

Recommendations from the
IHMF Management Strategies Workshop
and 5th Annual Meeting

Editors: Professor PD Griffiths
Dr A Volpi

PROGRESS WITH DIAGNOSTIC TESTS AND VACCINES FOR ALPHA- HERPESVIRUSES



IHMF
International
Herpes Management
— Forum —

Participants

CO-CHAIRS:

- Professor PD Griffiths** Professor of Virology, Department of Virology,
The Royal Free Hospital, Pond Street, London, NW3 2QG, UK
- Dr A Volpi** Researcher, Department of Public Health,
University of Rome 'Tor Vergata', Via di Tor Vergata 135, 00133 Roma, Italy

SPEAKERS:

- Dr DWG Brown** Virus Reference Laboratory, Central Public Health Laboratory, 61 Colindale Avenue, London,
NW9 5HT, UK
- Dr R Ashley** University of Washington, c/o Children's Hospital, CH-82, Virology, Room G-815,
4800 Sand Point Way N.E., Seattle, WA 98105, USA
- Dr R Patel** Department of GU Medicine, Royal South Hampshire Hospital, Brintons Terrace, Southampton,
Hampshire, SO9 4PE, UK
- Dr J Breuer** Consultant Virologist, Department of Microbiology, Royal London NHS Trust,
Whitechapel, London, E1, UK
- Dr L Stanberry** Professor of Pediatrics, Division of Infectious Diseases, Children's Hospital Research Foundation,
3333 Burnet Avenue, Cincinnati, OH 45229-3039, USA
- Dr SE Straus** Chief, Laboratory of Clinical Investigation, Division of Intramural Research,
National Institute of Allergy & Infectious Diseases, National Institute of Health (NIH), Building 10,
Room 11N-228, Bethesda, MA 20892, USA
- Dr L Corey** Fred Hutchinson Cancer Research Center, Program in Infectious Diseases, 1124 Columbia Street,
Seattle, WA 98104-2092, USA

DISCUSSANTS:

- Professor RJ Whitley** Professor of Pediatrics, Microbiology & Medicine,
The University of Alabama at Birmingham, Suite 616 Children's Hospital, 1600 7th Avenue South,
Birmingham, AL 35233-0011, USA
- Dr S Kroon** Consultant Physician in Dermatology and Venereology,
Pederstrup Vej 40, DK-5210 Odense NV, Denmark
- Dr S Barton** Clinical Director, HIV/Genitourinary Medicine,
St Stephen's Centre, Chelsea and Westminster Hospital, 369 Fulham Road, London, SW10 9NH, UK
- Dr A Simmons** Head, Herpes Research Infectious Diseases Laboratories, Institute of Medical & Veterinary Science,
Box 14 Rundle Mall PO, Frome Road, Adelaide SA 5000, Australia
- Professor E Petersen** Universitäts-Frauenklinik Freiburg, Hugstetter Strasse 55, 79106 Freiburg, Germany

The above were participants in the *Management Strategies* Workshop.

The contribution of the participants at the *5th Annual Meeting of the IHMF* is also acknowledged.

The *International Herpes Management Forum* (IHMF) was established to improve the awareness, understanding, counselling and management of infections caused by herpesviruses. Steered by an IHMF Board of Professor Richard Whitley, Dr Martin Wood, Dr Larry Corey, Professor Paul Griffiths, Dr Susanne Kroon and Dr Antonio Volpi, the IHMF involves international opinion leaders in all aspects of medical management of herpesvirus infections.

The tenth IHMF workshop was held on 1–2 March 1997 to discuss progress with diagnosis and vaccines for alpha-herpesvirus infections. Diagnosis has been the topic of a previous IHMF workshop and a *Management Strategies in Herpes* publication. However, the wealth and breadth of research in these areas has led to significant advances in both diagnosis of herpesvirus infections, and in the development of potential vaccines. The aim of the tenth IHMF workshop was therefore to review the current data and to develop guidelines in areas where they did not exist previously.

Summary	5
Chapter 1 Diagnosis of Herpes Simplex Virus Infections and Varicella Zoster Virus Infections	6
◆ The Importance of Testing	6
◆ Diagnosis of HSV Infections	6
◆ Tests for Diagnosis of HSV	6
◆ Direct Detection of HSV	7
◆ Diagnosis of VZV Infections	10
Chapter 2 The Serological Diagnosis of Genital Herpes Simplex Virus Infections	12
◆ Introduction	12
◆ What is Serological Testing?	12
◆ The Use of Serological Screening in Pregnancy	15
◆ Types of Serological Tests	17
Chapter 3 Varicella Zoster Virus Vaccines	21
◆ Introduction	21
◆ Development and Production of Oka Strain VZV Vaccine	21
◆ Differences Between the Oka Strain Vaccine and Wild-Type Varicella Zoster Virus	22
◆ Clinical Studies with Oka Strain Varicella Vaccine	23
◆ Immune Responses to Varicella Vaccine	25
◆ Booster Doses	28
◆ Dosage of Vaccine	28
◆ Incidence of Herpes Zoster after Vaccination	30
◆ Oka Strain Varicella Vaccine in Combination with Measles-Mumps-Rubella Vaccine	31
◆ Strategies for Vaccination	32
◆ Economic Analyses of Vaccination	34
◆ Benefits and Disadvantages of Vaccination Strategies	35
◆ Vaccination to Reduce the Incidence of Herpes Zoster	36

Chapter 4	Immunotherapeutic Vaccines for Herpes Simplex Virus Disease	38
◆	Introduction	38
◆	Approaches to the Immunotherapy of HSV	38
◆	Glycoprotein Vaccines	41
Chapter 5	Current Status of Vaccines for the Prophylaxis of Herpes Simplex Virus Disease	47
◆	Introduction	47
◆	Immunology of HSV Infection	47
◆	Requirements of a Prophylactic HSV Vaccine	49
◆	Support for the Concept of a Prophylactic HSV Vaccine	50
◆	Potential HSV Vaccines	51
Chapter 6	Conclusions	58
◆	Diagnosis of Genital HSV Infection	58
◆	Diagnosis of HSV Encephalitis	59
◆	Diagnosis of Varicella Zoster Virus Infection	59
◆	Diagnosis of Ocular HSV Infections	59
◆	Diagnosis of Orolabial HSV Infections	60
◆	The Use of Diagnostic Tests and Serodiagnosis for Genital HSV Infections in Primary Health-Care Settings	60
◆	What are the Advantages of Improving Diagnosis?	62
◆	Vaccines	63
	References	64
	Acknowledgements	67

- ◆ For cases of suspected herpes simplex virus (HSV) encephalitis, it is strongly recommended that the physician should obtain a sample of cerebrospinal fluid (CSF) for polymerase chain reaction (PCR) analysis as quickly as possible, and treat the patient empirically with aciclovir until the diagnosis becomes clear.
- ◆ Genital herpes is a sexually transmissible infection. Infection with HSV is much more common than disease. Of infected individuals, 80% will either have no symptoms or have unrecognizable disease. However, clinically apparent disease can be managed. Atypical presentations are very common.
- ◆ On first clinical presentation, about 50% of patients with HSV infection appear to be misdiagnosed in primary healthcare settings as suffering from *Candida* or other causes of vulvitis.
- ◆ Any patient presenting with suspected genital HSV infection should have a swab taken including those with classic symptoms. Thus, every patient with genital herpes should receive a laboratory-confirmed diagnosis at least once.
- ◆ In people who present with initial genital herpes, every effort should be made to have the virus typed because it gives important prognostic information.
- ◆ Serial swabs may be useful for monitoring an immunocompromised patient.
- ◆ If an immunocompromised patient presents with disseminated vesicular lesions, which the clinician suspects are due to varicella zoster virus (VZV) infection, the diagnosis should be confirmed by taking a smear of cells and using immunofluorescent staining with antibodies against VZV.
- ◆ Clinical diagnosis of a typical case of herpes zoster in the elderly immunocompetent individual is usually sufficient. However, if the patient has disseminated lesions or atypical presentation, then testing by smear and staining may be helpful.
- ◆ It is recommended that a patient who presents with suspected ocular HSV should have a swab taken for culture, although a negative result does not exclude the diagnosis.
- ◆ For labial HSV infections and primary gingivostomatitis, a clinical diagnosis is often sufficient, although a swab for HSV culture may be helpful, to diagnose atypical or difficult cases of gingivostomatitis or recurrent orolabial lesions.
- ◆ Classic cases of labial HSV should be confirmed by culture only in immunocompromised patients.
- ◆ From a public health standpoint, genital ulcerative disease is associated with increased risk for acquisition of HIV. For high-risk individuals, testing for HSV type 2 (HSV-2) infection could help prevent acquisition of HIV.
- ◆ Serological tests for HSV-2 can be valuable, particularly in selected patient settings such as pregnant women. Newer tests will be approved in the near future.
- ◆ Serological testing may also be useful in the patient who has chronic recurrent genitourinary symptoms for which there is no apparent aetiology, and also where there is a concern about transmission in discordant couples.
- ◆ Vaccines for HSV are under investigation. While they offer great potential for the individual, in public health terms nothing will be ready for use in clinical practice in the near future.

Diagnosis of Herpes Simplex Virus and Varicella Zoster Virus Infections

The Importance of Testing

The need for accurate and rapid diagnosis

Why do we need diagnostic tests for herpes simplex virus (HSV) infection? The guiding principle for use of a test should be whether it will improve the management of the patient. Clinical diagnosis of both varicella zoster virus (VZV) and HSV infections is accurate and sufficient in many cases. However, there are situations where diagnosis is difficult, for example, if lesions are atypical, or if the lesions have healed before the patient consults a physician; also in the immunocompromised host, where herpetic lesions may resemble other infections such as *Candida*. In such cases there is a need for laboratory confirmation. Some individuals may have subclinical asymptomatic infection where laboratory tests are the only way to confirm infection. In some life-threatening conditions, such as herpes encephalitis and herpesvirus infections in immunocompromised individuals or neonates, confirmation of the diagnosis with accurate and rapid tests is essential. Two articles (Users' Guides to the Medical Literature, *JAMA*, 1994) may help physicians to assess the value of different laboratory tests.^{1,2}

The natural history of genital HSV type 1 and type 2 (HSV-1 and -2) varies. HSV-2 infections recur more frequently than HSV-1 infections;³ this observation has implications for prognosis and patient management – an important part of the management of genital herpes is to inform patients of the likely prognosis, and to counsel them appropriately. Therefore, accurate diagnosis of HSV type is important, although it may not be routinely performed because of lack of availability of the test(s).

Diagnosis of HSV Infections

HSV-1 and -2 and VZV are alpha-herpesviruses. This sub-family is characterized by having a wide range of susceptible cells *in vitro*, a relatively fast replicative cycle, cytopathic effects, and establishing latent infection in sensory ganglia. Following primary infection, the virus reactivates from time-to-time and can result in clinical illness. The majority of primary infections with HSV-1 and -2 are subclinical, but infection can result in a wide spectrum of disease ranging from the mild (e.g. herpes labialis) to the severe (e.g. herpes encephalitis), see Table 1.

The diagnosis of the mucocutaneous manifestations of HSV is mainly clinical, but laboratory investigations contribute to effective patient management in several clinical situations.

Tests for Diagnosis of HSV

There are several ways to confirm a diagnosis of a viral infection within a laboratory:

- ◆ Direct cytological examination. Cells from lesions can be examined using histology and microscopy which reveal characteristic cell changes due to infection. The sensitivity and specificity of this approach is limited. This method is not widely used because it is technically demanding and has been superseded by methods to detect virus

<p>◆ Mucocutaneous infections</p>	<p>Oropharyngeal infections Genital herpes Herpes gladiatorum Herpetic whitlow</p>
<p>◆ Central nervous system disease (CNS)</p>	<p>Encephalitis Myelitis Meningitis</p>
<p>◆ Other manifestations</p>	<p>Atypical distribution autoinoculation Erythema multiforme Herpes keratitis</p>
<p>◆ Neonatal infection</p>	<p>Skin, eye and mouth infections (SEM) Encephalitis (CNS involvement) with or without SEM Disseminated infection with or without SEM infection</p>
<p>◆ Immunocompromised</p>	<p>Oesophagitis Pneumonitis</p>

Table 1: The spectrum of diseases caused by HSV

- ◆ Detection of virus, viral products or virus-induced cell changes:
 - culture from the lesion for live virus
 - detection of viral antigens:
 - on fluids using enzyme immunosorbent assays (EIA)
 - on smears or tissues (using monoclonal antibodies)⁴
 - detection of viral nucleic acids (polymerase chain reaction [PCR]) may be available at some centres
 - detection of virus particles by electron microscopy
- ◆ Detection of an immune response by identifying HSV-1 or HSV-2 specific antibodies (serological tests), by detecting specific antibodies to glycoprotein G-1 or -2.

Deciding on an appropriate test

There are a number of issues that can affect which test a physician orders from the laboratory:

- ◆ Value of the test for patient management
- ◆ Is the test reproducible? For example, the Western blot technique for detection of HSV antibodies has not so far proved easy to set up in many laboratories
- ◆ Cost. Immunoassays are significantly cheaper than PCR tests, and there are no commercially available PCR tests for alpha-herpesvirus infections
- ◆ Laboratory management issues, i.e. turnaround time and quality control.

Direct Detection of HSV

The characteristics of the four different approaches commonly used for direct detection of HSV are shown in Table 2.⁵⁻⁸

	Test			
	Virus culture	Antigen detection (immuno-fluorescence on smears)	Antigen detection (EIA)	Nucleic acid detection (PCR)
◆ Source	Swab/scraping	Smear/tissue section	Swab/scraping	Swab/scraping
◆ Sensitivity	High; >90% from lesions	>80%	80%	Highest
◆ Specificity	High (virtually 100%)	High	High	Controls for cross-contamination important
◆ Advantages	Allows virus typing and antiviral sensitivity	Inexpensive	Cost and speed	Allows virus typing and has high sensitivity
◆ Disadvantages	Sample transport, labour intensive, expensive	Not 100% sensitive, requires trained individuals	Not 100% sensitive	No commercial assay available, expensive

Table 2: Laboratory tests for the direct detection of HSV (adapted from references 5–8)^{5–8}

Virus culture

Virus culture has been the mainstay of HSV detection, and has the advantages that it allows the virus to be typed, and provides an isolate that can be used for testing antiviral sensitivity. There are a number of important technical issues which affect virus isolation; one of them is the requirement to collect an appropriate sample and actually inoculate it into the cell culture promptly. Correct transportation of the sample is therefore important. Virus culture is an expensive technique because it requires skilled laboratory staff to run the test.

Key factors that determine success for HSV isolation include the type of swab transport media and the importance of getting specimens to the laboratory without great delay.

Antigen detection

The antigen detection tests are simple and rapid to perform. There are two types of antigen detection method, use of smears and staining or enzyme immunoassays. Several enzyme immunoassays are commercially available, including IDEIA and Wellcozyme. However, there are variable reports in the literature on the sensitivity of such tests, which may depend on the type of assay and on the range of specimens. In general, the sensitivity of antigen detection is high for typical genital or ocular HSV lesions. It is more sensitive than culture for late samples, but less sensitive than culture for cervical and urethral swabs.^{5–8} Some or many of these assays cannot distinguish between HSV-1 and HSV-2.

PCR

PCR is the most sensitive technique for detecting HSV. Contamination can be an issue due to the high sensitivity of the technique, and the use of appropriate controls is essential to ensure that results are accurate. PCR can also be used for typing virus but is a research tool. There is no commercial assay available at the moment and it is potentially expensive.

Sensitivity of PCR analysis depends on the type of clinical sample. PCR assays have been described that can detect one virus genome reliably in 100 µl of CSF.⁹ Extraction and storage of CSF specimens are important issues, as is the need to detect both virus types.

Appropriate tests for HSV infections

A summary of available tests and their uses is shown in Table 3. While many mucocutaneous HSV infections do not require laboratory confirmation, with genital infection there is a need to counsel the patient and give specific advice about HSV-1 and -2, because the natural history and prognosis is different.³ For this reason, the standard test is still virus culture preferably with virus typing. In many clinics, only immunoassays are available. These can be used successfully in a large number of cases

Mucocutaneous infections	
Oropharyngeal	virus culture
Genital	virus culture
	EIA
Immunocompromised host	
	virus culture antiviral sensitivity
Other manifestations	
Herpetic whitlow	virus culture
Herpes keratitis	EIA or virus culture
Encephalitis	PCR (CSF)

Table 3: Appropriate tests for HSV infections according to different clinical presentations

to confirm the diagnosis, but do not provide information on virus type. While there may be a future role for PCR in the diagnosis of genital herpes (for example, for detection of subclinical virus shedding), it is not routinely used at the moment.

In the immunocompromised patient, culture of virus has been the standard approach used for all presentations of HSV, and is important for detecting and monitoring virus sensitivity to antiviral therapy in this patient group.

Laboratory tests also have a role in the management of HSV in pregnancy. This is a complex area and readers are referred to the IHMF *Management Strategies* monograph, *Can We Improve the Management of Perinatal Herpes Simplex Virus Infections?* for a wide-ranging discussion.¹² Although the rate of HSV infection in the neonate is low, serological confirmation of HSV status has a potential role in preventing transmission of the virus to the neonate. When pregnant women first present to the clinic, one of the issues that should be addressed is whether they or their partners have evidence of genital herpes. Serology may be the most direct way to answer that question with seronegative women/seropositive partners forming a high-risk group.

If the woman is infected with HSV-2, and has clinically obvious lesions during delivery, then Caesarian section is one option that can be considered. For babies born to mothers with genital HSV infections, culturing for virus in a number of potential sites in the baby should be considered.

Detection of HSV antibody in saliva

The use of saliva as an alternative to blood for detecting antibody is well accepted for epidemiological surveys but not for diagnosis in an individual patient, because reliable methods for demonstrating that the saliva specimen is of an adequate quality (i.e. it contains suitable levels of IgG) have not yet been developed. A suitably validated saliva test for HSV antibody has a potentially important place in identifying

subclinically infected individuals and may have a useful role in targeting future interventions (such as vaccines).

Diagnosis of HSV encephalitis

The diagnostic laboratory has a major role in the investigation and management of herpes encephalitis. The detection of viral genome by PCR in cerebrospinal fluid (CSF) is clearly established as the primary diagnostic approach in cases of suspected herpes encephalitis¹⁰ when prompt aciclovir therapy may improve the management of the patient.

Prior to the development of PCR, the detection of intrathecal antibody was used – that is, local production within the CSF of HSV-specific antibody. This technique is not reliable in the early stages of infection and cannot provide a diagnosis early enough to be used to make treatment judgements. The sensitivity of PCR and intrathecal antibody detection by day after the onset of encephalitis is shown in Table 4 and Figure 1. This demonstrates that PCR is reliable and highly sensitive within the first week after onset, and that it also has an advantage over the older techniques of antibody production detection. Likewise brain biopsy, coupled with culture for HSV, which was used in the early studies, has now been superseded by PCR.^{10,11}

Day after onset	Sensitivity (%)	
	PCR	Antibody
0–3	100	0
4–6	92	44
7–9	90	66
10–12	50	88
13–14	62.5	100
15–30	8	100

Table 4: Diagnosis of HSV encephalitis by PCR and intrathecal antibody detection from 42 patients¹⁰

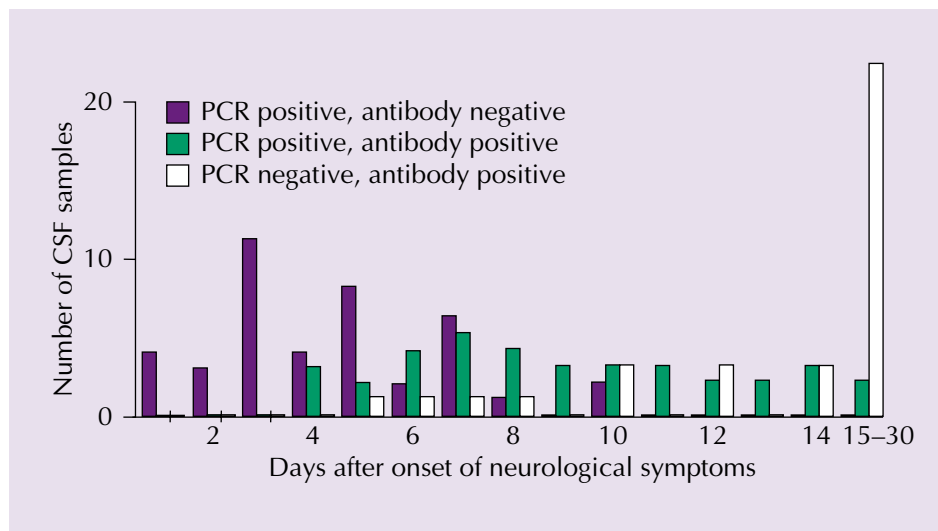


Figure 1: PCR detection of HSV in CSF from 42 patients with herpes encephalitis¹¹

Diagnosis of VZV Infections

Like HSV, VZV is an alpha-herpesvirus, which establishes a latent infection following primary infection. Reactivation occurs with increasing frequency in the immunocompromised and with increasing age. Infection results in a wide spectrum of clinical manifestations. Primary infection can cause varicella (chickenpox in the fetus, neonate, child and adult). Reactivation of VZV can cause herpes zoster (shingles) and may lead to post-herpetic neuralgia, motor weakness (including Ramsay-Hunt syndrome), and ophthalmic zoster.

Laboratory diagnosis of VZV contributes to patient management in several clinical settings such as for diagnosis in the immunocompromised individual. A range of diagnostic assays are available for detecting virus directly or detecting anti-VZV antibodies. A summary of the important features of the available methods for virus detection is shown in Table 5.

Test	Cytology	Electron microscopy	Culture	Antigen detection	PCR
Specimen	Vesicular fluid and cells	Vesicular fluid and cells	Vesicular fluid and cells	Vesicular fluid and cells	Vesicular fluid. Throat swab. Whole blood
Advantages	Rapid	Rapid		Rapid	Rapid. Sensitive
Disadvantages	Does not distinguish HSV/VZV. Low sensitivity	Does not distinguish HSV/VZV. Expensive	Technically demanding. Slow. Expensive		No commercial test available. Expensive

Table 5: Appropriate tests for detection of VZV

Virus culture of VZV is more technically demanding than that of HSV. Virus isolation is slow, and specimens must be transported to the laboratory rapidly. It is important to sample cells as well as vesicular fluid when attempting virus isolation. PCR tests have been described and shown to be more sensitive than culture. PCR is sufficiently sensitive to detect virus in a range of other specimens, but there are no commercial tests currently available.

VZV IgM is a reliable marker of recent infection and can also be detected during virus reactivation. It is not always detectable during the first week of varicella.¹² VZV IgG antibody tests are widely used to target herpes zoster immunoglobulin in high-risk patient groups, and a large range of commercial tests are available. Testing can also be useful for prophylaxis if the patient is susceptible, for example, in recommending the administration of zoster immune globulin to seronegative pregnant women exposed to varicella.

The Serological Diagnosis of Genital Herpes Simplex Virus Infections

Introduction

Genital herpes simplex virus (HSV) infections are common and increasing in prevalence worldwide. The clinical symptoms of genital HSV infections are often ignored or mistaken and patient history is diagnostically non-specific. Many infected individuals are without symptoms – they are asymptomatic most of the time. This broad range of clinical presentation, the high prevalence of atypical lesions, and the limited availability of accurate type-specific serological tests, explain why genital HSV infections are so under-diagnosed.

What is Serological Testing?

For patients with classic symptoms of genital herpes, including lesions from which swabs can be obtained, culture is recommended. However, some patients present with atypical lesions or present too late to detect virus. In such patients, serology may be useful, because it does not rely on the patient having developed antibodies (seroconversion).

Serological assays detect antibodies to HSV in a blood sample; thus, they indicate evidence of past infection. Assays include classic HSV neutralization as well as the newer, semi-automated tests such as enzyme immunoassays (EIA) and immunofluorescence assays (IFA). Serological tests to identify HSV type-specific antibodies are not widely available, and the commercially available EIA and IFA tests have limitations in diagnosis. No serological test can tell who gave what to whom or when.

Commercially available tests do not accurately distinguish between HSV type 1 and 2 (HSV-1 and HSV-2) antibodies. Three different commercial serological tests have been compared, using culture isolation of HSV as the standard (Table 1).¹ In primary episodes of genital HSV, these tests had varying ability to correctly diagnose the virus type and differed in their ability to detect virus type within the first 21 days. Cases of non-primary first episode herpes, that is, people who have pre-existing HSV-1 antibodies and who become infected with HSV-2, present a more difficult diagnostic situation because of the extensive cross-reactivity of the two viruses. With two commercial assays, the results were inaccurate on all samples; there was a rise in HSV-1 titre, without enough HSV-2 positivity to register in the particular algorithm used in the test. As most people have HSV-1 antibodies, these tests have no real use at the moment, except to distinguish the completely seronegative individual from the individual who has antibody to either HSV-1 or -2. They cannot distinguish between HSV-1 and -2, and should not be used that way. Moreover, commercial tests were as much as 80% inaccurate for subjects who were infected with only HSV-1, giving false positives for HSV-2.

Should physicians diagnose subclinical herpes if the patient is not troubled by the infection? Mertz and colleagues found that most people who transmit HSV have subclinical or unrecognized disease.^{2,3}

Clinical category	n	Commercial kit		
		A	B	C
Primary episode:	28			
Correct diagnosis		13 (46%)	18 (64%)	21 (75%)
False negative		12 (43%)	3 (11%)	1 (4%)
Positive, wrong type		3 (11%)	7 (25%)	6 (21%)
Non-primary, HSV-2:	12			
Correct diagnosis		0	0	1 (8%)
Incorrect diagnosis		12 (100%)	12 (100%)	11 (92%)
Recurrent HSV-2:	45			
Correct		28 (62%)	44 (98%)	33 (73%)
False negative		1 (36%)	0	0
Positive, wrong type		16 (36%)	1 (2%)	12 (27%)

Table 1: Comparison of three commercially available serology tests in symptomatic genital herpes (abstracted from reference 1)¹

Analysis of genital swabs by culture or by polymerase chain reaction (PCR) showed that women with lesions cultured daily in the first 6 months after a first episode may be PCR positive from the genital tract for as many as 75% of all days.⁴ Further studies have shown that the frequency of HSV shedding declines over time (Table 2).⁵ It is not yet known if the virus is always infectious, although it is a likely explanation for the observed rate of transmission.

Site	Time from end of primary episode		
	0–12 months	13–24 months	25–36 months
	← n/N (%) →		
Cervical site*	54/1749 (3.1)	6/464 (1.3)	3/226 (1.3)
Vulval site	36/1540 (2.3)	7/448 (1.6)	3/224 (1.3)
Days of shedding at any genital site*	78/1820 (4.3)	11/482 (2.3)	5/234 (2.1)

* $P \leq 0.005$; random effects model using time as a covariate. n/N: numbers of days with positive culture/total number of days sampled.

