Prevention and control of herpesvirus diseases*
Part 2. Epidemiology and immunology

A WHO MEETING

This is the second of two articles summarizing the information currently available on herpes virus diseases. The first described the different members of the herpesvirus group, clinical expression of infection, laboratory diagnosis, and chemotherapy. This article dealing with the epidemiological and immunological aspects of herpesvirus infections also brings up the special problems caused by virus latency and risks associated with congenital infection and immunodeficiency or immunodepressive treatment. The prospects of preventing herpesvirus infections by vaccination and treatment with immune sera are also discussed.

HERPES SIMPLEX VIRUSES IN THE HUMAN POPULATION

Epidemiology

Infected humans are the sole reservoir of herpes simplex virus (HSV) available for dissemination in the human population. A key factor in the epidemiology of HSV-1 and HSV-2 is the ability of these viruses to remain latent in the sensory and autonomic ganglia, as described below. The reactivated virus, whether in recurrent clinical lesions or as virus shed from a subclinical lesion, is an important factor in dissemination of the disease.

HSV-1 and HSV-2 are unstable and do not survive for long periods in the external environment. Infections are acquired by susceptible individuals through contaminated secretions, usually exchanged during close personal contact. Thus, specific environmental factors do not significantly influence the incidence of primary infections that occur all the year round with little seasonal variation. When outbreaks involving several people are observed, they are usually in families or in nursing or hospital environments, and genetic "fingerprints" of viral isolates clearly indicate that large-scale epidemics of HSV infections do not occur.

The incubation period for both HSV-1 and HSV-2 is 3–9 days and virus shedding is minimal during this time. Virus shedding may continue for 14–21 days after the appearance of lesions caused by the primary infection. High titres of virus are also found in the vesicles of recurrent herpetic lesions, when virus shedding lasts 3–7 days after appearance of the lesion. This implies a greater dose of virus when the exposure is to a person with an active lesion than to an asymptomatic virus shedder. Thus, the risk of infection will be greater following exposure to an active lesion than to virus shed in the absence of a lesion. The importance of the various sources of virus may vary with the social setting.

Asymptomatic shedding is relatively common and 1–5% of adults sampled in a cross-sectional survey have been found to be excreting herpes simplex virus in oral secretions in the absence of lesions. The prevalence of virus in genital secretions of women has been
found to depend upon age and sexual behaviour. Among women undergoing routine cervicovaginal cytological examinations, less than 0.5% shed the virus. A similar prevalence was observed among female university students and pregnant women at delivery. The rates of virus shedding among women attending sexually transmitted disease clinics were considerably higher and varied from 1.6% to 6.9%. Thus, asymptomatic shedding is sufficiently prevalent to account for a substantial source of virus infections in susceptible individuals.

Socioeconomic conditions influence the age-specific incidence of HSV infections. About 90% of children living in primitive conditions have acquired antibodies to HSV-1 by 10 years of age and essentially all individuals in these groups become infected by adulthood. Among individuals residing in cities in the developed countries, infections with HSV-1 are acquired later in life and a substantial proportion of the population reaches adulthood without being infected. The proportion of each age group infected decreases as socioeconomic conditions improve. The highest rate of infection occurs in the first 5 years of life in all populations examined and the second highest rate has been observed with the onset of sexual maturity among those who escape infection during childhood.

The existence of two types of herpes simplex viruses has interesting implications. While no differences exist between the viruses with respect to tissue tropism associated with initiating the primary infection, isolates from recurrent oral lesions have been mostly HSV-1 while those from recurrent genital lesions have been mostly HSV-2. The selective factors fostering the development and maintenance of the two types are very likely minute differences in the structure and function of HSV-1 and HSV-2, which ensure selective advantages for the establishment of latency and reactivation in ganglia innervating oral and genital tissues respectively. One cannot exclude the possibility that HSV-2 can develop sufficiently by unique antigenic determinants that enable it to grow in hosts previously infected with HSV-1.

The two types of HSV produce clinically distinguishable illnesses, each with its own unique epidemiology. For example, Kaposi’s varicelliform eruption is a generalized form of cutaneous herpes occurring primarily in persons suffering from atopic eczema. Herpetic whitlow occurs commonly among physicians, dentists and nurses. The majority of reported cases of anorectal herpes have been in male homosexuals. Aseptic meningitis is an occasional complication of genital herpetic infections and occurs mostly in young adults. The vast majority of infections, however, result from infections initiated in the oral cavity or genital tract. While oral and genital infections account for the greatest morbidity, mortality is most commonly seen in neonatal infections, infections of the brain, and infections of immunocompromised adults. In addition, ocular disease constitutes a major cause of blindness.

The incidence of neonatal infection is largely unknown since neonatal infections may occur without producing the severe form of generalized disease. The major source of neonatal infections is thought to be maternal genital tract secretions contaminated with virus. In Canada, the incidence of severe neonatal herpes is estimated to be 1 per million. The disease may be more frequent in the USA.

Herpetic encephalitis is the most common form of fatal encephalitis in the Western world. The disease occurs in all age groups and equally in both sexes. There is no seasonal variation in incidence. In a recent collaborative study, 70% of patients with biopsy-proven herpes encephalitis were seropositive at the onset. However, of those who were seronegative, 78% seroconverted during the course of the illness (24). This and other observations confirm earlier suggestions that reactivation of latent virus as well as infection with virus from an exogenous source may produce encephalitis (26).

