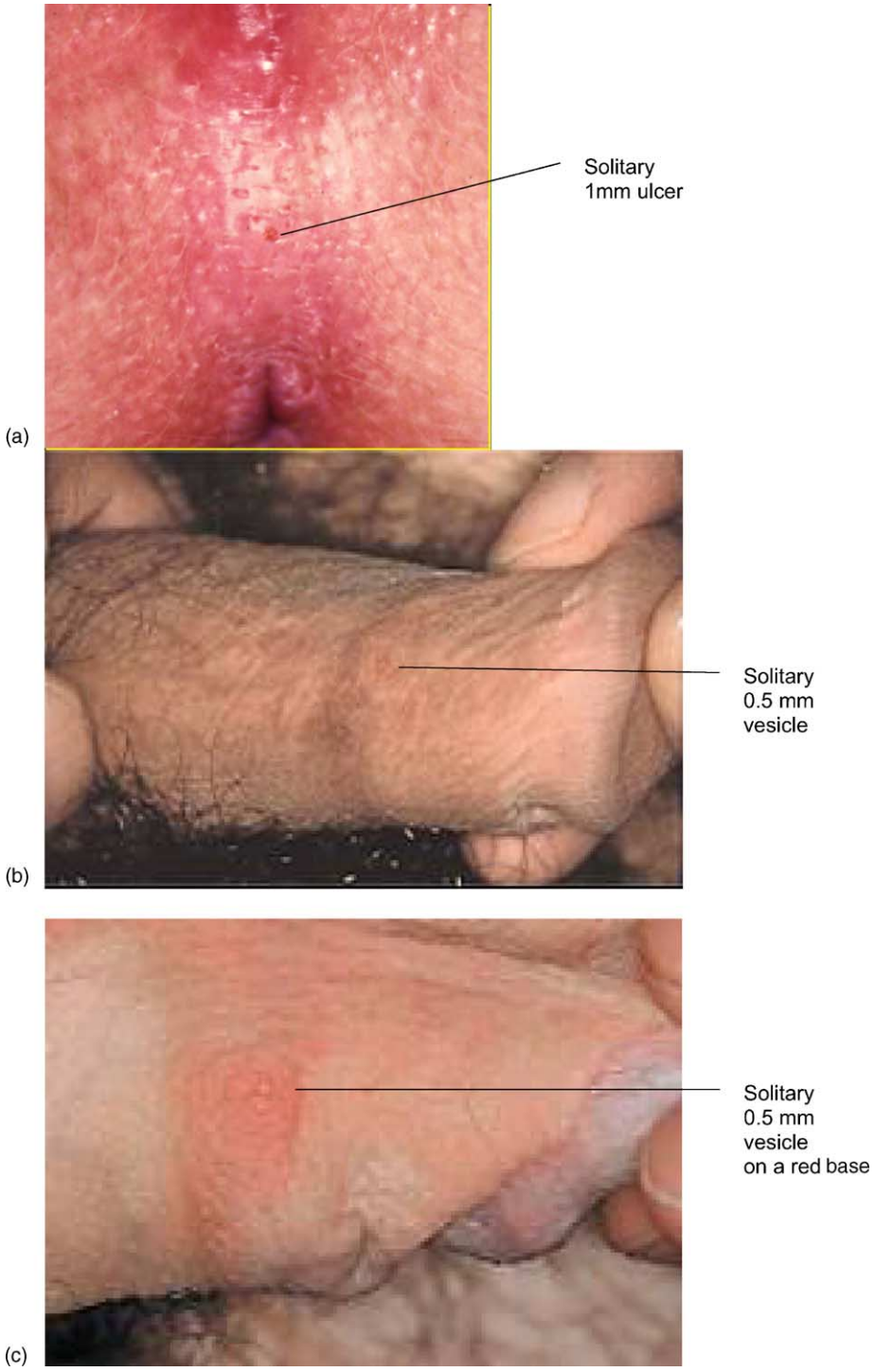

Fig. 3. Genital herpes lesions.

Most women who transmit HSV from mother to child are HSV-2 seropositive, but have no history of infection or symptoms at delivery (Whitley, 1988) Similarly, most men and women who transmit HSV to a sexual partner are HSV-2 seropositive, but have no history of infection (Mertz, 1993). However, when the individuals in this study were questioned, they gave histories suggestive of symptomatic infection and, potentially, of prodromal or mild lesions at the time of intercourse.