Table 2: HSV is shed more frequently in the first 12 months following acquisition⁵

Asymptomatic shedding is not necessarily lower in patients without a clinical history of infection. Figure 1 shows results from a study of volunteers with a history of clinically evident infection, compared with those who were diagnosed by serology only. The pattern of subclinical shedding is very similar between the two groups, the rate being only slightly increased in the case of those with symptoms.⁶

A number of studies show that if people are counselled about the clinical symptoms of genital herpes, about half begin to recognize their recurrences time.⁷ Figure 2 shows that 30% of subclinical shedding episodes occur as clusters.⁸ Having been taught to identify a clinically apparent lesion, or a symptomatic episode, the patient can usually

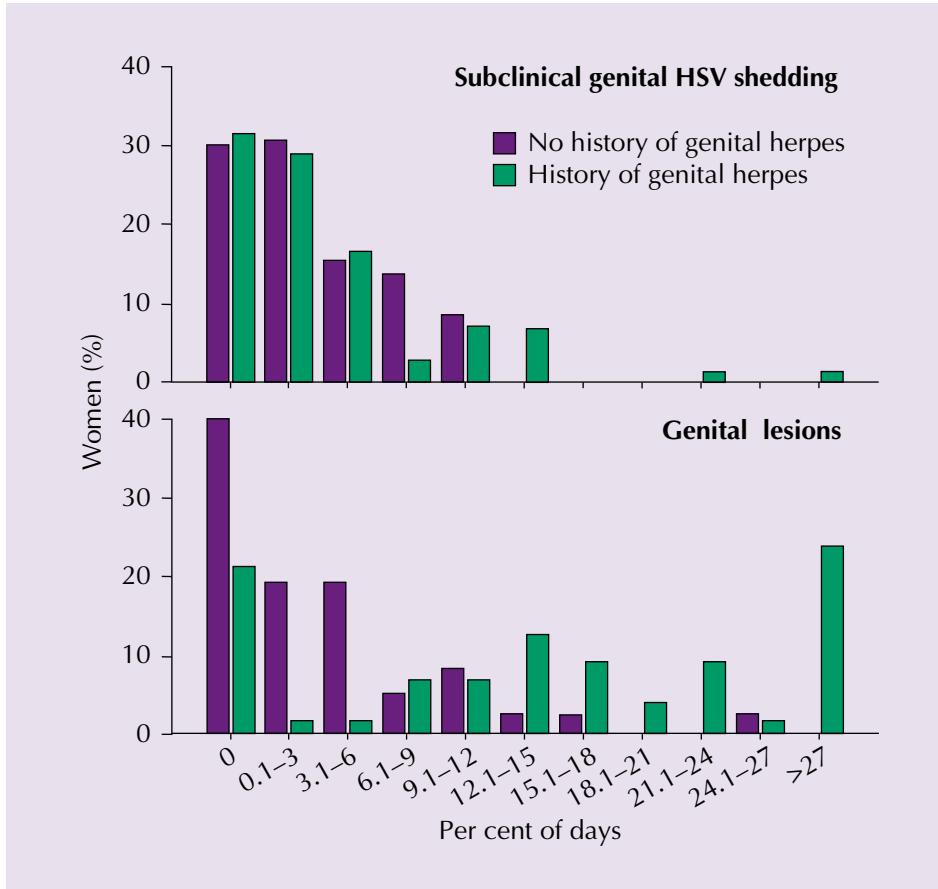


Figure 1: HSV shedding rate in women with and without a history of infection^a

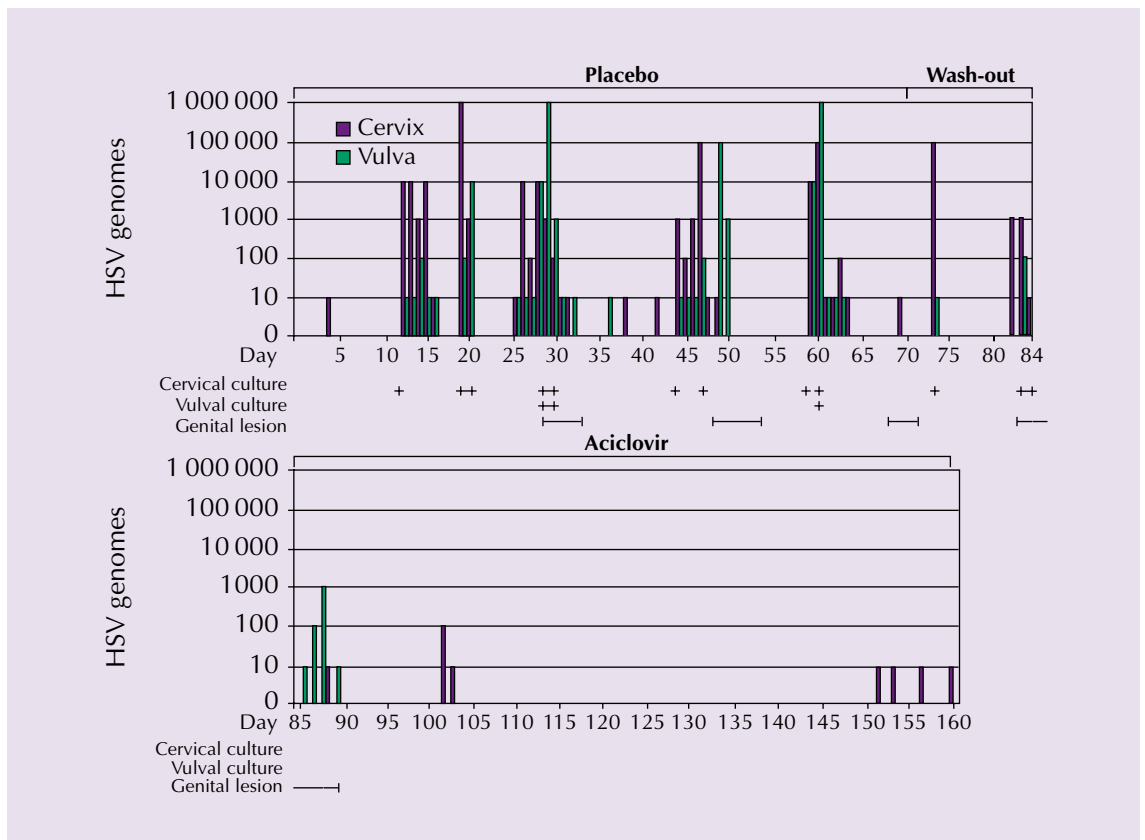


Figure 2: Subclinical HSV shedding occurs in clusters^a

identify a period of time when they are at risk for asymptomatic shedding. However, asymptomatic shedding also occurs beyond the window of symptoms.

Suppressive aciclovir therapy reduces the incidence of lesional episodes in people with genital herpes, the number of asymptomatic episodes and also the number of genomic copies of virus found by PCR found during these episodes.⁹ If they know they have genital herpes patients may be given a choice of behavioural changes, and/or consider antiviral therapy. Either course may reduce the risk of transmission.

The Use of Serological Screening in Pregnancy

Type-specific serologies could be particularly useful in a number of groups (Table 3), particularly pregnant women.

<p>◆ To identify subclinical HSV-2 carriers</p> <p>Candidates for behavioural intervention</p> <p>Candidates for antiviral therapy</p> <p>Potentially reduce transmission</p>
<p>◆ To identify pregnant women at risk</p> <p>Those who are uninfected but have serologically discordant partners</p> <p>Those already infected</p>
<p>◆ To identify asymptomatic individuals in other groups</p> <p>Semen donors</p> <p>Patients entering immunosuppressive therapy</p> <p>Patients with HIV infection</p>
<p>◆ To identify candidates for vaccines</p>

Table 3: Potential populations in whom HSV type-specific serology could be useful

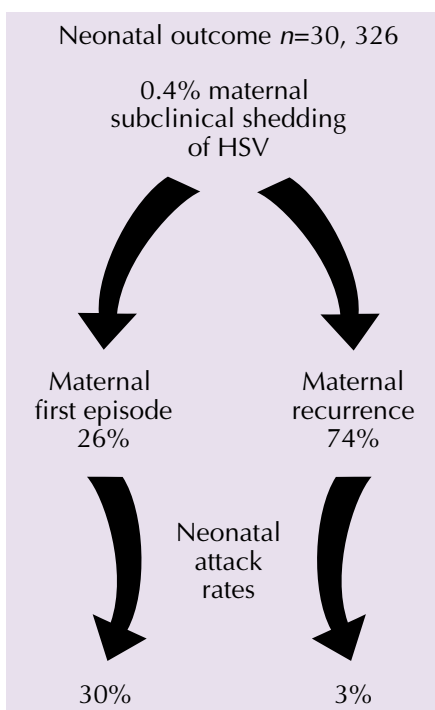


Figure 3: Exposure to genital HSV at birth

The issues with pregnancy are numerous. The neonatal infection rate from a mother who is asymptotically shedding HSV at term, as detected by virus culture, is higher than the rate from mothers with recurrence of genital HSV infection. The figures may vary, but the rate is about 10 times higher in the mother who has seroconverted, or developed genital HSV infection close to term, versus the mother who has had a more long-standing genital HSV infection (Figure 3).

How often do women seroconvert during pregnancy? In one study the adjusted rate was 2% (Table 4).¹⁰ In this study, a higher proportion of HSV seronegative mothers seroconverted to HSV-1 than to HSV-2 due to a higher exposure rate to HSV-1. No HSV-1 infections occurred in HSV-2-positive mothers.

The risk of seroconversion during pregnancy relates to the serostatus of the partners. The seronegative mother with a type 2-positive male partner has a markedly higher risk of acquiring HSV-2 than the other scenarios that were noted (Table 5).⁶

	Seroconversion	Adjusted rate for entire pregnancy
● HSV-	⇒ Any positive	3.7%
● HSV-	⇒ HSV-1+	2.3%
● HSV-	⇒ HSV-2+	1.4%
● HSV-1+	⇒ HSV-1+ & HSV-2+	1.7%
● HSV-2+	⇒ HSV-1+ & HSV-2+	0
● Overall seroconversion		2.1%

Table 4: HSV seroconversion prior to the onset of labour among 7046 HSV-susceptible women (n=94)¹⁰

Seroconversion event	Number of couples with the woman at risk	Number of maternal seroconversions
Negative ⇒ HSV-2+	6	2 (33%)
Negative ⇒ HSV-1+	50	2 (4%)
HSV-1+ ⇒ HSV-1&2+	19	1 (5%)
HSV-2+ ⇒ HSV-1&2+	38	0

Table 5: Risk of seroconversion in pregnancy⁶

If people are made aware of genital herpes – if physicians are trained to conduct a very detailed history, with a motivated subject during pregnancy – it seems logical to hope that there will be an improved diagnosis. However, a recent study by Brown *et al* (1995)¹¹ showed that detailed interviews with pregnant women who denied that they had genital herpes is poorly predictive of HSV-2 serostatus (Table 6). Twenty-eight per cent of patients with no symptoms suggestive of genital herpes still had HSV-2 antibodies; conversely, half of those thought by a trained interviewer to be highly likely to have genital herpes were seronegative for HSV-2.

Symptoms of genital herpes	Number in group	HSV-2 seropositive
None	109	31 (28%)
Somewhat suggestive	50	15 (30%)
Definitely suggestive	18	9 (50%)

Table 6: Detailed interview of pregnant women denying genital herpes is poorly predictive of HSV-2 serostatus¹¹

This study suggests that clinical presentation and even careful history do not adequately identify genital herpes, or the risk of acquiring it. Identifying pregnant women who are not infected with HSV but have a seropositive partner would be useful for the physician; the infected partner could be more closely monitored for lesions at term, and the physician would have a higher index of suspicion for neonatal HSV and any problems with the infant.

Formal studies are required to determine whether providing information would lead to behavioural changes. Such studies could involve the counselling of partners; for example, counselling seronegative women and their seropositive partners about the risks of

transmission of HSV to the neonate at term. Other groups that may benefit from screening include people who may be immunocompromised or HIV-positive individuals.

Types of Serological Tests

There are two ways to type antibodies to HSV, Western blot and glycoprotein G (gG)-specific tests.

The Western blot

The Western blot separates denatured proteins in the sample by molecular weight on a polyacrylamide gel. The proteins are then transferred to a nitrocellulose filter and incubated with the patient's serum antibodies. The bound antibodies are then themselves detected with a second antibody linked to an enzyme which catalyses a visible colour change.

Figure 4 shows a Western blot of three sera from individuals with HSV-1 infection, HSV-2 infection and both types of infections, respectively. The gG band, which is marked in the HSV-2 lane, is visible in profiles from sera with HSV-2 antibodies but is absent from the HSV-1 sample. This difference helps distinguish the antibodies to HSV-1 from those to HSV-2.

The HSV antibody type(s) on Western blots are reasonably easy to read in around three-quarters of cases. The other 25% of sera require pre-absorption and repeat testing to clarify the type-specific antibody profiles.

Figure 5 shows how seroconversion can be monitored by Western blot analysis.¹² Screening Western blots were performed on sera drawn 1, 3, 4 and 14 weeks after onset of symptoms due to genital HSV-2 infection. Sera were reacted with HSV-1 proteins (labelled "1" in the figure) or with HSV-2 proteins (labelled "2"). Detectable antibodies appear at Week 3 but the profile is not complete enough to be definitive for HSV-2. By Week 4, the profile is considerably more complex and includes the type-2 specific glycoprotein gG-2. The lack of antibody to ICP35 suggests recent infection. The Week 3

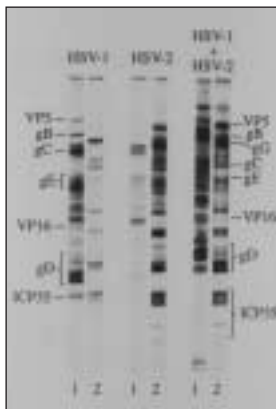


Figure 4: Western blot analysis of sera from an HSV-1 seropositive individual (left), an HSV-2 seropositive individual (centre) and an individual who is positive for both types (right)

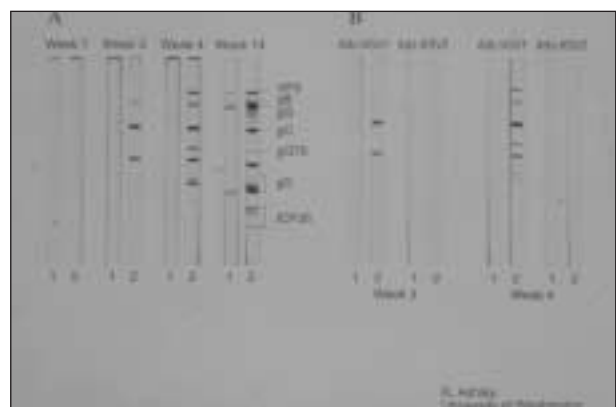


Figure 5: Seroconversion in an individual recently infected with HSV-2¹²

and 4 sera were pre-adsorbed against either HSV-1 ("Ads:HSV-1") or against HSV-2 ("Ads:HSV-2") proteins and rerun against fresh blots to confirm that HSV-2 antibodies were present. As shown, pre-adsorption against HSV-2 protein completely eliminates

reactive antibodies while pre-adsorption against HSV-1 proteins leaves antibodies which are capable of binding to HSV-2 blots (“type specific antibodies”). In isolation, the Week 3 profile would be considered positive for HSV but of indeterminate subtype. When the Week 3 and 4 profiles are read together, the progression of the profiles is diagnostic for seroconversion to HSV-2.

Some of the advantages of the Western blot technique are shown in Table 7. Western blot can detect antibodies to multiple proteins and can accurately distinguish HSV-2 and HSV-1 antibodies. Antibody profiles mature with time; while time of infection cannot be determined with this test, a fully developed Western blot profile in a patient describing symptoms for the first time indicates a first symptomatic recurrent episode. Western blot testing of over 200 subjects presenting to a Seattle clinic with *first episodes* showed that 20% of subjects had actually been infected for some time. This may affect counselling of patients and their current partners.

Type-specific assays using gG-1 and gG-2 glycoproteins

As shown in Figure 5 (Western blot with gG labelled figure), glycoprotein gG-2 is an important marker of infection with HSV-2. For this reason, investigators around the world have devised tests to detect antibodies to gG-2 and to the HSV-1 counterpart, gG-1. For the most part, these tests follow a format which binds gG-1 and gG-2 onto a solid surface in much the same way as the multiple proteins are bound onto nitrocellulose for Western blot. Serum is added and bound antibodies are detected by a second, enzyme-linked antibody directed against human immunoglobulin (enzyme-linked immunosorbent assay or ELISA).

Table 8 lists the chronology of the published methods that have been developed for detecting type-specific antibodies to HSV-1 or HSV-2. The names reflect different methods for binding the gG and, in some cases, different ways of detecting the patient’s bound antibody. The immunodot enzyme assay was one of the first to be published, and is still in use on a research basis in the USA. In Australia, an indirect gG2 ELISA is used.¹⁸ A

Multiple type-specific markers for HSV-1 and HSV-2

- Allows accurate antibody typing
- Allows recognition of HSV-2 in HSV-1-positive patients
- Allows recognition of HSV-2 in patients who are seronegative for gG-2

Antibody profiles mature with time

- Can differentiate first symptomatic recurrent episodes from true primary infection

Table 7: Advantages of HSV Western blot

Name	Abbreviation	Reference
Immunodot enzyme assay	gG-IEA	Lee <i>et al</i> , 1985 ¹³ ; Lee <i>et al</i> , 1986 ¹⁴
Western blot	WBA	Ashley <i>et al</i> , 1988 ¹⁵ ; Ashley <i>et al</i> , 1991 ¹
Immunoblot gG assay	gG-blot	Sanchez-Martinez <i>et al</i> , 1991a ¹⁶ ; Sanchez-Martinez & Pellet, 1996 ¹⁷
Indirect gG-2 ELISA	gG-2 EIA	Ho <i>et al</i> , 1993 ¹⁸
Monoclonal antibody blocking RIA	MAb-RIA	Slomka <i>et al</i> , 1995 ¹⁹
Chiron RIBA™ Strip Immunoblot Assay	RIBA™-SIA	Alexander <i>et al</i> , 1996 ²⁰ ; Ashley <i>et al</i> , 1997 ²¹
Gull gG-EIA	Gull EIA	Ashley <i>et al</i> , 1998 ²²

Table 8: Summary of type-specific tests developed for HSV antibodies

comparative study with Western blot has shown the accuracy of a monoclonal antibody radioimmunoassay (MAB-RIA) used in the UK.¹⁹ Recently, an EIA version has been developed. More recently, the RIBA™ strip immunodot assay, or RIBA™-SIA, has been developed, which appears to be accurate. However, the company does not plan to market this test. An alternative EIA format is under development. The Gull gG EIA which is also considered accurate, has been submitted for FDA approval and is available in Europe.²¹

When the Gull assay was compared with Western blot, 93% of results were concordant. The discordance was mainly due to differences in sensitivity, rather than wrong typing. The assay performed well with HSV-1 antibody, and very well for HSV-2 antibody. Of the test results that were equivocal by EIA, Western blot could be used to type or obtain a definitive answer for most of them. In the future, it is likely that some laboratories will use EIA to gG1 and gG2, with Western blot or other tests on equivocal samples.

The Gull EIA is an easy, fast, automatable test and has the familiar format of a microwell EIA. The cost is likely to be reasonable. A new test from Diagnology (Belfast, Northern Ireland, UK) – POCkit-HSV-2 – is a rapid gG-2-based test that can be performed in clinical settings. Early studies show that it is very accurate for HSV-2 antibody detection. HSV-1 antibodies are not detected. Western blot has limited availability, but it is accurate and will retain a place for some reference uses. Other EIA formats may come on the market (Table 9).

Accurate, widely available type-specific serological tests	
HSV gG-ELISA	Gull Laboratories, Salt Lake City, Utah, USA*†
POCKit-HSV-2	Diagnology, Belfast, Northern Ireland, UK*†
HSV-1 and HSV-2 IgG differentiation immunoblot	Microbiology Reference Laboratory (MRL), Cypress, California, USA
Accurate, type-specific serological tests (more limited availability)	
Competitive EIA or RIA	Central Public Health Laboratory, London, England, UK
HSV Western Blot	Virology Laboratory, University of Washington, Seattle, Washington, USA
*Note: Gull and Diagnology supply test kits to laboratories but do not provide testing services. MRL, the Central Public Health Laboratory and the University of Washington test sera in their laboratories.	
†Not yet available in the USA pending clinical trials.	

Table 9: Current availability of validated HSV type-specific antibody tests

Other serological assays for HSV

While type-specific assays that can confirm recent or past HSV-2 infection are becoming more widely available, it is important to emphasize that currently available tests do not accurately distinguish between HSV-1 and HSV-2 antibodies (type-common or non-typing tests) as previously noted. These tests are based on multiple proteins from HSV-1 and HSV-2 preparations. The proteins are bound to a solid surface and the patient's serum antibodies are added. Secondary antibodies against human immunoglobulins are then added and, if linked to an enzyme, the presence of HSV-antibodies is detected by colour change (ELISA or enzyme immunoassay; EIA). If the secondary antibody is linked to a fluorescing molecule, the antibody is detected by fluorescence (IFA: immunofluorescent antibody). These tests can tell whether a person has been infected with HSV but because antibodies against one type of HSV can readily react with proteins of the other type, tests using *bulk* protein preparations cannot determine which type of HSV the antibodies are directed against. These *non-*

typing tests must be used with caution – if at all – since their value is limited to the diagnosis of true primary infection.

Benefits and drawbacks of serological testing for HSV-2

When considering the use of serological diagnosis, the physician should be aware of the benefits and drawbacks of current methodology (shown in Table 10).

Benefits	Drawbacks
<ul style="list-style-type: none"> ➤ Better diagnosis of symptomatic patients ➤ Improving perinatal management where neonatal herpes is prevalent ➤ Alerts patients to unrecognized infections ➤ Defines susceptibility to infection ➤ Can help to resolve concerns about relationships 	<ul style="list-style-type: none"> ➤ Specificity is not complete ➤ Poor predictive value in low prevalence populations ➤ Cannot diagnose genital HSV-1 infection ➤ Increased anxiety and concerns ➤ Cost of testing ➤ Cost and effort of counselling

Table 10: Benefits and drawbacks of serological testing for HSV-2

Suggested guidelines for ordering tests

Type-specific serological tests are expensive. Suggested guidelines for ordering of HSV serological tests are shown in Table 11. A symptomatic patient is ideally diagnosed by culture from lesions. If the patient is symptomatic but the culture has been negative or cannot be obtained, a serum pair (that is, two serum samples taken from the same patient some time apart) to look for seroconversion can be useful, particularly if typing is available. Another common situation is the asymptomatic person who may have recently been exposed to genital herpes. If this individual is within 8 weeks of exposure, genital herpes is difficult to diagnose, as many assays are most sensitive when the antibody profile has fully developed (usually after 6–8 weeks [Figure 5]). A typing test cannot correlate the infection to an exposure, but it can tell the patient whether they are HSV-seropositive or not.

Situation	Serological test(s)	Comments
➤ Symptomatic, virus positive	None	A virological test is always better
➤ Symptomatic, virus negative	Serum pair (non-typing) Serum pair (typing)	Misleading in HS Very helpful
➤ Asymptomatic, worried, >8 weeks post-exposure	Non-typing Typing	Helps only if seronegative Positive test cannot correlate infection to exposure event
➤ High risk, history negative	Non-typing	Helpful only if negative

Table 11: Suggested ordering guidelines for HSV serological tests

In summary, the use of accurate type-specific serological tests should increase recognition and possibly decrease transmission rates if they are used appropriately along with counselling. At least two FDA approved type-specific serological tests will be available in the near future (the RIBA™-SIA and the Gull gG EIA tests). The real challenge, when they become available, will be for the patient and clinician to ensure that the gG-based tests, not the inaccurate EIA tests now available, are being used.

Varicella Zoster Virus Vaccines

Introduction

Primary varicella zoster virus infection (VZV) is generally regarded as a mild, self-limiting disease in countries in which the majority of infections are in children. In otherwise healthy children, complications of varicella are rare, with less than two deaths per 100 000 cases in children aged from 1–14 years.¹ In contrast, varicella is more frequently associated with complications and death in adults. For example, clinically apparent varicella pneumonia occurs in one per 400 cases. An even more serious complication is varicella encephalitis, which has a 10% mortality rate.¹

Serious consequences of infection can also arise when varicella occurs during pregnancy. Maternal infection in the first 20 weeks of pregnancy causes a 2% risk of fetal abnormalities.² Congenital varicella syndrome is characterized by unilateral limb hypoplasia, skin scarring and neurological damage (Table 1).

Maternal infection later in pregnancy may result in neonatal varicella.³ This infection of the newborn can lead to rapid dissemination of infection and visceral involvement.

Another group at particular risk from varicella are immunocompromised patients with severe impairment of cell-mediated immunity. For example, in bone marrow transplant recipients, the mortality rate from varicella can approach 15%.⁴ The virus may be transmitted to these patients from health-care workers or from individuals who are in close contact.

The VZV vaccine has demonstrated clinical efficacy for the prevention of varicella in children and adults. It is, therefore, an option to reduce some of the burden of varicella. The two commercial varicella vaccines (produced by Merck and by SmithKline Beecham Biologicals) are both derived from the Oka strain of VZV.

Development and Production of Oka Strain VZV Vaccine

The Oka strain of the live attenuated varicella vaccine is a commercially available vaccine which originated from the varicella vesicular fluid of a 3-year-old boy whose family name was Oka. The virus was attenuated by inoculating it into human and guinea pig tissue cultures and then performing over 30 passages at 34°C and 37°C (see Figure 1). This process of culturing the virus in non-primate cells and at a low temperature attenuated the virus. It is this attenuated virus that is used in the commercially available varicella vaccines.

Embryopathy associated with varicella infection

◆ Skin	scarring
◆ Limb	hypoplasia of bone and muscle
◆ CNS	microencephaly, mental retardation, sphincter dysfunction
◆ Eye	cataract, chorioretinitis, microphthalmia

Table 1: Fetal abnormalities associated with varicella infection during pregnancy

Manufacture of Oka strain varicella vaccine

The manufacturing process for the varicella vaccine ensures that vaccine lots produced over a period of years have identical properties with regard to purity, safety, genetic stability and immunogenicity.⁵ Identical vaccine virus in each production lot can only be obtained by using standardized conditions for replicating the virus. Therefore, the growth medium, cell substrate used, incubation temperature and time, as well as the passage level of the vaccine strain is carefully controlled⁵ (Figure 1).

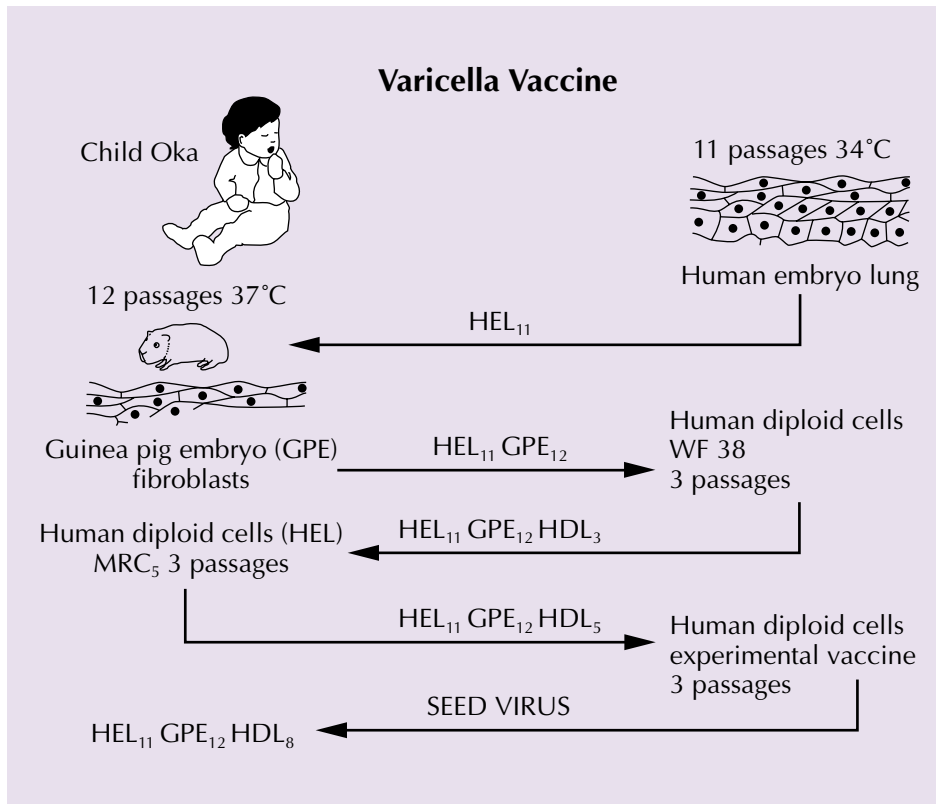


Figure 1: Manufacturing process for Oka strain VZV vaccine

There are three preparations of the vaccine available. Two preparations are stable at -20°C and should be stored for long periods at this temperature, but a recently developed preparation can be kept between 2°–8°C.

