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24. Supported by the National Institutes of Health grants AI35703-01, AI37542-01, and AI21089 and by the United Nations Development Program/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. We thank L. Hall for helpful discussions and E. Nardin for reviewing the manuscript.

Vaccines for Varicella-Zoster Virus and Cytomegalovirus: Recent Progress

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Despite more than 20 years of research effort, there are no human herpes virus vaccines licensed for use in the United States. Concerns persist about the safety and efficacy of such vaccines, in part because of the complexity of the virus life cycle, which includes latency and reactivation. Human pathogens in this group of DNA viruses include Herpes simplex types 1 and 2, Epstein-Barr virus, cytomegalovirus (CMV), and varicella-zoster virus (VZV), as well as the lesser known herpesviruses 6 and 7. Only CMV and VZV will be discussed here.

Varicella-Zoster Virus

VZV, the causative agent of varicella (chickenpox), is transmitted primarily by aerosolization of droplets from skin lesions. After initial replication in the respiratory tract, the virus moves to the bloodstream and ultimately to the epithelium, where infection is manifested as varicella blisters or vesicles on the skin. The cellular immune system—in particular, T cells and natural killer cells—is critical in suppressing viral replication (*1*). A deficient cellular immune response results in prolonged viral replication in the skin and in the viscera, accompanied by lung and liver disease (Table 1).

Latent VZV infection is established in dorsal root ganglia by ascending infection along sensory nerves from the skin. The site of latency is thought to be the satellite cells around the neurons rather than the neurons themselves (*2*). The latent state is maintained by varicella-specific cellular immunity until the host is immunocompromised by age, disease, or therapy administered for some underlying condition. Under those circumstances, VZV reactivates to cause zoster, a localized varicella restricted to the skin segments innervated by the ganglia in which viral reactivation occurred. In elderly patients, zoster can cause severe and persistent nerve pain.

The greatest progress in prevention of VZV-associated disease has been made not with a high-technology vaccine, but with a classical live attenuated VZV (the Oka strain) that was modified by passage in guinea pig and human cell culture in Japan during the 1970s (*3*). In the United States, a license application for a vaccine made from this virus is now being reviewed by the Food and Drug Administration. The Oka strain has already been licensed for normal (immunocompetent) children in Japan and Korea, where over 2 million have been vaccinated, and it is used on a limited scale in immunosuppressed children in the United States and Europe.

Abundant studies, conducted in thousands of children in the United States by Merck and in Japan by the Biken Institute, have shown that the Oka vaccine induces antiviral antibodies, lymphocyte proliferation responses, and cytotoxic T lymphocyte (CTL) responses (*1*, *3*, *4*). Antibodies persist for at least 8 years and lymphocyte proliferation responses persist for at least 6 years (*5*). Nevertheless, this vaccine does not prevent preschool and school-age children frequently exposed to VZV infection from developing "breakthrough" illness; postvaccination chickenpox occurs at a rate of 1 to 3% per year. Fortunately, the postvaccination illness is almost always mild (*6*). Thus, although 98% of vaccinated children will be protected from severe disease, only 70 to 85% will be completely protected against any form of varicella.

Whether a vaccine is needed to protect normal children against varicella is under debate by pediatricians and public health officials. Although varicella is normally viewed as a mild disease, a small percentage of complications is equivalent to a large number of children, given the millions of cases that occur each year. Furthermore, children who receive modest doses of steroids for diseases such as asthma may die of disseminated varicella. Cost-benefit analyses conducted by the Centers for Disease Control (*7*), as well as purely medical considerations, have convinced both the American Academy of Pediatrics and the

Host status	T cell immunity	Circumstance	Clinical outcome
Immunocompetent			
Susceptible	Normal	Exposure to VZV	Varicella
Immune	Normal	Exposure to VZV	Asymptomatic; boost in T cell immunity
Immune, aged	Poor	Reactivation	Zoster
Immunodeficient			
Susceptible	Poor	Exposure to VZV	Disseminated varicella
Immune	Poor	Reactivation	Disseminated zoster

Table 1. Relation between cellular immunity to VZV and clinical outcome. [Modified from (*1*), copyright 1992, The University of Chicago]

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Advisory Committee for Immunization Practices to recommend routine vaccination of susceptible children, adolescents, and adults in the United States, if the vaccine is licensed.

Opponents of universal varicella vaccination of children cite two major concerns: that the vaccine virus itself will become latent and cause zoster later, and that if vaccine-induced protection does not endure, adults vaccinated as children will become susceptible to the more severe form of varicella seen in patients older than 15 years. Indeed, there is evidence that the vaccine virus can be reactivated as zoster, but these cases have been mild and have occurred at a rate lower than that after natural infection. In vaccinated leukemic children, the rate of zoster is about 25% the rate observed after natural disease (*8*), and in vaccinated normal children the rate thus far appears to be lower than that observed after natural disease (*9*). Whether age-related immunologic debility increases the rate of vaccine-caused zoster remains to be seen. Loss of immunity by vaccinees is possible, but so far the evidence is to the contrary, and a second vaccination in adolescence (combined with the already recommended booster dose of measles, mumps, and rubella vaccine) would be feasible.