The usual clinical manifestation of HSV-1 infection in infants and children is acute gingivostomatitis. Children who have herpetic gingivostomatitis present with fever
following a brief incubation period and then develop lesions on the hard palate, gingiva and tongue (22). A subclinical form, not requiring medical attention or not recognized as oral herpes, accounts for the majority of infections in children and only about 1 in 5 infections are clinically recognized.

Among individuals who reach adulthood without any HSV infection, primary HSV-1 infections are commonly manifested as an acute upper respiratory tract infection. In a study of university students, only 30% of the students possessed antibodies to HSV upon entry and 10% seroconverted annually. Of the students requiring admission to the infirmary for acute respiratory illness, 11.5% were associated with HSV infections. Most of these students represented cases of pharyngitis or tonsillitis (21).

Infections of the genital tract in adults are usually acquired through sexual contact and the infections may be asymptomatic or may produce illness of varying severity. The greatest age-specific incidence of infection is between 20 and 29 years of age. Individuals with several sex partners are at greater risk of genital infections than individuals with few sex partners. Early age at first coitus is also associated with an increased risk of genital herpetic infections. Low income and limited formal education place women at increased risk of HSV-2 infections; however, in one study income and education of males did not significantly affect the occurrence of past HSV-2 infections, as detected serologically. These factors are, in part, interdependent.

In most populations, the majority of genital infections are asymptomatic, yet there has recently been an increase in the incidence of severe clinical herpes genitalis, especially among the middle class. The factors responsible for the varying severity of the clinical manifestations of the infections are poorly understood. Genital infections with either virus type in seronegative individuals are more likely to be clinically apparent, which suggests that the immune responses to infection with HSV-1 in childhood may modify the severity of subsequent genital infections (23, 25). However, decreased vaginal secretions and damage to the cells lining the vagina and cervix may also play a role. Genital infections may be caused by either HSV-2 or HSV-1 and the proportions of genital herpetic disease caused by these two virus types have been found to differ in different populations. HSV-2 has been isolated from over 90% of lesions developing in individuals of lower socioeconomic classes, while in some studies as many as 50% of the isolates from lesions in individuals of the middle socioeconomic class have been HSV-1. An increased number of visits by patients to their doctor on account of genital herpes has been recorded over the past decade and it was estimated that about 250,000 patients were seen in the USA for genital herpes in 1979. While an enhanced awareness of the disease might account for part of the increased number of visits, it is likely that a real increase in genital herpes incidence has occurred.

Recurrent lesions

The acute vesicular eruptions of recurrent herpes resemble the lesions associated with the initial infection except that they tend to be more localized and heal more rapidly. The occurrences of recurrent herpes labialis have been determined by retrospective surveys of defined populations, mainly by the use of questionnaires. The second approach has been to identify patients with primary herpetic lesions and then follow them prospectively for the occurrence of recurrent lesions. The prospective studies provide confidence in correctly recording the recurrences and accurately define the natural history of the disease but provide no information about recurrence among patients with asymptomatic or mild initial infections.

An initial survey of recurrent herpes labialis among university students revealed 38.2% suffering from recurrent cold sores. Subsequent studies of white adult populations yielded similar findings and it can be concluded that about one-third of individuals in industrial
countries suffer periodically from the disease. Lower rates have been found among students in South America and Asia (20).

The rates of recurrent genital herpes determined from prospective studies of patients with primary genital disease have been higher than those recorded for herpes labialis. Between one-half and two-thirds of the patients seen by physicians for initial episodes of herpes suffered from recurrences.

A number of factors have been incriminated as initiators of recurrences. These include fever, trauma to the trigeminal nerve root, dental extraction, exposure to sunlight, and use of certain immunosuppressive drugs. These factors account for only a small fraction of recurrent episodes. Emotional stress, psychological factors, and changes associated with the menstrual cycle have been suggested as precipitating factors but prospective studies of these factors have failed to yield profiles that predict recurrent episodes.

It has been repeatedly demonstrated that the severity of the first episode, especially a high antibody level recorded at that time, correlates well with the frequency of recurrent episodes.

Latency

An important property of HSV-1 and HSV-2 is their ability to remain latent for the life of the host after the first infection. The sequence of events is as follows: in the course of virus multiplication at the portal of entry into the body, the virus infects the nerve endings and ascends to the dorsal root ganglia and, less frequently, to the autonomic ganglia. Once in the ganglion, the virus remains latent in the neurons. Operationally, the latent state can be readily differentiated from the lytic state because in the latter case the virus is readily recovered in tissue homogenates, whereas latent virus can be recovered only by cocultivation of ganglionic cells with growing, susceptible cells. The epidemiological significance of latency is indicated by the fact that physical or emotional stress may induce the latent virus to multiply. The activated virus is then transported via the axon to the original portal of entry where it may cause a lesion. Except when it causes lesions at the portal of entry, the latent and induced virus appears to be shielded from the immune system of the host. The latent virus is, therefore, a reservoir of potentially infectious virus, shielded from the immune system of the host and available for infection of susceptible individuals who come in physical contact with persons harbouring the virus.

Widespread dissemination of HSV-1 and HSV-2 in populations is believed to be due to the ability of these viruses to remain latent and be reactivated. In the case of HSV-1, the first infection usually results in stomatitis, the virus remaining latent in the trigeminal or superior cervical ganglia. Under stress, recurrent vesicles carrying the virus appear, usually at the mucocutaneous junction of the lips, and this virus is ready to infect a susceptible person by direct contact. Similarly, the virus infecting the genital organs usually remain latent in the sacral ganglion.