3.2. *Redefining shedding in the era of PCR*

Subclinical reactivation of HSV in symptomatic people was first reported in the 1970s (Rattray et al., 1978). By the

Table 1
Effect of site on shedding of HSV as measured by viral culture (Wald et al., 2000)

Site	Days with symptomatic shedding (%)	Days with asymptomatic shedding (%)
Vulva	1.4	1.2
Cervico-vagina	1.6	0.9
Rectum	1.5	1.6

Table 2
Risk of subclinical HSV-2 shedding as measured by culture decreases over time (Wald et al., 2000)

	Time post-HSV acquisition			
	<6 months	6–24 months	2–10 years	>10 years
N	33	33	15	15
Days cultured				
Median	76	86	106	110
Range	50–246	52–382	60–451	55–259
Odds ratio[a]	1.0	0.5	0.4	0.3
95% CI		0.3–0.8	0.3–0.9	0.1–0.6

[a] By logistic regression.

Table 3
Effect of history on subclinical shedding as measured by culture (Wald et al., 2000)

	No history ($n = 37$)	With history ($n = 46$)	*P*-value[a]
Shed	27 (73%)	42 (91%)	0.03
Shed subclinically	26 (70%)	31 (67%)	0.8
Shed clinically	14 (38%)	42 (98%)	<0.001

[a] χ^2.

mid-1980s, subclinical shedding was recognized from two separate perspectives:

- unrecognized lesions, i.e., ulcerations in anatomically difficult-to-see areas such as peri-rectal and cervical;
- microscopic ulcerations in recognized areas (colposcopy generated some increase in visibility of lesions).

Definitions of subclinical HSV shedding have been put forward and are now widely accepted (Wald et al., 2000). These include:

- subclinical HSV shedding—the detection of HSV on genital mucosa on days without lesions consistent with genital herpes;
- unrecognized or unapparent genital herpes—serologically documented HSV-2 infection in persons who deny any history of genital herpes.

PCR has had a considerable impact on the understanding of HSV-2 shedding. Subclinical shedding of HSV-2 occurs commonly when PCR is utilized, detecting HSV DNA on 20–25% of all days. PCR has also shown that: (1) shedding occurs at many anatomical sites, (2) mucosal reactivation is 4–5 times more frequent than previously thought, and (3)

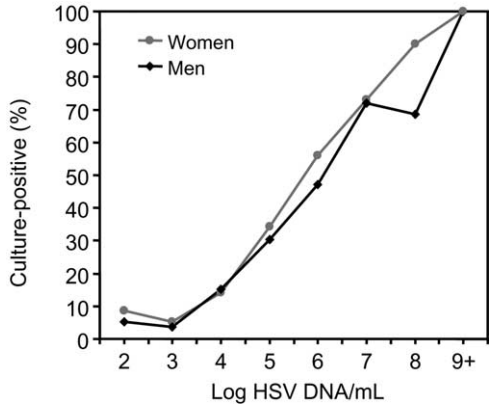

Fig. 4. Comparison of PCR with culture for the detection of HSV-2. Reprinted from Wald et al. (2003) with permission from the University of Chicago Press.

all HSV-2 seropositive people are infectious. Detection of HSV-2 by PCR and culture has been directly compared.

The results (Table 4) demonstrate that PCR is 3–4 times more sensitive for detecting HSV on mucosal surfaces than culture, independent of the presence of lesions or the immune status of the patient (Wald et al., 2003). Notably, patients who were PCR-positive but culture-negative for HSV-2 remained infectious. The 50% culture-positivity rate corresponds to 10^6 copies of DNA in the PCR assay (Fig. 4)

Days on which genital lesions were present were associated with higher copy numbers of HSV-2 DNA and longer duration of the shedding episode. Furthermore, the maximum copy number of viral DNA increases with the duration of a PCR-positive episode of shedding. In pregnant women, the high sensitivity of PCR has shown that subclinical shedding decreases with the time since acquisition of initial infection.

The advent of PCR detection of HSV-2 DNA helped to explain the high false negative rate of cultures from mothers delivering infected newborns, and the frequent sexual transmission of HSV-2 among sexual partners (Brown et al., 1997; Mertz et al., 1988).

