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REVIEW

The history of vaccination against cytomegalovirus

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Abstract Cytomegalovirus vaccine development started in the 1970s with attenuated strains. In the 1980s, one of the strains was shown to be safe and effective in renal transplant patients. Then, attention switched to glycoprotein gB, which was shown to give moderate but transient protection against acquisition of the virus by women. The identification of the pp65 tegument protein as the principal target of cellular immune responses resulted in new approaches, particularly DNA, plasmids to protect hematogenous stem cell recipients. The subsequent discovery of the pentameric protein complex that generates most neutralizing antibodies led to efforts to incorporate that complex into vaccines. At this point, there are many candidate CMV vaccines, including live recombinants, replication-defective virus, DNA plasmids, soluble pentameric proteins, peptides, virus-like particles and vectored envelope proteins.

Keywords Vaccination · Vaccine trials · Towne strain

Here I wish to commemorate two events: First that the domain of cytomegalovirus has reached a maturity that now commands the attention of many investigators, and second is the 60th birthday of Matthias J. Reddehase, one of those responsible for that growth to maturity. The late Monto Ho wrote a paper in this journal on the history of CMV some years ago [1], and here, I reproduce the first references of his paper. Table 1 notes that German scientists were the first to call attention to this agent, before it was identified as a virus. So it is not surprising that the study of CMV has flourished in Germany. If I may characterize it, it is the school of Ulrich H. Koszinowski, of which Matthias has been one of the stars. In Table 2 [2], I list some of their accomplishments.

The task given to me is to review the status of efforts to vaccinate against CMV. First, let us remember why we need a vaccine. Aside from the two main targets for disease prevention: congenital infection and post-transplant disease, Table 3 lists some of the many conditions to which CMV is suspected of contributing [3–8].

By the 1970s, the importance of CMV had become clear to some. The first efforts to develop a vaccine were focused on attenuated strains, developed almost simultaneously by Elek and Stern in London and my group in Philadelphia [9, 10]. It should be remembered that there had been great successes just before then in the development of other attenuated virus vaccines. Table 4 gives the succession of CMV vaccine development until 2005. That table, however, does not give the full story, for in the early years there was great skepticism in industry about the need for and viability of a CMV vaccine. That is why both Merck and GSK abandoned projects they had started during those years [11]. It is greatly to the credit of the people at Chiron in California that they launched an effort to develop gB as a vaccine in the 1990s, but even they abandoned it [12]. My own personal move to Sanofi Pasteur in the 1990s resulted in a transfer of the Chiron, now Novartis, CMV project to the French company.

However, the event that proved to be what is called a game changer in American slang was the publication in 2000 of an analysis by the US Institute of Medicine that placed CMV in the top priority for vaccine development

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Table 1 Early German contributions to discovery of HCMV

1. Ribbert H (1904) Ueber protozoenartige Zellen in der Niere eines syphilitischen Neugeborenen und in der Parotis von Kindern. Zbl All Pathol 15:945–948
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Table 2 Some seminal contributions of Matthias J. Reddehase to CMV research

CTL responses	1984
Importance of responses to IE	1984, 1987, 1989
Recombinant vaccine	1988, 1991
Latency	1993, 1994, 1997, 1999
T cell immunotherapy	1985, 1988, 1998
Immune evasion	1989
Memory inflation	2000

"Man lernt nichts kennen als was man liebt"—Goethe

Table 3 Medical conditions suspected of being caused by CMV aside from congenital infection and post-transplant diseases

Atherosclerosis
Glioblastoma
Immunosenescence
Deterioration while in intensive care
Biliary atresia

Table 4 History of CMV vaccine development until 2005

1974–1979	Attenuated AD-169 (Elek + Stern with Merck)
1975–1979	Attenuated Towne (Plotkin with GSK)
1980–1985	Attenuated Towne (Plotkin with Merck)
1985–1990	Towne (Plotkin with NIAID)
1991–2001	gB (Chiron)
1995	Canarypox vector (Sanofi)
1996	Towne–Toledo recombinants (MedImmune)
1997	Peptides (City of Hope)
2001	gB (Sanofi)
2005	DNA plasmids (Vical)

[13]. This caused industry to realize that a vaccine would be used if developed.