Vaccination of adolescents and adults with no history of varicella (whose number may be increasing) will be an important feature of vaccination recommendations, in order to protect against the severe varicella seen after childhood. Because immune responses to vaccine are mitigated in adults, two doses of vaccine, 1 or 2 months apart, may be needed to confer the same protection seen in vaccinated children.

Paradoxically, the least controversial use of the live VZV vaccine is in children receiving immunosuppressive therapies for cancer or other conditions (*4*). Before the development of antiviral drugs, varicella killed 7 to 30% of infected patients in such populations. The vaccine is administered in two doses during a short interruption of chemotherapy, and although there is a high incidence of rash, the vaccine has generally been well tolerated and effective.

Perhaps the most interesting use of the VZV vaccine from a biological viewpoint is its potential application as a "therapy" to prevent zoster. Zoster is due to weakened cellular immunity to the virus; thus, it may be possible to restimulate immunity by an injection of live virus. One population that might benefit from such therapy is the elderly. If the incidence of varicella in children is reduced, the incidence of zoster in grandparents could paradoxically increase because they would no longer receive the natural boosts to VZV immunity that grandparents currently receive. A recent study showed that injection of the Oka strain in elderly volunteers did augment proliferative responses to VZV antigens, restoring them almost to the levels seen in healthy young adults (*10*). These responses appeared to be durable, and although some of the vaccinees have subsequently developed zoster, the symptoms were relatively mild. A placebo-controlled trial in an aged population is being planned.

Efforts are also being directed toward the production of protein-based subunit vaccines, particularly because killed VZV may work as well as live VZV in boosting the immunity of the elderly (*11*), and because the viral glycoproteins bearing neutralizing epitopes are known and the viral DNA has been sequenced. Viral glycoproteins gp1 and gp2 appear to be the principal targets for neutralizing antibodies, and the immediate-early protein IE62 the principal target for cellular immunity (*12*). Vaccines in which VZV genes are expressed in vectors such as poxviruses are also being explored.

Variable	Total number
Live births per year	4,000,000
Newborns with CMV infection (rate, 1%)	40,000
Symptomatic disease at birth among infected newborns (rate, 7%)	2,800
Fatal disease (rate, 12%)	336
Survivors with sequelae (rate, 78%)	2,184
Survivors without sequelae (rate, 10%)	280
Asymptomatic infections among infected newborns (rate, 93%)	37,200
Sequelae (rate, 15%)	5,580
No sequelae (rate, 85%)	31,620
Total with sequelae or death	8,100

Table 2. Public health consequences of congenital CMV infection in the United States. [Modified from (*14*), copyright 1992, Massachusetts Medical Society]

Cytomegalovirus

The natural history of human CMV is even more complicated than that of VZV. The virus can be transmitted by salivary contamination, sexual intercourse, blood transfusion, or organ transplantation. Its pathological manifestations vary according to the host: In normal children it is usually asymptomatic; in normal adults it can be asymptomatic or cause an infectious mononucleosis-like syndrome; in fetuses it can cause severe damage (see below); and in immunosuppressed individuals, it can cause pneumonia, hepatitis, encephalitis, and death. After primary infection, CMV almost always becomes latent. The site of latency, although still controversial, is thought to be endothelial cells, macrophages, or macrophage precursors. Reactivation occurs frequently, but is asymptomatic except in the immunosuppressed.

Intrauterine infection is the major public health problem caused by CMV (*13*). In developing countries, CMV is transmitted early in life, either through breast milk or through contacts with infected children; thus, nearly all women of childbearing age are immune. In developed countries, many women escape infection and are susceptible to CMV infection during pregnancy. If a pregnant woman is infected, there is a 40% risk of CMV transmission to the fetus. The prevalence of the virus is such that about 1% of all fetuses are infected. Although few infants are symptomatic at birth, about 20% will suffer damage to the brain or cochlea, which makes CMV the most common infectious cause of congenital defects (*14*) (Table 2).

CMV is also the most important infection of patients receiving transplants. In solid organ transplants, the most dangerous situation is when the recipient is CMV seronegative and the donor CMV seropositive. As the virus is almost always latent in the donor organ, transmission is efficient and about 30% of recipients develop severe CMV disease. Bone marrow transplant recipients frequently develop CMV-induced pneumonia, usually caused in that situation by reactivation of the patient's own virus.

Work on CMV vaccines began 20 years ago, when two groups developed live attenuated viruses by passage in cell culture (*15*). Each strain produced asymptomatic infection (except for a local reaction at the inoculation site) and induced antibodies and cellular immune responses. One of the strains, the Towne virus (*16*), which was attenuated in human diploid fibroblasts, has been given to nearly 1000 volunteers, mostly renal transplant recipients. This vaccine induc-

es a low level of antibodies measurable by standard techniques, and also induces $CD4^+$ and $CD8^+$ T cell responses.