There are many unanswered questions about the mechanism by which latency is established and the virus is reactivated. It has been suggested that HSV-1 and HSV-2 encode functions dedicated to the establishment of latency specifically in the neuronal cells and that unidentified host factors are responsible for both establishing and terminating the latent state. In experimental systems, it has been observed that the concentration of prostaglandins in the area of the skin innervated by sensory ganglia carrying latent virus tends to increase just prior to or during reactivation. Stimuli associated with reactivation, enumerated earlier in the text, do not readily lend themselves to a common pathway. In individuals with multiple latent viruses, reactivation may be synchronous (i.e., occurs simultaneously) in different sites or may be localized to a particular site. These studies suggest that there are factors in the circulation that affect reactivation, but experimental
studies also show that trauma to the sensory nerves and skin nerve-endings may induce the latent virus to multiply.

The pathogenesis of HSV infections rests largely on the capacity of HSV to establish and maintain a latent infection. From this point of view, elucidation of the mechanisms by which latent infections are established, maintained, and triggered to induce virus multiplication is of paramount importance.

**Immunology**

Following the infection, there are both humoral and cell-mediated immune responses lasting for many years. HSV-1 and HSV-2 possess shared antigenic determinants, so that infection with one of these viruses results in the production of antibodies and T-lymphocytes which will react with the heterologous virus type. In addition, the immune responses influence the pathogenesis of the infections and determine the lesion's severity. This appears to be especially true for cell-mediated immune responses where it has been suggested that specific periodic deficiencies may account for recurrent herpetic lesions in otherwise healthy individuals. More thoroughly documented is the evidence that unusually severe herpetic disease is associated with congenital or acquired cell-mediated immune defects. With the increased use of immunosuppressive drugs in patients receiving organ transplants, there has been a rise in the proportion of hospitalized patients suffering from severe or protracted illnesses caused by herpes viruses.

Many newborns have passively transferred maternal antibodies, which are gradually lost during the first 6 months of life. HSV-1 antibodies begin to appear in the population in early childhood; by adolescence they are present in most persons. HSV-2 antibodies appear with increased sexual activity during adolescence and early adulthood.

Primary infection usually yields a rise in antibody titres to HSV-1 or HSV-2. Peak titres of antibodies are reached about 4–6 weeks after these infections and the titres generally persist at relatively stable levels thereafter. Virus-specific IgM antibodies are produced in the early stages of the initial infection and usually persist for 6–8 weeks. In individuals with pre-existing antibodies, recurrence or reinfecion with the same or a different type of virus is not associated with a dramatic change in antibody titre, nor are these infections accompanied by an IgM response. However, when such infections are extensive there may be an IgM response and a rise in the titres of IgG antibodies in the convalescent period.

Analysis of viral isolates with restriction enzymes indicates that persons with a prior infection with HSV-1 or 2 may become superinfected at the same or different site with another virus of the same serotype. These data cannot be explained by antigenic variation of the virus.

The immunological mechanisms at work during herpes simplex infection are being studied in animal models, especially in mice. At present, it is clear that antibodies are important in preventing the invasion of the central nervous system by virus. Both complement-mediated lysis and antibody-dependent cytotoxicity of virus-infected cells have been described. Specific T-lymphocyte populations appear to be involved in the immune response. Cytotoxic cells appear transiently early in the primary infection but are promptly recalled on virus challenge. T-cells involved in delayed hypersensitivity (DH) appear after 3 days and persist for life. Four types of suppressor lymphocytes have been identified and they also persist for life. These include B-cells that accumulate in the lymph node draining the primary site of infection and that suppress the expression of specific DH reactions to HSV, two kinds of T-cells suppressing induction of specific DH, and a T-cell that specifically suppresses expression of DH. It appears that delayed hypersensitivity is important in protective immunity. The induction of the suppressor T-cell is dependent upon the route by which the antigen is administered.
Possible role of HSV-2 in cervical cancer

There is a consensus that cervical cancer is caused by a sexually transmitted carcinogen and most of the epidemiological evidence suggests the involvement of a viral agent. Since 1968, HSV-2 has been the agent most often suspected to be an essential factor in cervical neoplasia. The causal relationship between HSV-2 and cervical carcinoma was materially strengthened by retrospective seroepidemiological case-control studies indicating—chiefly in low socioeconomic groups—a higher prevalence of HSV-2 antibody in the patients than in the matched controls; however, this is not suggested by recently completed prospective studies on middle-income healthy women in Prague and Houston (39, 40).

Although normal mammalian cells exposed to virus yield cells capable of causing malignant metastatic growth in hamsters and mice, the role of the viral gene products in initiating and maintaining the transformed state is unclear. The absence of viral genes or gene products from transformed cells has led to the postulation of an hypothesis for a hit-and-run effect. It has also been suggested that HSV might be mutagenic.

Vaccines

The objectives of vaccination are two-fold: to prevent primary infection and the establishment of latency, and to prevent or mitigate clinical manifestations of disease. Thus far, there is no convincing evidence available that a vaccine would be of benefit for patients already suffering from recurrent lesions.

In the past, three kinds of vaccines have been tested: live viruses, inactivated whole-virion vaccines, and subunit vaccines (Table 10). The studies of these vaccines have been carried out in an attempt to reduce the incidence of recurrent lesions as well as to prevent or mitigate the effects of a primary infection. Studies in individuals deliberately reinfected with their own virus have shown that reinfection is possible despite evidence of prior herpesvirus infection.