Differences between the Oka Strain Vaccine and Wild-Type Varicella Zoster Virus

It is important to distinguish the wild-type virus and the Oka strain of VZV when studying the efficacy and safety of VZV vaccination. The Oka strain has various attributes that allow it to be differentiated from wild-type virus. These are explained below.

Biological differences

The Oka strain varicella differs from wild-type VZV in two respects. The first difference is that the Oka strain grows less well in cell culture at 39°C as compared with wild-type VZV.⁶ The second difference is that the Oka strain has a greater affinity for guinea pig embryo fibroblasts. Other than these factors, it is not possible to identify any biological differences between the Oka strain and wild-type VZV.

Genetic differences

Although the Oka strain of varicella is attenuated, there is no evidence that it has undergone any specific mutations that alter the expression of viral genes.⁷ Differences between the Oka strain and wild-type VZV can be detected when the viruses are incubated with endonuclease enzymes. These enzymes cut the DNA at particular sites known as restriction sites; each strain of virus has its own pattern of restriction sites. The Oka strain has a particular pattern of restriction sites, but the differences from wild-type virus result from geographical variation and are not markers of attenuation.⁷

The pattern of restriction sites can be useful to distinguish wild-type VZV from the Oka strain in most clinical circumstances.⁸ For example, with the *Pst*I enzyme, there are restriction sites present in the Oka strain that are not seen in wild-type virus from the USA and UK (Table 2).⁸⁻¹⁰

	Percentage of strains identical to Oka (%)	Viruses with a specific restriction site found in Oka (%)			
		<i>Pst</i> I ⁻	<i>Bgl</i> II ⁺	R5A	R2 ₇
◆ VZV and reference:					
Japanese wild-type ¹⁰	7	37	n/k	13	10
USA wild-type ⁸	0	0	20	n/k	n/k
UK wild-type ⁹	0	0	20	95	16

Table 2: Genetic similarities between Oka strain and wild-type VZV; n/k = not known⁸⁻¹⁰

Clinical Studies with Oka Strain Varicella Vaccine

Measures of vaccine efficacy

Clinical studies indicate that vaccination offers good protection against varicella. However, there has been only one placebo-controlled study of the varicella vaccine. In the study, 468 children were immunized with a dose of 17000 plaque forming units (pfu) and 446 were given placebo.¹¹ Over the following 9 months, there were 39 cases of varicella, all of which occurred in the placebo recipients, giving a vaccine efficacy of 100%. During the second year of the study, one vaccinated child developed varicella, giving an efficacy rate of 98%. The children who originally received placebo were then vaccinated. During 7 years of follow-up, it was estimated that 95% of the vaccinees remained free of varicella (Table 3). The high degree of protection observed may be accounted for by the titre of the vaccine (17000 pfus) used. This titre is the highest dose ever used but is impractical to produce on a commercial scale.⁷ However, even at this high titre, the vaccine was found to be well tolerated.¹¹

Breakthrough varicella

It is possible to assess the efficacy of vaccination by calculating the rate of varicella in vaccinated individuals exposed to varicella. The cases of varicella that occur in these individuals is known as breakthrough varicella and the higher the attack rate (i.e. number of cases of breakthrough varicella), the lower the degree of protection. In unimmunized, susceptible adults, the attack rate is approximately 90% after household exposure. Vaccinated adults have a lower degree of protection than children; their attack

Interval	Number of cases	Attack rate ^b (%)	Mean number of lesions
Year 1 ^c	0	0.0	–
Year 2	1	0.2	17
Year 3	1	0.2	286
Year 4	5	1.0	43
Year 5	9	1.9	49
Year 6	3	0.6	57
Year 7	4	0.9	22
Total	23	–	53

^a Excludes one case reported but not laboratory confirmed; ^b Based on person-years at risk; ^c From the time of vaccination to 14 June 1983. Vaccine was administered between 9 September 1982 and 8 February 1983.

Table 3: Varicella cases^a after vaccination¹¹

rate after household exposures to varicella is 30–40%¹² compared with leukaemic children with an attack rate of about 13%¹³ and healthy children with an attack rate of about 10%.¹⁴

In all these groups, the breakthrough infections are milder in nature than natural infections. The incidence of fever and the number of lesions are lower in both vaccinated leukaemic and healthy children.^{11,15}

	Follow-up (years)	Rash or fever	Seroconversion	Protection: 1–3 yrs	Protection: >3 yrs
Healthy children	2–16	5%	95%	89–94%	88%
Adults		10%	>90% (two doses)	70%	
Leukaemic children	2–16	5–50%	95% (two doses)	86%	86%
Other immunosuppressed	2–3	5–50%	74–95% (two doses)	80%	

Table 4: Results of clinical trials with Oka strain VZV vaccine^{12,16–20}

Safety of Oka strain varicella vaccine

Several clinical studies have documented that the Oka strain varicella vaccine is safe and provides effective long-term immunity (Table 4).^{12,16–20} The main symptom of varicella is a minor skin rash. The frequency of rash reported after immunization in healthy children is about 5% and there are very few skin lesions (generally less than 10).^{21,3} Other common adverse events of the vaccine include fever (15%), temporary discomfort at the injection site (19–24%) and rash at the injection site (3–4%).^{21,3}

Similar mild adverse events are seen in healthy adolescents and adults although two doses of the vaccine are given. However, the rate of vaccine-associated rash in adults is 10%, twice that seen in healthy children.⁷

In vaccinated leukaemic children, the incidence of adverse events in the first 6 weeks after immunization is, however, significantly higher than in healthy children. A vaccine-associated rash was the most common adverse event reported after immunization. Fifty per cent of leukaemic children receiving chemotherapy developed rash compared with 5% of children no longer receiving chemotherapy in a study of 548 leukaemic children. Other adverse events were reported less frequently, in about 5% of these children.⁷

Immune Responses to Varicella Vaccine

In addition to evaluating efficacy, the immune response to vaccination with the Oka strain of varicella has also been studied. Both the humoral and cellular immune responses to the Oka strain vaccine have been explored in different populations.

Seroconversion

The rate of seroconversion following vaccination is generally high although the actual rate varies according to the population studied. Healthy children show the highest rate of seroconversion with rates of about 95% after one dose of the vaccine.^{11,21-23}

In contrast to the high rate of seroconversion seen in healthy children, only 80–85% of children with leukaemia seroconvert after one dose. Consequently, it is recommended that leukaemic children are given two doses of varicella vaccine which will achieve seroconversion rates greater than 90%.

It has been found that adults, including young adults, do not respond as well to the vaccine as children, even leukaemic children. Seronegative healthy adults have been successfully vaccinated in a number of studies but, as with leukaemic children, most investigators found that two doses of vaccine are required to achieve a seroconversion rate of more than 90%.

Persistence of antibody response after seroconversion

There is a high degree of persistence of antibodies to varicella after vaccination and it is greatest in children. In a 20-year follow-up after immunization, 25 out of 26 young adults remained seropositive.¹⁷ Similarly, of over 500 healthy children who were followed for up to 6 years, 95% remained seropositive.⁷ The immune response, however, is less persistent in immunized adults and leukaemic children than in healthy children (Figures 2 and 3).³

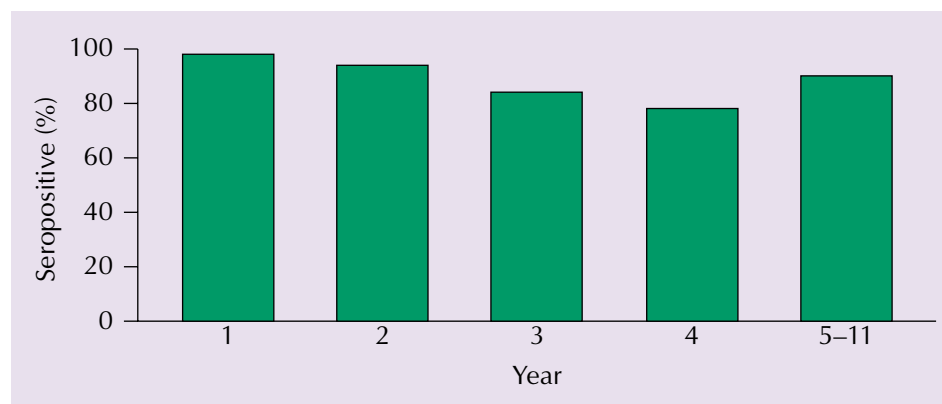


Figure 2: Percentage of leukaemic children seropositive after varicella vaccination (based on data from reference 3)³

Although adults do not respond as well to vaccination as children, 86–88% of healthy adults have persistence of antibodies to varicella from 7 to 13 years after immunization (Figure 3).³

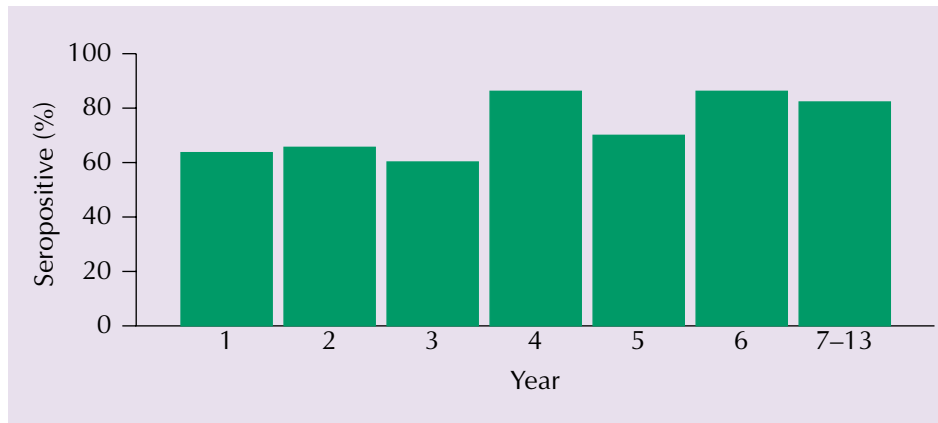


Figure 3: Percentage of adults seropositive after varicella vaccination (based on data from reference 3)³

Cell-mediated immunity

Cell-mediated immunity to VZV is mediated by antigen-specific T-cells that are elicited during a primary infection.^{24,25} VZV-specific cellular immunity is important in controlling viral replication in healthy and immunocompromised individuals with varicella or herpes zoster. Failure to produce T-cells that can recognize VZV antigens is correlated with a risk of persistent viraemia and life-threatening dissemination in immunocompromised children.²⁶ Clinical experience has also shown that absent, weak or delayed cell-mediated immune responses correlate with severe manifestations and complications of VZV in the newborn, elderly, otherwise healthy individuals and in patients with primary or secondary deficiencies of cellular immunity.²⁷

In children, good cell-mediated immune responses are produced after one dose of vaccine. In contrast for adults, two doses are required to produce the same degree of cellular immune response. This difference may be explained by the changes in the immune system that accompany ageing.

Memory T-cells that recognize VZV antigens are maintained at frequencies of approximately 1:40 000 peripheral blood mononuclear cells in adults^{28,29} and this level is similar to that seen after vaccination.³⁰ Ageing is associated with a decreased ability of T-cells to recognize VZV antigens and this waning appears to begin at about 40 years of age.³¹ This is manifested as decreases in delayed hypersensitivity reactions to VZV skin test antigen and in the frequencies of memory T-cells that recognize VZV antigens (Figure 4).³¹ Although there is a decrease in cellular responses, antibody levels remain positive into the ninth and tenth decades of life (Figure 5).³¹

As cell-mediated immunity is required to maintain the balance between the host and VZV, this diminished ability to respond to the virus is associated with an increased risk of herpes zoster in the elderly.³² A similar correlation occurs in patients receiving immunosuppressive therapy.^{13,33} However, reactivation of VZV in elderly and immunosuppressed individuals does not correlate with decreasing titres of VZV IgG antibodies.¹³

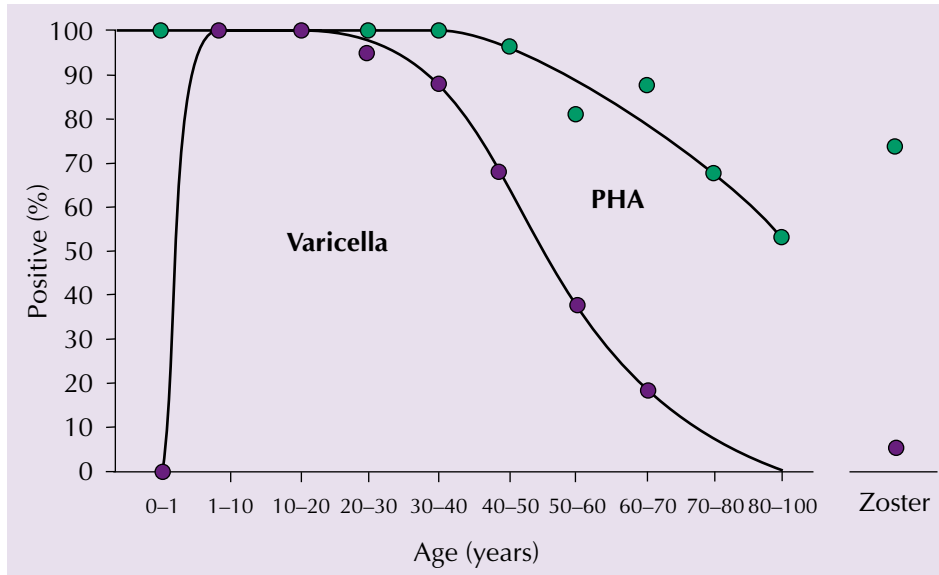


Figure 4: Percentage of positive skin test responses to VZV (●) and phyto-hemagglutinin (PHA) (●) according to age. 'Zoster' indicates values in patients with herpes zoster³¹

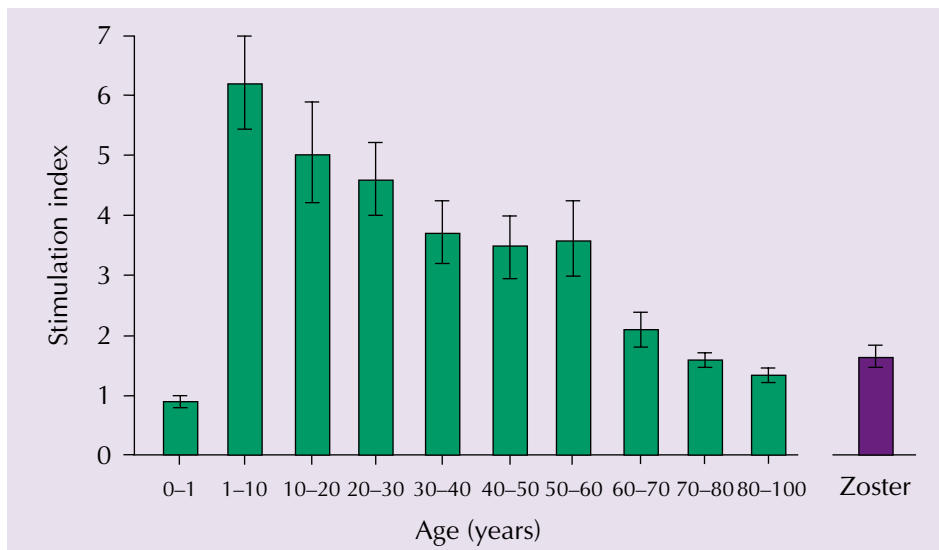


Figure 5: In vitro VZV-induced lymphocyte stimulation according to age and expressed as stimulation index: counts per minute of ³H-thymidine uptake for lymphocytes incubated with VZV divided by counts per minute after incubation with medium alone. 'Zoster' indicates values in patients with herpes zoster³¹

Correlates of protection

It is uncertain which responses to vaccination correlate with protection. In leukaemic and healthy children there is a trend towards improved protection in those who have detectable humoral and cell-mediated immune responses at the time of household exposure compared with those who do not manifest these responses.³ The relative importance of these two arms of the immune response is difficult to interpret. There is good evidence that cell-mediated immunity correlates with protection but there is less consensus that the presence of antibody is protective.²⁷ The severity or incidence of varicella did not increase with time for leukaemic children who had originally seroconverted after vaccination but became seronegative over a period of up to 11 years.⁸ In contrast, the attack rate of breakthrough varicella following household exposure in 83 children was 8% in seropositive children and 29% in seronegative children.¹³ Furthermore, protection

correlated with the titre of VZV-specific antibody at 6 weeks post-vaccination in a study of 4042 healthy children and adolescents (Figure 6).²²

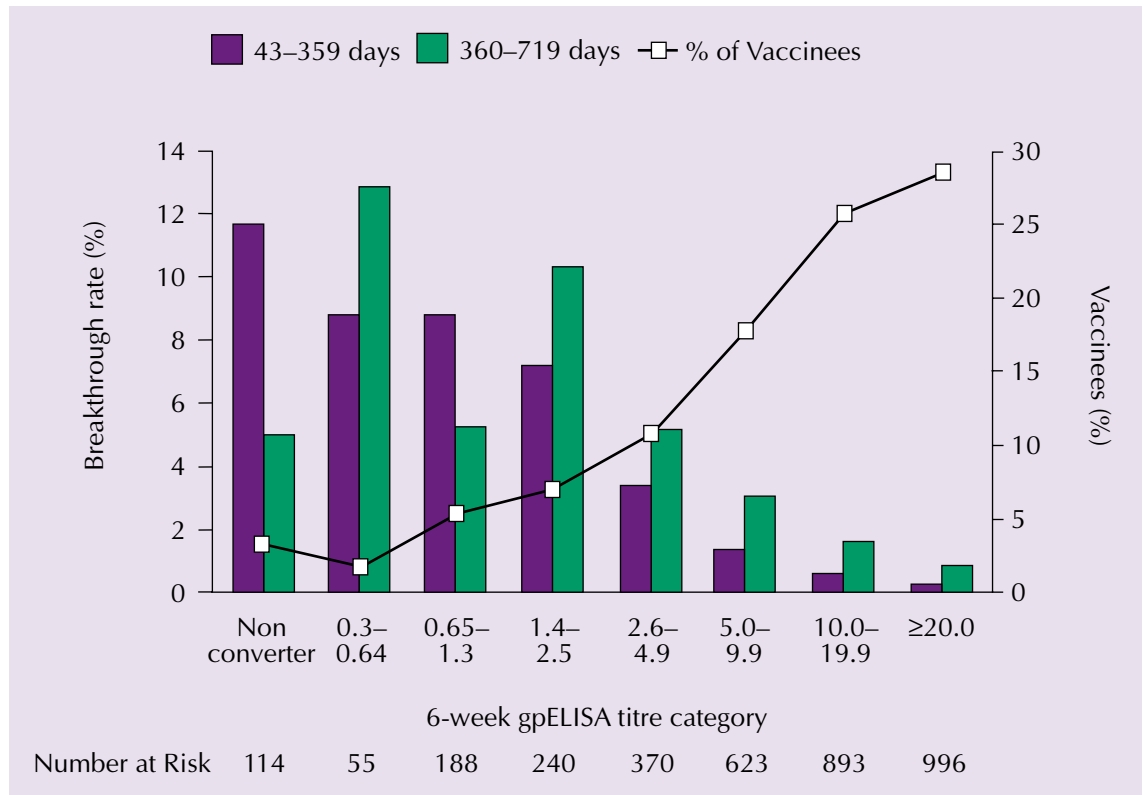


Figure 6: Rate of varicella cases in Years 1 and 2 post-vaccination by 6-week antibody category (gpELISA) production²²

Booster Doses

Natural boosting can occur because of periodic re-exposures during the annual varicella epidemics and is one reason why immune responses may persist.²⁶ Immunity to VZV can also be maintained by subclinical reactivations of latent virus resulting in endogenous re-exposure to viral antigens.³⁴

Vaccination has also been shown to give prolonged immune responses; this may be due to the vaccine itself or because it boosts secondary exposure to VZV in the community. However, the persistence of immunity has been questioned and there are concerns about the risk of varicella outbreaks in non responders if the vaccine is used widely. Therefore, studies have evaluated the responses to booster doses of vaccine given several years after initial immunization. Booster doses are associated with increases in humoral³⁵ (Table 5)³⁶ and cellular immunity³⁶ in immunocompetent individuals and are well tolerated.^{35,36} However, while it seems likely that booster doses will increase protection, there are as yet no data to support this contention.

Dosage of Vaccine

The intensity of immune response and protective efficacy is correlated with the titre of vaccine. A high titre of infectious virus is associated with a greater likelihood of seroconversion.^{35,37,38} The rate of seroconversion also appears to correlate positively with titre of virus.^{39,40} In addition to antibody response correlating with dose, T-cell proliferation also positively correlated with dose in two studies^{38,39} but not another.³⁷

Time	Seropositivity rate	
	% response (no. responding/no. tested)	Geometric mean titre
Before booster	98.8 (414/419)	25.7
Days 5–16	100.0 (302/302)	143.6
6 weeks (subset)	100.0 (74/74)	218.8
3 months	100.0 (358/358)	119.0

Table 5: Humoral response to varicella vaccine booster³⁶

However, in the study in which no correlation was found, although the virus titre of each dose was different, the antigen content was the same. It is possible that viral antigen content can affect cell-mediated immune responses to varicella vaccines and therefore mask any dose relationship.³⁷

The titre of virus is also correlated with the degree of protection offered by the vaccine. One study evaluated seroconversion and breakthrough varicella in 513 10–30-month-old children receiving either a high titre (non-heat-exposed) vaccine (10 000–15 850 pfu), a low-titre (heat-exposed) vaccine (630–1260 pfu) or placebo.¹⁶ There were statistically significant differences in the number of breakthrough cases of varicella between the groups; there were five cases of breakthrough varicella in the high-titre group compared with 19 in the low-titre group and 24 in the placebo group ($P=0.05$ for each comparison [Figure 7]¹⁶). Thus, the efficacy of the high-titre vaccine was 88% versus 55% in the low-titre vaccine group. There was also a trend to a higher rate of seroconversion with higher titre of virus, but the correlation between level of post-vaccination antibody and protection was poor.

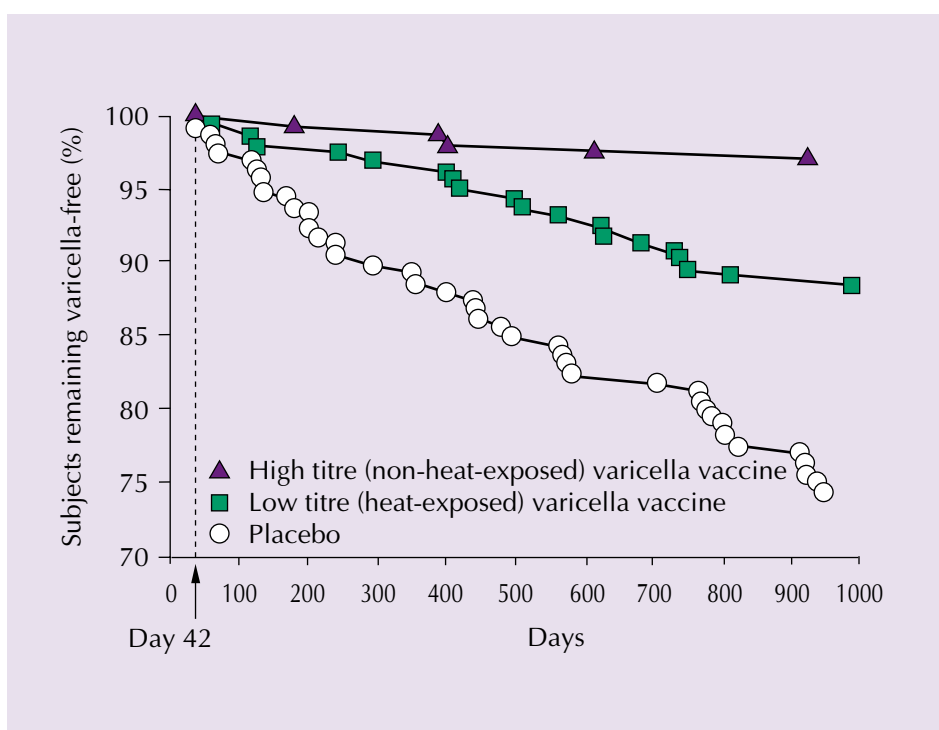


Figure 7: Percentage of subjects remaining varicella-free¹⁶

The relationship between virus titre and protection (or seroconversion) has implications for the storage and handling of the vaccine. The vaccine is quite labile and sensitive to light and, therefore, should be stored and used according to the manufacturers instructions. In this way, it will retain full potency and generate maximal immune responses.

Incidence of Herpes Zoster after Vaccination

There has been concern that vaccination would result in premature or a changing pattern of herpes zoster. The incidence of herpes zoster has not increased in healthy vaccinated children.²² However, the incidence of herpes zoster is low in children and it is difficult to draw any firm conclusions about the effect of vaccination. In contrast, the rate of herpes zoster is higher in children with leukaemia and, therefore, the incidence of herpes zoster after immunization has been studied in this patient group.