In the renal transplant patients, vaccination was followed by a period of immunosuppression at the time of surgery. Although latent, natural CMV reactivates frequently in this situation, the Towne vaccine virus did not, and indeed replication of the Towne virus was not detectable except temporarily at the injection site (*15*). The induction of antibodies by vaccination to nonvirion antigens that are expressed only during viral replication, and the failure of inactivated Towne virus to immunize, provide evidence that replication of the attenuated strain is needed for the immune response.

Tests of vaccine efficacy have been performed in three ways. First, an unattenuated low-passage CMV was used to challenge 30 healthy volunteers who were CMV-susceptible, CMV-immune, or vaccinated with the Towne virus. In susceptible individuals, 10 plaque-forming units (PFU) of the challenge virus produced an infectious mononucleosis syndrome, whereas among the naturally immune, symptoms developed only in those volunteers receiving 1000 PFU of virus. Vaccinees were completely protected against a 10 PFU challenge, but at 100 PFU ~50% became infected, albeit with little or no symptoms (*15*).

A second test of vaccine efficacy consisted of three double-blind placebo-controlled studies of CMV-seronegative kidney transplant patients who received kidneys from seropositive donors. In all three studies, vaccination did not affect the incidence of CMV infection, but did reduce the occurrence of severe illness by 80 to 100% (*15*).

Finally, the vaccine was tested in mothers of children in day-care centers, who have high rates of contact-acquired CMV infection. Towne vaccination did not affect the rate of infection (*17*), although naturally immune women who had a significantly higher titer of neutralizing antibodies were resistant to infection.

Meanwhile, efforts to develop CMV vaccines that are based on newer technologies are also burgeoning. The entire viral genome has been sequenced and the envelope glycoproteins that induce neutralizing antibodies have been identified, as have the antigens that induce CTLs. The main candidate for a subunit vaccine is an envelope glycoprotein known as UL55 or gB, which is synthesized as a 130- to 140-kD precursor that is cleaved to proteins of 116 and 55 to 58 kD (*18*). Several domains, notably the immunodominant epitope on the 55- to 58-kD protein, account for most of the neutralizing antibodies in serum from convalescent patients, and purified gB has been shown to induce neutralizing antibodies and lymphocyte sensitization in both animals and humans (*19*, *20*).

The gene encoding gB has been inserted into several different vectors. Baculovirus-produced gB, in combination with the adjuvant QS21, is a potent immunogen in mice. Substantial quantities of gB can now be produced in animal cell culture (*21*). The gB gene has also been inserted into a deletion mutant of adenovirus type 5, and this replication-competent virus was shown to be immunogenic in small animals (*22*). A poxvirus from canaries, which gives only an abortive replication in mammals, is also being tested as a vector for gB. Phase I clinical trials of some of these candidates are being planned.

Other candidates for a CMV subunit vaccine include the gH glycoprotein, which also carries neutralizing epitopes. The gH glycoprotein is difficult to produce in vitro because it requires a chaperone (*23*), but antibodies against it may complement those induced by gB. Internal viral proteins such as the immediate-early proteins or matrix proteins may be the best inducers of CTLs. The importance of CMV-specific CTLs in transplant recipients is well known [for example, see (*24*)]. Thus, complex subunit or vectored vaccines may be needed to elicit good T cell responses. Other possible approaches to CMV vaccines include synthesis of peptides that mimic key epitopes, production of anti-idiotypic antibodies, and genetic manipulation of the Towne attenuated virus to augment its immunogenicity.

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25. The author thanks C. J. White and R. L. Burke for sharing unpublished information.

Pneumococcal Disease: Prospects for a New Generation of Vaccines

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During the early 1900s, pneumococcal pneumonia was a much feared disease with a high mortality rate. The advent of effective antibiotics provided a new means to treat the disease, and as a result, early vaccine efforts were abandoned.

Nevertheless, *Streptococcus pneumoniae* (pneumococcus) remains the most common cause of bacterial pneumonia in the United States today. Disease rates are particularly high in young children, in the elderly, and in patients with predisposing conditions such as asplenia, chronic medical conditions (heart, lung and kidney disease, diabetes, alcoholism) or immunosuppressive illnesses, particularly AIDS (*1*). These same groups are at greater risk of pneumococcal spread to the bloodstream and to the central nervous system (meninges), and thus have a greater risk of death. In countries like the United States, which have introduced universal immunization against *Haemophilus influenzae* b (Hib), the pneumococcus is the most common cause of bacterial meningitis. On a global level, the pneumococcus is believed to be the most common bacterial cause of acute respiratory infections, which are estimated to result in more than 1 million childhood deaths each year (*2*). It is also associated with middle ear infections and sinusitis, which, though less severe illnesses, nevertheless incur substantial medical costs (*3*).

Recently, antibiotic-resistant strains of pneumococcus have emerged throughout

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