Subunit vaccines consisting of purified glycoproteins extracted from infected cells have been developed in several countries and controlled studies have been planned. The genes specifying some of the protective antigens have been cloned and are being expressed in E. coli and eukaryotic cell cultures. Synthetic peptides capable of eliciting antibodies are also being constructed. Such preparations should be available shortly for trials on the prevention of primary infections. It is not yet clear whether these vaccines will provide sufficiently long-lasting immunity.

Table 10. Prospective HSV vaccines

<table>
<thead>
<tr>
<th>I. Subunit vaccines</th>
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<tbody>
<tr>
<td>(a) purified glycoproteins from infected eukaryotic cells;</td>
</tr>
<tr>
<td>(b) purified glycoproteins made in E. coli;</td>
</tr>
<tr>
<td>(c) glycoproteins made from expression vectors in eukaryotic cells;</td>
</tr>
<tr>
<td>(d) synthetic peptides.</td>
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<table>
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<tr>
<th>II. Live vaccines</th>
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<tbody>
<tr>
<td>(a) antigens expressed by non-HSV (e.g., vaccinia) vectors;</td>
</tr>
<tr>
<td>(b) heterologous herpesviruses;</td>
</tr>
<tr>
<td>(c) mutants;</td>
</tr>
<tr>
<td>(d) genetically engineered variants.</td>
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</tbody>
</table>
Research is also being conducted with live vaccines. These are of three sorts:

1) Heterologous herpes viruses to produce partial immunity, just as turkey herpesvirus protects against Marek’s disease.
2) The production of vaccinia virus containing genes coding for herpes glycoproteins.
3) The use of genetically engineered live herpes viruses lacking pathogenicity.

It is hoped that these approaches may yield a vaccine giving a more durable immunity than that likely to be produced from a non-living immunogen.

EPIDEMIOLOGY, IMMUNOLOGY, AND CONTROL OF VARICELLA

Experimental work has been much impeded in the past owing to the difficulties in handling the varicella zoster (VZ) virus in the laboratory, its high degree of specificity for man, and the consequent lack of animal models. In spite of this, progress has been made towards controlling this disease.

Epidemiology

Varicella, a highly contagious and therefore a ubiquitous acute childhood infection, is characterized by fever, malaise, and a generalized papular vesicular rash. It is usually a benign disease but is severe in adults and may be life-threatening in the immunocompromised host. Infection during pregnancy presents a risk for the fetus and the neonate (32). The spread of the infection varies, depending upon climate and the extent of urbanization. Transmission, usually by the respiratory route, is so effective that in temperate climates most children contract chickenpox during the first 6-8 years of life. In tropical areas the disease is often acquired later in life. Seroepidemiological studies show that in temperate climates 90% of 14-year olds possess specific IgG antibodies and only 5% of the women in the childbearing age lack immunity. Cases of zoster occur sporadically throughout the year and affect about 0.3-0.5% of the population annually. The incidence increases with age so that more than half of the cases occur in those over 50 years old.

Immunology

Following varicella, immunity to exogenous reinfection is lifelong so that second attacks of the disease are virtually unknown. Antibody develops to reach a peak titre between 10 and 28 days after onset of the exanthem. On the other hand, it has long been suspected that cell-mediated immunity is important in recovery from varicella zoster (VZ) infections and in protection against the reactivation of latent VZ virus. It appears that the immunocompetent state of the host seems to be the major factor in determining the subsequent induction of specific cellular immunity in primary varicella and the maintenance of latency in later life. Despite this immunity, the virus persists in a latent state in the sensory ganglia, often for decades. When cell-mediated immunity wanes with age, or following immunosuppressive therapy, the virus may reactivate, resulting in zoster.

The humoral immune response is characterized by the development of IgM, IgA and IgG antibodies in the first few days after appearance of the rash. The IgG antibodies persist for life and their presence is correlated with protection against varicella (30). The cell-mediated immunity can be detected at the time that the rash appears by means of the lymphocyte proliferative assay, the direct and antibody-dependent cytotoxicity assays, or a specific skin test. Although the importance of cell-mediated immunity is generally recognized,
research in cellular immunity in varicella zoster lags far behind the knowledge of humoral responses.

Control of varicella

To control varicella, especially in immunocompromised persons, immune plasma prophylaxis and active immunization by vaccination can be considered. At present, only zoster immunoglobulin is available.

Life-threatening varicella has become an increasing problem with the development of increasingly potent and frequent forms of immunosuppressive treatment of children with leukaemia and other malignancies. Since circulating antibodies are known to be effective,

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**Table 11. Management of varicella-zoster infections in pregnancy**

<table>
<thead>
<tr>
<th>Varicella-zoster</th>
<th>Antibody status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact at once</td>
<td>VZV-ELISA IgG (5h)</td>
</tr>
<tr>
<td>Seronegative</td>
<td>5.2% IgG 1: &lt; 64</td>
</tr>
<tr>
<td>within 24-72 h</td>
<td>Zoster immunoglobulin (ZiG), 0.2 ml/kg IgG 1:256 000</td>
</tr>
<tr>
<td>Seropositive</td>
<td>94.8% IgG 1: ≥ 128</td>
</tr>
<tr>
<td>No ZiG</td>
<td></td>
</tr>
</tbody>
</table>