4. Reducing viral shedding may reduce transmission

4.1. Antiviral drugs

Daily antiviral therapy has been shown to markedly reduce clinical and subclinical reactivation rates of HSV-2, and breakthrough shedding in patients on antiviral therapies is associated with greatly reduced titers of HSV (Wald et al., 1997). Both aciclovir and valaciclovir are effective for the prophylaxis of herpes labialis in the normal host (Rooney et al., 1993; Baker and Eisen, 2003). Aciclovir has also been used as short-term prophylaxis in stress situations. Skiers with a history of sun-induced recurrences were effectively treated with prophylactic aciclovir administered before their

Table 4
Comparison of PCR with culture for the detection of HSV-2 (Wald et al., 2003)

Group	Specimens	Positive specimens				Ratio of positive PCR/culture	Mean number of HSV DNA copies
		Culture		PCR			
		n	Percentage	*n*	Percentage		
Total	36,471	1,087	3	4,415	12	4.1	4.6

Table 5
Can HSV-1 shedding be used as a surrogate marker for transmission?

	Asymptomatic shedding demonstrated	Antiviral prophylaxis decreases clinical events	Antiviral prophylaxis decreases viral shedding	Antiviral prophylaxis decreases transmission
Oral herpes	YES	YES	ND	ND
Genital herpes	YES	ND	ND	ND
Ocular herpes	YES	YES	ND	ND

ND: no data.

vacation. Only 7% of those treated with aciclovir developed herpes labialis, compared with 26% of those treated with placebo (Spruance et al., 1988).

4.1.1. HSV-1

Could HSV-1 shedding be used as a surrogate marker for transmission of HSV-1 infection? Although asymptomatic shedding of HSV-1 has been demonstrated clinically (Table 5), a direct link has not been demonstrated between the effects of antiviral prophylaxis on clinical events, or on virus shedding and transmission. There are no studies looking at transmission of HSV-1 between partners and families. Moreover, there are no studies of reduction of virus by antivirals as measured by PCR (Table 5).

4.1.2. Suppression of HSV-2 shedding with aciclovir

A crossover study of 34 women clearly showed that a regimen of suppressive aciclovir therapy reduced the shedding of HSV-2 by 94%. In addition, the percentage of days with asymptomatic shedding were reduced from 5.8% on placebo to 0.37% on aciclovir ($P < 0.001$) (Wald et al., 1995). A subset of 24 of these women was evaluated by PCR, with

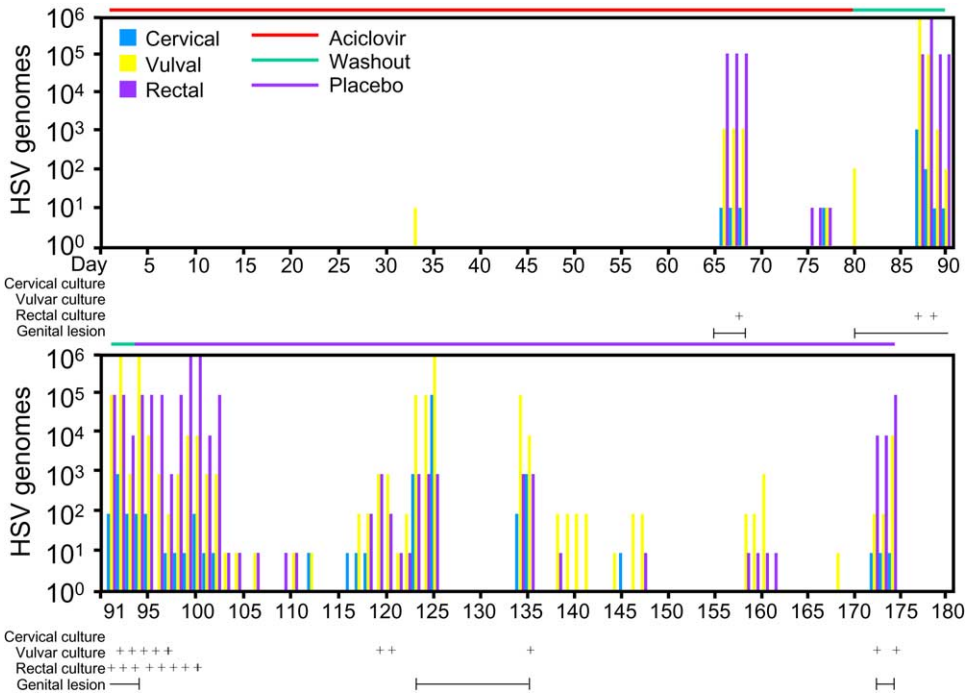

Fig. 5. Suppressive aciclovir reduces asymptomatic shedding of HSV-2 (Wald et al., 1997).