To return briefly to attenuated viruses, the AD-169 strain was not developed further by Merck. During the 1970s and 1980s, the Towne strain, which was the result of 125 passages in human diploid fibroblasts, was tested in many human clinical trials [14–24]. The summary of those trials is shown in Table 5. On the safety side, there were no systemic reactions, no excretion of virus even when vaccinated renal transplant patients were immunosuppressed, and no depression of cell-mediated immunity. Interestingly, there was a local injection site reaction at about a week after injection, probably as the result of cellular immune responses to the local deposition of antigen. On the immunogenicity side, neutralizing antibodies were induced at a similar level to that found in convalescent sera and both CD4+ and CD8+ cells directed against CMV were generated. Of course, now we know that during cell culture passage Towne and other CMV strains lost the ULb' region of the genome, in which the genes permitting entry into epithelial cells and those influencing latency are found [25, 26].

During that time, an interesting challenge study was done, showing that there was a gradation of immunity against subcutaneous challenge with a wild CMV strain (Table 6) [27, 28]. Whereas even a 10-PFU dose of challenge virus infected seronegative volunteers, naturally seropositive individuals could be infected if given 500–1000 PFU. Towne strain vaccines were intermediate, in that 100 PFU could infect them. So resistance varied over a three log10 range.

Even more interesting were the results of studies to prevent CMV disease in recipients of renal transplants. As is well known, the high-risk group is seronegative recipients of kidneys from seropositive donors, as the virus is latent in those kidneys. Figure 1 shows the results. About a third of recipients given placebo developed CMV disease, only about 5 % of Towne vaccinees did so [28, 29]. The protection afforded was similar to that in naturally seropositive recipients in the study who received a kidney from a seropositive donor. Even more important, the transplant rejection rate was reduced by 50 % in the vacinees. This was the first evidence that CMV disease could be prevented by vaccination. In addition, the vaccine virus did not establish latency [27].

However, when Stuart Adler did a study of mucosal acquisition of CMV in mothers exposed to children infected in day care, vaccination did not prevent the mothers from being infected [30]. Thus, immunity was incomplete. MedImmune, now a subsidiary of AstraZeneca, made

Table 5 Neutralizing titers to CMV in adults after natural infection or Towne vaccine

Group	Virus strain	No. of doses/subject	Subjects	Reciprocal antibodies geometric mean neutralizing titer
Females	Wild type	0	15	488 (256–2048)
Males	Towne	1	23	270 (128–1024)
Males	Towne	2	43	402 (128–2048)
Males	Towne	3	12	512 (256–1024)

Adler et al. PIDJ 17:200–206, (1998)

Table 6 Doses of subcutaneous CMV challenge required to infect or cause disease in 50 % of different groups

	Infection	Disease
Seronegatives	<10 PFU	<10 PFU
Natural seropositives	≈500 PFU	1000 PFU
Vaccinees	100 PFU	>100 PFU

Plotkin, J Clin Virol (2002);25:S14

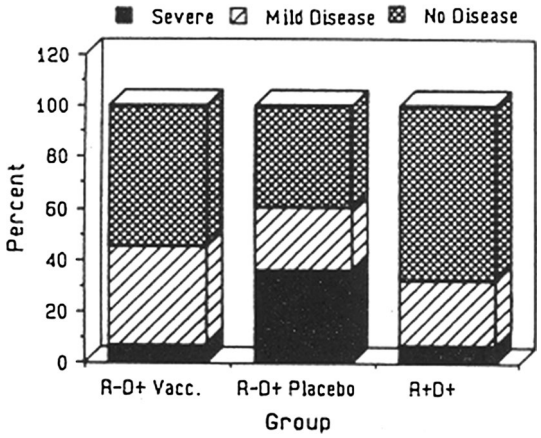

Fig. 1 Outcome of exposure to transplanted kidney from a CMV seropositive donor (D+) in renal transplant recipients. Reproduced from Plotkin et al. Rev Inf. Dis (1990);12, Suppl 7, S827–S838

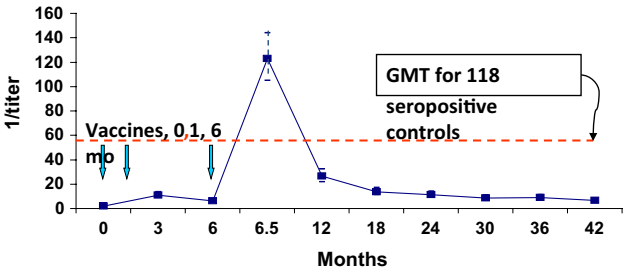

Fig. 2 Neutralizing antibody to gB: 136 recipients of three injections of CMV gB vaccine, GMT and 95 % CI, N from 92 to 136

recombinants of Towne and a low-passage strain I gave them called Toledo [31]. Unfortunately, owing to hesitancy at the US FDA and loss of interest by MedImmune, those recombinants are just now being tested clinically in seronegatives by Adler.