**Varicella**

<table>
<thead>
<tr>
<th>1st to 5th month of gestation</th>
<th>Risk of congenital defects: low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Termination of pregnancy: no</td>
<td></td>
</tr>
<tr>
<td>Fetaloscopy: no</td>
<td></td>
</tr>
<tr>
<td>Detection of IgM and IgA antibodies in fetal blood: questionable</td>
<td></td>
</tr>
</tbody>
</table>

**Varicella**

<table>
<thead>
<tr>
<th>Rate of infection</th>
<th>Fatality rate</th>
<th>ZiG in mother</th>
<th>ZiG in child</th>
</tr>
</thead>
<tbody>
<tr>
<td>Around term</td>
<td>25%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>From 30 to 5 days before delivery</td>
<td>17%</td>
<td>32%</td>
<td>0.2 ml/kg (before delivery)</td>
</tr>
<tr>
<td>From 4 days before to 2 days after delivery</td>
<td>17%</td>
<td>32%</td>
<td>2 ml/kg (within 24 h after birth)</td>
</tr>
<tr>
<td>Isolate mother and child with varicella.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control immune status of women around term who are in contact with a varicella case.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Zoster**

<table>
<thead>
<tr>
<th>In pregnancy</th>
<th>No risk of congenital anomalies.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No immunoglobulin (ZiG) present.</td>
<td></td>
</tr>
<tr>
<td>No chemotherapy needed.</td>
<td></td>
</tr>
<tr>
<td>No steroids (parenteral) needed.</td>
<td></td>
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</tbody>
</table>

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prompt administration of zoster immunoglobulin after contact can prevent the disease, but such protection is transient (Table 11).

The vaccine OKA strain developed by Takahashi in Japan has been extensively studied in field trials. This virus was adapted to human embryonic lung cells, then transferred to primary guinea pig cells, and the vaccine was finally prepared in human diploid cell lines. The strain is characterized by three stable markers: (1) its ability to grow equally well in guinea pig and human cell culture, (2) restricted growth at 39 °C, and (3) the restriction endonuclease digestion pattern (27). Requirements for the production and control of varicella vaccine (live) have been formulated by WHO.

The vaccine has now been administered to nearly 8000 seronegative persons in whom it has proved effective and safe. The frequency of mild local reactions at the site of inoculation is about 1% in both normal healthy and immunocompromised persons. A general reaction to the vaccine, mainly rash or mild varicella, is related to the degree of immunosuppression and may appear in 40% of severely immunosuppressed patients. Vaccination should not be performed in patients with low lymphocyte counts or a poor delayed-hypersensitivity response. Maintenance chemotherapy must be interrupted for at least one week before and after vaccination. Some years after vaccination, zoster may occur in the vaccinated population, with a similar incidence as in naturally infected people, but the results to date indicate that the disease is of a milder nature (28, 29).

Seroconversion, after vaccination, in healthy seronegative children is over 90%. The immunocompromised respond to a similar extent, although some 10% tend to lose measurable antibodies 6–12 months after vaccination. The cell-mediated immune response to vaccination is equally good and there is no evidence of its waning, so far. Over 90% of vaccine recipients develop a solid protective immunity to varicella (31).

Though the vaccine may be used with advantage in all seronegative individuals, its special value is for certain risk groups, in particular for seronegative children receiving immunosuppressive therapy.

**EPIDEMIOLOGY AND IMMUNOLOGY OF CMV INFECTION**

**Mode of transmission**

Cytomegalovirus (CMV) is a ubiquitous agent which has been found in all populations thus far tested, including the remote Tiriyo Indians of Brazil who even lack evidence of past measles and influenza infections (36). Infection by CMV is species-specific, and there are no known vectors in the natural transmission cycle. In most populations, only a minority of individuals escape infection with CMV during their lifetime; however, the age of acquisition differs in various geographical and socioeconomic settings. CMV maintains a delicately balanced parasitic relationship with the host, which is probably the result of long-standing coevolution. As with other herpesviruses, despite the development of a vigorous immune response by the adult host, viral shedding after a primary infection usually persists for several months, until the infection eventually becomes latent (33, 35). In contrast, following congenital and perinatal infections, continuous viral excretion commonly persists for between four and eight years (4). In both adults and children, the latent state may be interrupted by periodic recurrences, the mechanisms of which are poorly understood. Thus, intermittent shedding occurs in a significant proportion of young seropositive adults. Sources of virus include saliva, urine, cervical and vaginal secretions, semen, breast milk, tears, blood, and grafted organs. In the vast majority of cases, the CMV infections are subclinical and infected individuals remain for a long time capable of transmitting the virus to other susceptible people. It is clear that a
large reservoir of CMV exists in the population at all times. Because of the lability of CMV to various environmental factors, close and prolonged personal contact is required for horizontal spread. Although the presence of CMV in oropharyngeal secretions might suggest the possibility of respiratory spread, evidence for airborne transmission is still lacking. CMV infections have no seasonal variations. However, some poorly defined socioeconomic factors influence the rates of both vertical and horizontal transmissions.

**Congenital infection**

Congenital CMV infection occurs in approximately 1% of all newborn infants (4). However, its incidence varies between 0.2% and 2% in different populations. The incidence of congenital infections is higher in the poorer socioeconomic sectors of developed countries and in developing nations of the world. CMV can be transmitted to the fetus after reactivation of latent maternal infection as well as after primary maternal infection.