Table 6
Famciclovir reduces asymptomatic HSV shedding (Sacks, 2004)

	Famciclovir 125 mg TID	Famciclovir 250 mg TID	Placebo
Number of patients	60	59	58
Ratio of days	32/6198	24/5788	177/5732
Days with asymptomatic virus shedding (%)	0.52%	0.41%	3.09%
Odds ratio	6.1	7.7	
95% CI	(3.5, 10.8)	(4.0, 14.5)	
P-value	<0.0001	<0.0001	

similar findings. The PCR data from a representative subject are shown in Fig. 5.

4.1.3. Suppression of HSV-2 shedding with famciclovir

In a randomized, double-blind, placebo-controlled study, the proportion of days of asymptomatic virus shedding during treatment with either famciclovir 125 mg TID or 250 mg TID and placebo were compared in women with recurrent genital HSV-2 infection (Sacks, 2004). This study demonstrated that treatment with famciclovir at either dose significantly reduced asymptomatic shedding at all sites (Table 6). These data were obtained by measuring HSV using culture rather than the more sensitive PCR.

Famciclovir treatment dramatically reduced the rate of shedding of HSV in patients with HIV infection (Schacker et al., 1998). In a double-blind, placebo-controlled, crossover trial, 48 patients who were HIV positive (median CD4 cell count 384 cells/mm^3) and HSV seropositive were randomly assigned to receive famciclovir, 500 mg orally twice daily, or placebo for 8 weeks. They then received the other regimen after a 1-week washout period. Daily cultures of perirectal, urethral, oral, and genital areas were obtained. Overall, the percentage of days with HSV-2 shedding was reduced from 9.7 to 1.3%, a reduction of 87%.

4.1.4. Suppression of HSV-2 shedding with valaciclovir

In a double-blind, randomized, crossover study lasting 6 months, valaciclovir 500 mg BID was compared with aciclovir 400 mg BID and placebo. HSV shedding was detected using culture and PCR (Gupta, submitted). Sixty-nine people entered the study, during which HSV was detected in 90% of subjects by culture and 98% by PCR. Both antiviral drugs reduced shedding (clinical and subclinical) of HSV dramatically (Table 7).

Suppressive antiviral therapy also reduces the titers of viral DNA shed during a breakthrough episode (Gupta, submitted).

5. Vaccines

The parallels between use of antivirals and vaccines for prevention of transmission between HSV-2 discordant couples, and particularly the publication of the recent two trials in these populations, will inevitably raise the question of comparisons. The effects of antivirals are often considered only in the context of shedding from the donor rather than variability in susceptibility of the recipient (this occurred in the HIV field for many years until CCR5 Delta 32 was discovered). As vaccination is practiced on the potential recipient of a viral infection, this emphasizes the second side of the equation. It is relevant in view of some of the recent discussions about post exposure prophylaxis of HSV-2 infection and is already in practice in the HIV field.

The rationale for the development of HSV vaccines is to produce an agent that is either prophylactic (against primary infection) or therapeutic (against recurrent infection). Prophylactic vaccines aim to reduce or eliminate viral replication in mucosa and prevent entry into nerves, thereby reducing disease, whereas therapeutic vaccines aim to prevent virus reactivation or epithelial replication of virus, and reduce recurrences of disease.

The Chiron gB2/gD2 HSV vaccine was the first prophylactic vaccine to be tested in clinical trials, and although neutralizing antibody levels exceeded natural infection, the efficacy as measured by time to HSV-2 infection was poor. A second (GSK) vaccine, gD2 MPL HSV, was the first to be partially successful for prophylaxis of genital herpes. It was effective in a guinea pig model, and induced Th1 response in humans. Two clinical studies with a primary endpoint of genital herpes disease showed that the vaccine had significant efficacy in preventing disease in HSV negative women, but not in men or in HSV-1 seropositive women. It also produced a trend towards a lower rate of infection in women, although the difference did not quite ($P = 0.06, 0.07$) reach significance in either study.

Studies with these two vaccines provided valuable lessons for future therapeutic and prophylactic vaccines:

- Gender differences were noted in the protection against disease.
- Individuals who were seropositive for HSV-1 were afforded no additional protection against HSV-2 by the vaccine.
- The greater effect of the vaccines on protecting against disease rather than infection suggested that it is easier to control than to prevent infection.

