Immunotherapeutic Approaches To Prevent Cytomegalovirus-Mediated Disease

EDITH A. SEEDAH,1 ZACHARY P. FRYE,2 and JENNIFER A. MAYNARD2

1Department of Biochemistry, University of Texas at Austin, Austin, TX 78712; 2Department of Chemical Engineering, University of Texas at Austin, Austin, TX 78712

ABSTRACT Human cytomegalovirus (CMV) is the major cause of congenital neurological defects in the United States and also causes significant morbidity and mortality for hematopoietic and solid organ transplant patients. Primary infection in immunocompetent individuals rarely causes disease but resolves as a life-long latent infection, characterized by sustained antibody and cellular responses. Despite considerable efforts over the last 40 years to develop live attenuated and subunit vaccines, none is close to receiving regulatory approval. However, there is evidence that antibodies can prevent primary infection and cytotoxic T cells can suppress secondary infection. Prior maternal infection decreases the risk a fetus will contract CMV, while adoptive transfer of virus-specific CD8+ T cells is highly protective against CMV disease in hematopoietic stem cell transplant recipients. As a result, three polyclonal immunoglobulin preparations are approved for clinical use and one monoclonal antibody has reached phase III trials. Enhanced understanding of the viral life cycle from a biochemical perspective has revealed additional targets for neutralizing antibodies in the gH/gL/UL128-131 pentamer. Until an effective vaccine is licensed, passive immunotherapeutics may present an alternative to maintain viral loads and prevent CMV disease in susceptible populations. This review summarizes the progress and potential of immunotherapeutics to treat CMV infection.

INTRODUCTION
First visualized in 1904 as large inclusions in tissue sections from luetic infants and isolated in 1957 (1), the human cytomegalovirus (CMV) is a remarkably successful pathogen. Worldwide, there is a 50 to 90% probability of infection by age 50 without any clear markers of genetic susceptibility. Primary infection results in life-long latency, requiring continuous vigilance by the host immune system and characterized by serum antibody titers and a strong cytotoxic T-cell response. While most individuals will be infected with at least one strain of CMV, infection rarely leads to disease in immunocompetent individuals. However, CMV is a primary cause of congenital neurological defects and causes disease in those with compromised immune systems, such as transplant patients, with only limited therapies available.

Symptomatic CMV disease is often observed in infants who received the virus from an infected mother in utero, patients receiving hematopoietic stem cell or solid organ transplants, and patients with immunosuppressive diseases such as human immunodeficiency virus (HIV) and AIDS (Table 1). Primary maternal infection during gestation represents an ~40% risk of intrauterine transmission to the fetus, with congenital viral infection affecting 0.5 to 3% of all births (~40,000 per year in the United States)—more children than are affected with Down syndrome, fetal alcohol syndrome, and spina...
**TABLE 1** Treatment spectrum for high-risk CMV demographic groups

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Infection risk</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy individual</td>
<td>Primary infection, followed by latency; occasionally mononucleosis syndrome similar to that caused by Epstein-Barr virus</td>
<td>None; typically asymptomatic</td>
</tr>
<tr>
<td>Fetus of CMV+ mother, newborn</td>
<td>Primary infection with high risk of serious neurological effects (e.g., hearing loss, retardation, microcephaly, seizures in 10–17%); high mortality rate of those with symptomatic disease (~30% reported)</td>
<td>Antiviral therapy or IVIG provided to mother during gestation to prevent primary infection</td>
</tr>
<tr>
<td>CMV+ solid organ transplant patient</td>
<td>Primary infection risk if the donor is CMV+; historically, 10–50% develop symptomatic disease including leukopenia, retinitis, tissue-invasive disease (e.g., nephritis, pancreatitis); increased graft rejection; ‘early’ disease during the first 3 months has been reduced significantly due to prophylactic antiviral therapy</td>
<td>IVIG to prevent primary infection; Cellular immunotherapy with genetically modified T cells expressing CMV-specific TCRs</td>
</tr>
<tr>
<td>CMV+ solid organ transplant patient</td>
<td>Lower risk of secondary infection due to reactivation of latent infection while immunosuppressed; risk of acquiring a different CMV strain if the donor is CMV+; increased graft rejection</td>
<td>Antiviral therapy</td>
</tr>
<tr>
<td>CMV+ and CMV- stem cell transplant patient</td>
<td>Primary infection (~30% CMV+ recipients) and secondary infection (~70% CMV+ recipients) due to reactivation of latent infection while immunosuppressed; most common form of disease is CMV pneumonia with a 30–60% mortality rate</td>
<td>Antiviral therapy for first 100 days posttransplant; high mortality rate if CMV disease appears after the first 100 days (~50%)</td>
</tr>
<tr>
<td>HIV/AIDS patient</td>
<td>Primary or secondary infection when CD4+ T cell count is &lt;50/μl; retinitis, pneumonitis</td>
<td>Adoptive transfer of CMV-specific T cells isolated from donor material to prevent infection; Some evidence to support IVIG; Antiviral therapy</td>
</tr>
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The consequences of congenital CMV disease are severe: vision and hearing loss, mental retardation, and even death, amounting to an estimated $4 billion per year in the United States alone. The rate of vertical transmission is reduced to ~2% in pregnant women who have previously been infected with CMV and have circulating anti-CMV antibodies (3), suggesting a protective role for antibodies against primary infection. Solid organ transplantation, particularly of a CMV+ organ into a CMV seronegative (CMV-) organ into a CMV seronegative (CMV-) recipient, with the associated immunosuppressive regimen, greatly increases patient susceptibility to primary CMV infection and transplant failure, while secondary infection may result due to reactivation of latent CMV in a CMV+ recipient. Similar risks are observed for HIV and AIDS patients with T-cell counts of <50/μl and high-risk elderly with reduced naïve T-cell receptor (TCR) diversity (4) but are less common given the prevalence of antiretroviral therapies.

