SUPPLEMENT ARTICLE

Cytomegalovirus Vaccines

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An effective cytomegalovirus (CMV) vaccine could prevent the majority of birth defects caused by congenital CMV infections. Candidate vaccines in clinical evaluation include live attenuated, protein subunit, DNA, and viral-vectored approaches. Subunit approaches have focused on the CMV proteins pp65 and IE1 as important inducers of cytotoxic T cells and glycoprotein B (gB) as an important inducer of neutralizing antibodies. A vaccine comprised of recombinant gB protein with MF59 adjuvant reduced the incidence of primary infection by 50%. Recent revelations regarding CMV entry pathways into different cell types suggest a possible course for improvement. A 5-subunit pentameric complex is uniquely required for endothelial and epithelial cell entry. Sera from naturally infected subjects contain high-potency neutralizing activities specific for this complex, whereas the gB/MF59 vaccine fails to induce comparable neutralizing activities. A vaccine's ability to induce salivary antibodies that neutralize epithelial cell entry may be especially important for preventing oral transmission as the first cells infected are presumably epithelial cells of the oral mucosa. In addition, recent evidence suggests that antibodies can inhibit postentry CMV spread between endothelial and epithelial cells. Such activities may serve to limit viral replication in tissues or impair dissemination to the placenta and fetus. Thus, inclusion of epitopes derived from the pentameric complex may provide enhanced efficacy by inducing potent neutralizing/spread-inhibiting antibodies that target virus replication in a broad spectrum of cell types. Next-generation vaccine candidates in preclinical development incorporate peptides, subunits, or multisubunit complexes representing parts or all of the pentameric complex. Approaches include peptides, recombinant proteins, DNA, replication-defective viral vectors, genetically disabled CMV, and inactivated CMV virions. The diversity of novel strategies under development engenders optimism that a successful candidate will emerge.

Keywords. cytomegalovirus; congenital infection; vaccine.

Cytomegalovirus (CMV) is among the largest and most complex of the known viruses that cause human disease. The 235-kb genome encodes at least 165 proteins [1], but CMV vaccine research has focused on a limited number of viral proteins that dominate cellular or humoral immune responses during natural infection. The pp65 protein is a major target of the cytotoxic T-cell response [2]. Located within the tegument between the capsid and the viral envelope, pp65 is the most abundant protein in CMV virions. The IE1 protein is also an important cytotoxic T-cell target that is not in the virion but is abundantly expressed in cells after infection. On the virion surface and embedded in the envelope are several glycoprotein complexes that mediate host cell entry. A heterodimer comprised of glycoprotein M and glycoprotein N is believed to initiate host cell interaction by binding to heparin. A second heterodimer comprised of glycoprotein H and glycoprotein L (gH/gL) may mediate receptor interactions that culminate in the triggering of conformational changes in glycoprotein B (gB) that drive fusion of the viral envelope with the target cell membrane [3]. All of these complexes are important targets for humoral immunity as they contain epitopes that bind a select class of antibodies known as neutralizing antibodies [4–7].

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By binding to viral entry mediators, neutralizing antibodies can block cell entry and hence neutralize virus infectivity.

LIVE ATTENUATED VACCINES

Live attenuated vaccines present potential advantages for complex viruses such as CMV. By expressing a full or nearly full complement of viral antigens, they can induce both humoral and cellular immune responses that closely mimic those induced by natural infection. However, safety is a significant concern for a live CMV vaccine, especially if inadvertently administered during pregnancy. The live Towne vaccine consists of a CMV isolate that was attenuated by serial passage on cultured human fibroblasts. It has been safely administered to nearly 1000 volunteers and induces both cellular and humoral responses. When used at a low dose, the Towne vaccine failed to protect against primary maternal infection [8]. However, a higher dose was subsequently shown to be more immunogenic; efficacy of this dose has not been reported.

In an attempt to improve the immunogenicity of the Towne vaccine while retaining its safety profile, regions comprising approximately 25% of the Towne genome were replaced with corresponding sequences from the low-passage Toledo strain to produce 4 different "Towne-Toledo chimeras." Four live attenuated vaccines, each containing a different chimera, had no vaccine-related adverse events when administered to CMV-seropositive volunteers but did not boost CMV immunity above preexisting levels [9]. A phase 1 trial of the 4 chimera vaccines in CMV-seronegative volunteers is in progress.

SUBUNIT VACCINES

Subunit vaccines provide potent, focused immune responses to select viral immunogens. The simplest subunit vaccines combine subunit immunogens (eg, recombinant proteins) with an adjuvant, whereas vectored delivery systems rely on genetic programming of host cells (via uptake of naked DNA or infection with replication defective viruses) to express the desired immunogen(s) in vivo. Subunit vaccine strategies have focused primarily on pp65 and IE1 to induce cellular immunity and gB to induce neutralizing antibodies. The gB/MF59 vaccine developed by Sanofi combines a recombinant soluble form of gB with MF59, an oil and water adjuvant [10]. In phase 1 trials, the gB/MF59 vaccine was both safe and highly immunogenic [10]. In a phase 2 trial, it reduced the incidence of primary infection by 50% [11]. A subsequent study showed that patients who received the gB/MF59 vaccine prior to renal transplantation had reduced duration of CMV viremia and required fewer days of antiviral treatment [12]. A similar vaccine comprised of recombinant gB protein adjuvanted with AS01 has been developed by GlaxoSmithKline. It was safe and immunogenic in a phase 1 trial.