**Perinatal infection**

Perinatal CMV infection is principally a result of maternal-to-infant transmission (4). The two main maternal sources of transmission are infected breast milk, which results in approximately 60% of perinatal infection, and the infected genital tract in late gestation, which is associated with transmission in 25–50% of cases.

There is considerable difference in the rate of perinatal acquisition of CMV throughout the world (4). Younger seropositive women who breast feed are more likely to transmit the virus to their infants, especially in lower socioeconomic groups. The current renewed interest in breast-feeding, particularly among women of middle to upper socioeconomic backgrounds in developed countries, is resulting in a more rapid acquisition of infection. The consequence of this phenomenon will not be fully understood for several years.

**Postnatal infections**

After the neonatal period the rate of infection increases slowly but with a wide variation between populations (4, 37). Early studies performed in western Europe, the United Kingdom, and the USA demonstrated that 20–80% of children are infected by the time they reach puberty. In other areas of the world, 90–100% of the population may be infected during childhood, at even as early as 5 years of age. Studies conducted in populations of different socioeconomic backgrounds suggest that a poor standard of living is associated with higher rates of antibody prevalence and early acquisition of infection. However, more recent studies indicate crowding as a more important factor. The most convincing data in this regard were derived by comparing the prevalence of antibodies to CMV in urban Jewish children, in kibbutz children, and in bedouin children (38). Kibbutz children live under high hygienic standards and socioeconomic conditions but by the second year of life, 76% of them were seropositive while only 44% and 54% of urban Jewish children and bedouin children, respectively, were seropositive. In the kibbutz, by the second year of life, 87% of those children who slept in the children’s houses with their peers were seropositive in contrast to 69% of those sleeping at home. For urban children, a higher prevalence of CMV infection is also significantly associated with crowding. A significant rise in seropositivity occurs when children attend day-care nurseries or schools.

Similar findings occur in children of middle and upper income backgrounds who attend day-care centres in developed countries. The rate of excretion may range from 9% in infants less than one year of age to 83% among toddlers in their second year of life. Children in day-care centres often take things to the mouth and commonly come into close contact with one another and play with the same toys. CMV has been isolated from plastic
toys that had been mouthed by children known to be excreting virus, suggesting that this is a potential vehicle for transmission of CMV. The fact that the infection rate is lowest in infants under 12 months of age suggests that spread through aerosols or transmission by employees is very unlikely, at least in the setting of the day-care centres. Recent data show that the risk of infection for health care workers is not greater than the risk for individuals in the community (8). As breast-feeding regains popularity, the incidence of perinatal CMV infection will increase, thus creating a sizeable pool of infected children. As children become mobile and engage in close interpersonal contact, CMV can be expected to spread rapidly among them. This phenomenon should lead to significant changes in the epidemiology of CMV within the next decade.

Infection during pregnancy

In general, in developing areas of the world 90–100% of the population are infected with CMV during childhood, at even as early as 5 years of age (35). However, in the developed countries CMV infection is acquired at a lower rate and in some population groups there is a burst of infection during the early childbearing years. This observation and the findings of higher rates of genital excretion of virus in women of low-income backgrounds, higher rates of infection in promiscuous women, shedding of virus into saliva, and high levels of prolonged excretion of CMV in semen suggest that the infection may be spread by kissing and sexual contact.

CMV infection is generally minor, but may have a major impact if acquired during pregnancy. Prospective studies of primary CMV infection were performed on more than 35 000 pregnant women, of whom over 11 000 were seronegative (35); the average rate of seroconversion among these women was 1.6% for the full 40 weeks of gestation or approximately 2.0–2.2% per year. In these studies only 39% (range, 26–50%) of the cases of primary CMV infection resulted in congenital infection. Although the information is still limited, it appears that gestational age exerts no influence on the rate of intra-uterine transmission following primary CMV infection.

High-risk groups

Blood transfusion is now recognized as an important iatrogenic cause of CMV infection (35), the risk of such transmission being particularly troublesome when it occurs at an early age since infected infants become chronic excretors. Among infants of seronegative mothers exposed to seropositive blood the rate of infection is approximately 15% (7). Among infants of seropositive mothers exposed to seropositive blood the rate of infection may be similar; however, disease in infants is rare and, when present, is of minor consequence. The risk may be as high as 24% for patients who receive more than 50 ml of packed red blood cells and who are exposed to at least one seropositive blood donor. In infants with low birth weight (1500 g), disease is common and mortality may be as high as 40%. In contrast, no infection occurs in infants of seronegative mothers exposed to blood from seronegative donors.

In adults, post-transfusion CMV infection is a frequent complication, particularly in patients undergoing extracorporeal blood circulation. The risk of CMV infection has been estimated to be 2–3% per unit of seropositive blood transfused. The problems associated with organ transplantation, on the other hand, are far more complicated and less amenable to control since infection may be transmitted not only by blood transfusions but by the grafted organ itself.

In renal transplant recipients, up to 25% of all deaths and approximately 20% of all graft rejections are associated with CMV infection (5). Among bone marrow transplant
recipients, 50% of all graftees become infected by CMV (6); of these, 33% develop interstitial pneumonia with a death rate of 88% and a mean survival time of 3 weeks or less.