Meanwhile, as previously mentioned, the first clinical studies of gB were being done by Chiron. The choice of that glycoprotein as a candidate vaccine was suggested by the demonstration in my laboratory and elsewhere that neutralizing antibodies, as detected by the prevention of infection in fibroblasts, were mainly directed against it [32–34]. Chiron combined gB with their oil in water adjuvant, MF-59, and showed that after three doses given at 0, 1 and 6 months, a reasonable titer of neutralizing antibodies was generated in adults [12, 35, 36]. Toddlers given the vaccine developed titers six times higher [37]. However, gB antibodies waned within <1 year, revealing a compromised durability of response.

Chiron, now Novartis, launched a randomized, placebo-controlled trial of gB/MF-59 under the direction of Bob Pass at the University of Alabama, which was continued under the auspices of Sanofi Pasteur [38, 39]. The trial was conducted in young women who were exposed to CMV by both respiratory and sexual routes. As is well known, the trial did show a 50 % reduction of CMV acquisition over a period of 42 months, but almost all the protection occurred in the first 18 months of the trial. The probable explanation for this emerged when the neutralizing antibody data were examined: Such antibodies peaked at 6.5 months just after the third dose and faded quickly thereafter (Fig. 2) [35, 40].

Recently, Bernstein et al. [41] have performed a similar study in Cincinnati. They achieved protection of only 45 %, but it can be said that the study confirmed that gB when used alone as a vaccine has a moderate degree of efficacy against acquisition of CMV by a mucosal route. Meanwhile, GSK has adopted the same antigen, gB, but added to it an adjuvant containing MPL, a stimulator of TLR receptor 4 (Arnaud Marchant, personal communication, 2014). This study has also not yet been published, but the data presented at meetings showed a good neutralizing antibody response that persisted longer than after the gB/MF-59 vaccine. Both the gB/MF-59 and gB/AS01 vaccines generated CD4+ T cell responses [39] (Arnaud Marchant, personal communication, 2014).

Meanwhile, Paul Griffiths and his colleagues in London tested gB/MF-59 as a prophylactic in recipients of kidney or liver transplants [42]. The recipients, whether CMV