The two studies conducted to date have found that fewer vaccinated children with leukaemia developed herpes zoster compared with those who acquired varicella naturally. In one study, none of the 34 vaccinated leukaemics but 15 (21%) of 73 unmatched controls developed herpes zoster ($P=0.0170$).⁴¹ In the second study, 96 vaccinated leukaemic children were matched with 96 leukaemics who had experienced natural varicella.¹³ The rate of herpes zoster was 2% in the vaccinees and 15% in the controls ($P=0.02$); Figure 8.¹³

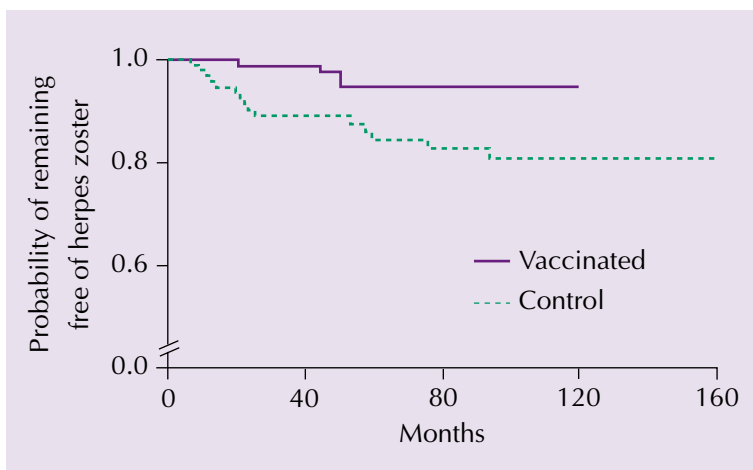


Figure 8: Probability of remaining free of herpes zoster in leukaemic children¹³

Further evidence that vaccination appears to lower the incidence of herpes zoster comes from a study in which 268 healthy adults were immunized. The average age of the adults was 27 years and they received two doses of vaccine, 3 months apart. Of these vaccinees, only one developed herpes zoster. The calculated incidence of herpes zoster is 12.8 cases per 100 000 person-years compared with an expected rate of 77 cases per 100 000 person-years.⁴²

There are a number of hypotheses to explain why herpes zoster is less common after vaccination than natural infection. It may be that the virus is attenuated or because vaccination seldom causes skin lesions, the virus cannot gain access to the sensory nerves as it is thought to do during natural infection when lesions are present. Support for the importance of a skin rash comes from studies of vaccinated leukaemic children in which the risk of developing herpes zoster was nearly six times higher if the vaccinee had a history of VZV-related rash than if no rash had occurred.¹³

The apparent importance of a skin rash for the development of herpes zoster has led to the prediction that the incidence of herpes zoster will be lower in healthy vaccinees than those who have had natural infection. This is because a vaccine-associated rash and breakthrough varicella are unusual in healthy vaccinated adults or children.¹²

Oka Strain Varicella Vaccine in Combination with Measles-Mumps-Rubella Vaccine

The licensing of the Oka strain varicella vaccine in the USA has led to the suggestion that the addition of the varicella vaccine to the measles-mumps-rubella (MMR)-combination vaccine would make universal vaccination more probable.^{40,43} Furthermore, a combined vaccine would be more acceptable to the patient, parent and practitioner.

A preparation which contained both the MMR vaccine and the varicella vaccine (MMRV) has been compared with the MMR vaccine and varicella vaccine injected at separate sites given at the same visit (MMR+V) in children 15 months of age.⁴³ In both groups there were 100% varicella seroconversion rates and lymphocyte proliferation responses at 6 weeks and 1 year after immunization (Table 6). While the antibody titre to varicella was lower in the MMRV group at 6 weeks, at 1 year there was no difference compared with the MMR+V group. The seroconversion rates for measles, mumps and rubella were 100% in both groups. There was no statistical difference in lymphocyte proliferation responses between the two groups.⁴³

Time after immunization (weeks)	Humoral immune responses							
	Group 1				Group 2			
	<i>n</i>	GMT in gpELISA	SD	Seroconversion (%)	<i>n</i>	GMT in gpELISA	SD	Seroconversion (%)
0	48	<.625	0	NA	49	<.625	0	NA
6	48	7.0	2.4	100	45	15.3	1.6	100
52	42	19.0	3.3	100	41	24.1	2.4	100

Time after immunization (weeks)	Cell-mediated immune responses, mean					
	Group 1			Group 2		
	<i>n</i>	SI	SE	<i>n</i>	SI	SE
0	24	1.1	0.1	30	1.3	0.1
6	21	26.4	4.3	24	35.0	6.1
52	18	14.9	3.7	21	29.0	8.7

GMT = geometric mean titre, gp = glycoprotein, NA = not applicable, SI = stimulation index (for lymphocyte proliferation), SD = standard deviation, SE = standard error

Table 6: Humoral and cell-mediated immune responses to varicella virus after immunization with combined vaccine (measles, mumps, rubella and varicella [Group 1]) ; or measles-mumps-rubella-vaccine and varicella vaccine administered simultaneously at separate sites (Group 2)⁴³

Another trial compared the MMRV vaccine with the MMR vaccine followed 6 weeks later by the varicella vaccine.⁴⁰ Both immunization schedules stimulated 100% seroconversion to all the component viruses. The antibody titres were similar for all the viruses in both groups.

The two studies found that the frequency of local and systemic reactions were similar after administration of the MMRV vaccine preparation and when the MMR vaccine and varicella vaccine were given separately.

In summary, the results of the two studies suggest that MMRV is effective in stimulating immune responses. However, long-term follow-up is required to determine the persistence of both cellular and humoral immunity over time after immunization with this investigational preparation.

Strategies for Vaccination

There are several different strategies proposed for vaccinating susceptible individuals (Table 7), each with its own advantages.

- ◆ Universal childhood vaccination (with MMR)
- ◆ Universal adolescent (12 years)
- ◆ Catch-up
- ◆ Targeted

Universal vaccination

There are two types of universal vaccination: vaccination of young children or vaccination of children at 12 years of age. The catch-up strategy is a combination of these two different approaches to universal vaccination. In contrast, targeted vaccination involves immunizing only those individuals who are at risk of severe or complicated varicella.

Table 7: Vaccination strategies

Universal vaccination of all children aged 12–18 months is recommended by the Advisory Committee on Immunization Practices (ACIP) in the USA (see Table 8).⁴⁴ Such routine vaccination is considered essential as without universal vaccination, cohorts of children would reach adulthood without exposure to the virus and, therefore, would be susceptible to varicella at an age when the risks for severe disease and complications are highest.⁴⁵ Because of these risks, the ACIP also recommends vaccinating children aged 19 months to 12 years who have not previously been vaccinated and lack a reliable history of varicella. It suggests that children are vaccinated before 13 years of age because two doses of vaccine are required after the thirteenth birthday.

ACIP Recommendations

- ◆ 12–18 Months
1 dose varicella vaccine with MMR at the same time
- ◆ 19 months – 12 years
Negative history of chickenpox: 1 dose varicella vaccine
- ◆ ≥13 years: test for IgG antibodies
Target immunization to high-risk groups
- ◆ Susceptible contact immunocompromised
health-care workers
family
- ◆ Susceptible health-care workers
- ◆ Susceptible persons working with children/in institutional settings/colleges
- ◆ Susceptible women of childbearing age but not pregnant
- ◆ Susceptible travellers abroad

Table 8: ACIP recommendations⁴⁴

For people older than 13 years, the ACIP recommends immunizing those at highest risk of exposure and for transmitting the disease to others. Vaccination is advocated for susceptible individuals who have close contact with people at high risk of serious complications. This group includes health-care workers and family or close contacts of immunocompromised individuals, especially transplant recipients.

In addition to those in close contact with people at risk of complications, other groups who are a high priority for immunization include those who work in environments where there is a high likelihood of transmission. Under this category, the ACIP includes susceptible individuals working with children, in institutions and in colleges. Susceptible women of childbearing age and travellers abroad are also recommended as candidates for vaccination.

Targeted vaccination

However, in the UK and many other countries, the emphasis is on targeted vaccination. In the UK, where a varicella vaccine has not been licensed, the candidates for targeted vaccination would be non-pregnant women of childbearing age, the immunocompromised, adults and health-care workers.

Current UK Department of Health guidelines recommend post-exposure prophylaxis with varicella zoster immune globulin or aciclovir for immunocompromised patients at risk of severe varicella infection and susceptible health-care workers.⁴⁶ It has been proposed that, instead, there is targeted pre-exposure vaccination of these groups. This suggestion is controversial but such an approach may simplify infection control measures, reduce the risk of severe varicella infection in immunosuppressed patients and also reduce the costs of controlling outbreaks and days lost from work.⁴⁷

There is also a strong argument for the targeted vaccination of women of childbearing age. Susceptible pregnant women are at high risk of exposure to varicella from children in their household as most cases of varicella occur in those under the age of 5 years. Furthermore, the attack rate of varicella is extremely high with household contact and, therefore, a substantial number of women with a negative history are susceptible.

Varicella in these women causes severe maternal morbidity, and 10–20% of infected women develop varicella pneumonia with mortality as high as 40%.⁴⁸ Furthermore, if the mother develops rash near the time of delivery or shortly after this may result in neonatal varicella which has a mortality of up to 30%.⁴⁹ In addition, the overall rate of teratogenicity is 2%.⁵⁰ Although this is a lower percentage than fetal damage resulting from rubella infection in pregnant women (85% rate), in the UK, the absolute number of babies born with birth defects due to varicella is similar to the number of cases of congenital rubella syndrome now that rubella has been reduced by immunization.

The ideal candidates for targeted vaccination have yet to be confirmed. If targeted vaccination is chosen as a strategy, then the choice of candidate groups must depend on an understanding of the risks and benefits of this strategy for each patient group.

Economic Analyses of Vaccination

The choice of a targeted vaccination policy or universal vaccination can be influenced by many factors. The costs and benefits of both vaccination strategies have been explored and, in the case of universal vaccination, have been a contributing factor in the choice of this particular strategy in the USA.

Universal vaccination

Universal vaccination is only considered to be cost-effective if both medical and non-medical costs are taken into account.⁵¹⁻⁵³ An analysis of the cost-effectiveness of vaccination conducted over 10 years ago found that if the loss of income resulting from parents having to take time off work to care for their sick children was taken into account along with medical costs, then vaccination was cost-effective.⁵⁴ A more recent study reported that for every dollar spent on immunization, US\$5 dollars was saved.⁵³ However, as with the earlier study, this saving was only made if the costs of lost work by parents were considered; if only medical costs were considered, then the universal vaccination would cost US\$2 for every varicella case prevented.

Another analysis has also found that universal vaccination is cost-saving mainly because it reduces the burden of parental work loss associated with this common childhood disease.⁵² It was estimated that vaccination would yield net economic benefits of US\$66.47 per vaccinee.

The cost-effectiveness of universal vaccination determined in these studies compares favourably with other health-care interventions. Vaccination has a similar cost-effectiveness to hepatitis B vaccines and pertussis vaccines for infants, and it is more cost-effective than hypertension screening for middle-aged men.⁵⁴

Most of the analyses of the cost-effectiveness of vaccination have been performed in North America and, as a result, are heavily dependent on North American resource utilization data. As the results of these analyses indicate that the cost-effectiveness is heavily reliant on non-medical costs, the finding that vaccination is a relatively cost-effective use of resource cannot be readily applied to other countries. Studies should be performed in other countries to establish local cost-effectiveness of vaccination.

One study has examined the cost-effectiveness of routine varicella vaccination in Germany.⁵⁵ It compared three vaccination strategies with no vaccination: vaccination of all 15-month-old children, vaccination of susceptible 12-year-olds and a combination of these two strategies (catch-up). The strategy of vaccinating 12-year-olds was the only one which resulted in direct medical cost savings (Figure 9). However, although this strategy was the most cost-effective, it was felt that the approach may be less acceptable from a medical or organizational standpoint. Consequently, a second option was considered which was to start immunizing the 15-month-old children and to use the catch-up vaccination of 12-year-olds for 11 years. Then from Year 12, only 15-month-old children would be routinely immunized. This combination of strategies was also found to be cost-effective. In the first 11 years of this combined strategy, for every one Deutschmark (DM) spent, DM4.72 would be saved, while from Year 12 onwards (when only 15-month-old children are vaccinated), one Deutschmark spent on vaccination would save DM4.60.⁵⁵

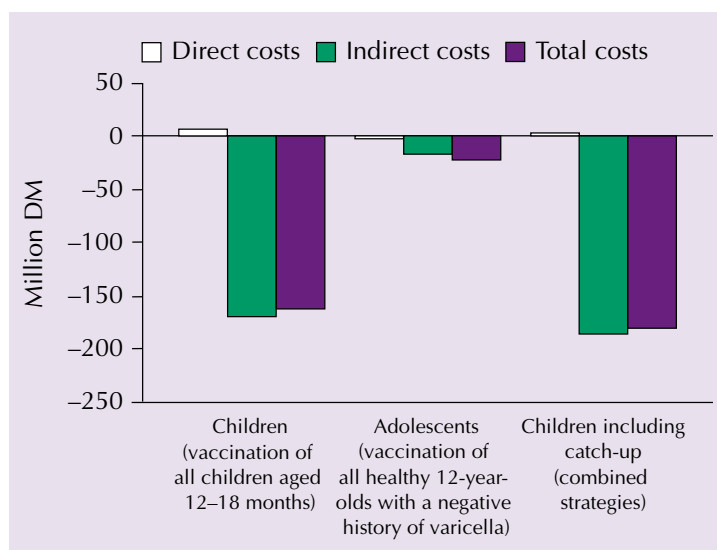


Figure 9: Incremental costs of varicella vaccination and no vaccination⁵⁵

These findings further support the cost-effectiveness of vaccination but, once again, these results cannot be directly extrapolated to other countries. The analysis used resource data from Germany where the costs of treatment of varicella are high. However, results from a scenario in which the costs of treatment were halved, to better reflect the situation in other European countries, did not seem to alter the general conclusions of the economic benefits of vaccination.⁵⁵

Targeted vaccination

In addition to findings that universal vaccination is cost-effective, targeted vaccination has also been reported to be cost-effective. For example, the vaccination of susceptible leukaemic children is cost-effective compared with no vaccination. In a study of 472 leukaemic children, it was 11–13 times cheaper to vaccinate all children than to treat some of them.⁵⁶

The cost-effectiveness of targeted vaccination for women of childbearing age has been calculated in the USA as US\$7000 per case of varicella prevented. This finding was based on the assumption that 95% of women were seropositive, the incidence of varicella in pregnancy was three women per 1000 and that all women were screened regardless of disease history.⁵⁷ It was estimated that 2000 women would be screened and 100 vaccinated for every case of varicella prevented in pregnancy at an overall cost of US\$70 000. However, it was suggested that there may be savings in diagnosis and treatment and, in the cases of congenital varicella, the costs related to fetal morbidity such as blindness and neurological defects.

A similar analysis demonstrated that vaccination was cost-saving when epidemiological data from an actual clinical population was used.⁵⁸ In this population, 80% of women were seronegative and the incidence of varicella in pregnancy was 7.7 cases per 1000 women. Therefore, vaccination may be more cost-effective in populations where there is a low proportion of seropositive women.

Benefits and Disadvantages of Vaccination Strategies

Universal vaccination

One advantage of universal vaccination is that there is the potential to eradicate varicella, although this will be more difficult than for measles, mumps and rubella because VZV persists in the form of herpes zoster which represents a reservoir for exposure to children.⁵⁹ Furthermore, as the Oka strain of varicella is attenuated, any herpes zoster that develops may be less severe. However, the disadvantages are that the cost-benefit of universal vaccination has not been established fully. There is also a

concern that long-term immunity may wane but results to date are encouraging and suggest that immunity persists.

Another significant problem is the potential for a universal vaccination programme to shift upwards the age at which susceptible individuals acquire varicella.⁴⁵ This could result in cohorts of individuals being exposed to varicella in adulthood when the risks of severe varicella and complications are greatest. It has also been suggested that the reduction in natural boosting that would accompany the reduction in incidence of natural varicella infection may increase the incidence of herpes zoster in the older population.⁴⁵

Targeted vaccination

Targeted vaccination is less costly than universal vaccination while protecting high-risk groups. It also has the advantage that the benefit of natural boosting is not lost. The major problem with targeted vaccination is its implementation as it depends largely on the vigilance and commitment of the practitioner. In addition many of the populations at risk require two doses of vaccine which adds to the logistical problems. There may also be people who have severe immunosuppressive disorders that make them unsuitable for vaccination but who are still at risk of naturally acquired varicella. Finally, as with universal vaccination, there is the potential waning of immunity over time.

Vaccination to Reduce the Incidence of Herpes Zoster

Herpes zoster results from the reactivation of VZV in sensory ganglia. The incidence and the severity of herpes zoster increase with age.⁶⁰⁻⁶² The most frequent complication of herpes zoster in older individuals is zoster-associated pain which can be severe and disabling.

Immunity to VZV plays a vital role in limiting viral reactivation. The increase in incidence of herpes zoster is associated with a decline in cell-mediated immunity and it has been suggested that herpes zoster develops because VZV-specific cell-mediated immunity falls below a certain level.⁶³

The Oka strain vaccine can stimulate cell-mediated immunity in children and adults. The vaccine can also stimulate the waning cell-mediated immune responses in elderly subjects to levels observed in younger individuals.⁶³ The vaccine was administered to 200 elderly individuals (mean age 67 years). The responder cell frequency increased from 1 per 70 000 peripheral blood mononuclear cells (PBMCs) to 1 per 40 000 PBMCs. This response is comparable to that seen after natural infection and to that observed in 40-year-old individuals. The increase in VZV-specific cell-mediated immunity was long-lasting, with a half-life of 54 weeks (Figure 10).³² In the 4 years of observation, only one case of herpes zoster was virologically confirmed and two cases were immunologically confirmed. None of the cases was associated with prolonged zoster-associated pain or extensive rash.⁶³

It is, therefore, reasonable to hypothesize that the restoration of cell-mediated immunity may reduce the frequency and severity of herpes zoster and its complications.

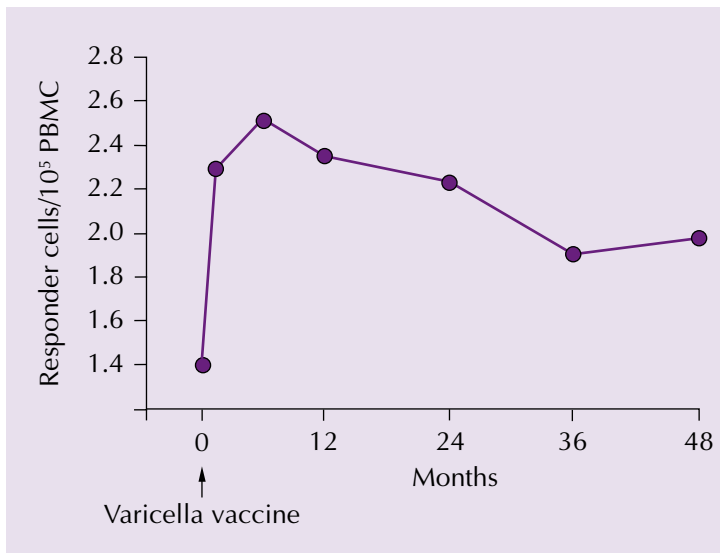


Figure 10: VZV-specific cell-mediated immune responses in elderly individuals after vaccination with Oka strain VZV vaccine (based on data in reference 32)³²

To test the hypothesis, a large-scale placebo-controlled trial is ongoing. This study has a very large sample size, 27 000 subjects, because of the relatively low incidence of herpes zoster and zoster-associated pain. The trial will follow these elderly individuals for a minimum of 3 years.³²

Immunotherapeutic Vaccines for Herpes Simplex Virus Disease

Introduction

Herpes simplex virus (HSV) infects man by penetrating mucous membranes or injured skin. It then replicates in epithelial cells causing lysis and initiating an inflammatory response. The virus spreads via sensory nerves to establish a latent infection in ganglia. As a neuron is an immunologically privileged site and is a difficult sanctuary to breach, latency is a barrier to vaccine efficacy. If a vaccine is to be effective as a therapy, it must limit the reactivation of the virus or limit its spread. To do this, a vaccine should augment the host's specific immune responses.

Important targets for vaccine development are the glycoproteins (g). There are 11 HSV glycoproteins of which gB, gC, gD and gE trigger potent immune responses;¹ they are inserted into the envelope of the virus and perform important biological functions. For example gB plays a central role in the absorption of the virus and penetration into the host cell (Figure 1).¹

The glycoproteins elicit both cellular and humoral immune responses.² Neutralizing antibodies to HSV are predominantly directed to gB and gD^{3,4} and monoclonal antibodies to these glycoproteins protect guinea pigs from experimental challenge with HSV.⁵ In addition, immune responses to other glycoproteins have been shown to be protective.⁶ The importance of the HSV glycoproteins has led to the development of vaccines directed against them.

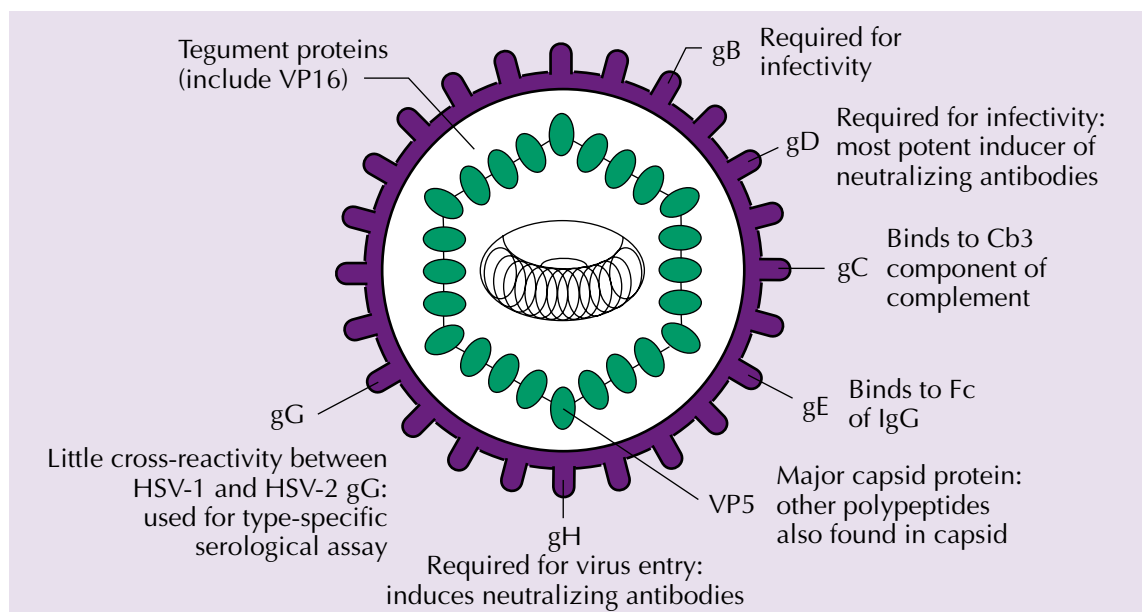


Figure 1: An overview of the structure of HSV (for clarity, many proteins are not shown)

Approaches to the Immunotherapy of HSV

A number of different approaches has been employed for the immunotherapy of HSV infection (see Table 1). Many of the trials of these potential vaccines have had significant

- ◆ Non-specific live vaccines
- ◆ Autoinoculation of live HSV
- ◆ Whole inactivated vaccines
- ◆ Inactivated subunit vaccines
- ◆ Glycoprotein vaccines

Table 1: Vaccines evaluated for the immunotherapy of HSV infection

(vaccinia) vaccines were used in open clinical trials. The theoretical basis for the trials was that HSV and vaccinia stimulate cross-protective immunity, an assumption that was later disproved.⁸ The bacillus Calmette-Guerin (BCG) has also been used as a non-specific immune stimulant but failed to show efficacy in a double-blind placebo-controlled trial.⁹

Autoinoculation of live HSV

Inoculation of an individual with their own vesicular fluid was used to treat HSV recurrences in the 1950s and 1960s. This *natural* infection did not prevent lesions and, in some cases, lesions recurred at the injection site as well as at the sites of the original lesions.^{10,11}

Whole, inactivated vaccine

The safety concerns associated with the use of live vaccines prompted the development of killed-virus preparations. These were prepared by chemical or physical inactivation of the virus

One of the first clinical trials of an inactivated vaccine for the treatment of recurrent HSV infection was performed by Frank in 1938.¹² Suspensions of formalin-inactivated, HSV-infected rabbit brain were repeatedly injected into 14 volunteers, 13 of whom reported subjective improvement in the interval between recurrences. However, a placebo group was not included and the vaccine was not immunogenic.

The study design by Frank was the model for a large number of therapeutic studies conducted between 1946 and 1982. These studies evaluated the efficacy of inactivated vaccines in the treatment of recurrent HSV disease. In these studies, from 0 to 100% of patients reported improvements. However, the assessments of efficacy were subjective and, as with the Frank study, there were no placebo controls.

The first placebo-controlled studies were conducted by Kern and Schiff¹³ using an inactivated whole virus vaccine. An initial trial assessed the vaccine's efficacy over a 6-month period.¹⁴ It found that the number of recurrences was reduced in 10 of the 12 treated subjects (83%) and in two of 11 placebo recipients (18%). In a second study using the same vaccine but in which the patients were followed from 6 to 36 months, Kern and Schiff reported that 16 of 23 vaccine recipients (70%) had fewer recurrences and 22 of 29 placebo recipients (76%) also experienced improvements (Table 2).¹³ The significant placebo effect observed in this study highlights the importance of appropriate controls when evaluating vaccine immunotherapy of HSV infection.

methodological flaws that have rendered their interpretation difficult. However, recent advances in molecular biology have led to the development of new vaccines which have undergone more rigorous clinical trials.⁷

Non-specific live vaccines

Non-specific live vaccines were first used in the 1920s when smallpox

Author (year)	Vaccine preparation	Therapeutic trials no. improved/total (%)			
		Vaccine		Placebo	
Kern and Schiff (1964) ¹³	Whole virus, formalin-inactivated	16/23	(70%)	22/29	(76%)
Weitgasser <i>et al</i> (1977) ¹⁷	Whole virus, heat-killed	28/34	(82%)	18/60	(30%)
Kutinova <i>et al</i> (1988) ¹⁹	Subunit, lectin-purified	18/24	(43%)	15/42	(35%)

Table 2: Results of early double-blind human trials of inactivated vaccines for the treatment of HSV infection (adapted from reference 18)¹⁸

Yet, despite the importance of controls in clinical trial design, large-scale open trials were still being conducted 20 years after Kern and Schiff's findings. Heat-killed, whole virus vaccines from HSV-1 (Lupidon H) and HSV-2 (Lupidon G) have been reported to produce significant improvement in 81% of 1059 participants in an uncontrolled, open trial.¹⁵ Similarly, a formalin-inactivated vaccine was given to over 2000 individuals and reported to give significant improvements for 65–80% of patients.¹⁶

The reports of success in uncontrolled trials led to the evaluation of two Lupidon vaccines in a double-blind, placebo, controlled trial.¹⁷ Ninety-four patients were enrolled and had HSV lesions in various locations. Improvement was defined as prolongation in the disease-free interval or a decrease in severity of recurrence. Over the 6 months of the study, 28 of 34 vaccinees (82%) and 18 of 60 of the control group (30%) were reported as improved (Table 2).¹⁸ Although this difference was significant, the study has several weaknesses: there was a diverse study population; there were many treatments given; there was a failure to report the method of assignment to treatment group and the method of outbreak assessment.

Inactivated, subunit vaccines

These subunit vaccines contain components of the virus rather than the whole virus. They were initially adopted because of a suggested link between HSV and cervical cancer and, fortuitously, because of the advances in protein purification at the time. The inactivated, subunit vaccines for HSV have largely contained HSV glycoproteins with or without adjuvants. As these glycoproteins have important functions and are highly immunogenic, they are important components of candidate HSV vaccines. The adjuvants (e.g. alum) have been used to enhance the immune response to the vaccines.