The prevalence of primary CMV infections in seronegative renal transplant recipients receiving a kidney from a CMV seropositive donor varies from 20% to over 80%. The risk of disease among those infected is in the range of 40–100%, CMV infection usually occurring within 1–2 months after transplantation. The prevalence of recurrent infection in seropositive recipients varied from 75% to 90% and the risk of disease among those with recurrent infection ranges from 0% to 86%. In recurrent infections, graft rejection usually occurs within one month before the onset of CMV excretion or the appearance of a significant antibody rise (usually detected between 2 and 3 months after transplantation).

**Humoral immunity**

Specific antibodies appear to be directed against the viral envelope and infected cell membranes, as well as against internal antigens induced within the host cells (immediate early, early, and late antigens) (33–35). A low level of CMV antibodies persists for long after the infection and these antibodies can be detected by some serological tests (indirect haemagglutination and enzyme immunoassay) but not all (complement fixation). CMV-specific antibodies are markers of past infection and can be used as an indicator of possible sources of infection, i.e., in the selection of blood donors.

Some studies suggest that the presence of antibodies is of limited efficacy in reducing the acquisition of CMV infection. Maternal antibodies do not prevent either reactivation of latent maternal virus or transmission of virus to the fetus. The development of CMV-specific complement-fixing antibodies precedes the onset of virus shedding by several months in children. Perinatal infection can occur despite the presence of maternally conferred antibodies and chronic virus excretion can continue for years in the presence of antibody. Antibody production in bone marrow recipients does not correlate with prolonged survival or recovery from CMV infection and high antibody titres are found in renal transplant patients who succumb to CMV disease.

On the other hand, there are indications that the presence of CMV-specific antibodies can attenuate the severity of CMV infection. Although the incidence of post-transfusional CMV infection remains the same, infants with maternally acquired antibodies have reduced morbidity and mortality. In adults, the risk of infection following transfusion is 3–6 times higher when the recipient is seronegative. The presence of CMV antibodies in transplant recipients lowers the risk of death.

**Cell-mediated immune response**

Usually, CMV infection has an initial depressive effect on cell-mediated immune responses (33, 35). The depressed response is mostly indicated by a lack of reactivity to CMV antigens, either in lymphoproliferation or interferon production. When CMV infection occurs in an already immunocompromised individual (e.g., renal or bone marrow transplant recipients), it results in further depressions of lymphocyte reactivity to antigens other than those of CMV and to mitogen stimulation. Deficiency in cytotoxic T-cell activity is paralleled by depressed natural killer-cell responses. Such CMV-induced depression is reflected at the clinical level by a reversal of the normal suppressor/helper T-cell ratio, and an increased susceptibility to bacterial and fungal infections. Convalescence is accompanied by a return to a normal ratio and recovery of lymphocyte reactivity. The disease outcome is related favourably and directly to the development of CMV-specific cytotoxic and natural killer-cell responses, as well as to reduction of immunosuppressive therapy.
CMV seropositive individuals are permanently sensitized to CMV antigens, as can be detected by in vitro lymphocyte proliferation. There is evidence that the CMV-induced immunodepressive effect may be the result of direct infection of cell components involved in the immune response. During acute infection, virus can be isolated from peripheral white blood cells. The presence of active and latent virus in blood components is clearly reflected by the high rate of CMV infection in leukocyte-granulocyte transfused patients and by the decreased incidence of post-transfusional CMV infection when leukocyte-depleted blood is administered.

Immunoprophylaxis

Immunoprophylaxis using pooled gamma globulin proved effective, whereas prophylactic administration of different interferon preparations (see below) and transfer factor did not. In controlled trials using hyperimmune plasma from renal transplant patients or intramuscularly or intravenously administered gamma globulin prepared from seropositive normal blood donors, there was a significant reduction in mortality from CMV infection and a decrease in the incidence of CMV interstitial pneumonia and asymptomatic infection in bone marrow recipients. However, the therapeutic effects were only evident in patients not receiving granulocyte therapy.

Interferon has been administered with only marginal therapeutic benefit. When given prophylactically or immediately post-transplantation, interferon delayed the onset of CMV excretion and seroconversion, and lowered the level of viraemia. The future application of interferon therapy must take into account the adverse effects of high doses of leukocyte interferon, which have been common.

Vaccination

Vaccination against CMV infection should induce a humoral and cellular immune response comparable to that obtained from natural infection (35). The vaccine should contain viral elements capable of inducing resistance to a variety of CMV strains, thereby providing protection against reinfection with wild viral strains. When live attenuated virus is used, it should not give rise to clinical illness, it should not replicate in the vaccinee to the point that this person becomes a source of contamination, it should not become latent or persist in the host, and, finally, it should not be oncogenic.

Populations at risk, such as women of childbearing age and candidates for organ transplants, are well defined and may benefit even if protection is for a limited period of time. For these populations, subunit vaccines—those that are likely to induce a shorter immunity—may be considered as a possible alternative to attenuated strains.

In seronegative volunteers, a live, attenuated experimental vaccine induced both humoral and cellular immune responses typical of primary natural infection within 2–4 weeks of subcutaneous inoculation. Specific lymphoproliferation in vitro and production of anti-complement immunofluorescent antibodies, complement-fixing antibodies, and neutralizing antibodies to CMV antigens were observed. Contrary to what is encountered following natural infection, helper/suppressor T-cell ratios and lymphocyte proliferation to mitogen stimulation remained normal. In no instance was virus isolated from serum, the throat, urine, buffy coat, semen, or cervical secretions. The only clinically apparent side-effects were transient, local erythema and swelling at the site of inoculation and these required no medical attention.