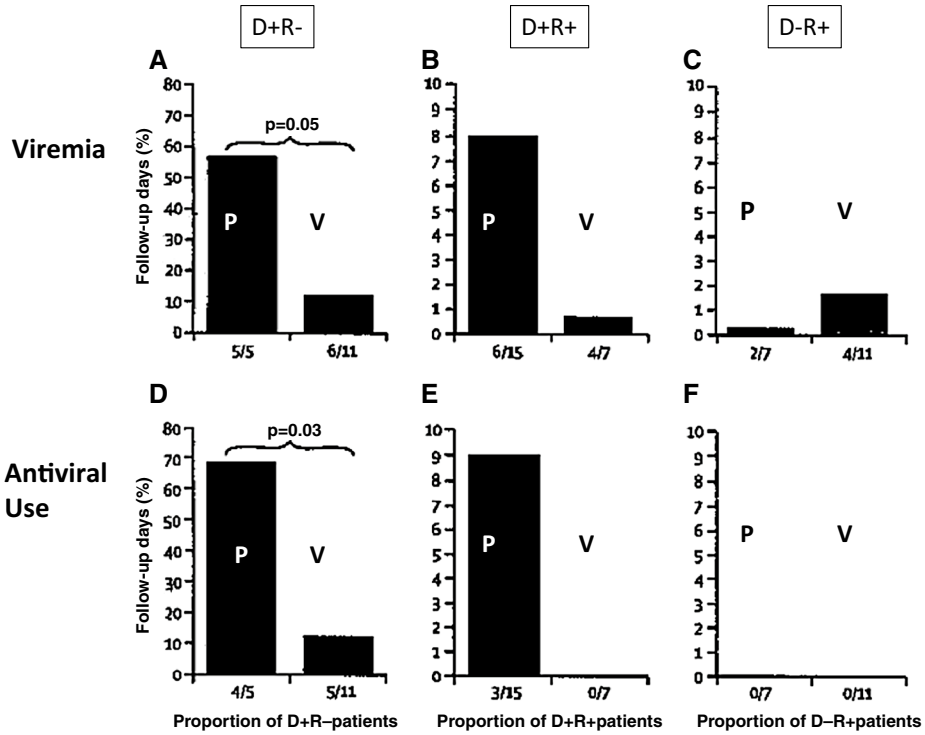

Fig. 3 Sanofi Pasteur gB/MF-59 in kidney or liver transplant patients. Proportion of days that patients in the three subgroups at risk of CMV infection spent with viremia or received antiviral treatment. P: placebo, V: vaccine. Modified from Griffiths et al. (2011) Lancet, 377:1256

seronegative or seropositive, were given three doses of the vaccine before transplant. The results were astounding in that both recipients infected by the transplanted organ (R-D+) as well as those in whom the source of the infection could not be identified (R+D+), revealed a marked reduction of CMV viremia and therefore antiviral use (Fig. 3). This implies that there is a stage when the virus reactivates from the donated organ and must spread via viremia to the rest of the body, during which gB antibodies prevent that viremia.

However, aside from solid organ transplantation, there is also the problem of CMV disease in recipients of hematopoietic stem cell transplants. In that population, the problem is different: Virus reactivates in the recipient who has had prior natural infection, but in whom immunosuppression ablates cellular immunity, particularly CD8+ T cells [43]. Although cytotoxic T cells after natural infection are directed against many proteins of CMV, most individuals have CTL specific for the major tegument protein, pp65 [44–46]. In the late 1990s, the group at City of Hope Hospital led by Don Diamond had experimented with CMV peptides that could elicit CTL responses [47]. A pp65/gB combination DNA vaccine formulated in a poloxamer nanoparticle adjuvant was developed by Vical and licensed to Astellas. In a controlled trial, Vical showed that T cell responses were generated against pp65, but there were relatively poor antibody and cellular responses against the gB component [48–50]. Nevertheless, CMV viremia was reduced by prior vaccination. A phase III trial is in progress. Also, the biotech Inovio has reported promising results in mice using DNA plasmids and electroporation [51].

Another approach to generating both antibodies and cellular responses was taken by Novartis. They used replicons based on Venezuelan equine encephalitis virus containing the gB, pp65 and IE1 proteins. Responses were generated against all three, best against gB and worst against IE1, but those responses were not particularly high [52–54].

That brings us to the present: gB continues to be of interest as a vaccine antigen, but the discovery of the gH/gL/UL128-131 pentameric complex and the link shown by the group in Pavia between response to the pentamer in infected pregnant women and prevention of CMV transmission to the fetus has caused all parties to reconsider the constitution of a CMV vaccine [55–59]. Merck has chosen to pursue a replication-defective virus strategy [60–62]. They have linked two CMV proteins, UL51 and IE1/2 to a protein domain that renders stability of these viral proteins dependent on the small molecule Shield 1 (Shld 1). In cell culture containing Shld 1, the virus replicates and is produced in quantity. However, when injected, the virus cannot replicate fully and only defective virus is produced. Nevertheless, the defective virus expresses the pentameric complex as well as all other AD169 strain proteins, including gB. Monkeys inoculated with the replication-defective virus have developed both good antibody and cellular responses. A phase 1 trial is ongoing.

Table 7 CMV vaccines in development

Live CMV vaccines in development	
Attenuated strain (Towne)	Med coll VA
Recombinants with wild virus (Towne–Toledo)	MedImmune
Replication-defective virus	Merck
Alphavirus replicon	Novartis
Vectored: pox, adeno, LCMV	Sanofi Pasteur, City of Hope Queensland Inst., Paxvax, Hookipa
Non-living CMV vaccines in development	
Recombinant gB glycoprotein with adjuvant	Sanofi Pasteur, GSK
DNA plasmids	Vical, Inovio
Self-replicating RNA	Novartis
Peptides	City of Hope
Dense bodies	Vaccine project management (Germany)
Virus-like particles	Variations Biotech, Redbiotec
Soluble pentamers	Humabs

