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Development and Clinical Status of Cytomegalovirus Vaccines

Stuart P. Adler

Division of Infectious Diseases, Children's Medical Center, Medical College of Virginia, Richmond, Virginia, USA

Summary

Neonates and immunodeficient patients need protection against cytomegalovirus (CMV) disease. Neonates who acquire CMV either via transfusion or transplacentally are protected against severe CMV disease if their mothers had antibodies to CMV prior to pregnancy, suggesting that immunisation will provide protection for neonates. For immunocompromised patients, protection against severe CMV-associated disease is afforded by immunity acquired either by naturally acquired infection prior to immunosuppression or by passive or active immunisation.

In 3 randomised placebo-controlled studies, live attenuated CMV vaccine successfully protected seronegative recipients of seropositive renal transplants from severe CMV disease by efficiently inducing humoral and cellular immunity. The vaccine virus is neither excreted nor latent. Noninfectious subunit vaccines under study include the CMV glycoproteins gB and gH, each of which contains neutralising epitopes. Given all of these facts, safe and effective CMV vaccines are possible.

This article briefly reviews recent trials of cytomegalovirus (CMV) vaccine in renal transplant recipients as well as the natural history of CMV infection in neonates, demonstrating that vaccination against both cytomegalovirus infection and disease is feasible. Several recent reviews have covered additional aspects of this same topic in greater detail.[1-3]

1. Renal Transplant Recipients

Prophylaxis of CMV infection in transplant recipients provides major evidence for the success of CMV vaccination. Many studies over the last 20 years have found that CMV infection prior to transplantation of organs protects the recipient from CMV disease post-transplantation.[4] Thus, severe CMV disease occurs almost exclusively in the seronegative recipient who receives an organ from a seropositive donor. This observation was confirmed in 3 vaccine studies that used a live attenuated CMV strain (Towne) for immunisation.[5-7]

In these and other similar studies CMV disease was defined using a scoring system. Clinical symptoms of CMV disease after transplantation include fever, leukopenia, thrombocytopenia, hepatitis, pneumonia, gastrointestinal bleeding, central nervous system manifestations, renal insufficiency, arthritis, superinfection and death. Each of these symptoms except for death (given a score of 4) when they occurred in a study patient was given a score of 1, 2 or 3 based on strictly defined levels of severity. This type of scoring system depends upon

the weight given to each symptom, and it is very difficult to know which of the manifestations are more serious or frequent. For example, should pneumonia and central nervous system infection be given equal weight? Nevertheless, once established, this type of system is easy to use in a clinical trial and provides a reproducible protocol that can be used at multiple centres.

The first study, a double-blind randomised study of the Towne strain by Plotkin et al.,[5] was published in 1991 even though this vaccine was first developed in 1975. Phase I, II and III studies in renal transplant recipients required 16 years. In this study, 124 patients received a single dose of the Towne vaccine [approximately 10^3 plaque-forming units (PFU)/dose] and 113 received placebo. Regardless of whether an individual was CMV seropositive before transplantation or whether the donor was infected with CMV, vaccination had no effect on the 50% rate of CMV infection post-transplantation. For CMV disease, however, there was an effect of vaccination.

Using the scoring system described above, Plotkin et al. found that the effects of vaccine on CMV disease occurred only in seronegative recipients of kidneys from seropositive donors.[5] In this category of patients there was a mean disease score of 4.9 for the 31 placebo recipients and 2.3 for the 36 vaccine recipients ($p < 0.05$), with a marked shift toward higher scores among placebo recipients compared with vaccine. Receipt of CMV vaccine was also associated with significant prolongation of graft survival for recipients of cadaveric kidneys: 9 of 14 vaccine recipients retained functioning kidneys compared with only 4 of 16 placebo recipients.

A second randomised double-blind study of renal transplant patients included 117 patients who received vaccine. Of these, 35 were seronegative patients who received seropositive kidneys.[6] In this study there was again no effect of vaccination on infection rates, but there was an effect of vaccination on disease. Five of 6 seronegative recipients of seropositive kidneys who received placebo developed either severe or lethal disease, compared with only 2 of 8 vaccine recipients. The p value in this study was 0.1.

A third study which used the Plotkin protocol was completed at 3 other centres. This study included only seronegative recipients of seropositive kidneys.[7] The results of this study confirmed those of the first 2 studies, also finding more severe disease among the 24 recipients of placebo than among the 37 recipients of vaccine.