Over a short follow-up period (18 months), all vaccinees displayed positive enzyme immunoassay reactivity and lymphoproliferative responses in vitro despite loss of complement-fixing and anticomplement fluorescent antibodies (35). Antibodies neutralizing a variety of CMV strains persisted in all but one case. A longer follow-up study
(8 years) revealed that only half of the vaccinees retained positive enzyme immunoassay reactivity or neutralizing antibodies, and lymphocyte proliferative responses.

The response of renal transplant candidates to vaccination differed from that of normal seronegative volunteers. Only about half these candidates had an immune response at all to vaccination. Among those responding, antibody responses were of lower geometric mean titre; 20% had only a cell-mediated response. Lymphoproliferative responses were also depressed, but this was true for the naturally infected dialysis patients as well.

Post-transplantation immunotherapy initially eliminated cell-mediated immunity in both vaccinated and naturally infected patients. Ultimately, the positive effects of vaccination were seen, e.g., less illness and milder intensity.

In this limited study, none of the offspring of three previously vaccinated women showed signs of infection for as long as the first year of life. Although CMV has been isolated from vaccinated renal transplant recipients, in only one case was the virus believed to be of the same vaccine strain.

RESEARCH NEEDS

1. Current technology for the isolation, identification, typing, and genetic fingerprinting of herpesvirus isolates are cumbersome and difficult to carry out in general hospital laboratories. Further research is needed for the development of techniques for rapid identification of herpesviruses in clinical specimens and for serotyping and, where necessary, for the development of simplified procedures for genetic fingerprinting of isolates for epidemiological studies.

2. Current knowledge of the association of herpesviruses with human malignancy remains unsatisfactory. It is imperative that prospective studies should be pursued using advanced techniques of molecular biology, immunology, and epidemiology.

3. Notwithstanding attempts to determine why some individuals suffer from recurrent disease while others do not, the basis of the predisposition to recurrence is unknown. The great strides being made in our understanding of factors involved in human immunology should permit a detailed investigation of the role of humoral and cell-mediated components of the immune system as determinants of the frequency and severity of herpesvirus disease.

4. Differentiation between clinical episodes caused by exogenous and reactivated virus remains problematic. The development of sensitive markers of primary and reactivated infection is highly desirable. Such markers would allow an evaluation of the role of exogenous superinfection in congenital CMV disease in regions of the world differing with respect to antibody levels in childbearing age groups.

5. The protection of high-risk populations (e.g., renal and bone marrow transplant recipients) against devastating episodes of herpesvirus infection remains a major challenge. It is important to compare the beneficial effects of immunoglobulin treatment, vaccination, and chemotherapy as measured by the reduction of morbidity and mortality.

6. Transmission of CMV through blood products remains a major problem. Further research is needed to determine the optimal procedures for freeing blood products of latent and contaminating virus.

7. In the light of the development of numerous live vaccines for the prevention of herpesvirus diseases, it is critical that the construction, testing, and evaluation of risks and benefits should be standardized. It is particularly recommended that all live vaccines should contain markers to differentiate vaccine viruses from wild type strains, thereby allowing evaluation of the reactivation of vaccine strains in a clinical situation.

In principle, the vaccine studies should be organized as double-blind. Vaccinated test populations should be prospectively followed up to determine the efficacy and durability
of vaccine-conferred immunity. Furthermore, particular attention should be paid to wild type isolates obtained from vaccinees to determine if antigenic drift does occur.

8. An enormous amount of work is in progress for the development of new antitherpes agents. Efforts should be made to collect, centralize, and disseminate information on the efficacy and safety of these drugs, the diseases at which they are targeted, and the modalities of treatment. Particular attention should be devoted to the occurrence of resistant virus strains and the long-range consequences of chemotherapy.

9. Since the development of drug resistance may be accompanied by alteration of other properties, e.g., pathogenicity, it is important to characterize resistant strains fully. Studies on the characterization of these virus strains should therefore be encouraged.

10. Combination antiviral chemotherapy is a viable approach for the treatment of herpesvirus infections. Combination of antiviral drugs with interferons should be attempted. The possible toxic side-effects of this combination therapy should be carefully controlled.

RECOMMENDATIONS

At the international level, much can be done to promote the application of diagnostic methods for herpesvirus diseases, the development, control, and application of viral vaccines, and the application and control of chemotherapy, such as the following:

1. Workshops should be held to teach laboratory techniques for the production and quality control of reagents.
2. Reference reagents should be supplied to laboratories providing working reagents so that the latter may be standardized.
3. Studies should be coordinated on the evaluation of new, simple and rapid techniques and diagnostic reagents.
4. The production and quality control of reagents should be aided by the provision of relevant information to all concerned.
5. Countries should be encouraged to carry out epidemiological surveillance of herpesvirus diseases.
6. Collaborative studies should be organized to assess the impact of herpesviruses on populations living in different social and regional settings.
7. The future development of both subunit and live-virus vaccines should be aided by formulation of the requirements for the production and control of such vaccines.
8. With regard to the future application of chemotherapy and chemoprophylaxis of herpesvirus infections, an international collaborative programme should be coordinated to formulate a standardized procedure for assaying the sensitivity of herpesviruses to antiviral agents in cell culture systems. International collaborative centres should be encouraged to establish a library of selected herpesvirus mutants resistant to chemotherapeutic agents, including well-characterized laboratory virus strains with which the mutants arising from treatment of patients with herpesvirus infections can be compared.

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