However, the procedures required to produce subunit vaccines are labour intensive and costly. They include detergent extraction, DNase treatment, and affinity chromatography to isolate the subunits. Furthermore, the yield from these procedures is often low and the product is difficult to reproduce and standardize. Despite this, several studies have explored subunit vaccines for HSV immunotherapy.¹⁹

As with the earlier whole, inactivated vaccines, the interpretation of most of these clinical studies has also been made difficult because of design flaws. For example, there were fewer and less frequent recurrences in 59 volunteers receiving a subunit vaccine

containing HSV-2 envelope glycoproteins and alum than in 33 unimmunized control patients.²⁰ When recurrences did occur in the vaccinated group, they were of shorter duration and less severe. Unfortunately, there may have been a placebo effect as the control group knew they were not receiving vaccine. In addition, the study group comprised individuals with genital herpes and those with orolabial herpes. These two diseases have unique natural histories and should not be combined for analysis.²¹

Similar design flaws are also associated with trials of an HSV-1 glycoprotein vaccine adsorbed to alum for the treatment of recurrent genital or orolabial herpes.¹⁹ Comparable decreases in the frequency of recurrences were seen in the vaccine recipients (43%) and the placebo recipients (35%); thus, there was a significant placebo effect (Table 2). Moreover, the vaccine was found to be minimally immunogenic, producing little or no humoral response. The combination of the placebo effect and the poor immunogenicity of the vaccine may explain why no clinical benefit was demonstrated in the trial.

An HSV subunit vaccine, called the *Skinner vaccine* was produced by Skinner and colleagues. It consists of mixed HSV-1 glycoproteins prepared by formalin inactivation and detergent extractions. A series of uncontrolled studies has claimed efficacy for prophylaxis in over 100 sexual partners at risk of HSV infection and has also claimed therapeutic efficacy.^{22,23} The Skinner vaccine has since been assessed in a double-blind, placebo-controlled trial in 316 patients with frequently recurring genital herpes.²⁴ The vaccine did not significantly reduce the number of recurrences during the first 6 months after vaccination compared to placebo and neither did it reduce the median number of days to first recurrence. The vaccinees had fewer lesions and symptoms during outbreaks but the healing time was unaffected. The vaccine recipients had an increase in neutralizing antibody titre at 1 month but no change in lymphocyte proliferation responses to HSV-1 or HSV-2.²⁴

Thus, the studies of inactivated, subunit vaccine, and of whole, inactivated virus do not provide definitive evidence of the efficacy of immunotherapeutic HSV vaccines. However, the indications of efficacy in two of these studies^{14,17} suggest that immunotherapy is a valid concept. The advent of recombinant DNA technology and the development of an animal model have led to the evaluation of several different recombinant glycoprotein vaccines.

Glycoprotein Vaccines

Guinea pig model of genital herpes

The experimental testing of immunotherapeutic vaccines has been aided by the recent development of a guinea pig model of recurrent genital herpes.²⁵ The model mimics human disease in several respects (see Table 3) and has been used to study this persistent viral infection. However, there are no reagents available to characterize fully the immune responses in the guinea pig.

Other experimental models used in vaccine immunotherapy include rabbit and mouse ocular herpes.^{26,27}

The current generation of glycoprotein vaccines are produced by recombinant DNA technology. It allows large quantities of glycoproteins to be obtained easily and relatively inexpensively.

- ◆ Self-limited acute illness
- ◆ Quantifiable vesiculoulcerative genital disease
- ◆ Quantifiable HSV replication in the genital tract
- ◆ Spontaneous recurrent infections:
 - clinically-observable lesions
 - viral shedding in the absence of observable lesions
- ◆ UV radiation-induced recurrent infections
- ◆ Definable humoral, cell-mediated and cytokine responses

Table 3: Characteristics of guinea pig model of genital herpes

Immunotherapy with glycoprotein vaccines has been shown to significantly reduce the frequency and severity of recurrent genital HSV infections in the guinea pig model. Guinea pigs who had recovered from a primary infection were inoculated with either recombinant HSV-1 glycoprotein B from (gB1) and gD1 with complete Freund's adjuvant. The recurrences during the first 3 weeks after vaccination were reduced by 45% and following a booster on Day 29, were reduced by 68% over the second 3-week observation period. The improve-

ments in genital HSV infections were accompanied by a 4–244 fold increase in antibody titre.²⁸ This was the first controlled study to indicate that immunotherapy can alter the natural history of genital HSV infection. Subsequently, treatment with a vaccine containing HSV-1 glycoproteins B and D has been shown to reduce the frequency of recurrences and asymptomatic HSV shedding in infected guinea pigs.²⁹

The guinea pig model has since shown that a variety of factors influence the efficacy of immunotherapy (Table 4), including dose, type of immunogen and timing of inoculation relative to the primary infection.³⁰ Of all the factors tested, the single most important factor was the adjuvant formulation used. Potent (immunogenic) adjuvants, such as complete Freund's, muramyl tripeptides (MTP) in oil and liposomal formulations were effective, while poor adjuvants such as aluminium hydroxide (alum) were largely ineffective.³⁰

- ◆ Timing of administration relative to primary infection
- ◆ Dose and route of administration
- ◆ Adjuvant
- ◆ Immunogen:
 - HSV glycoproteins
 - HSV gD
 - HSV gB + gD
 - HSV ICP35
 - HSV gD gene (DNA)
 - HSV-2 DISC virus

Table 4: Factors influencing efficacy of HSV immunotherapy in guinea pigs

Although animal studies indicate that complete Freund's adjuvant and MTP are the most potent, they are too reactogenic to be used in man. For this reason, alum is frequently used in man. Newer agents are being tested to identify a compromise between reactogenicity and potency and one candidate is MF59. This is a microfluidized oil-in-water emulsion that is highly potent in animals and has been shown to be immunogenic in man. Other adjuvants include squalene, polysorbate 80 and sorbitan trioleate.

Clinical trials of glycoprotein vaccines

A gD2-alum vaccine increased HSV-specific antibody titre in a Phase I trial. The increases were larger than those reported in any previous trial.³¹ Twenty-four individuals with or without prior HSV-1 or -2 infection were vaccinated four times with 30 µg or 100 µg of gD2 in alum. The peak EIA (enzyme immunoassay) titres in seronegative individuals were higher than the initial titres in seropositive individuals.

The HSV-2 seropositive volunteers had 6–10-fold increases in their EIA titres and 6–7-fold increases in neutralizing antibody titres (Figure 2).³¹

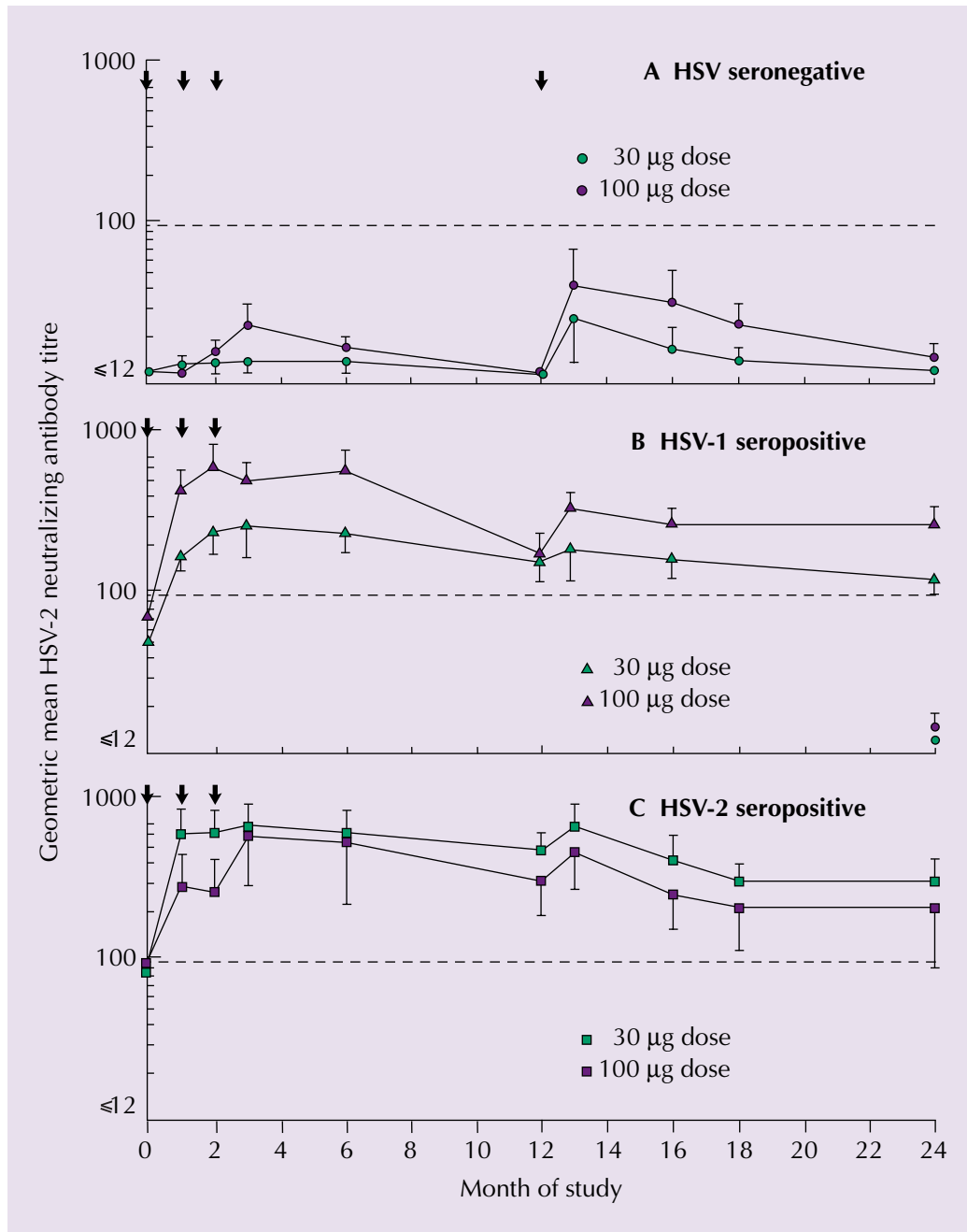


Figure 2: Neutralizing antibodies to HSV-2 in vaccine recipients. Arrows indicate vaccine administration. Dashed line indicates baseline mean gD2 titres in HSV-2-positive subjects³¹

The success of this preliminary trial led to a double-blind, placebo-controlled trial of the gD2-alum vaccine in 98 patients with recurrent genital herpes.³² These individuals had between 4 and 14 recurrences per year. They received injections of 100 µg gD2 in alum or a control of alum alone at entry into the study and at 2 months. The patients were followed for a year and their recurrences were confirmed by examination and virus culture.

By several criteria, the gD2 vaccine recipients experienced fewer genital herpes recurrences than placebo recipients. The gD2 recipients reported 24% fewer recurrences per month than the control group ($P=0.055$) and 36% fewer virologically confirmed

recurrences per month ($P=0.019$). Over the year of the study, there was a lower median number of recurrences in the vaccinees ($P=0.039$). The vaccine also increased neutralizing antibody titres 4-fold and gD2-specific EIA titres 7-fold (Figure 3).³²

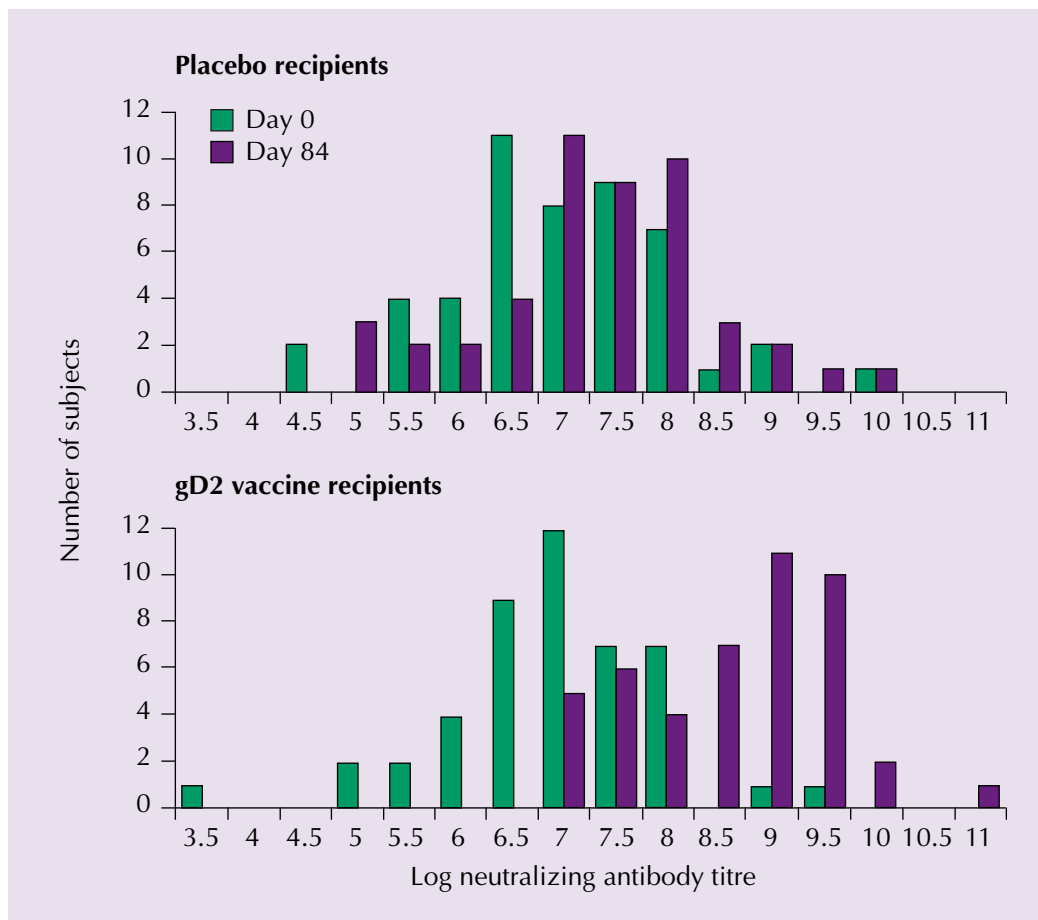


Figure 3: Neutralizing antibody titres to HSV-2 at baseline and 84 days after first vaccination³²

This was the first placebo-controlled trial in man to demonstrate that a vaccine could modify the course of a chronic viral disease. However, in this study, no critical determinant of the immune response was identified. Although there was a rise in antibody titre, this did not correlate with recurrence rates, which corresponds with the observations in guinea pigs.²⁸

The alum used in the trial is not a potent (immunogenic) adjuvant and, in an attempt to improve upon the vaccine, gD2 was combined with other adjuvants and glycoproteins. Animal models had shown that gB2 was immunogenic and effective in therapy of HSV infection. Therefore, the two glycoproteins were combined in one vaccine. It was also observed that the MF59 adjuvant boosted immunogenicity without unacceptable reactogenicity, whereas an MTP-PE adjuvant was excessively toxic.³³ Consequently, a vaccine containing gB2 and gD2 with MF59 was evaluated clinically.

The peak antibody titres in seronegative individuals following vaccination with the gB2gD2-MF59 vaccine were higher than those with the gD2-alum vaccine and the mean neutralizing antibody titre was higher than in individuals with naturally acquired HSV-2 infection. There were also sustained increases in T-cell responses with the gD2gB2-MF59 vaccine that were not observed with the gD2-alum vaccine.

As a consequence of these promising studies, the gB2gD2-MF59 vaccine entered several Phase III clinical trials. One of these trials was a double-blind, placebo controlled therapeutic study in 202 individuals with recurrent genital herpes.³⁴ Half of the participants received vaccine (gB2gD2-MF59) at 0, 2 and 12 months and placebo (MF59 only) at 14 months. The other half received placebo at 0 and 2 months and vaccine at 12 and 14 months. The participants were followed for 18 months, during which recurrences and cellular and humoral responses were measured.

Preliminary results indicate that the vaccine did not significantly reduce the monthly rate of herpes recurrences. However, a number of parameters were significantly reduced during vaccination: duration of symptoms (from 6.7 to 2.7 days, $P=0.002$) new lesion formation (from 6.9 to 4.1 days, $P=0.04$) and the duration of healing (from 8.8 to 6.8 days, $P=0.004$). In contrast, the duration of viral shedding was not significantly reduced (3.8 versus 2.7 days, $P=0.16$).³⁴ These results led the manufacturer to stop trials of the vaccine as therapy for HSV infection.

Another recombinant glycoprotein vaccine is currently undergoing clinical trials for immunotherapy of HSV infection. The vaccine consists of 20 µg of truncated gD2 vaccine adsorbed onto alum and administered with or without the adjuvant monophosphoryl lipid A (MPL). Initial trials established that the vaccine was well tolerated, stimulating humoral and cellular immune responses in seropositive and seronegative subjects. Another study in seronegative individuals showed that higher antibody levels were produced when the vaccine was combined with MPL.³⁵ The effect of MPL was confirmed in a 1-year follow-up study which showed that the vaccine-MPL combination engendered higher antibody levels, T-cell responses, IL-2 and γ -interferon levels than in the MPL-only group.³⁶ The vaccine is currently undergoing a Phase II/III trial for the immunotherapy of HSV infection.

Future Directions for HSV vaccine immunotherapy research

Many of the HSV immunotherapeutic vaccines have not been successful and there is currently no immunotherapeutic vaccine available with proven efficacy (see Table 5 for an overview)³⁷. Several reasons have been suggested for the failure of these vaccines.

	Inactivated vaccines	Live attenuated vaccines	Recombinant viral vaccines	Recombinant glycoproteins
◆ Safety				
Infectivity	No	Yes	Yes	No
Reactivation	No	Yes	No	No
Recombination	No	?	No	No
Oncogenicity	?	?	No	No
◆ Immunogenicity				
Potency	++	+++	+++	++
Durability	+	+++	++	+
Production cost	Moderate	Moderate	Low	Low

Table 5: Relative advantages and disadvantages of different types of HSV vaccines³⁷

One significant reason is that the critical immune responses important in controlling HSV reactivation are unknown. This lack of knowledge is a significant impediment to the rational development of effective vaccine immunotherapy. Many of the experimental vaccines have induced substantial rises in antibody as measured by ELISA (enzyme-linked immunosorbent assay) or by neutralization. However, the antibody response does not appear to predict the therapeutic efficacy of a vaccine. This may be because the assays do not measure the critical antibody (or antibodies) or it may be that other mechanisms are important for immunotherapy of recurrent infections. Animal studies have shown that vaccines which are effective in controlling recurrent infection also enhance specific lymphoproliferative cytotoxic and cytokine responses.^{38,39} Although caution must be applied before extrapolating the results of animal studies to man, it may be that identifying a vaccine that better stimulates cellular immunity may be more worthwhile.

It is also possible that immunotherapeutic vaccines fail because despite the fact that they may potentiate immune responses, this vaccine-induced immunity cannot deter repeated, high-level exposures and reactivating virus. In any case, the efficacy, cost and convenience of any of the vaccines developed to date are not competitive with preventative (suppressive) antiviral therapy.

Lessons can be learned from the experience of the development of immunotherapeutic vaccines. The ideal therapeutic vaccine must boost critical immune responses and the response induced must be durable. The vaccine must be safe and of low reactogenicity. Preferably, the vaccine should contain antigen or non-infectious virus. If a live virus is used, it should be of low virulence and be capable of inhibition by an antiviral drug.

Despite the discouraging history of the development of therapeutic vaccines, there is still much activity in the area. Trials results are awaited from the gD2-MPL vaccine and a number of new approaches are being explored, including live attenuated, defective and replication-incompetent viruses. In any case, it is important to define the protective and therapeutic immune responses to HSV as these can guide the development of vaccines for prophylaxis of HSV infection.

Current Status of Vaccines for the Prophylaxis of Herpes Simplex Virus Disease

Introduction

The development of vaccines for herpes simplex virus (HSV) infection has been aided by an understanding of the immune response to HSV. Innate and acquired immune mechanisms have been reported to play an important part in controlling the resolution of HSV infections. The growing knowledge of the biology of HSV and importance of the components of the acquired immune response have led to several development strategies for vaccines to enhance the acquired immune response and thereby combat HSV infection.

Immunology of HSV Infection

Immune responses to HSV infection involve both innate and acquired (adaptive) immune mechanisms. The innate responses serve to limit the virus early on in infection before the acquired immune response develops. The important components of the innate immune response include natural killer cells, cytokines and macrophages. The natural killer cells lyse certain target cells that are infected with the virus and, as they are effective without prior exposure, they are an important defence mechanism in primary infection. The relatively low natural killer cell function and reduced production of some cytokines may contribute to the susceptibility of newborn babies to herpesvirus infections.¹

The acquired immune response consists of two arms; the cellular immune response and the humoral response. The relative importance of these two components is unresolved.

Humoral response

Following HSV infection, antibodies develop to a variety of virus-specific antigenic targets which include nonstructural proteins (e.g. immediate early gene products) and structural proteins (e.g. major and minor glycoproteins). The antibody neutralization of extracellular HSV may be particularly important early on in infection. Antibodies may also play a part in resolving established HSV infection.

Type-specific antibodies protect against vertical transmission of HSV type 2 (HSV-2) from mother to infant, an observation which lends further support to the importance of antibody. In an extension of a study first published by Brown *et al*, 1991,² 31 800 women had a herpes culture and a serum sample taken within 48 hours of delivery and asymptomatic shedding was detected in 122 women.³ Results from Western blot serotyping were available for 116 of these 122 women (Figure 1).

Among these 116 women, 96 women had evidence of reactivation of HSV at delivery: 89 with HSV-2 and seven with HSV type 1 (HSV-1). There were no cases of neonatal herpes in the 89 infants born to the women with HSV-2. Of the seven women with reactivation of genital HSV-1 disease, there was one case of neonatal herpes. In contrast, among women acquiring first-episode infection at or near term, neonatal herpes was common. Of six primary infections (three HSV-1 infections, three HSV-2

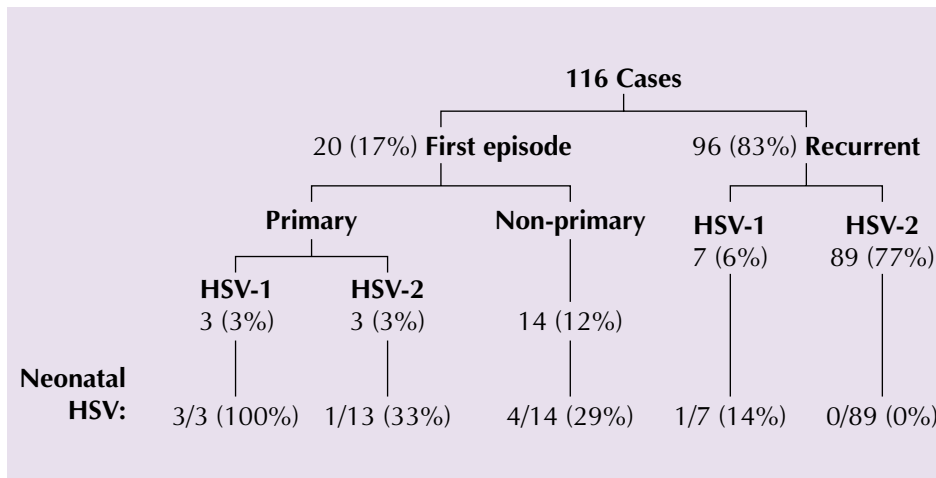


Figure 1: The incidence of neonatal herpes in children born to seropositive women³

infections), there were four instances of transmission to the neonate (three of three HSV-1 infections; one of three HSV-2 infections). In a non-primary first episode infection (i.e. an HSV-2 infection in an HSV-1 seropositive mother) there were 14 births and four cases of neonatal herpes (see Figure 1).³ These data suggest that the presence of HSV antibody is associated with a lower risk of neonatal herpes. This is confirmed by previous studies that have shown an inverse association between maternal neutralizing antibodies and acquisition of HSV-2 infection in infants.^{4,5} The perceived importance of antibodies has led to the development of vaccines that preferentially stimulate the humoral immune response.

Cellular immune responses

It is unlikely that the humoral response alone is sufficient to prevent HSV infection or disease; HSV can spread from cell to cell in the presence of antibodies and some studies have noted little association between antibody levels and clinical status.⁶ However, support for the importance of another effector mechanism is provided by the observation that B-cell suppressed mice can clear primary HSV-1 infections.⁷ Adoptive transfer experiments have shown that CD4 and CD8 T-cell subsets can clear virus from the site of infection and effect recovery from infection.⁸ Furthermore, adoptively transferred HSV-immune T-cells homed to the urogenital mucosa and protected mice from lethal challenge against wild type HSV-2.⁹

Other evidence for the importance of T-cells comes from observations that clinical disease is most severe in persons with depressed T-cell immunity and other defects in cell-mediated immunity, but that agammaglobulinaemics can respond well to HSV infection with normal patterns of recurrences.¹⁰

The relative importance of CD4 cells and CD8 cells in the response to HSV is unresolved. Adoptive immunotherapy in different animal models has shown protection with both CD4 and CD8 cell types.

Measurements of T-cell responses in genital lesions reveal that resolution of the lesions does not begin until cytotoxic T-cell (CTLs) are recruited into the lesions. This HSV CTL activity is detected very early on in the course of a lesion and increases over time.¹¹ CTLs, which have the CD8 phenotype, recognize the viral antigens in

association with major histocompatibility complex (MHC) class I gene products and destroy the infected cell. For a vaccine to induce a CTL response, it must have the appropriate epitopes (antigenic sites) and present the antigens in such a way that it can be taken up by cells bearing MHC class I molecules.

CD4 T-cells are also important in mounting immune responses to HSV infection. They are vital in stimulating the CTL and antibody responses. These CD4 cells also develop antigen-specific responses which result in cytokine release or cytotoxic responses that can aid immunity. The CD4 cells also mediate delayed type hypersensitivity which is thought to be important in recovery from cutaneous HSV disease.¹²

Importance of humoral and cell-mediated immunity

It is likely that both humoral and cellular immunity are important in controlling the response to virus infection and it is unlikely that any one immune response alone is *protective*. It may be that antibody responses are important for controlling primary infection and that cell-mediated immunity is more important once an infection is established. The aim of vaccine therapy must be to augment the immune response. The approaches developed to date have explored modulation of one or both arms of the immune response in order to make a discernible clinical difference.

Requirements of a Prophylactic HSV Vaccine

There are three ideal requirements for a prophylactic (preventative) vaccine for HSV infection. It should:

- ◆ Prevent infection (a difficult goal)
- ◆ Prevent clinical manifestations of the infection, i.e. prevent clinical disease
- ◆ Prevent the establishment of viral latency and thus the reactivation source to decrease the potential infectivity over time.

Prevention of infection

The pathogenesis of primary genital HSV infection is shown in Figure 2.¹³ Prevention of infection must block immediate viral replication upon initial exposure to the infecting antigen, i.e. sterilizing immunity. In addition, an effective vaccine must prevent replication before virus becomes latent in the sensory ganglia, where it is relatively protected from the immune system.

There are several challenges for the immune system in mounting a protective immune response. First, unlike many other viruses (e.g. primary varicella zoster virus [VZV] infection), HSV does not have a viraemic phase that leads to its dissemination or its subsequent reactivation. Second, the time in which to develop an effective immune response upon exposure to HSV infection is shorter than following VZV and other herpesviruses infections. These two factors combine to make HSV a difficult virus to develop a prophylactic vaccine for; if the vaccine is to be effective, the level of immunity that exists on encountering the wild-type virus must be high.

