Vaccine Development to Prevent Cytomegalovirus Disease: Report from the National Vaccine Advisory Committee

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Cytomegalovirus (CMV) infection is the most common intrauterine infection in the United States, and it exacts a heavy toll when it infects children and immunocompromised individuals. A CMV vaccine was assigned the highest priority by the Institute of Medicine in its 1999 assessment of targets for vaccine development. The priority was based on the cost and human suffering that would be alleviated by reducing the disease burden of congenital CMV infection. The National Vaccine Advisory Committee and invited experts examined the prospects for a CMV vaccine and the actions needed to bring about successful vaccine development at a National Vaccine Program Office workshop in October 2000. This article summarizes information about the changing epidemiology of CMV and immune responses to infection and immunity, and it reviews the current status of several vaccine candidates. Support of government agencies for CMV vaccine research and development is critical to address this need.

In a recent review of priorities for vaccine development, the Institute of Medicine ranked a vaccine to prevent cytomegalovirus (CMV) disease at the highest priority on the basis of the economic costs that would be avoided and the years of life and disability that would be saved by a successful vaccine. Congenital CMV disease is not reportable to the Centers for Disease Control and Prevention (CDC), but it has been estimated to impact thousands of children born each year. The National Vaccine Advisory Committee (NVAC) and invited experts examined the prospects for a CMV vaccine and the actions needed to bring about successful vaccine development at an NVAC/National Vaccine Program Workshop at the CDC (Atlanta, GA) in October 2000. An effective vaccine would be certain to reduce the substantial burden of congenital CMV infection (i.e., deafness and other neurological diseases due to infection of the fetus or infant). Increased support from the National Institutes of Health (NIH), the CDC, and the US Food and Drug Administration (FDA) will be necessary to find vaccine candidates and to assure that they are developed.

This report reviews information about the changing epidemiology of CMV and about immune responses to infection and to experimental vaccines, and it lists unanswered questions about CMV infection and immunity and summarizes the status of several vaccine candidates tested in 2003. Support of government agencies for the development of such a vaccine is critical.

PUBLIC HEALTH IMPACT

CMV causes a spectrum of disease syndromes in children and adults. CMV is a cause of mononucleosis in immunocompetent individuals and is a well-known cause of serious morbidity and sometimes fatal infections in immunocompromised patients, especially recipients of solid-organ or hematopoetic cell allografts and individuals with advanced AIDS.

The impact of congenital CMV infection as a public health problem is not widely recognized and, when recognized, cannot be addressed adequately either by education or prevention. Intrauterine CMV infection is often inapparent and undiagnosed in newborns. On the basis of population sampling, CMV has been estimated to be the leading infectious cause of damage to the developing fetus in utero in the United States, as well as in Europe and other developed areas of the world where im-

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This meeting was planned and the manuscript was prepared by present and past members of the National Vaccine Advisory Committee.

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proved hygiene has delayed acquisition of CMV to the childbearing years. The disease burden for congenital CMV infection is similar to that for congenital rubella before vaccination was introduced to control this disease.

Congenital CMV infection is associated with a range of clinical manifestations, but relatively few infected infants are severely ill at birth. In many, the signs and symptoms may be subtle or nonspecific during the newborn period and during early childhood, even as the child experiences progressive hearing damage and as serious consequences of infection, including mental retardation, cerebral palsy, and impaired vision, become apparent. CMV is one of the most important causes, infectious or noninfectious, of deafness in children. Children who are born with symptoms of CMV infection frequently have late-onset hearing loss. Of congenitally infected children who appear to be healthy at birth, ~15% will have progressive hearing loss when examined over the course of several years, and this damage may eventually become profound. Congenital CMV infection is believed to be responsible for one-third of cases of sensory neural hearing loss in children [1–3]. An estimated 8000 newborns have health problems each year as a result of congenital CMV infection [1]. In the 1990s, the overall disease burden associated with congenital CMV infection was estimated to cost the US health care system at least $1.86 billion annually [4], with a cost per child of more than $300,000, compared with an estimated cost of $200,000 per child for rubella had it not been controlled by vaccination (figures are not adjusted for 2003 dollars) [5]. Thus, the problem of congenital CMV infection is enormous, and the damage affects the very young, resulting in long-term morbidity.