PLASMID DNA AND VIRAL-VECTORED VACCINES

The TransVax vaccine was developed by Vical for use as a therapeutic vaccine in transplant patients. It is comprised of plasmid DNAs encoding pp65 and gB formulated with poloxamer adjuvant. In a phase 2 trial, TransVax reduced posttransplant CMV viremia in patients undergoing hematopoietic cell transplant [13]. TransVax has been licensed to Astellas Pharma Inc and has entered a phase 3 trial in hematopoietic cell transplant patients. Vical's congenital CMV vaccine, CyMVectin, is in late preclinical development. CyMVectin is comprised of plasmids that express pp65 and gB formulated with Vaxfectin, an adjuvant that promotes robust cellular and humoral responses.

AVX601 is an RNA virus–vectored vaccine developed by Alphavax. This platform uses replication-defective Venezuelan equine encephalitis virus to express gB and a pp65/IE1 fusion protein [14]. The vaccine has been licensed by Novartis and was evaluated in a phase 1 trial.

THE PENTAMERIC COMPLEX

The focus on gB as a vaccine immunogen arose from studies indicating that gB-specific antibodies comprise the majority of the neutralizing activity in CMV-seropositive sera [4]. These studies measured the ability of antibodies to neutralize virus entry into fibroblast cells. More recent studies revealed that CMV entry mechanisms are cell-type specific—whereas gB and the gH/gL dimer are necessary for fibroblast entry, endothelial or epithelial cell entry requires 3 additional viral proteins, UL128, UL130, and UL131A, which interact with gH/gL to form a pentameric complex [15]. That the pentameric complex is fully dispensable for fibroblast entry suggested that fibroblast-based neutralizing assays are incapable of detecting neutralizing antibodies that target epitopes unique to the pentameric complex. To address this issue, fibroblasts and epithelial cells were used to measure the neutralizing activities of CMV-seropositive sera. On average, neutralizing activities against epithelial entry were 48-fold greater than those against fibroblast entry, suggesting that a large component of neutralizing activity was missed by previous fibroblast-based assays. When epithelial neutralizing titers were measured for Towne and gB/MF59 vaccine recipients, neither vaccine came close to inducing epithelial entry neutralizing titers comparable to those induced by natural infection—gB/MF59 was 15-fold lower, whereas Towne was 28-fold lower [16]. Subsequent studies showed that the majority of the epithelial-specific neutralizing response is directed against epitopes within the pentameric complex [17].

These findings suggest that induction of robust epithelial/endothelial-specific neutralizing activities may be necessary for an effective CMV vaccine. As most CMV infections are

Table 1. Experimental Cytomegalovirus Vaccines in or Approaching Clinical Evaluation

Type	Vaccine	Developer	Components	Status
Live attenuated	Towne	Wistar	Whole virus	Phase 2
	Towne-Toledo 1, 2, 3, 4	Aviron/MedImmune	Whole virus	Phase 1
Subunit protein	gB/MF59	Chiron/Sanofi	gB/MF59	Phase 2
	GSK1492903A	GlaxoSmithKline	gB/ASO1	Phase 1
Subunit DNA vectored	TransVax	Vical	gB, pp65	Phase 2
	CyMVectin	Vical	gB, pp65	Late preclinical
Subunit viral vectored	AVX601	Alphavax/Novartis	gB/pp65-IE1	Phase 1

acquired orally, epithelial-specific neutralizing activities in saliva could block viral transmission by preventing entry of inoculum virus into epithelial cells of the oral mucosa [18]. In cases where salivary antibodies are ineffective, serum antibodies may serve to limit CMV replication and spread within tissues or to impair dissemination to the placenta, and ultimately, the fetus. In vitro, antibodies can inhibit CMV spread between endothelial or epithelial cells but not fibroblasts [19]. Thus, antibodies that inhibit epithelial/endothelial entry/spread may be important for preventing maternal infection or, failing that, for impairing spread and/or dissemination in vivo, thereby reducing the risk of transmission to the fetus.

NEW VACCINE STRATEGIES

Vaccines designed to generate neutralizing responses that target both fibroblast and epithelial/endothelial cell entry are in preclinical development. However, the extent to which conformational and/or multisubunit-dependent epitopes dominate the "neutralizing epitome" of the pentameric complex remains unclear. That it may be necessary to represent the complete pentameric complex in its conformationally native state is suggested by a study of monoclonal antibodies isolated from naturally infected subjects: of 17 pentameric complex–specific neutralizing antibodies, all but 1 recognized multisubunit-dependent epitopes [6]. At the other end of the complexity spectrum, a UL130 subunit DNA vaccine induces epithelial-specific neutralizing responses and immunization with 2 short peptides from UL130 and UL131 induces neutralizing titers in rabbits that exceed those of most seropositive human sera [20].

A variety of vaccine approaches are under study, including simple peptides or subunits; recombinant multisubunit complexes such as gH/gL or the complete pentameric complex; inactivated CMV virions containing native pentameric complex, gB, pp65, and other viral antigens; genetically disabled CMV expressing native pentameric complex, which potentially combines the immunogenicity of a live vaccine with the safety of a killed vaccine; replication-defective viral vectors (eg, pox, adenovirus, alphavirus, and others) expressing subunits or multisubunit complexes; and prime/boost combinations of the above.

CONCLUSIONS

Several experimental CMV vaccines have advanced to clinical evaluation (Table 1) and one, gB/MF59, has some protective efficacy. Incorporation of epitopes derived from the pentameric complex may provide additional efficacy by inducing potent neutralizing/spread-inhibiting antibodies that target virus replication in a broad spectrum of cell types. Next-generation vaccine candidates that incorporate epitopes of the pentameric complex are in preclinical development. The diversity of novel strategies under development engenders optimism that a successful candidate will emerge.

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