Preventing disease

Preventing the manifestations of clinical disease may not be a major requirement for an HSV vaccine. Only 20% of HSV-2 seropositive persons seek medical attention for

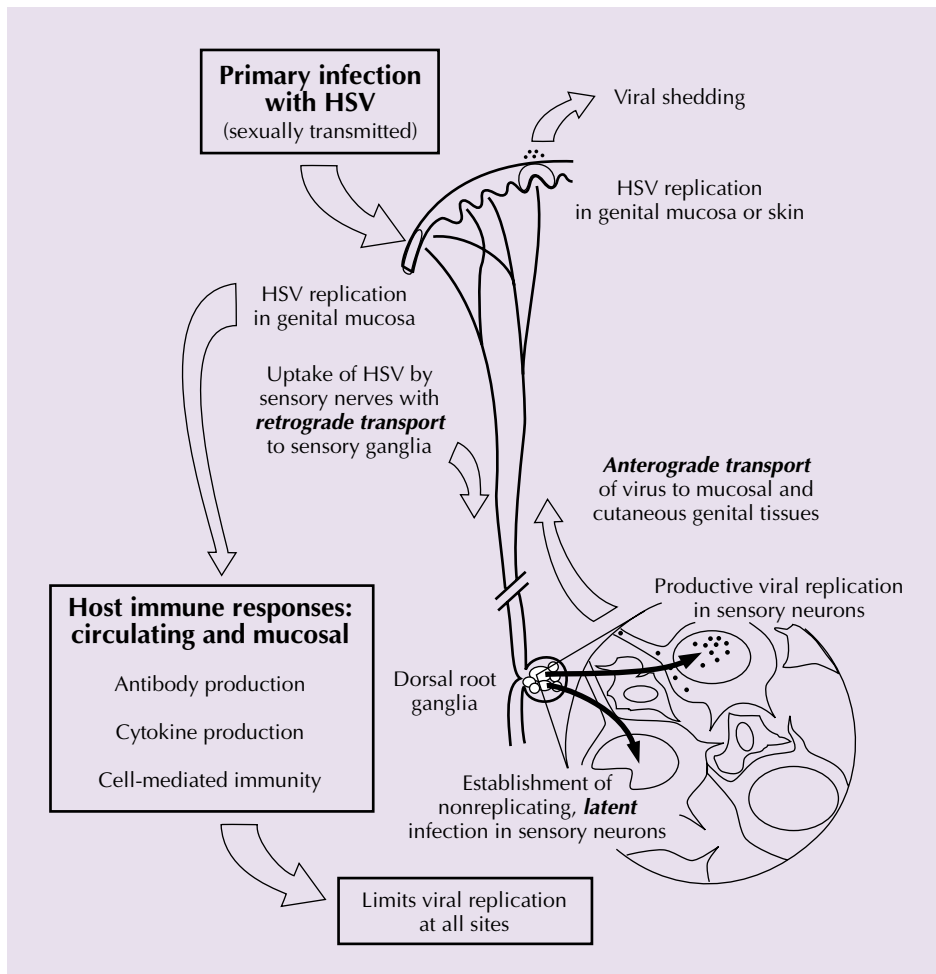


Figure 2: Pathogenesis of primary genital HSV infection¹³

primary infection^{14,15} even though 80% of first-episode acquisitions are symptomatic.¹⁶ Furthermore, a vaccine that prevented symptoms but did not alter the long-term natural history of reactivation, or had no effect on asymptomatic shedding, may be detrimental overall. The outcome could be an increase in the reservoir of people with undiagnosed infection, so leading to higher rates of transmission.

However, a disease-preventing vaccine that did not prevent latency or change the natural history of the disease may be useful for selected patient populations such as seronegative pregnant women and seronegative transplant recipients, and to prevent neonatal herpes. In these instances where HSV disease can cause serious complications, prevention of the disease would have obvious benefits even though HSV may establish latency.

Support for the Concept of a Prophylactic HSV Vaccine

There are some observations which support the protective effect of pre-existing immune responses and suggest that it is feasible to produce a disease-preventing vaccine. One line of evidence is that reinfection by the same subtype at the same anatomical site is rare.^{17,18} Further support comes from the observation that transmission to the neonate is uncommon in HSV-2 seropositive mothers, suggesting that maternal immunity reduces the frequency and severity of the disease.^{2,19,20}

In addition to these observations that pre-existing immunity is important, the targets against which the immune system is directed are remarkably constant in HSV. There is little strain differentiation in the major surface proteins of HSV (and other herpesviruses), in contrast to the persistent RNA viruses which use antigenic change as a major mechanism of their persistence.²¹ For example, glycoprotein (g) D is a highly conserved protein.²² Importantly, gD is a confirmed target of neutralizing antibody, antibody-dependent cellular cytotoxic responses and of virus-specific immune responses mediated by CD4 and CD8 cells.²³ In addition to showing similarity between strains, gD has been sequenced over time from people who have a reactivation and has shown no sequence changes.²⁴ Therefore, stimulating immune responses to HSV antigens may offer protection against infection with HSV.

Potential HSV Vaccines

There is a variety of potential vaccines for prophylaxis of HSV infection and disease. These vaccines have been investigated in animal models and several have undergone clinical trials in man (Table 1).

- ◆ Attenuated live virus vaccines
- ◆ Disabled infectious single cycle (DISC) virus vaccines
- ◆ Modified live virus subunit vaccines

Table 1: Types of vaccines in development

Attenuated live virus vaccines

Attenuated live virus vaccines cause an infection and establish latency in the ganglia. Ideally there should be little or no reactivation even during severe immunosuppression. One attenuated live virus vaccine is R7020, an HSV-1 strain that has been genetically attenuated by deleting sequences of viral DNA.²⁵ The deletions reduced the ability of the virus to establish

latency and also created space for insertion of sequences encoding the glycoproteins G and D. The gG sequence was inserted to act as a marker of successful immunization and the gD sequence was added as it was known to be immunogenic.²⁵

The modified virus was found to be stable and attenuated in a number of animal models. In the same models, R7020 protected against wild-type challenge and latency was established at a reduced rate (Table 2).²⁵ However, in man it was poorly immunogenic and as a consequence, serial administration of very high (10^5 plaque forming units [pfus]) doses of R7020 were required to elicit an immune response. As well as its poor immunogenicity, R7020 was not well tolerated in HSV-1 seropositive individuals in whom it caused adverse events that were severe enough to stop the clinical trial.²⁶

Although, to date, success with live attenuated vaccines is limited, if a less attenuated construct can be developed, this option offers the best immunological approach. However, there are issues about the stability of attenuation as genetic recombination could occur with wild-type virus. There is also the concern that the attenuated vaccine may reactivate from latency in the immunocompromised patient.

Disabled infectious single cycle virus vaccines

Disabled infectious single cycle (DISC) virus vaccines are also known as replication defective viruses. They are a new approach in the development of virus vaccines and are derived from a virus that has had an essential gene deleted (Figure 3). The viruses

Test measurements	No. positive for presence of latent virus/ total no. tested after immunization			
	Control	Intradermal		Intramuscular
		HSV-2 (G) (10 ⁷ pfu)	R7020 (10 ⁸ pfu)	R7020 (10 ⁸ pfu)
Vaginal shedding	10/10	1/5	9/10	10/10
Genital lesions	9/10	1/5	7/10	7/10
Paralysis	7/10	0/5	0/10	1/10
Death	5/10	0/5	0/10	0/10
Latency:				
Total	5/5	1/5	2/10	2/10
L3–L6	2/5		0/10	2/10
S1–S3	4/5		2/10	1/10

*L, lumbar; S, sacral

Table 2: Protection of guinea pigs against intravaginal challenge with HSV-2 (G) after immunization with R7020 (adapted from reference 25)²⁵

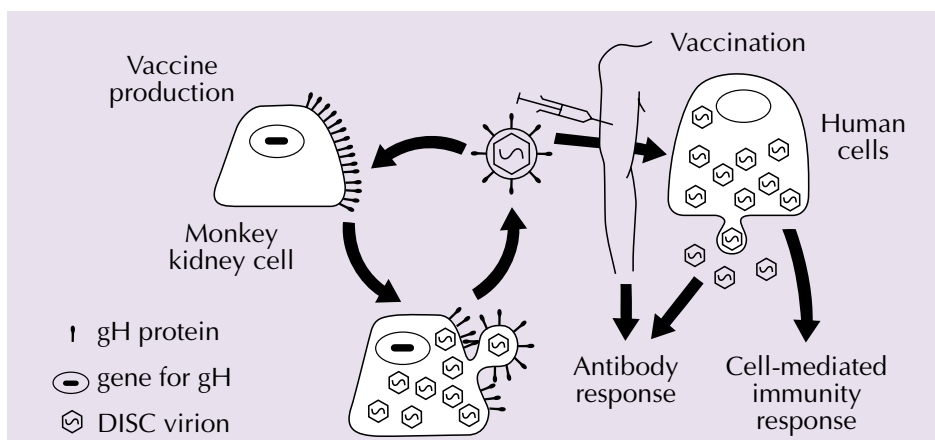


Figure 3: How DISC vaccines work²⁷

with the gene deletion are then cultured in monkey kidney cells which have been genetically engineered to express the missing gene, which allows the assembly of a fully functional virus. After these modified viruses infect host cells, the viral replication cycle will proceed to a point where the missing gene product is required.²⁸ If this is early in the cycle, then viral proteins will accumulate; if it is later in the cycle, then most of the cycle may be completed but no infectious virus will be produced.

The DISC viruses have several features that make them attractive candidates for vaccines. They should be unable to spread in the host because of the deletion of an essential gene and because they are inactivated they may be given in relatively large amounts to generate humoral immune responses. After inoculation, because the DISC virus has most of the structural proteins, it will stimulate an immune response against all these proteins rather than the selected antigens that are used in subunit vaccines. Another important property is that the viral antigens that are produced after the single cycle of replication can be presented in conjunction with MHC class I molecules and stimulate a broad cell-mediated response to viral infection.

There are two versions of DISC virus vaccines currently in development. In one version, either the ICP-27 gene or the ICP-8 gene is deleted. Both these genes are essential for enabling late gene expression. Although these genes have been deleted, the DISC virus can enter the host cell and begin to replicate. However, the replication cycle stops at the point where the deleted gene is required and so the earlier gene products accumulate. These gene products can be processed by antigen presenting cells and stimulate immunity.

In the other type of DISC vaccine, the glycoprotein (gH) gene has been deleted in an HSV-1 and -2 strain. This gH gene encodes a glycoprotein essential for virus entry into cells and thus it is necessary for the vaccine to be propagated in cells which supply the missing function. However, despite this deletion, the virus can complete a single cycle of replication, leading to the synthesis of all the viral proteins except gH. The result is the assembly and release of fully formed but non-infectious progeny virions.²⁹

The DISC vaccines produced have been shown to be immunogenic. They stimulate antibody responses and T-cell responses. Although the cellular immune response is low, it appears quickly upon challenge. The DISC vaccines have also been shown to ameliorate disease upon challenge and reduce latency in animal models. Guinea pigs vaccinated with the gH deleted HSV-1 or HSV-2 DISC viruses have been shown to be protected from disease when challenged with wild-type HSV-2 (Figure 4).^{28,30,31}

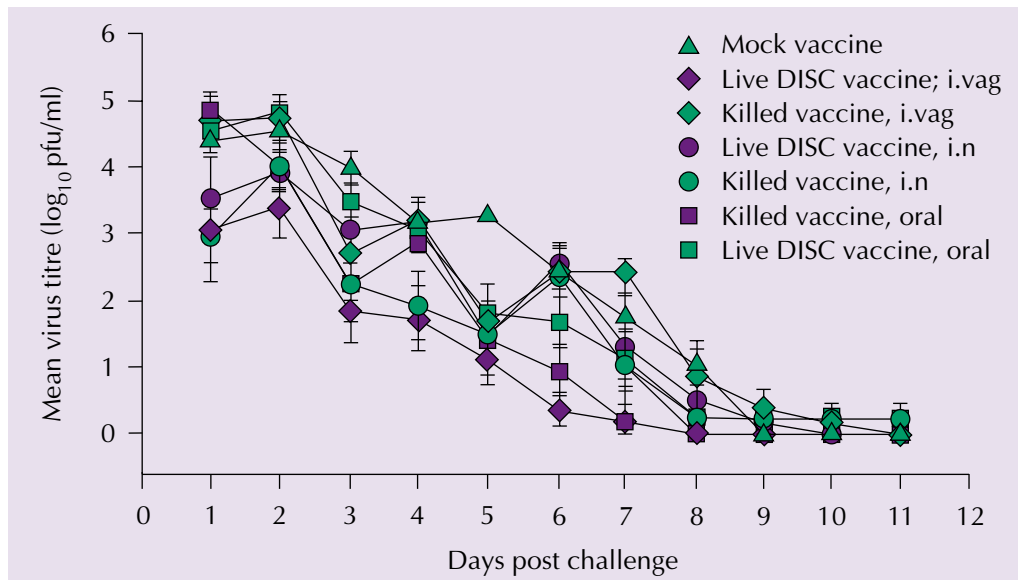


Figure 4: Mean virus titre in guinea pigs challenged with HSV-2 after immunization with HSV-2 DISC vaccine.³⁰ i.vag = intravaginal; i.n. = intranasal

Animals vaccinated with the HSV-1 DISC virus showed substantial but not complete protection against primary and recurrent disease.²⁸ Higher levels of protection against primary and recurrent disease were provided in the same model using the HSV-2 DISC vaccine^{30,31} (Figure 5).³¹ In animals receiving two doses of HSV-2 DISC virus (10^7 pfu), there was complete protection against primary disease and a 98.6% reduction in recurrent disease symptoms compared with the control group ($P < 0.0001$). Furthermore, there was also complete protection against primary disease even when the guinea pigs were challenged 6 months after vaccination. In animals receiving one dose of the HSV-2 DISC vaccine (10^7 pfu/dose), this long-term protection was greater than 99% when

compared with the control group ($P < 0.0001$). The protection from disease symptoms was 100% after double- and triple-dose vaccinations ($P < 0.0001$ in both cases). In addition, virus shedding was also reduced after challenge with wild-type virus.³¹ The DISC virus vaccine has also generated CTL responses against HSV antigens.³⁰

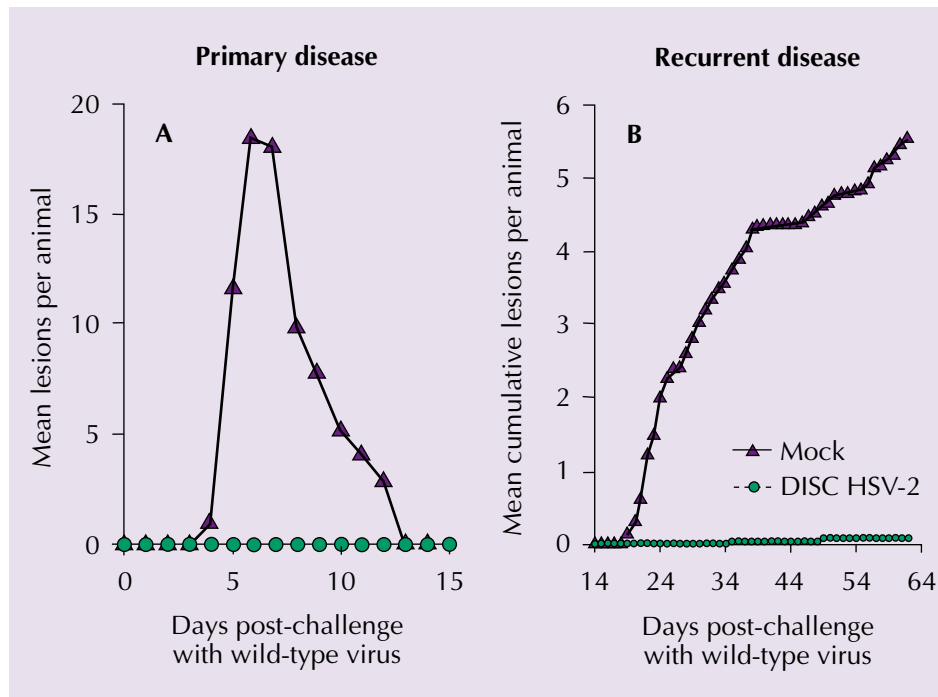


Figure 5: Protection against primary and recurrent genital herpes in an animal model after vaccination with HSV-2 DISC virus vaccine³¹

Thus, the HSV-2 DISC virus vaccine provided high levels of protection against disease symptoms and this response was long-lasting and substantially reduced replication of challenge virus.^{30,31} The duration of immunity is clearly of critical importance to any vaccine but the reduction in viral shedding also provides the theoretical advantage of a decreased risk of transmission.

Following these promising studies, the DISC virus vaccines are currently undergoing trials to evaluate their safety and immunogenicity in man. Initial results indicate that the DISC viruses are immunogenic, suggesting that this is an attractive approach to development of a prophylactic vaccine.³²

Modified live virus subunit vaccines

An alternative approach is to incorporate the genes for subunits of HSV into another virus. This virus would infect the host and express its own proteins and those of the HSV subunit genes inserted into it.

Vaccinia virus has been used as a vector for HSV genes in several studies. These vaccines have been shown to be effective in preventing infection in animal models. A vaccinia virus expressing gD protected mice against a lethal challenge of HSV-1 and prevented latency in two-thirds of ganglia.^{33,34} The protection and T-cell response was greater if the vaccinia-gD vaccine used an early promoter that produced gD within 2 hours after inoculation than if the gD was under control of a late promoter.³⁵ The responses to the vaccine lasted for over a year and could be boosted.³⁵

Despite its apparent efficacy, there are concerns about the safety of the vaccinia-gD vaccine in man.³⁶ Immunocompromised individuals and those with skin diseases are susceptible to severe vaccinia infection, and there may be spread from vaccinees to these individuals. In addition, vaccinia can produce rare but severe disease in healthy people.

An alternative vector is the adenovirus. A recombinant adenovirus expressing gB, when given intraperitoneally, protected mice from a lethal challenge of HSV-2.⁹ When the adenovirus-gB vaccine was given intranasally, it also protected against intranasal challenge and stimulated mucosal and cell-mediated immune responses.³⁷

There are other viruses that are potential vectors (see Table 3), one of which is canarypox. A cloned isolate of an attenuated canarypox vaccine strain has been designated ALVAC. Although canarypox does not replicate in mammalian species, an ALVAC recombinant can be as potent at stimulating immune responses in mammalian target species as a replication competent vaccinia virus recombinant.³⁸ In HIV vaccine trials it has resulted in CD8 and CTL responses that are comparable to responses to the vaccinia virus. ALVAC has been proposed as a candidate vector for an HSV vaccine.






	Vaccinia virus
	Pox viruses
	Rhinovirus
	VZV
	Poliovirus

Table 3: Candidate viruses for use as vectors in an HSV vaccine

Nucleic acid vaccines

Nucleic acid vaccines utilize the observation that when DNA is inoculated into muscle tissue the gene encoded in the DNA is expressed, resulting in presentation of a conformational protein or peptide which is processed by antigen-presenting cells and transported to the regional lymph nodes where it can stimulate antibody and cellular responses (Figure 6).³⁸ The response that is stimulated by injection of DNA (100 µg of DNA may contain approximately 13×10^9 copies of a gene) may be greater than if an equivalent amount of protein is injected.

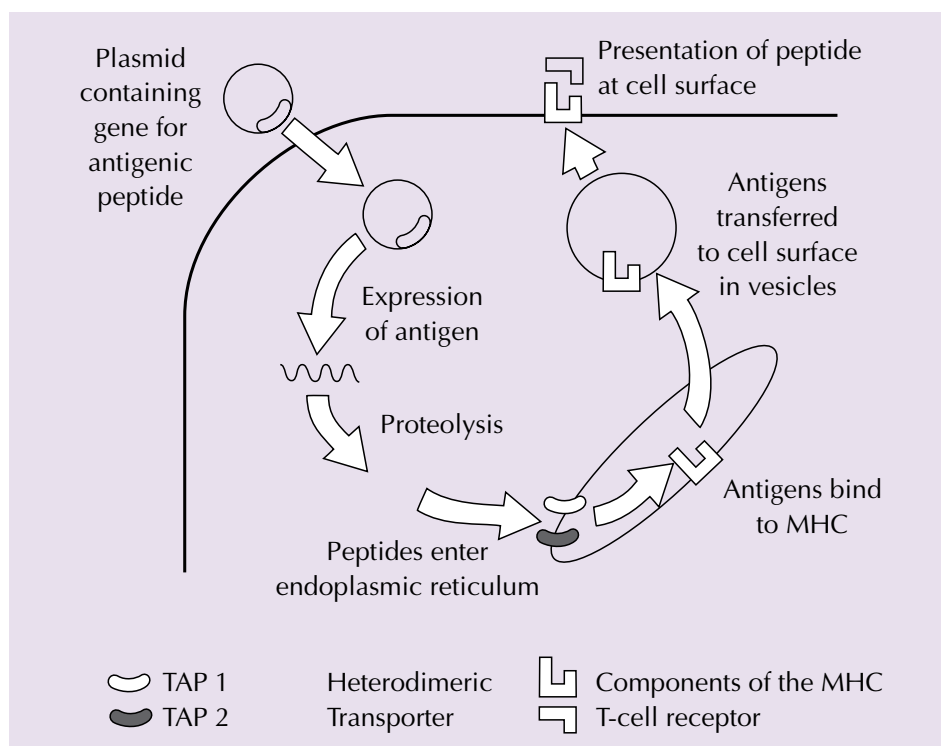


Figure 6: Antigen presentation after DNA injection (adapted from reference 39)³⁹

The advantages of a nucleic acid-based immunogen is that it allows presentation for class I and class II major histocompatibility complex (MHC) responses. This will result in a broader, and perhaps more effective, immune response. Multiple epitopes (antigenic sites) can also be presented in a single formulation and so stimulate a wide variety of responses. The nucleic acid vaccine approach has been investigated for Hepatitis B, HIV, influenza and as potential vaccines for HSV.

Plasmid DNA containing the ICP-27 gene of HSV-1 (pc-ICP 27) has been shown to induce a protective immune response in mice (Table 4).⁴⁰ Mice were immunized twice with intramuscular injections of pc-ICP 27. They were then challenged with HSV-1 and the nature of the immune response measured. There was protection, as measured by lesion development, in 70–80% of immunized mice. The mice also exhibited HSV-specific lymphoproliferation, MHC I restricted CTL activity and type I cytokine production. Adoptive transfer studies revealed that recipients of CD4 cells but not CD8 cells were protected from subsequent challenge.⁴⁰

Immunogen	Days post-challenge (% of mice developing lesion) ^a			
	Day 6 ^b	Day 10	Day 14	Day 21
pc-ICP 27	0	30 (2.3)	30 (NS) ^c	30 (NS)
Vector	10 (1.0)	100 (3.3)	100 (NS) ^d	100 (NS)
HSV-1 (KOS)	0	0	0	0
None	20 (1.0)	90 (3.9)	100 (NS)	100 (NS)

^a BALB/c mice (3–4 weeks old) were immunized with 90 µg of DNA or with HSV-1 (Strain KOS) (10⁷ TCID₅₀) in the quadriceps muscle on Days 0 and 7. On Day 14, the mice were challenged with 10 ID₅₀ (10⁴ TCID₅₀) of HSV-1. The table represents one of the three experiments done using 10 mice per group. The values in parentheses indicate mean severity scores of the animal with positive lesions. Mortality of the animals began at 10 days post-challenge and all were dead by Day 14. The lesions were graded as 1+ to 4+ depending on severity.

^b Day 6 = 6 days post-virus challenge.

^c All 30% of the mice which developed lesions died previously.

^d All the mice died previously.

Table 4: HSV lesions in mice immunized with p-ICP 27⁴⁰

Although the injection of pc-ICP 27 was protective, the level of protection was not as great as that after vaccination with live virus.⁴⁰ This may have been due to quantitative differences in cellular immune responses observed and because injection of pc-ICP 27 failed to induce an antibody response. Antibody may play a role in clearing virus and therefore, the effectiveness of pc-ICP 27 in conjunction with gB DNA, which induces antibody, is being investigated to see if efficacy can be improved.⁴⁰

Nucleic acid vaccination with plasmids containing the DNA sequence for HSV-2 gD2 (pc-gD2) has been shown to be immunogenic and provides protection against HSV-2 induced disease in mice (Figure 7).⁴¹ Three immunizations with pc-gD2 resulted in lower virus titres from vaginal secretions than in control animals. Addition of a single injection of 0.1 µg of Vitamin D3 at the beginning of the vaccination series appeared to enhance protection but did not significantly enhance levels of antibody. The vaccinated animals also produced gD2-specific serum antibodies and HSV-2 neutralizing antibodies which were not seen in the controls. Cellular immune responses were not studied in this model.⁴¹

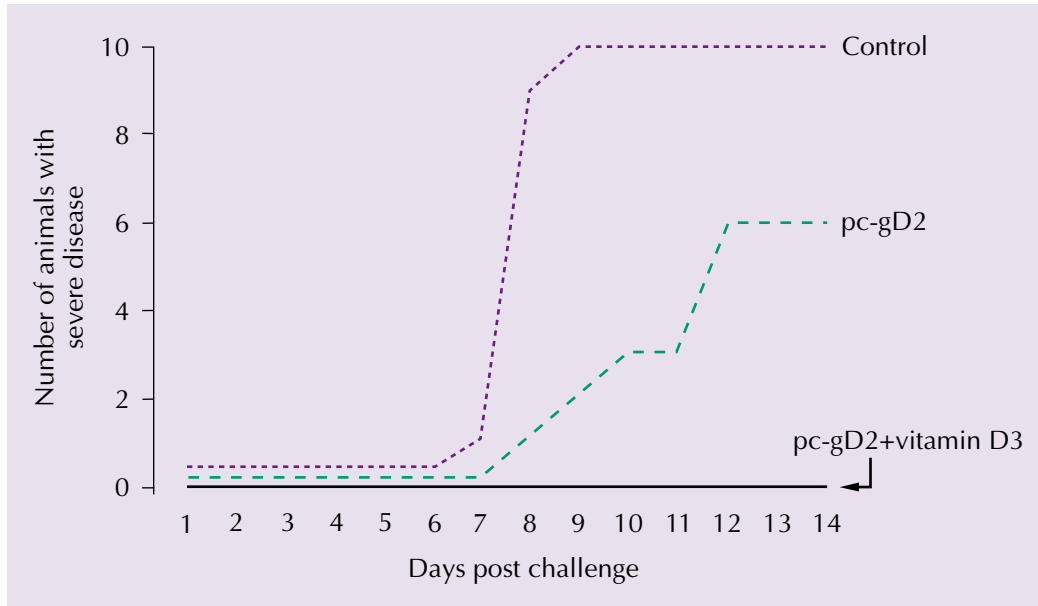


Figure 7: Time course of severe disease in animals injected with pc-gD2⁴¹

Another nucleic acid vaccine that also encodes gD2, but is under the control of the cytomegalovirus immediate-early gene promoter, also offered protection against intravaginal HSV-2 challenge in a guinea pig model.⁴² The vaccinated animals exhibited protection from primary genital HSV-2 disease and there was little or no development of lesions. After recovery from primary infection, the guinea pigs had significantly fewer recurrences and significantly less latent HSV-2 as judged by the amount of genomic DNA detected in the sacral dorsal root ganglia.⁴² Nucleic acid vaccines show promise in animal models for the prophylaxis of HSV infection and are at an early stage of investigation in man.

Conclusions

The first step to diagnosis of suspected herpes simplex virus (HSV) infection is to obtain a sample from the infected area to be analysed by a laboratory. This is a general principle which should be applied when herpesvirus infection is suspected.

Diagnosis of Genital HSV Infection

Any patient presenting for the first time with suspected genital HSV infection should have a swab taken for virus culture. If the patient has a symptom or a sign (lesion, blister, ulcer), this is the first step of diagnosis.