EVOLVING EPIDEMIOLOGY

Molecular evolutionary analysis of the CMV genome suggests that this virus has coevolved with mammals and then with the human species. CMV infection is life-long, but viral replication is usually well controlled by the immune system. Transmission of CMV occurs in situations in which direct contact with body fluids occurs (e.g., breast-feeding, intimate contact, and care of young children). When breast-feeding is widely practiced and most mothers are seropositive, the majority of infants acquire CMV during the first year of life. CMV is transmitted transplacentally by 0.5%–1.0% of CMV-seropositive women, but perinatal and early postnatal transfer of infectious virus through breastfeeding is much more efficient. Rates of >30% have been documented in infants of seropositive mothers who were breast-fed for ⩾3 months [6] Because young children with CMV infection shed virus for years, preschool-aged children who are in close contact, such as those in day care centers, often acquire CMV from each other. Infected infants shed the virus in high titers and are a source for CMV spread to any susceptible children or adults who are in close contact [79]. CMV is also spread by sexual contact. Importantly, rates of primary CMV infection are high in adolescent mothers, and rates of congenital CMV infection are several-fold higher than the national average in their offspring.

From the standpoint of CMV persistence in the human population, perinatal and breast-milk–associated transmission may be regarded as the primary routes of transmission, whereas shedding in infant urine and transfer of infectious virus by sexual contact may be viewed as "back-up" measures that guarantee nearly universal infection. The pattern of CMV transmission has been interrupted by the wide acceptance of formula feeding of infants. As a result, 30%–50% of women of childbearing age in the United States and Europe are susceptible to CMV infection. These women may acquire primary CMV infection during pregnancy if they have close contact with healthy breast-fed infants of CMV-seropositive mothers or with their own toddlers if they have been infected by exposure to healthy infected children (e.g., at day care). Women may also acquire CMV infection via sexual contact with a CMV-seropositive partner who is shedding the virus in saliva or semen. Because the highest risk of serious consequences for fetal development appears to be associated with primary CMV infection during gestation [1], these perturbations in CMV transmission help to explain the growing importance of congenital CMV disease in developed countries.

CMV PATHOGENESIS

CMV molds cell functions to support its replication and displays tropism for differentiated human cells that are critical for its "life cycle." CMV destroys infected cells by active lytic replication. CMV genes block apoptosis (programmed cell death), interfere with the expression of immune recognition molecules and HLA on the surface of infected cells to avoid lysis by natural killer or cytotoxic T cells, and inhibit the antiviral effects of IFNs [10–13]. CMV gene products that attract phagocytic cells may promote transfer to cells of the monocyte/macrophage lineage, and other genes facilitate establishment of latency and persistence in these cells. The virus infects placental cytotrophoblasts, and through placental infection, it may be transferred to the fetus. With this genetic repertoire, CMV achieves high levels of infectious virus in peripheral blood and in epithelial cells during primary infection. Over time, the immune response suppresses replication, although CMV is not eliminated. During chronic CMV infection, the quantity of virus in peripheral blood may increase intermittently, and the virus can replicate actively at epithelial sites throughout the lifetime of the host. Indicators of virus load in the blood, such as quantitative PCR, provide important insights into pathogenesis and confirm the persistent nature of CMV infections [14].

Little is known about the mechanisms by which CMV harms the fetus [15]. Recent work suggests that the virus infects ma-

ternal endothelial cells, from which there is spread to placental cytotrophoblasts and, ultimately, from infected placental cells to the fetal circulation [16, 17]. Important unanswered questions about congenital CMV infection are the relationship of maternal viremia (virus load and duration of viremia) to fetal infection and the role of immunopathology in fetal disease. Studies of the mechanisms that permit or block placental transfer should be expanded.