Table I summarises the 3 renal transplant studies and lists the rate of reduction of severe disease by CMV immunisation. Overall, for seronegative recipients of seropositive kidneys, the receipt of vaccine prior to transplantation reduced severe disease, defined by a score of 7 or more, by 89%. The trials revealed that:

- about 90% of vaccine recipients developed humoral and cellular immunity;
- the virus was neither latent nor excreted;
- the vaccine protected seronegative renal transplant recipients from severe disease associated with CMV infection;
- the vaccine may prolong graft survival, particularly for recipients of cadaveric kidneys.

Table I. Summary of results of 3 trials of Towne vaccine in cytomegalovirus (CMV) seronegative recipients of seropositive renal transplants. Severe CMV disease is defined as a score of 7 or more in the scoring system described in the text

Trial location	Number of patients	Rate of all CMV disease (%)		Rate of severe CMV disease (%)		Reference
		vaccine	placebo	vaccine	placebo	
San Diego, Michigan, Oxford	61	38	59	0	17	7
Pennsylvania	67	39	55	6	35	5
Minnesota	35	33	43	5	36	6
All	163	37	54	3	29	

2. Neonates and Congenital Infection

2.1 Transfusion Studies

In the early 1980s, transfusion studies in neonates first showed that antibodies to CMV were likely to be protective against CMV disease.[8,9] These studies found that premature neonates of CMV seronegative mothers developed symptomatic CMV infections acquired from transfused blood products, but premature neonates of CMV seropositive mothers remained asymptomatic after receiving the same blood products.

After birth, maternal antibody to CMV declines rapidly. By 8 weeks of age only between 10 and 20% of maternal antibody to CMV remains.[10] Even at this age, although not protected against CMV infection, neonates are protected against developing severe disease due to CMV. Later, when maternal antibody is at very low levels, some neonates will develop symptomatic infections although their severity is generally less than that observed in seronegative infants.[8,10] These observations indicate that maternal antibody modulates CMV infection.

2.2 Congenital Infection

Another observation showing the importance of CMV immunity for disease prevention comes from studies of the natural history of congenital infections and pregnancy. These studies show that maternal immunity protects against symptomatic congenital infection.

In 1992, Fowler et al.[11] found that of 125 infants with congenital infection who were born of mothers with a primary CMV infection during pregnancy, sequelae occurred in 25% of the infants. This contrasted with an 8% rate for 64 infants with congenital infection but born of mothers who were CMV seropositive prior to pregnancy. More importantly, none of the infected infants born of seropositive mothers developed severe sequelae such as bilateral hearing loss or mental retardation, as defined as an intelligence quotient of less than 70. Such sequelae occurred only among the infants born of mothers who had a primary infection during pregnancy. These observations suggest that immunity to CMV prior to pregnancy will prevent the majority of severe sequelae associated with congenital infection.

Fowler et al.[11] estimated that about 8000 infants in the US are born annually with severe sequelae or die because of congenital CMV infection.[11] A cost-benefit analysis[12] estimated that $US834 million would be saved annually by preventing these 8000 cases, assuming complete immunisation of all women aged 15 to 24 with a vaccine costing between $US8 and $US16 per dose (the cost of other live virus vaccines).

2.3 Can Vaccination Protect Against Infection During Pregnancy?

To help determine if a vaccine can protect women from acquiring a primary CMV infection during pregnancy, a collaborative study asked if immunisation with Towne vaccine and natural immunity can protect women of childbearing age against secondary infection.[13] We studied mothers who had a child less than 3 years old who was shedding CMV. We used a double-blind randomised protocol where seronegative mothers received either placebo or vaccine and seropositive mothers received placebo. The women were then monitored for at least 1 year and in most cases for 2 years post-vaccination. The characteristics for the 3 groups were similar for maternal age, age of the child, and the duration of viral shedding by the child.

We found a 42% infection rate (defined as a 4-fold rise in titre to CMV, or viral shedding) among those women who received vaccine and a 47% infection rate among those women who received placebo. Among those women who were naturally seropositive the infection rate was only 2.7%.

Why did the Towne strain vaccine fail to protect? We compared lymphoproliferative responses, enzyme immunoassay (EIA) titres and neutralising titres to CMV antigens in individuals who received vaccine and were secondarily infected with those in individuals who received vaccine but remained uninfected. Women who remained uninfected had

the same EIA titres and lymphoproliferative responses following vaccination as did women who became infected. However, the mean neutralising antibody titre was highest among those who remained free of secondary infection following vaccination compared with those secondarily infected after vaccination.

We also compared lymphoproliferative responses, EIA titres and neutralising titres to CMV antigens of vaccine recipients with those in women who experienced a naturally-acquired infection. For lymphoproliferative responses there were no significant differences between those vaccinated or infected naturally. However, a single dose of the Towne strain produced EIA or neutralising titres 10- to 20-fold lower than observed either in women who had a primary infection or who were seropositive prior to entering the study.