Tests are important for the subsequent management of the patient. Following the first presentation with genital herpes, the physician should have the test results before that patient returns for the next consultation. Accuracy with diagnosis of mucocutaneous herpesvirus infections in the immunocompetent adult is important, but as long as the test result is back before the next patient visit, it is not necessary to have the result immediately.

Many people who have genital HSV infection but with symptoms suggesting a history of herpes who have now just been diagnosed may have previously had a negative swab test result. Physicians must recognize that a swab may give a false negative for HSV and so a negative test result does not rule out genital HSV infection.

Should physicians routinely take a swab, even if a patient has typical lesions and clinical diagnosis seems straightforward? If the patient presents with presumed genital herpes and on examination has classic vesicles, a swab should still be taken to confirm the diagnosis. Every patient with genital herpes should receive a laboratory-confirmed diagnosis, regardless of whether it is the first episode or not.

In a presentation of first episode genital herpes, the virus should be typed because this gives important prognostic information. Typing can assist in providing accurate information on frequency of recurrences to the patient.

If the first test is negative, the patient should be invited to return for another swab/culture as soon as possible when they next have symptoms in case it leads to a diagnosis of genital HSV.

If the patient has had one positive diagnosis of genital herpes and the physician has explained the natural history and implications, the patient is likely to return to the physician, with recurrent episodes. There is an indication for repeating the culture only if there is an unusual presentation or symptoms, because the patient may have an additional infection, or blistering dermatosis.

Serial swabs may be useful for monitoring an immunocompromised patient. For example, take the case of an individual with HIV infection and disease with a CD4 count of 2 cells/ μ l. The physician takes a swab from a perianal ulcer. The physician might initiate oral aciclovir therapy, but not be completely sure about the dose, and whether the patient will respond. The patient could be asked to return to the clinic

7–10 days later for a further swab which would indicate whether the culture had converted to negative. This, together with the clinical response, would tell the physician how much longer the patient should be treated, or whether reduced sensitivity to the drug is present.

Diagnosis of HSV Encephalitis

For cases of suspected HSV encephalitis, it is strongly recommended that the physician obtain a sample of cerebrospinal fluid (CSF) and ask for a polymerase chain reaction (PCR) analysis as quickly as possible.

- ◆ If PCR is not available or until PCR results are obtained, the infection can be confirmed by antibody assay; all patients with a positive PCR sample are recommended to be treated with aciclovir. The patient should be treated empirically until the test confirms the diagnosis.
- ◆ If the PCR sample is negative, but the physician feels that clinically the patient has encephalitis (possibly if the sample was not taken early enough after the onset of infection), empirical treatment should be continued.
- ◆ CSF can be taken 1 week after the initiation of aciclovir therapy as 80–85% of patients still have a CSF sample that is HSV-positive.
- ◆ If the PCR is negative, the CSF sampling should be repeated. If it is negative, twice and if there is an alternative diagnosis, aciclovir treatment can be stopped; if there is not an alternative diagnosis, repeat the CSF sampling and consider other diagnoses.

Diagnosis of Varicella Zoster Virus Infection

If an otherwise healthy child presents with classic varicella, is there ever any indication for taking a swab for analysis? Swabs are appropriate only if the child has been vaccinated; they have a history of having had varicella zoster virus (VZV) or if the presentation is atypical. In these cases the test of choice is immunofluorescence as the virus grows slowly in culture. However, in patients with a history of vaccination, culture is important to establish whether lesions are due to a reactivation of the vaccine virus, or if it is a wild type infection. Serology is less useful in patients with a history of VZV infection.

If an immunocompromised patient presents with widespread vesicular lesions which the clinician believes are due to VZV infection, the diagnosis should be confirmed. In these patients, cases may present which appear to be typical varicella, but which may be a result of drug reactions.

Lesion culture may assist in diagnosis of atypical presentations of herpes zoster or disseminated lesions in the elderly immunocompetent patient.

Diagnosis of Ocular HSV Infections

It is recommended that a patient who presents with suspected ocular HSV should have diagnosis confirmed by a swab although it can be difficult to culture virus. Who should take the swab depends on the way in which health-care is organized in that country and

other factors such as the availability of laboratory facilities. Taking a culture should be the first step in referral to an ophthalmologist.

Diagnosis of Orolabial HSV Infections

For labial HSV infections and primary gingivostomatitis, the diagnosis can usually be made by inspection. However, it can be a morbid infection in small children, who may become dehydrated and unable to eat.

With gingivostomatitis in children, the differential diagnosis includes Coxsackie virus infection. However, the most important issue relating to collection of a culture from a child with vesicular lesions of the oral cavity is whether it will impact on, or lead to, treatment. A swab may be particularly helpful in atypical or difficult cases of gingivostomatitis.

For pharyngitis, which paediatricians and medical practitioners dealing with adolescents see frequently, herpes is rarely considered as a diagnosis. However, pharyngitis may be caused by herpesvirus infection and, in certain populations, is frequently caused by it. The only way to establish this diagnosis is by culture, rather than by history. The symptoms associated with herpes pharyngitis can last weeks and should be treated.

Classic cases of orolabial HSV should be swabbed for culture only in immunocompromised patients. However, as aciclovir cream is now available over the counter (OTC) in some countries, there might also be value to know that infection is due to HSV, before the patient uses OTC medications. It is better clinical practice to help guide long-term self-medication.

Cultures should be obtained from those who have atypical or problematic recurrent orolabial lesions, and where the diagnosis is uncertain.

A clear indication for a diagnostic test is where it can demonstrate to both the physician and the patient that infection is due to HSV, rather than VZV or other infections, at sites other than the genitals, eye or oral cavity.

The Use of Diagnostic Tests and Serodiagnosis for Genital HSV Infections in Primary Health-Care Settings

Under-diagnosis of genital HSV infection is a problem. Serological studies show that the infection is common, but questionnaire studies of primary health-care physicians suggest that they never see it – they rarely make the diagnosis or recognize the disease.

On first clinical presentation in primary health-care settings, approximately 50% of patients with HSV infection appear to be misdiagnosed with *Candida* or other causes of vulvitis. In a study of patients in Sheffield, UK, about 30% had made three visits to their primary health-care physician before the diagnosis of HSV was made, and, in some cases, before this diagnosis was actually *considered* by their physician.

Under-diagnosis and misdiagnosis of genital HSV infection may result from physicians' perceptions and appreciation of the clinical spectrum of genital herpes, and their own

expertise and comfort in ordering and interpreting the tests. Often, when physicians are trained, they are shown typical lesions of genital herpes (Figure 1a), and to look for blisters and ulcers but atypical presentations are common (Figure 1b).

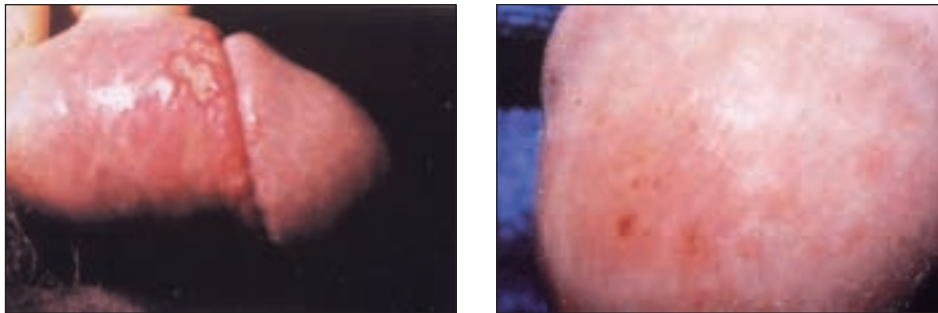


Figure 1: Typical (a) and atypical (b) presentations of genital HSV infection

Infection with HSV is very much more common than recognized disease. Of infected individuals, up to 80% will either have no symptoms or have unrecognized disease. However, clinically apparent disease can be managed.

What level of expertise is it reasonable to expect primary health-care physicians to have? What are the practical benefits for patient care from adopting diagnostic guidelines? More cases will be diagnosed, but will this lead to any real developments and advantages in patient care? What about the resource implications?

Practitioners should have simple protocol and guidelines that can be applied. Swabs need to be easy to take and to transport to a laboratory. Primary care physicians think that they do not see very many cases of genital HSV infection and therefore test kits need to have a long shelf-life. For serological tests, the physician should ideally have to take a single sample, the same test should be applicable for different disease stages, it should give a reliable result, and not be prohibitively expensive.

One way to approach the diagnosis of HSV infections would be to provide general practitioners with a kit containing a single swab and a single blood bottle, with instructions that if the patient presents with a lesion, it should be swabbed. If the physician suspects HSV infection, but there are no lesions present, they should take a blood sample. These samples should be sent to a laboratory and appropriate analysis based on the samples and what the physician has seen clinically. Results should be sent back or further samples requested. Protocols advising laboratories how to manage these requests would be necessary, as well as guidelines for the interpretation of results. More primary health-care physicians should be encouraged to consider a diagnosis of HSV infection and be prepared to take the tests in the first place.

Physicians should be encouraged to integrate tests into existing management protocols. An existing example of this strategy is the management of jaundice in primary care, physicians are not expected to be aware of the current spectrum of viral causes but instead they fill in clinical details, and send blood and stool samples to the laboratory, where the appropriate tests are performed. In contrast, if the physician sees a patient with vulvitis, they have to ask for each individual organism they want culturing and send the appropriate swab, so it is not surprising that they send a *Candida* swab and only ask for that.

What are the Advantages of Improving Diagnosis?

Genital HSV infection could be managed with fewer visits to the primary care physician. The diagnosis of herpes could be normalized, so that it becomes a disease which no longer requires a visit to a STD clinical for the full range of tests, counselling and long-term follow-up. If primary care physicians acquire the habit of diagnosing the disease, their skills in this field will improve.

The economic impact of increased testing and diagnosis has to be considered. Diagnostic tests have a cost, and counselling will require increased health-care provider time. There will also be a cost consequence of increased antiviral usage and, if there is poor management, more time will need to be spent in patient management.

From a broader public health standpoint, genital ulcerative disease is associated with increased risk for HIV acquisition. Therefore, for high-risk individuals with multiple sexual partners, knowing that they are seropositive for HSV type 2 (HSV-2) and may have unrecognized recurrent lesions, could be of value in the prevention of acquisition of HIV, particularly if they have unprotected sex.

The importance of serological screening of pregnant women for HSV, to prevent neonatal herpes may depend on its incidences in a particular country. For example, routine screening of pregnant women in countries where the incidence is low, such as the UK, may not be practical, whilst in other areas of high incidence, such as parts of the USA, routine screening may be valuable.

Before wider serological testing can be advocated, a number of questions should be considered. Serological tests represent a service expansion in many countries and for many physicians, and will have to be justified. Unanswered questions include what are the benefits for patient care, and how should serological tests be best applied? Tests will help to characterize the infection, and provide patients with prognostic information on likely recurrence frequency and the risks of transmission. Whether this will affect transmission is as yet unclear. The benefit of serodiagnosis is likely to vary among individuals depending on their social situation.

Another issue is HSV type 1 (HSV-1) – how should it be managed in areas of high incidence such as Japan and parts of Europe? Situations where serological screening will not be useful include areas where there is a high prevalence of HSV-1 genital infection. If serology is used to confirm a diagnosis of genital herpes in a culture-negative person, there is a possibility that they will be HSV-1 seropositive and HSV-2 seronegative, so the test will not be able to confirm that genital lesions were due to HSV.

There is a strong argument for positive virological diagnosis of a patient with lesions, using swabs and culture. However, in the situation where a patient who once had some symptoms of genital HSV infection, which were not confirmed by culture, but who later has positive HSV-2 serology, there is a danger that an assumption will be made that their blisters were due to HSV infection, rather than considering all possible diagnoses.

Serological testing may also be useful in the patient who has chronic recurrent genitourinary symptoms for which there is no apparent aetiology, and also where there is a concern about transmission in discordant couples.

Vaccines

A vaccine for HSV offers great potential for the individual and in public health terms. Given the lack of success in producing an effective HSV vaccine, it is recommended that pharmaceutical companies establish a working consortium to develop new vaccines. It should pool expertise and resources to reduce the commercial risks to individual companies that are associated with developing a vaccine.

There is a need to explore further the immune response to HSV to identify new approaches and targets.

A number of early HSV vaccines have been developed. However, the trials were not rigorous and many of these vaccines cannot be considered to be effective or to offer any likelihood of benefit. New technology and understanding of the molecular biology of HSV suggest a number of encouraging approaches. Several candidate vaccines are currently undergoing clinical trials. However, there is, as yet, no proven effective immunotherapeutic or immunoprophylactic vaccine,

A large number of subunit vaccines have been developed. The one that is furthest along in development consists of gD2-QS-21 plus an MPL-alum adjuvant. Clinical trials have shown it to be immunogenic and well-tolerated in Phase I/II trials. Larger Phase II/III trials in discordant couples have been undertaken.

CHAPTER 1

1. Jaeschke R, Guyatt GH, Sackett DL. Users' guides to the medical literature: III. How to use an article about a diagnostic test. B. What are the results and will they help me in caring for my patients? *JAMA* 1994a;**271**:703–707.
2. Jaeschke R, Guyatt GH, Sackett DL. Users' guides to the medical literature: III. How to use an article about a diagnostic test. A. Are the results of the study valid? *JAMA* 1994b;**271**:389–391.
3. Lafferty WE, Coombs RW, Benedetti J *et al*. Recurrences after oral and genital herpes simplex virus infection: influence of anatomic site and viral type. *N Engl J Med* 1987;**316**:1444–1449.
4. Volpi A, Lakeman AD, Pereira L, Stagno S. Monoclonal antibodies for rapid diagnosis and typing of genital herpes infections during pregnancy. *Am J Obstet Gynecol* 1983;**146**:813–815.
5. Baker DA, Pavan-Langston D, Gonik B *et al*. Multicenter clinical evaluation of the Du Pont Herpcheck HSV ELISA, a new rapid diagnostic test for the direct detection of herpes simplex virus. *Adv Exp Med Biol* 1990;**263**:71–76.
6. Cone RW, Swenson PD, Hobson AC *et al*. Herpes simplex virus detection from genital lesions: a comparative study using antigen detection (HerpCheck) and culture. *J Clin Microbiol* 1993;**31**:1774–1776.
7. Kudesia G, Van Hegan A, Wake S *et al*. Comparison of cell culture with an amplified enzyme immunoassay for diagnosing genital herpes simplex infection. *J Clin Pathol* 1991;**44**:778–780.
8. Kowalski RP, Gordon YJ. Evaluation of immunologic tests for the detection of ocular herpes simplex virus. *Ophthalmology* 1989;**96**:1583–1586.
9. Aurelius E, Johannsson B, Sköldenberg B *et al*. Rapid diagnosis of herpes simplex encephalitis by nested polymerase chain reaction assay of cerebrospinal fluid. *Lancet* 1991;**337**:189–192.
10. Brown D, personal communication.
11. Aurelius E. Herpes simplex encephalitis: early diagnosis and immune activation in the acute stage and during long-term follow-up. *Scand J Infect Dis* 1993;**89**:3–62.
12. Sundqvist V-A. Frequency and specificity of varicella zoster virus IgM response. *J Virol Methods* 1982;**5**:219–227.

CHAPTER 2

1. Ashley R, Cent A, Maggs V *et al*. Inability of enzyme immunoassays to discriminate between infections with herpes simplex virus types 1 or 2. *Ann Intern Med* 1991;**115**:520–526.
2. Mertz GJ, Jones CC, Mills J *et al*. Long-term acyclovir suppression of frequently recurring genital herpes simplex virus infection: a multicenter double-blind trial. *JAMA* 1988;**260**:201–206.
3. Mertz GJ, Benedetti J, Ashley R *et al*. Risk factors for the sexual transmission of genital herpes. *Ann Intern Med* 1992;**116**:197–202.
4. Brock BV, Selke S, Benedetti J *et al*. Frequency of asymptomatic shedding of herpes simplex virus in women with genital herpes. *JAMA* 1990;**263**:418–420.
5. Koelle DM, Benedetti J, Langenberg A *et al*. Asymptomatic reactivation of herpes simplex virus in women after the first episode of genital herpes. *Ann Intern Med* 1992;**116**:433–437.
6. Ashley R, personal communication.
7. Langenberg A, Benedetti J, Jenkins J *et al*. Development of clinically recognizable genital lesions among women previously identified as having 'asymptomatic' herpes simplex virus type 2 infection. *Ann Intern Med* 1989;**110**:882–887.
8. Wald A, Corey L, Cone R *et al*. Frequent genital herpes simplex virus 2 shedding in immunocompetent women: effect of acyclovir treatment. *J Clin Invest* 1997;**99**:1092–1097.
9. Wald A, Zeh J, Barnum G *et al*. Suppression of subclinical shedding of herpes simplex virus type 2 with acyclovir. *Ann Intern Med* 1996;**124**:8–15.
10. Brown ZA, Selke S, Zeh J *et al*. The acquisition of herpes simplex virus during pregnancy. *N Engl J Med* 1997;**337**:509–515.
11. Brown ZA, Benedetti JK, Watts DH *et al*. A comparison between detailed and simple histories in the diagnosis of genital herpes complicating pregnancy. *Am J Obstet Gynecol* 1995;**172**:1299–1303.
12. Ashley RL. Current concepts of laboratory diagnosis of herpes simplex infections. In: *Clinical Management of Herpes Virus Infections* (Sacks SL, Straus SE, Whitley

- RJ, Griffiths PD, eds). Washington DC: IOS Press, 1995; pp 137–171.
13. Lee FK, Coleman RM, Pereira L *et al*. Detection of herpes simplex virus type 2-specific antibody with glycoprotein G. *J Clin Microbiol* 1985;**22**:641–644.
14. Lee FK, Pereira L, Griffin C *et al*. A novel glycoprotein for detection of herpes simplex virus type 1-specific antibodies. *J Virol Methods* 1986;**14**:111–118.
15. Ashley R, Milioni J, Lee F *et al*. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. *J Clin Microbiol* 1988;**26**:662–667.
16. Sanchez-Martinez D, Schmid DS, Whittington W *et al*. Evaluation of a test based on baculovirus-expressed glycoprotein G for detection of herpes simplex virus type-specific antibodies. *J Infect Dis* 1991;**164**:1196–1199.
17. Sanchez-Martinez D, Pellett PE. Expression of HSV-1 and HSV-2 glycoprotein G in insect cells by using a novel baculovirus expression vector. *Virology* 1996;**182**(1):229–238.
18. Ho DWT, Field PR, Irving WL *et al*. Detection of immunoglobulin M antibodies to glycoprotein G-2 by Western blot (immunoblot) for diagnosis of initial herpes simplex virus type 2 genital infections. *J Clin Microbiol* 1993;**31**:3157–3164.
19. Slomka MJ, Ashley RL, Cowan FM *et al*. Monoclonal antibody blocking tests for the detection of HSV-1 and HSV-2 specific humoral responses: comparison with Western blot assay. *J Virol Methods* 1995;**55**:27–35.
20. Alexander D, Dinella R, Ashley R *et al*. Simultaneous use of glycoproteins gB1, gG1, gD2 and gG2 as antigens for a type-specific serological assay to diagnose prior infection with HSV-1 or HSV-2. *96th ASM General Meeting*, New Orleans, 1996.
21. Ashley RL, Warford A, Gleaves C *et al*. Evaluation of the CHIRON®RIBA™ HSV type-1/type-2 strip immunoblot assay (SIA) for typing of HSV antibodies (Ab). 35th Inf Dis Soc Amer, San Francisco, CA, 1997.
22. Ashley RL, Wu L, Pickering JW *et al*. Pre-market evaluation of a commercial glycoprotein G-based enzyme immunoassay for herpes simplex virus type-specific antibodies. *J Clin Microbiol* 1998; **36**:294–295.

CHAPTER 3

1. Preblud SR. Age-specific risks of varicella complications. *Pediatrics* 1981;**68**:14.
2. Enders G, Miller E, Cradock-Watson J *et al*. Consequences of varicella and herpes zoster in pregnancy: prospective study of 1739 cases. *Lancet* 1994;**343**:1548–1551.
3. Gershon AA. Varicella-Zoster Virus: Prospects for Control. *Adv Ped Infect Dis* 1995;**10**:93–123.
4. Feldman S, Hughes T, Daniel CB. Varicella in children with cancer: seventy-seven cases. *Pediatrics* 1975;**56**:388–397.
5. D'Hondt E, Berge E, Colinet G *et al*. Production and quality control of the Oka-strain live varicella vaccine. *Postgrad Med J* 1985;**61**:53–56.
6. Hayawaka Y, Torigoe S, Shiraki K *et al*. Biological and biophysical markers of a live varicella vaccine strain (Oka): identification of clinical isolates from vaccine recipients. *J Infect Dis* 1984;**149**:956–963.
7. Arvin AM, Gershon AA. Live attenuated varicella vaccine. *Annu Rev Microbiol* 1996;**50**:59–100.
8. LaRussa P, Lungo O, Hardy I *et al*. Restriction fragment length polymorphism of polymerase chain reaction products from vaccine and wild-type varicella-zoster virus isolates. *J Virol* 1992;**66**:1016–1020.
9. Hawrami K, Breuer J. Analysis of United Kingdom wild-type strains of varicella-zoster virus; differentiation from the Oka vaccine strain. *J Med Virol* 1997; **53**: 60–62.
10. Takada M, Suzutani T, Yoshida I *et al*. Identification of varicella-zoster virus strains by PCR analysis of three repeat elements and a PstI-site-less region. *J Clin Virol* 1995;**33**:658–660.
11. Kuter BJ, Weibel RE, Guess HA *et al*. Oka/Merck varicella vaccine in healthy children: final report of a 2-year efficacy study and 7-year follow-up studies. *Vaccine* 1991;**9**:643–647.
12. Gershon AA, LaRussa P, Hardy I *et al*. Varicella vaccine: The American experience. *J Infect Dis* 1992;**166**:S63–S68.
13. Hardy I, Gershon AA, Steinberg SP *et al*. The incidence of zoster after immunization with live attenuated varicella vaccine. A study in children with leukemia. *N Engl J Med* 1991;**325**:1545–1550.
14. White CJ, Kuter BJ, Hildebrand CS *et al*. Varicella vaccine (VARIVAX) in healthy children and adolescents: results from clinical trials, 1987 to 1989. *Pediatrics* 1991;**87**:604–610.
15. Krause P, Klinman DM. Efficacy, immunogenicity, safety

- and use of live attenuated chickenpox vaccine. *J Pediatr* 1995;**127**:518–525.
16. Varis T, Vesikari T. Efficacy of high-titer live attenuated varicella vaccine in healthy young children. *J Infect Dis* 1996;**174**:S330–S334.
 17. Asano Y, Suga S, Yoshikawa T *et al*. Experience and reason: twenty year follow up of protective immunity of the Oka live varicella vaccine. *Pediatrics* 1994;**94**:524–526.
 18. Takahashi M, Iketani T, Sasada K *et al*. Immunization of the elderly and patients with collagen vascular diseases with live varicella vaccine and use of varicella skin antigen. *J Infect Dis* 1992;**166**:S58–S62.
 19. La Russa P, Steinberg S, Gershon AA. Varicella vaccine for immunocompromised children: results of collaborative studies in the United States and Canada. *J Infect Dis* 1996;**174**(Suppl 3):S320–S323.
 20. Meurice F, De Bouver JL, Vandevoorde D *et al*. Immunogenicity and safety of a live attenuated varicella vaccine (Oka/SB Bio) in healthy children. *J Infect Dis* 1996;**174**(Suppl 3): S324–S329.
 21. Weibel RE, Neff BJ, Kuter BJ *et al*. Live attenuated varicella virus vaccine: efficacy trial in healthy children. *N Engl J Med* 1984;**310**:1409–1411.
 22. White CJ, Kuter BJ, Ngai AL *et al*. Modified cases of chickenpox after varicella vaccination: correlation of protection with antibody response. *Pediatr Infect Dis J* 1992;**11**:19–23.
 23. Provost PJ, Krah DL, Kuter BJ *et al*. Antibody assays suitable for assessing immune responses to live varicella vaccine. *Vaccine* 1991;**9**:111–116.
 24. Giller RH, Winistorfer S, Grose C. Cellular and humoral immunity to varicella zoster virus glycoproteins in immune and susceptible human subjects. *J Infect Dis* 1989;**160**:919–928.
 25. Hickling JK, Borysiewicz LK, Sissons JG. Varicella-zoster virus-specific cytotoxic T lymphocytes (Tc): detection and frequency analysis of HLA class I-restricted Tc in human peripheral blood. *J Virol* 1987;**61**:3463–3469.
 26. Arvin AM, Koropchak CM, Wittek AE. Immunological evidence of reinfection with varicella-zoster virus. *J Infect Dis* 1983;**148**:200–205.
 27. Zoulek G. *Laboratory markers of immunity to varicella zoster virus*. *Postgrad Med J* 1985;**61**(Suppl 4):47–52.
 28. Hayward A, Villaneuba E, Cosyns M *et al*. Varicella-zoster virus (VZV)-specific cytotoxicity after immunization of nonimmune adults with Oka strain attenuated VZV vaccine. *J Infect Dis* 1992;**166**:260–264.
 29. Sharp M, Terada K, Wilson A *et al*. Kinetics and viral protein specificity of the cytotoxic T lymphocyte response in healthy adults immunized with live attenuated varicella vaccine. *J Infect Dis* 1992;**165**:852–858.
 30. Levin MJ, Murray M, Rotbart HA *et al*. Immune response of elderly individuals to a live attenuated varicella vaccine. *J Infect Dis* 1992;**166**:253–259.
 31. Burke BL, Steele RW, Beard OW *et al*. Immune responses to varicella-zoster in the aged. *Arch Intern Med* 1982;**142**:291–293.
 32. Oxman MN. Immunization to reduce the frequency and severity of herpes zoster and its complications. *Neurology* 1995;**45**:S41–S46.
 33. Myers JD, Flurnoy N, Thomas ED. Cell-mediated immunity to varicella-zoster virus after allogeneic bone marrow transplantation. *J Infect Dis* 1980;**141**:479–487.
 34. Wilson A, Sharp M, Koropchak C *et al*. Subclinical varicella-zoster virus viremia, herpes zoster, and T lymphocyte immunity to varicella-zoster viral antigens after bone marrow transplantation. *J Infect Dis* 1992;**165**:119–126.
 35. Sperber S, Smith BV, Hayden FG. Serologic response and reactogenicity to booster immunization of healthy seropositive adults with live or inactivated varicella vaccine. *Antiviral Research* 1992;**17**:213–222.
 36. Watson B, Rothstein E, Bernstein H *et al*. Safety and cellular and humoral immune responses of a booster dose of varicella vaccine 6 years after primary immunization. *J Infect Dis* 1995;**172**:217–219.
 37. Watson B, Piercy S, Soppas D *et al*. The effect of decreasing amounts of live virus, while antigen content remains constant, on immunogenicity of Oka/Merck varicella vaccine. *J Infect Dis* 1993;**168**:1356–1360.
 38. Bergen RE, Diaz PS, Arvin AM. The immunogenicity of the Oka/Merck varicella in relation to infectious varicella-zoster virus and relative viral antigen content. *J Infect Dis* 1990;**162**:1049–1054.
 39. Wiebel RE, Kuter BJ, Neff BJ *et al*. Live Oka/Merck varicella vaccine in healthy children: further clinical and laboratory assessment. *JAMA* 1985;**254**:2435–2439.
 40. Arbeter AM, Baker L, Starr SE *et al*. Combination measles, mumps, rubella and varicella vaccine. *Pediatrics* 1986;**78**:742–747.
 41. Brunell PA, Taylor-Wiedeman J, Geiser CF *et al*. Risk of herpes zoster in children with leukemia: varicella vaccine compared with history of chickenpox. *Pediatrics* 1986;**77**:53–66.
 42. Hammerschlag MR, Gershon A, Steinberg S *et al*. Herpes zoster in an adult recipient of live attenuated varicella vaccine. *J Infect Dis* 1989;**160**:535–537.
 43. Watson BM, Laufer DS, Kuter BJ *et al*. Safety and immunogenicity of a combined live attenuated measles, mumps, rubella and varicella vaccine (MMR(II)V) in healthy children. *J Infect Dis* 1996;**173**:731–734.
 44. Holmes SJ. Review of recommendations of the advisory committee on immunization practices, centers for the disease control and prevention, on varicella vaccine. *J Infect Dis* 1996;**174**:S342–S344.
 45. Gamett GP, Ferguson NM. Predicting the effect of varicella vaccine on subsequent cases of zoster and varicella. *Rev Med Virol* 1996;**6**:151–161.
 46. Department of Health. Immunisation against Infectious Disease 1996. London: HMSO, 1996
 47. Jones EM, Reeves DS. Controlling chickenpox in hospitals (editorial). *BMJ* 1997;**314**:4–5.
 48. Paryani SG, Arvin AM. Intrauterine infection with varicella-zoster virus after maternal varicella. *N Engl J Med* 1986;**314**:1542–1546.
 49. American Academy of Pediatrics: *Committee on Infectious Diseases*, 23rd edn. 1994; pp 510–517
 50. Katz VL, Kuller JA, McMahon MJ *et al*. Varicella during pregnancy. Maternal and fetal effects. *West J Med* 1995;**163**:446–450.
 51. Preblud SR, Orenstein WA, Koplan JP *et al*. A benefit cost-analysis of a childhood varicella vaccination programme. *Postgrad Med* 1985;**61**:17–22.
 52. Huse DM, Meissner HC, Lacey MJ *et al*. Childhood vaccination against chickenpox: an analysis of benefits and costs. *Pediatrics* 1994;**124**:869–874.
 53. Lieu TA, Cochi SL, Black SB *et al*. Cost-effectiveness of a routine varicella vaccination program for US children. *JAMA* 1994;**271**:375–381.
 54. Preblud SR. Varicella: complications and costs. *Pediatrics* 1986;**78**:728–735.
 55. Beutels P, Clara R, Tormans G *et al*. Costs and benefits of routine varicella vaccination in German children. *J Infect Dis* 1996;**174**:S335–S341.
 56. Lieu TA, Finkeler LJ, Soerl ME *et al*. Cost-effectiveness of varicella serotesting versus presumptive vaccination of school-age children and adolescents. *Pediatrics* 1995;**95**:632–638.
 57. Seidman DS, Stevenson DK, Arvin AM. Varicella vaccine in pregnancy (editorial). *BMJ* 1996;**313**:701–702.
 58. Breuer J, personal communication.
 59. Gamett GP, Grenfell BT. The epidemiology of varicella-zoster virus infections: the influence of varicella on the prevalence of herpes zoster. *Epidemiol Infect* 1992;**108**:513–528.
 60. Hope-Simpson RE. The nature of herpes zoster: A long-term study and a new hypothesis. *Proc R Soc Med* 1965;**58**:9–20.
 61. Ragozzino MW, Melton LJ, Kurland LT *et al*. Population-based study of herpes zoster and its sequelae. *Medicine* 1982;**62**:310–316.
 62. Donahue JG, Choo PW, Manson JE *et al*. The incidence of herpes zoster. *Arch Intern Med* 1995;**155**:1605–1609.
 63. Levin MJ, Murray M, Zerbe GO *et al*. Immune responses of elderly persons 4 years after receiving a live attenuated varicella vaccine. *J Infect Dis* 1994;**170**:522–526.