Table 8 Lessons learned from prior CMV vaccine trials

Neutralizing responses to gB can be elicited by live virus, subunit glycoprotein, poxvirus and alphavirus vectors
gB, if adjuvanted, is a protective antigen against CMV infection in seronegative women and solid organ transplant recipients
Live attenuated virus also protected immunosuppressed solid organ transplant recipients against severe disease
CTL responses to pp65 can be elicited by live virus, poxvirus vector, alphavirus vector and DNA plasmids
pp65 is a potent inducer of CD8+ and CD4+ T cell responses and together with gB reduces viremia and disease in seropositive stem cell recipients
Priming with DNA or vectors coding for CMV proteins followed by various boosts may improve response
Duration of protection is yet uncertain
Antiepithelial/endothelial cell entry antibodies induced by the gH/gL/UL128-131 pentamer may be important in the prevention of transmission to fetus

Meanwhile, Novartis has adopted the self-replicating RNA strategy, in which RNA for the major candidate antigens is produced in vitro and then injected in vivo, where transcription generates the proteins [63]. Data are awaited.

An approach developed in Germany by Bodo Plachter and colleagues involves the purification of dense bodies, subviral particles produced by CMV in cell culture [64, 65]. These enveloped particles contain virion tegument and envelope proteins, including gB, and are being modified to include the pentameric complex products.

Other strategies now actively explored include CMV peptides to generate cellular immunity in transplant patients and virus-like particles presenting gB and, in some cases, the pentamer on their surfaces [66, 67]. Variations Biotech in the USA, Redbiotec in Switzerland and Humabs in Switzerland have all had success in animal immunization experiments. Human data are anxiously awaited [68].

Table 7 lists all of the CMV vaccines in development known to me. What have we learned from all of this work, now going back almost 40 years? Table 8 summarizes the lessons learned, which are quite positive. In fact, there are multiple ways to generate immune responses to key CMV proteins, and a degree of protection has been shown with gB, pp65 and probably the pentameric complex. The clinical pathways to demonstrate efficacy in pregnant women and transplant recipients have been described.

So whom would we vaccinate with a CMV vaccine? The obvious targets would be prepubescent girls, who are already receiving other vaccines at ages 11–13; adult women who intend to have children; seronegative solid organ transplant recipients; and seropositive hematogenous stem cell transplant recipients. There are many ways in which efficacy might be demonstrated in the prevention of congenital infection. However, the way designated by the US FDA is by a placebo-controlled study in a cohort of women vaccinated before they become pregnant, with a follow-up of their newborns for the evidence of infection at birth. Fortunately, PCR on urine or saliva is highly sensitive and specific for fetal infection if done during the first 2 weeks of life [69].

There are more speculative targets for CMV vaccination such as prospective cardiac bypass patients to prevent restenosis and all adults to prevent immunosenescence, but there is a more real indication for routine immunization of all infants. This is because modeling shows that circulation of CMV in toddlers is the reason for most acquisition of the virus in women and that vaccination of those children would have a great protective effect for their mothers apart from the direct utility of vaccination before pregnancy [70]. Another advantage of toddler vaccination is that whereas the duration

Table 9 How to demonstrate efficacy of a CMV vaccine

Artificial challenge with low-passage virus
Prevention of infection of women whose children are in day care
Prevention of infection of children entered in day care
Prevention of disease or infection in solid organ and hematogenous transplant recipients
Cohort study in prepregnant women to prevent later fetal infection
Prevention of fetal disease

of protection by vaccination of prepubescent girls is a major uncertainty, a decrease in CMV circulation in toddlers could be protective through the induction of herd immunity even if the duration of immune response in them is short.

Table 9 lists the many ways in which the efficacy of a CMV vaccine could be demonstrated. Multiple possibilities exist to prove that maternal and fetal infection can be prevented by vaccination. To show the efficacy of CMV vaccination in the transplant situation would require only the monitoring of viral load, the use of antivirals, graft rejection and of course CMV clinical disease.

So, in summary, the antigens needed in a CMV vaccine have been identified, evidence that vaccination can protect is available, the targets of vaccination are largely known, and a path for licensure has been defined. This situation is the result of 40 years of work. To reach the goal of a CMV vaccine, we need now a concentrated effort to combine the important antigens and to generate durable responses that will protect for a significant period of time. I do think that goal is obtainable and I humbly hope that it will be obtained in my lifetime.

Acknowledgments Figure 3 is reprinted with permission from Elsevier (The Lancet, 2011, 377(9773):1256-1263).

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