CONTROL OF CMV BY THE IMMUNE SYSTEM

Both innate and adaptive arms of the immune response are important for the control of CMV infection, and within the adaptive response, both T cells and antibodies have been shown to protect from acquisition of CMV or from serious disease in different settings. Some studies suggest that transplacentally acquired or passively administered antibody protects against CMV disease in neonates and transplant recipients. Antibodies, presumably capable of neutralizing infectivity, can have a role in protection against infection or disease [18, 19], but they are not typically sufficient to control CMV infection. Virus-specific T cell immunity is the most important adaptive immune component responsible for suppressing CMV dissemination to lungs or other organs in which life-threatening damage may occur.

The host immune response does not eliminate CMV, although concentrations of virus at mucosal sites and in peripheral blood are reduced or become undetectable, and persistence and latency are established. Once infection has occurred, the role of the immune system is to suppress replication and to establish and maintain the balance such that intermittent reactivations of the persistent virus remain subclinical. What are the characteristics of this immune response? Because most CMV infections are asymptomatic (in adults as well as children), it has been difficult to characterize the early phases of host response to the virus [20]. However, critical experiments in immunocompromised bone marrow transplant recipients [21–24] and in a murine model demonstrate that CMV-specific cell-mediated immunity is essential to control the disease. The innate immune response may shape or augment the adaptive immune response, and the magnitude of the initial adaptive immune response is important in determining the numbers of antigen-specific memory T cells.

During the transition from an innate immune response to adaptive anti-CMV immunity, natural killer cells may be an important source of IFN-γ, facilitating the expansion of antigen-specific helper T cells that are critical for CMV control. During initial infection, the number of CMV-specific $CD8^+$ T cells increases to an extraordinarily high level. As suppression of viral replication is established, many CMV-specific T cells die, but by comparison with other common viral pathogens, the numbers of circulating T cells that recognize CMV peptides remain quite high [25, 26]. The high frequencies of $CD4^+$ and $CD8^+$ T cells associated with natural CMV infection may reflect "boosts" that result from CMV reactivation and replication at epithelial sites, or they may reflect antigen presentation by virus that persists in monocytes. An effective vaccine may not need to produce such high frequencies of circulating antigen-specific cells to provide protection from new exogenous exposures to the virus.

Analyses of immune responses to naturally acquired CMV indicate that many of the antigen-specific CD8 T cells recognize peptides of the pp65 protein, although the spectrum of antigen recognition may include other viral proteins [27–30]. CMV glycoprotein B and other viral glycoproteins are targets of IgG, IgM, and IgA antibodies and are also recognized by T cells [31]. Although antigens that induce neutralizing antibody and cytotoxic T cells have been identified, other immunogenic proteins should be investigated.

Whether the protein specificity of the initial immune "burst" and the subsequent memory T cell repertoire induced by CMV vaccines should mimic the patterns that are observed with naturally acquired immunity is a consideration for vaccine design and evaluations of immunogenicity. Immune responses to natural CMV infection are complex and seem to be overlapping and redundant in their functions. This redundancy makes it difficult to define immunologic correlates of protection from disease on the basis of assessments of humoral or cellular immunity in the naturally immune host. Animal models of infection with related viruses that are species-specific, such as murine, guinea pig, or rhesus CMV, provide unique opportunities to explore virus-host interactions, including disseminated or intrauterine infection. The contributions of components of the host response to the control of infection can be dissected using these models and may help to optimize strategies for human vaccines. The guinea pig CMV model is particularly useful in this respect and suggests that the gB analogue is protective [32]. How or whether other factors, such as host age or genetic make-up, may alter the outcome of CMV infection—and, thus, the potential response to CMV vaccines—remains to be determined.

GENETIC AND ANTIGENIC VARIATION OF CMV

Different "strains" or genotypes of CMV have been identified on the basis of neutralization by monoclonal antibodies to CMV glycoproteins and restriction enzyme analysis. Is a novel strain of CMV more likely to infect a person with preexisting immunity due to CMV infection? More importantly, from a practical point of view, will vaccination protect against all strains of CMV? Despite the consensus that most morbidity is associated with primary CMV infection, the role of reinfection or super-infection with different strains of CMV in CMV-related disease in the fetus, in newborns, and in immunocom-

promised patients requires further investigation [33]. The likelihood of super-infection after exposures to new CMV strains may be determined by the virus inoculum or the route of inoculation. If CMV is transmitted to a person with established immunity, infection may be asymptomatic, with a low virus load and limited spread within the host, reducing the risk of transfer from mother to fetus. Even vaccines that do not prevent infection may alter the dynamics of maternal infection in ways that prevent fetal disease due to CMV.