CHAPTER 4

1. Spear PG. Glycoproteins specified by herpes simplex viruses. In: *The Herpesviruses*, volume 3 (Roizman B, ed). New York: Plenum Press, 1985; pp315–356.
2. Stanberry LR, Burke RL, Myers MG. Herpes simplex virus glycoprotein treatment of recurrent genital herpes. *J Infect Dis* 1988;**157**:156–163.
3. Johnson RM, Spear PG. Herpes simplex virus glycoprotein D mediates interference with herpes simple virus infections. *J Virol* 1989;**63**:819–827.
4. Muggerridge MI, Robert SR, Isola VJ *et al*. Herpes simplex virus glycoproteins. In: *Immunochemistry of Viruses: The basis for serodiagnosis and vaccines* (von Regenmortel MH, Neurath AR eds). New York Elsevier;1990: pp 459–481.
5. Balachnadran N, Bacchetti S, Rawls SE. Protection against lethal challenge of BALB/c mice by passive transfer of

- monoclonal antibodies to five glycoproteins of herpes simplex virus type 2. *Infect Immun* 1981;**34**:192–199.
6. Ghiasi H, Nesburn AB, Wechsler SL. Vaccination with a cocktail of seven recombinantly expressed HSV-1 glycoproteins protects against ocular HSV-1 challenge more efficiently than vaccination with any individual glycoprotein. *Vaccine* 1996;**14**:107–112.
 7. Shanley JD, Stanberry LR. Immunotherapy of persistent viral infections. *Rev Med Virol* 1994;**4**:105–118.
 8. Schiff BL, Kern AB. Multiple smallpox vaccinations in the treatment of recurrent herpes simplex. *Postgrad Med* 1954;**15**:32–36.
 9. Douglas JM, Vontver A, Stamm WM *et al*. Ineffectiveness and toxicity of BCG vaccine for the prevention of recurrent genital herpes. *Antimicrob Agents Chemother* 1985;**27**:203.
 10. Lazar MP. Vaccination for recurrent herpes simplex infection: initiation of a new disease site following the use of unmodified material containing live virus. *Arch Dermatol* 1956;**73**:70–71.
 11. Blank H, Haines HG. Experimental human reinfection with herpes simplex virus. *J Invest Dermatol* 1973;**61**:223–225.
 12. Frank SB. Formolized herpes virus therapy and the neutralizing substance in herpes simplex. *J Invest Dermatol* 1938;**1**:267–282.
 13. Kern AB, Schiff BL. Vaccine therapy in recurrent herpes simplex. *Arch Dermatol* 1964;**89**:844–845.
 14. Hull RN. Immunization of experimental animals with vaccines produced in known oncogenic cell lines. *Natl Cancer Inst Monogr* 1966;**29**:503–509.
 15. Schmersahl P, Rudiger G. Results of treatment with the herpes simplex antigen Lupidon H, resp. Lupidon G. *Z Hautkr* 1975;**50**:105–112.
 16. Dundarov S, Andonov P, Bakalov B *et al*. Immunotherapy with inactivated polyvalent herpes vaccines. *Develop Biol Standard* 1982;**52**:351–358.
 17. Weitgasser H. Kontrollierte Klinische Studie mit den Herpes-Antigenen Lupidon H und Lupidon G. *Z Hautkr* 1977;**52**:624–628.
 18. McKenzie R, Straus SE. Therapeutic immunization for recurrent herpes simplex virus infections. In: *Antiviral Chemotherapy 4* (Mills J, Volberding PA, Corey L, eds). Plenum Press, New York, 1996, pp67–84.
 19. Kutinova L, Benda R, Kalos Z *et al*. Placebo-controlled study with subunit herpes-simplex virus vaccine in subjects suffering from frequent herpetic recurrences. *Vaccine* 1988;**6**:223–228.
 20. Cappel R, Sprecher S, deCuyper F *et al*. Clinical efficacy of a herpes simplex subunit vaccines. *J Med Virol* 1985;**16**:137–145.
 21. Lafferty WE, Coombs RW, Benedetti J *et al*. Recurrences after oral and genital herpes simplex virus infection: influence of anatomic site and viral type. *N Engl J Med* 1987;**316**:1444–1449.
 22. Skinner GRB, Woodman CBJ, Hartley CE *et al*. Early experience with “antigenoid”: vaccine Ac Nfu1 (S-) MRC towards prevention or modification of herpes genitalis. *Develop Biol Standard* 1982;**52**:333–344.
 23. Woodman CBJ, Buchan A, Fuller A *et al*. Efficacy of vaccine Ac Nfu1 (S-) MRC5 given after an initial clinical episode in the prevention of herpes genitalis. *Br J Vener Dis* 1983;**59**:311–313.
 24. Benson CA, Turyk ME, Wilbanks GD. A placebo-controlled trial of vaccination with a mixed glycoprotein herpes simplex virus type 1 vaccine for the modulation of recurrent genital herpes. In: IDSA 33rd Annual Meeting, San Francisco, CA. 1995:418.
 25. Stanberry LR, Kern ER, Richards ST *et al*. Genital herpes in guinea pigs: pathogenesis of the primary infection and description of recurrent disease. *J Infect Dis* 1982;**146**:397–404.
 26. Green MT, Rosborough JP, Dunkel EC. *In vivo* reactivation of herpes simplex virus in rabbit trigeminal ganglia: electrode model. *Infect Immun* 1981;**34**:69–74.
 27. Anderson JR, Field HJ. An animal model of ocular herpes. Keratitis, retinitis and cataract in the mouse. *Br J Exp Pathol* 1984;**65**:283–297.
 28. Stanberry LR, Burke RL, Myers MG. Herpes simplex virus glycoprotein treatment of recurrent genital herpes. *J Infect Dis* 1988;**156**:156–163.
 29. Myers MG, Bernstein DI, Harrison CJ *et al*. Herpes simplex virus glycoprotein treatment of recurrent genital herpes reduces cervicovaginal virus shedding in guinea pigs. *Antiviral Res* 1988;**10**:83–88.
 30. Stanberry LR, Harrison CJ, Bernstein DI *et al*. Herpes simplex virus glycoprotein immunotherapy of recurrent genital herpes: factors influencing efficacy. *Antiviral Res* 1989;**11**:203–214.
 31. Straus SE, Savarese B, Tigges M *et al*. Induction and enhancement of immune responses to herpes simplex virus type 2 in humans by use of a recombinant glycoprotein D vaccine. *J Infect Dis* 1993;**167**:1045–1052.
 32. Straus SE, Corey L, Burke RL *et al*. Placebo-controlled trial of vaccination with recombinant glycoprotein D of herpes simplex virus type 2 for immunotherapy of genital herpes. *Lancet* 1994;**343**:1460–1463.
 33. Langenberg AGM, Burke RL, Adair SF *et al*. A recombinant glycoprotein vaccine for herpes simplex virus type 2: safety and efficacy. *Ann Intern Med* 1995;**122**:889–898.
 34. Straus SE, Wald A, Kost RG *et al*. Immunotherapy of recurrent genital herpes with a recombinant herpes simplex virus type 2 glycoproteins D and B: results of a placebo-controlled vaccine trial. *J Infect Dis* 1997;**176**:1129–1134.
 35. LeRoux-Roels G, Moreau E, Verhasselt B *et al*. Immunogenicity and reactivity of a recombinant HSV-2 glycoprotein D vaccine with or without monophosphoryl lipid A in HSV seronegative and seropositive subjects. (Abstract 1209) 33rd ICAAC New Orleans USA, 1993
 36. LeRoux-Roels G, Moreau E, Desombere I *et al*. Persistence of humoral and cellular immune response and booster effect following vaccination with herpes simplex 9gD2t) candidate vaccine with MPL. (Abstract H57) 34th ICAAC, Orlando USA, 1994.
 37. McKenzie R, Straus SE. Vaccine therapy for herpes simplex virus infections: An historical perspective. *Rev Med Virol* 1996;**6**:85–96.
 38. Ho RYJ, Burke RL, Merigan TC. Antigen-presenting liposomes are effective in treatment of recurrent herpes simplex virus genitalis in guinea pigs. *J Virol* 1989;**63**:2951–2958.
 39. Bernstein DI, Harrison CJ, Jenks LJ *et al*. Cell-mediated immunologic responses and recurrent genital herpes in the guinea pig. Effects of glycoprotein immunotherapy. *J Immunol* 1991;**146**:3571–3577.

CHAPTER 5

1. Fitzgerald PA, Lopez C. Natural killer cells as mediators of resistance to herpesvirus infections in humans. In: *Human Herpesvirus Infections*. (C Lopez, B Roizman, eds). Raven Press, New York: 1985.
2. Brown ZA, Benedetti J, Ashley R *et al*. Neonatal herpes simplex virus infection in relation to asymptomatic maternal infection at the time of labor. *N Engl J Med* 1991;**324**:1247–1252.
3. L Corey, unpublished observations.
4. Yeager AS, Arvin AM, Urbani LJ *et al*. Relationship of antibody to outcome in neonatal herpes simplex virus infection. *Infect Immun* 1980;**29**:532–538.
5. Arvin AM. Relationships between maternal immunity to herpes simplex virus and the risk of neonatal herpesvirus infection. *Rev Infect Dis* 1991;**13**:S953–S956.
6. York LJ, Giorgio DP, Mishkin EM. Immunomodulatory effects of HSV2 glycoprotein D in HSV1 infected mice: implications for immunotherapy of recurrent HSV infection. *Vaccine* 1995;**13**:1706–1712.
7. Kapoor AK, Nash AA, Wildy P. Pathogenesis of herpes simplex virus in B cell-suppressed mice. The relative roles of cell mediated and humoral immunity. *J Gen Virol* 1982a;**61**:127–131.
8. Kapoor AK, Nash AA, Wildy P *et al*. Pathogenesis of herpes simplex virus in congenitally athymic mice: the relative roles of cell mediated and humoral immunity. *J Gen Virol* 1982b;**60**:225–233.
9. McDermott MR, Graham FL, Hanke T *et al*. Protection of mice against lethal challenge with herpes simplex virus by vaccination with an adenovirus vector expressing HSV glycoprotein B. *Virology* 1989;**169**:244–247.
10. Posavad CM, Koelle DM, Shaughnessy MR *et al*. Severe genital herpes infections in HIV-infected individuals with impaired HSV-specific CD8⁺ cytotoxic T lymphocyte responses (abstr). *22nd International Herpesvirus Workshop*, 1996.
11. Larry Corey, personal communication.
12. Nash AA, Phelan J, Wildy P. Cell mediated immunity in herpes simplex virus infected mice: H-2 mapping of the delayed type hypersensitivity response and the antiviral T cell response. *J Immunol* 1981;**126**:1260.
13. Stanberry LR. Genital and neonatal herpes simplex virus infections: epidemiology, pathogenesis and prospects for control. *Rev Med Virol* 1993;**3**:37–46.
14. Koutsky LA, Stevens CE, Holmes KK *et al*. Underdiagnosis of genital herpes by current clinical and viral-isolation procedures. *N Engl J Med* 1992;**326**:1533–1539.
15. Breinig MK, Kingsley LA, Armstrong JA *et al*. Epidemiology of genital herpes in Pittsburgh: serologic,

- sexual, and racial correlates of apparent and inapparent herpes simplex infections. *J Infect Dis* 1990;**162**:299–305.
16. Mertz GJ, Benedetti J, Ashley R *et al*. Risk factors for the sexual transmission of genital herpes. *Ann Intern Med* 1992;**116**:197–202.
 17. Schmidt OW, Fife KH, Corey L. Reinfection is an uncommon occurrence in patients with symptomatic recurrent genital herpes. *J Infect Dis* 1984;**149**:645–646.
 18. Maitland NJ, Smith IW, Peutherer JF. Restriction endonuclease analysis of genital isolates of herpes simplex virus type 2. *Infect Immun* 1982;**38**:834–842.
 19. Brown ZA, Benedetti J, Selke S *et al*. Asymptomatic maternal shedding of herpes simplex virus at the onset of labor: relationship to preterm labor. *Obstet Gynecol* 1996;**87**:483–488.
 20. Prober CG, Sullender WM, Yasukawa LL *et al*. Low risk of herpes simplex virus infections in neonates exposed to the virus at the time of vaginal delivery to mothers with recurrent genital herpes simplex virus infections. *N Engl J Med* 1987;**316**:240–244.
 21. Fauci AS. Multifactorial nature of human immunodeficiency virus disease: implications for therapy. *Science* 1993;**262**:1011–1018.
 22. Lasky LA, Dowbenko DJ. DNA sequence analysis of the type-common glycoprotein-D genes of herpes simplex virus types 1 and 2. *DNA* 1984;**3**:23–29.
 23. Langenberg AGM, Burke RL, Adair SF *et al*. A recombinant glycoprotein vaccine for herpes simplex virus type 2: safety and efficacy. *Ann Intern Med* 1995;**122**:889–898.
 24. Spear PG. Glycoproteins specified by herpes simplex viruses. In: *The Herpesviruses*. Volume 3 (Roizman B, ed). New York: Plenum Press 1985: pp315–356.
 25. Meigner B, Longnecker R, Roizman B. In vivo behavior of genetically engineered herpes simplex viruses R7017 and R7020: construction and evaluation in rodents. *J Infect Dis* 1988;**158**:602–614.
 26. Whitley RJ. Prospects for vaccination against herpes simplex virus. *Ped Ann* 1993;**22**:726–732.
 27. Fricker J. Herpes vaccines: spinning a new DISC. *Lancet* 1996;**348**:1576.
 28. McLean CS, Erturk M, Jennings R *et al*. Protective vaccination against primary and recurrent disease caused by herpes simplex virus (HSV) type 2 using a genetically disabled HSV-1. *J Infect Dis* 1994;**170**:1100–1109.
 29. Forrester A, Farrell H, Wilkinson G *et al*. Construction of a mutant herpes simplex virus type 1 with glycoprotein gH sequences deleted. *J Virol* 1992;**66**:341–348.
 30. McLean CS, Ni Challanain D, Duncan I *et al*. Induction of a protective immune response by mucosal vaccination with a DISC HSV-1 vaccine. *Vaccine* 1996;**14**:987–992.
 31. Boursnell ME, Entwisle C, Blakeley D *et al*. A genetically inactivated herpes simplex virus type 2 (HSV-2) vaccine provides effective protection against primary and recurrent HSV-2 disease. *J Infect Dis* 1997;**175**:16–25.
 32. Corey L, personal communication.
 33. Wachsman M, Aurelian L, Smith CC *et al*. Protection of guinea pigs from primary and recurrent herpes simplex virus (HSV) type-2 cutaneous disease with vaccinia virus recombinants expressing HSV glycoprotein D. *J Infect Dis* 1987;**155**:1188–1197.
 34. Cremer KJ, Mackett M, Wohlenberg C *et al*. Vaccinia virus recombinant expressing herpes simplex virus type 1 glycoprotein D prevents latent herpes in mice. *Science* 1985;**228**:737–740.
 35. Wachsman M, Aurelian L, Smith CC *et al*. Regulation of expression of herpes simplex virus (HSV) glycoprotein D in vaccinia recombinants affects their ability to protect from cutaneous HSV-2 disease. *J Infect Dis* 1989;**159**:625–634.
 36. Redfield PR, Wright DC, James WD *et al*. Disseminated vaccinia in a military recruit with human immunodeficiency virus (HIV) disease. *N Engl J Med* 1987;**316**:673–676.
 37. Gallichan WS, Johnson WC, Graham FL *et al*. Mucosal immunity and protection after intranasal administration with recombinant adenovirus expressing herpes simplex virus glycoprotein B. *J Infect Dis* 1993;**168**:622–629.
 38. Limbach KJ, Paoletti E. Non-replicating expression vectors: applications in vaccine development and gene therapy. *Epidemiol Infect* 1996;**116**:241–256.
 39. McCarthy M. DNA vaccination: a direct line to the immune system. *Lancet* 1996;**348**:1232.
 40. Manickan E, Yu Z, Rouse RJD *et al*. Induction of protective immunity against herpes simplex virus with DNA encoding the immediate early protein ICP 27. *Viral Immunol* 1995;**8**:53–61.
 41. Kriesel JD, Spruance SL, Daynes RA *et al*. Nucleic acid vaccine encoding gD2 protects mice from herpes simplex virus type 2 disease. *J Infect Dis* 1996;**173**:536–541.
 42. Bourne N, Stanberry LR, Bernstein DI *et al*. DNA immunization against experimental genital herpes simplex virus infection. *J Infect Dis* 1996;**173**:800–807.

Acknowledgements

We wish to thank the following publishers and individuals for permission to reproduce tables and figures in this publication:

CHAPTER 1

Figure 1 from Aurelius E. Herpes simplex encephalitis: early diagnosis and immune activation in the acute stage and during long-term follow-up. Reproduced with permission of *Scand J Infect Dis* 1993;**89**:3–62.

CHAPTER 2

Table 2 from Koelle DM, Benedetti J, Langenberg A and Corey L. Asymptomatic reactivation of herpes simplex virus in women after the first episode of genital herpes. Reproduced with permission of *Ann Intern Med* 1992;**116**:433–437.

Figure 1 Courtesy of R Ashley. University of Washington, Seattle, USA.

Table 4 from Brown Z *et al*. The acquisition of herpes simplex virus during pregnancy. Reproduced from *N Engl J Med* 1997;**337**:509–515. Copyright 1997 Massachusetts Medical Society; All rights reserved.

Figure 2 from Wald A *et al*. Frequent genital herpes simplex virus 2 shedding in immunocompetent women. Reproduced from the *Journal of Clinical Investigation*, 1997,**99**:1092–1097. By copyright permission of the Rockefeller University Press.

Figure 5 from Ashley RL. Current Concepts of Laboratory Diagnosis in Herpes Simplex Infection. In: *Clinical Management of Herpes Virus Infections*. Eds: Sacks SL, Straus SE, Whitley RJ, Griffiths PD. IOS Press, Washington DC. 1995. pp 137–171.

Acknowledgements

CHAPTER 3

- Table 3** from Kuter B *et al.* Oka/Merck varicella vaccine in healthy children: final report of a 2-year efficacy study and 7-year follow-up studies. *Vaccine* 1991;**9**:643–647. Copyright 1991, with kind permission from Elsevier Science Ltd, The Boulevard, Langford Lane, Kidlington OX5 1GB, UK.
- Table 5** from Watson B, Rothstein E, Bernstein H *et al.* Safety and cellular and humoral immune responses of a booster dose of varicella vaccine 6 years after primary immunization. Reproduced with permission of *J Infect Dis* 1995;**172**:217–219. Copyright 1995, The University of Chicago Press.
- Table 6** from Watson B, Laufer DS, Kuter B *et al.* Safety and immunogenicity of a combined live attenuated measles, mumps, rubella, and varicella vaccine (MMRIV) in healthy children. Reproduced with permission of *J Infect Dis* 1996;**173**:731–734. Copyright 1996, The University of Chicago Press.
- Figure 4 and 5** from Burke BL *et al.* Immune responses to varicella-zoster in the aged. Reproduced with permission of *Arch Intern Med* 1982;**142**:291–293. Copyright 1982, American Medical Association.
- Figure 6** from White CJ *et al.* Modified cases of chickenpox after varicella vaccination: correlation of protection with antibody response. Reproduced with permission of *Pediatr Infect Dis* 1992;**11**:19–23. Copyright 1992, Williams & Wilkins.
- Figure 7** from Varis T and Vesikari T. Efficacy of high-titer live attenuated varicella vaccine in healthy young children. Reproduced with permission of *J Infect Dis* 1996;**174**:S330–S334. Copyright 1996, The University of Chicago Press.
- Figure 8** from Hardy I *et al.* The incidence of zoster after immunization with live attenuated varicella vaccine. Reproduced with permission of *New Engl J Med* 1991;**325**:1545–1550. Copyright 1991, Massachusetts Medical Society.
- Figure 9** from Beutels P, Clara R, Tormans G *et al.* Costs and benefits of routine varicella vaccination in German children. Reproduced with permission of *J Infect Dis* 1996;**174**:S335–S341. Copyright 1996, The University of Chicago Press.
- Figure 10** from Oxman MN *et al.* Immunization to reduce the frequency and severity of herpes zoster and its complications. Reproduced with permission of *Neurology* 1995;**45**(12 Suppl 8):S41–S46. Copyright by Advanstar Communications Inc. Advanstar Communications Inc. retains all rights to this article.

CHAPTER 4

- Figure 2** from Straus SE *et al.* Induction and enhancement of immune responses to herpes simplex virus type 2 in humans by use of a recombinant glycoprotein D vaccine. Reproduced with permission of *J Infect Dis* 1993;**167**:1045–1052. Copyright 1997, The University of Chicago Press.
- Figure 3** from Straus SE *et al.* Placebo-controlled trial of vaccination with recombinant glycoprotein D of herpes simplex virus type 2 for immunotherapy of genital herpes. Reproduced with permission of *Lancet* 1994;**343**:1460–1463. Copyright by The Lancet Ltd.
- Table 5** from McKenzie R and Straus SE. Vaccine therapy for herpes simplex virus infections: an historical perspective. Reproduced with permission from *Rev Med Virol* 1996;**6**:85–96. Copyright John Wiley and Sons Ltd.

CHAPTER 5

- Figure 2** from Stanberry L. Genital and neonatal herpes simplex virus infections: epidemiology, pathogenesis and prospects for control. Reproduced with permission of *Reviews in Medical Virology* 1993;**3**:37–46. Copyright by John Wiley & Sons Limited.
- Figure 3** from Fricker J. Herpes vaccines: spinning a new DISC. Reproduced with permission of *The Lancet* 1996;**348**:1576. Copyright by The Lancet Ltd.
- Figure 4** from McLean CS *et al.* Induction of a protective immune response by mucosal vaccination with a DISC HSV-1 vaccine. Reprinted from *Vaccine* 1996;**14**(10):987–992, with kind permission from Elsevier Science Ltd., The Boulevard, Langford Lane, Kidlington, OX5 1GB, UK.
- Figure 5** from Bournnell ME *et al.* A genetically inactivated herpes simplex virus type 2 (HSV-2) vaccine provides effective protection against primary and recurrent HSV-2 disease. Reproduced with permission of *J Infect Dis* 1997;**175**(1):16–25. Copyright 1997, The University of Chicago Press.
- Figure 6** from McCarthy M. DNA vaccination: a direct line to the immune system. Reproduced with permission of *The Lancet* 1996;**348**:1232. Copyright by The Lancet Ltd.
- Figure 7** from Kriesel JD *et al.* Nucleic acid vaccine encoding gD2 protects mice from herpes simplex virus type 2 disease. Reproduced with permission of *J Infect Dis* 1996;**173**(3):536–541. Copyright 1997, The University of Chicago Press.
- Table 4** from Manickan E, Yu Z, Rouse, R *et al.* Induction of protective immunity against herpes simplex virus with DNA encoding the immediate early protein ICP 27. Reproduced with permission of *Viral Immunol* 1995;**8**:53–61.

The *International Herpes Management Forum* (IHMF) World Wide Web site has information on the IHMF, forthcoming meetings and internet versions of *Management Strategies in Herpes*.

Sponsored by the *International Herpes Management Forum* (IHMF).

The opinions and recommendations expressed by faculty and other experts whose input is included in the programmes are their own. They do not represent nor speak for Glaxo Wellcome plc or PPS Europe.

Written and produced by PPS Europe, a division of PAREXEL MMS Europe Ltd under an educational grant from Glaxo Wellcome plc.