That natural seropositivity protects against secondary infection was first observed in a challenge study done by Plotkin et al.[14] They found that 4 of 7 male healthy volunteers who received vaccine and were subsequently challenged with 100 PFU of a non-attenuated strain (Toledo) became infected, but that only 1 of 5 naturally seropositive men became infected after a subcutaneous challenge with the same dose.

These observations lead to the hypothesis that a vaccine that produces neutralising antibody titres equal to those caused by wild-type infection will protect healthy immunocompetent women from secondary infection. Using the live attenuated Towne strain or other vaccines, how might more potent vaccines be achieved? Studies of the Towne vaccine are currently in progress, evaluating immunoadjuvants and multiple doses.

3. Subunit Vaccines

Enhanced immunogenicity for CMV vaccines may be achieved by noninfectious subunit vaccines. Many investigators have defined the components of the CMV viral capsid that may serve as potential subunit vaccines. The recombinant hepatitis B vaccine, which produces neutralising antibodies and is protective, is a recent example of a successful application of this technology.

Table II. Envelope glycoproteins of cytomegalovirus containing neutralising epitopes

Glycoprotein	Epitope (amino acids)	Antibody	Reference
gB	1-513	Human sera	15
	27-84	Neutralising monoclonal	16
	484-588	Neutralising antibody	17
	645-700	Neutralising antibody	17
	780-907	Neutralising antibody	17
	589-645	Neutralising monoclonal	18
	798-805	Neutralising antibody	19
gH	34-43	Neutralising monoclonal	20,21
gCII	Native	Neutralising monoclonal	22

For CMV, table II summarises the human immune response to recombinant proteins expressed in a variety of vectors, including plasmids, vaccinia, baculoviruses and adenoviruses.[23-25] Neutralising antibodies are associated with envelope glycoproteins gH, gB and gCII.[15-23,25-31] For gB and gH the particular peptide epitopes with neutralising domains have been mapped using either monoclonal antibodies or human sera (table II). Many laboratories worldwide are engaged in characterising these proteins and peptides and cross-linking them with appropriate carriers or expressing them in appropriate vectors for evaluation as human vaccines.

The best studied and most promising potential vaccine is the disulphide-linked glycoprotein complex (gB) with components of 130 and 55kD.[23,26,32,33] This complex is a major component of the envelope glycoprotein of the CMV viral capsid. The gB complex contains at least 7 neutralising epitopes (table II). Antibodies to gB are abundant in sera from patients convalescing from CMV infection.[28,29] Monoclonal antibodies against gB neutralise both wild-type viral isolates and laboratory-adapted strains.[31] The gB proteins have been expressed in recombinant viruses using both adenovirus and vaccinia and these recombinants induce neutralising antibodies in animals.[24] The levels of neutralising antibodies induced by gB are proportional to the quantity of gB expressed

in recombinant vaccines or used directly for immunisation.[24,25] In both animals and humans, gB also contains epitopes that stimulate T lymphocytes cytotoxic for CMV.[15] Thus, gB is the most likely candidate for a subunit vaccine.

4. Conclusions

Over the past 16 years it has been established that seronegative recipients of organs from seropositive donors and women seronegative prior to pregnancy are the 2 major groups who need protection against CMV infection by vaccination. The live attenuated Towne vaccine protects seronegative transplant recipients against severe CMV disease post-transplantation. For seronegative women, a single dose of the vaccine lacks sufficient potency to protect against secondary infection. Whether the current vaccine has sufficient potency to protect against disease caused by intrauterine infection following a primary maternal infection remains to be determined. Thus, a great deal has been accomplished in terms of evaluating the Towne strain of CMV for safety, immunogenicity and efficacy. A great deal has also been accomplished with regard to an understanding of the viral components that contribute to the immune response and production of neutralising antibody.

The future holds many challenges. Despite its proven efficacy and safety, the Towne strain of CMV is unavailable for seronegative transplant patients because it lacks a commercial manufacturer. Thus, one challenge will be to convince pharmaceutical companies of the economic viability of CMV vaccines. Other future challenges will be: (a) to convince the public and public health officials of the importance of CMV disease; (b) to produce vaccine with the immunogenic potency of the wild-type virus; and (c) for congenital disease, to design field trials that will show not only protection against secondary infection but also protection of the fetus.

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Correspondence and reprints: Dr *Stuart P. Adler*, Division of Infectious Diseases, Children's Medical Center, Medical College of Virginia, Box 163, Richmond, VA 23298, USA.