The transcendent question is how much protection is afforded by prior infection, and whether that protection is based on antibody, cellular immunity, or both. The importance of strain heterogeneity must be determined. Do the antigenic differences noted among strains mean that vaccines must be multivalent? Are super-infections by novel strains more likely to cause intrauterine disease? This issue must be investigated not only with respect to antibody responses but also with respect to cellular immunity.

VACCINE CANDIDATES IN CLINICAL TRIALS

Five vaccine candidates have been or are being tested in humans (table 1). The first vaccine listed is a CMV attenuated by classical means; the rationale for this vaccine was that the most effective vaccines tend to be live attenuated vaccines. The second is a chimera between the attenuated CMV and wild-type virus; the rationale for this vaccine is that the attenuated virus appears to be erratic in efficacy and has lost segments of its genetic material, so that replacing some genes may produce a more robust vaccine. The third is a nonreplicating recombinant vector, canarypox, with either a gB envelope or a pp65 core antigen; the rationale is that canarypox is a safe, nonreplicating vector that directs synthesis of the CMV protein, thus priming for antibody production or inducing cytotoxic T cells. The fourth is an envelope glycoprotein vaccine made by recombinant technology; the rationale for this vaccine is that it is safe and induces neutralizing antibodies. The fifth is a mixture of synthetic peptides incorporating a T helper epitope, known $CD8^+$ cytotoxic T cell epitopes, and a lipid tail; the rationale for this vaccine is that it will induce cytotoxic/effector T cells. Currently, the attenuated CMV, the protein subunit, and the recombinant vector have been or are being tested in CMV-negative persons, and the chimeric vaccine has been tested in CMV-positive persons as a precursor to testing in CMV-negative persons. Other vaccine candidates that are proposed include a DNA vaccine and a recombinant vaccine.

Support by the National Institute of Allergy and Infectious Disease (NIAID) of the NIH for phase I and phase II trials of candidate CMV vaccines has been critical to providing preliminary safety and immunogenicity data. In addition, planning for efficacy testing must be initiated. The elements required for a successful efficacy trial are listed below.

1. Definition of the desired clinical trial end point: prevention of infection or illness, infections in day care attendees, infections in mothers of day care attendees, viremia in pregnancy, fetal infection or fetal disease, and disease in adults, such as transplant recipients. The logistical difficulty, required sample sizes, and length of clinical trials will vary enormously depending on the end point chosen.
2. Identification of sites and populations appropriate to the end point chosen, such as day care centers (for prevention of infections in infants) or CMV-negative women likely to become pregnant in the next year.
3. Standardization and validation of methods chosen to detect infection, such as virus isolation, PCR, and/or serological testing.
4. Raising consciousness concerning the risks of CMV infection, particularly intrauterine infection, among physicians and prospective trial volunteers. Studies to determine how to increase the diagnosis of CMV infection in neonates and follow-up to detect late-onset hearing loss would be important first steps.

THE PATH TO A CMV VACCINE

The NVAC and expert consultants agreed that research support by the NIH and the CDC, as well as by vaccine manufacturers, is critical for developing a CMV vaccine to prevent death, deafness, and CNS injury due to congenital CMV infection. The NIH is in a position to play a key role by funding early stage efforts towards CMV vaccine development. This report provides a justification for increasing the current levels of NIH support for such initiatives. There are several promising candidate vaccines against human CMV infection in development. However, vaccine developers are uncertain about the immunological basis of protection and the design and conduct of appropriate clinical trials. Because of this uncertainty and for lack of financial resources, none of the projects is progressing rapidly.

Opinion leaders agree that public resources should be devoted to CMV vaccine development. The Institute of Medicine recently assessed the priorities for new vaccine development on the basis of cost and human suffering that would be alleviated. CMV vaccine was ranked the first priority. The development of a vaccine is unlikely to occur without active support of government agencies.

RECOMMENDATIONS

A CMV vaccine to prevent congenital infections, neurologic damage, and deafness should remain a high priority, as recommended by the Institute of Medicine. To accomplish this goal, the strong support of government agencies will be required.