Table 7
Reduction of HSV shedding by aciclovir and valaciclovir as detected by PCR (Gupta, submitted)

	Placebo	Aciclovir	Valaciclovir
Total shedding	1336/3340 (40.0%)	271/3253 (8.5%)	248/3321 (7.5%)
Subclinical shedding	706/2630 (26.8%)	215/3151 (6.8%)	199/3214 (6.2%)

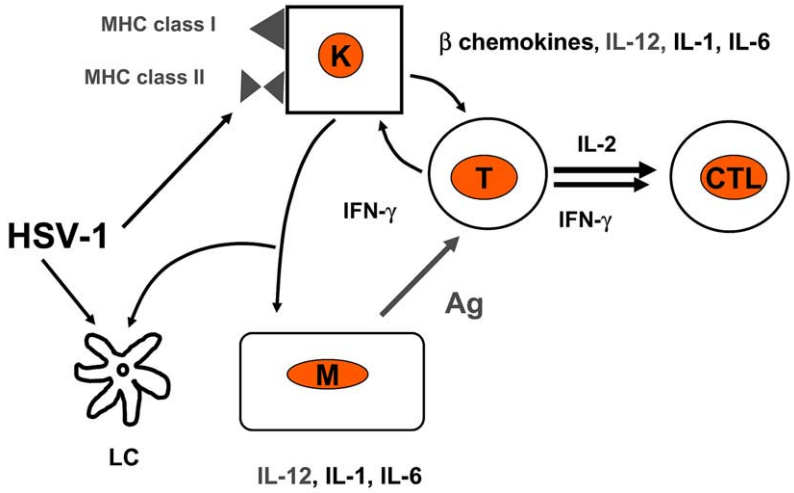

Fig. 6. Immune responses during a recurrent episode of HSV. Reprinted with kind permission from Cunningham et al. (2004).

- High levels of neutralizing antibodies did not guarantee protection against infection.
- Different adjuvants may produce different cytokine responses and may be important in influencing vaccine efficacy (Stanberry et al., 2002).

Knowledge of the immunology of HSV infection provides important insights into vaccine action. For example, sterilizing versus controlling immunity may be natural or vaccine-induced; this is also a big question in HIV infection. A potential hierarchy of effects can be considered—to prevent infection of keratinocytes, eliminate all infected keratinocytes, control keratinocyte infection and prevent or reduce infection of axon termini (Milligan et al., 1998; Stanberry et al., 2000). Can prophylactic or therapeutic vaccines, or the development of natural resistance, convert HSV-2 disease to asymptomatic infection, with or without virus shedding? Innate immune responses that could be involved in the prevention of infection include interferons, collectins, complement and macrophages; antibodies and cytokines could be involved in adaptive processes. Responses able to eliminate infections include cytokines, natural killer cells, and cytotoxic T cells (Schultz et al., 1999; Cerny and Chisari, 1999). All of these immune responses are likely to play a role in the elimination of HSV.

There is a difference between immunization and natural immunity. Viable HSV can down-regulate MHC class I on the surface of keratinocytes, which can be restored by interferon gamma. Epithelial dendritic cells are killed by HSV. Although they are probably taken up by bystander dendritic cells which carry HSV antigens back to the lymph node to stimulate the immune response, this process results in immunodelay, providing the virus with sufficient time to replicate (Fig. 6) (Jones et al., 2003). Therefore, immunization has the advantage of bypassing this pathway of natural events.

Reports of elimination of other viral infections (such as HIV) have implications for therapy of HSV infections. In particular, they suggest that transmission does not always occur when there is viral shedding. As shedding occurs episodically, the timing of sex in relation to shedding has to coincide for transmission to occur. That only a specific reduction in shedding may be needed to prevent transmission implies that infection thresholds for mucosal immune defenses exist. It may not be necessary to completely eliminate shedding, providing viral loads can be kept below the threshold. It would be useful to be able to define the threshold; however, this is likely to be variable due to specific factors, including the host immune status, genetic variation, gender, hormonal variations, status of the stratum corneum, and other coexistent infections.

6. Conclusions

- HSV is shed asymptomatically from multiple anatomical sites.
- Symptomatic and asymptomatic HSV-2 shedding from the genital and anal region is common and more frequent than with HSV-1 infection of the genital tract.
- HSV-2 shedding is affected by the site and time since acquisition of infection.
- Sensitive PCR techniques have shown that the magnitude and frequency of viral shedding is higher than shown previously with viral culture techniques.
- Suppressive (daily) antiviral therapy reduces clinical and subclinical reactivation rates.

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