CMV is also an important cause of disease in adults—par-

Table 1. Clinical trials of cytomegalovirus (CMV) vaccines.

Vaccine name	Type	Sponsor/manufacturer	Population	Approximate no. of subjects	Study is ongoing	Vaccine found to be safe	Neutralizing antibodies	T cell immunity	Efficacy	NIH support	Reference(s)
Towne	Live attenuated	None currently	Transplant recipients	500	No	Yes	…	…	Partial	Yes	[33]
			Children	16	Yes	Yes	…	…	NT	Yes	[34]
			Women	300	Yes	Yes	…	…	NT	Yes	[33, 35]
Chimera	Towne/wild type recombinant	Aviron/Medimmune	CMV-seropositive adults	25	Yes	Yes	NA[a]	Yes[a]	TBD	Yes	[36, 37]
ALVAC	Canarypox pp65	Aventis Pasteur	CMV-seronegative adults	40	Yes	…	No	Yes	NT	Yes	[35, 38]
Subunit	Recombinant envelope (gB/MF59 or gB/alum)	Chiron	CMV-seronegative adults	420	No	Yes	Yes	Yes	NT	No	[39–41]
			CMV-seropositive adults	60	No	Yes	Yes	NT	NT	No	[42]
			Women who are planning to become pregnant	400	Yes	Yes		NT	TBD	Yes	[43]
			Toddlers	15	No	Yes	Yes	NT	NT	No	[34]
ALVAC-Subunit	Canarypox followed by Gb/MF59	Aventis Pasteur and Chiron	CMV-seronegative adults	90	No	Yes	Yes	Yes	NT	Yes	[44]
Peptide fusion of CMV-CTL epitope	Peptide fusion of A2 and pp65 with helper peptide	City of Hope; Bachem	Transplant recipients	Phase I	2Q,2004	NA	TBD	TBD	TBD		[45]
Particles	Dense bodies	Gutenberg University	CMV-seronegative adults	…	…	…	…	…	…	No	[46]
DNA	Plasmid	Vical	Transplant recipients	…	Yes	…	…	…	…	No	Not published

NOTE. Table updated to June 2003. Some trials were supported by the National Institutes of Health (NIH), and others were supported by industry.

[a] The findings of studies involving persons with preexisting CMV immunity may be difficult to interpret.

ticularly immunosuppressed adults—and CMV infection in mothers is an important cause of congenital CMV infection. Efforts also should be made to develop a CMV vaccine to prevent adolescent and adult CMV infection and disease.

The NIH should continue to fund studies of the epidemiology of CMV infection and disease, especially of delayed neurologic damage in infants who appear normal at birth, the immune responses that protect against infection or disease, and the role of CMV in adult diseases, such as cardiovascular disorders. A better of understanding of the natural history of CMV infection in healthy individuals, including patterns of viremia and viral shedding, is important for determining how immunity modulates the consequences of CMV infection. Population-based data on the incidence and risk factors for congenital CMV from multiple localities will be required for the planning of pivotal efficacy trials.

The support provided for phase 1 and 2 trials of candidate vaccines is essential for research in this field. The NIH should prepare to support a large efficacy trial if a candidate vaccine proves to be sufficiently promising.

The CDC should expand its epidemiological surveillance to gather population-based data and to include data on late effects of congenital CMV infection. The CDC should implement the planned education programs to promote behavioral prevention of infection during pregnancy.

Trial sponsors should seek early input from the FDA to help them develop workable clinical trial designs and clinical trial programs that will lead to expeditious approval if products are safe and efficacious. Practicing physicians should educate themselves, their peers, and their patients about the importance of avoiding CMV infection during pregnancy and diagnosing congenital CMV infection, including inapparent infection, which may lead to insidious loss of hearing and perhaps other neurologic handicaps.

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Conflict of interest. S.P. is a consultant to Aventis Pasteur, which is developing a vaccine against CMV. A.M.A. is a member of the Scientific Advisory Board of Medimmune, which has a candidate CMV vaccine in testing. P.F., M.M., and R.R.: No conflict.

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