For these reasons, when the Institute of Medicine was commissioned in 1999 to prioritize vaccine development based upon quality-adjusted life years, a vaccine to prevent CMV was judged “head and shoulders” above all other potential vaccines with regard to cost effectiveness (estimated at $300,000 per affected child) (5). Remarkably, while a CMV vaccine has been a top priority for nearly 40 years (5, 6), none of the candidates evaluated has been licensed. One challenge has been the lack of a clear serological correlate of protection, which may be different for the different patient groups. In the meantime, antiviral therapeutics are the standard of care, with high-titer CMV immunoglobulin and adoptive T-cell transfer emerging as alternatives. Considering the success of antibody therapeutics to treat some viral diseases (2), the apparent protective role of antibodies against CMV and recent identification of neutralizing epitopes in CMV, antibody therapeutics present an attractive option until a vaccine becomes available. This review focuses on the current use and potential of antibody therapeutics to treat CMV disease.

**THE INFECTION**

**Virus Structure and Life Cycle**

CMV is a ubiquitous beta herpesvirus type 5, related to the herpes simplex viruses, Epstein-Barr virus, and varicella zoster, the causative agent of chicken pox. Specialized strains appear to have coevolved with and become restricted to their respective mammalian hosts. The human CMV genome is among the largest and most complex animal virus genomes, with a large 235-kb double-stranded linear DNA genome encoding ~165 open reading frames (8). The genome and some viral
FIGURE 1 Human CMV structure. The 235-kb double-stranded linear DNA genome is surrounded by an icosahedral nucleocapsid, enveloped by the viral tegument proteins (including pp65, which harbors a dominant cytotoxic T lymphocyte epitope) and lipid bilayer, which is studded with at least 20 glycoproteins. The fusogenic glycoprotein gB binds some cell surface receptors and appears to be immunodominant, but neutralizing antibodies recognizing this protein only block viral entry into fibroblasts. The gH/L dimer appears to bind specific receptors and potentiate gB-membrane fusion. When occurring as a gH/L/O complex, it is also involved in entry into fibroblasts, a process that appears to occur via direct membrane fusion. In contrast, the gH/L/UL128-131 pentameric complex is required for entry into epithelial and endothelial cells, a process mediated by endocytosis and low-pH fusion. The gM/N complex is the most abundant on the virion surface, initiating adsorption to cells by binding heparin sulfate proteoglycans. The gN may be heavily glycosylated to shield the virion against antibody recognition. doi:10.1128/microbiolspec.AID-0009-2013.f1

mRNAs are surrounded by an icosahedral nucleocapsid, enveloped by the viral tegument proteins and a lipid bilayer, which is studded with at least 20 different glycoproteins forming a number of complexes. The whole unit spans 200 to 300 nm in diameter (Fig. 1). CMV can spread directly from cell to cell but destroys infected cells by lytic replication, resulting in a high serum viral load which correlates with end-organ disease. CMV disease is monitored by quantitative PCR to detect CMV DNA in leukocytes or serum and detection of the tegument structural protein pp65 in peripheral blood leukocytes by immunostaining.

The virion contains several major glycoprotein complexes at the cell surface. (i) Two molecules of glycoprotein B (gB), in which each 160-kDa monomer is cleaved and then joined by a disulfide bond to include a 115-kDa surface unit and a 55-kDa transmembrane component. The gB has been reported to bind epidermal growth factor receptors and integrins and is a type 3 fusogen, which initiates membrane fusion. (ii) A disulfide-linked heterodimer formed by the gM and gN proteins is the most abundant complex and may initiate adsorption to cells by binding heparin sulfate proteoglycans. (iii) The gH/gL/gO disulfide-linked trimer is notable, as all herpes viruses use a gH/gL complex to mediate fusion of the viral envelope with the cellular membrane, although the CMV gH/gL cocrystal structure does not resemble a typical fusogen (9). The gH is an 86-kDa glycoprotein, present in nuclear and cytoplasmic membranes of infected cells and in the viral envelope. While gH binds the αvβ3 integrin, the 32-kDa gL forms extensive contacts with gH and is required for gH to fold and localize properly at the cell surface. The gO does not appear to be crucial for membrane fusion but appears to chaperone gH/gL incorporation into new virions (10). (iv) A second gH/gL complex combined with the trimeric products of UL128, 130, and 131 (UL128-131) has recently been characterized in greater detail, revealing critical roles during invasion of endothelial and epithelial cells (11).

In vivo, the virus infects a broad spectrum of cells, including macrophages, fibroblasts, epithelial, neural, and muscle cells, allowing it to affect multiple organ systems and cause a wide range of diseases, including pneumonia, gastrointestinal disease, retinitis, hepatitis, and encephalitis. CMV entry into cells is a complex process initiated by adsorption onto the cell surface, followed by specialized invasion strategies for different cell types (Fig. 2). In particular, fibroblast entry is mediated by the fusogenic gB and potentiated by the gH/gL/gO complex, possibly involving β1 integrins and epidermal growth factor receptors, followed by direct fusion of the viral envelope with the cell membrane (11, 12). In contrast, infection of endothelial cells, epithelial cells, and macrophages requires the gH/gL/UL128-131 pentamer. When associated with the cell surface, this complex triggers endocytosis, followed by a gB- and gH-dependent fusion of the viral membrane with the endosomal membrane (13, 14). In addition to the gH/gL/UL128-131 pentamer, viral entry into these cells appears to require a different gB conformation to trigger fusion (12). The ability of CMV strains to invade fibroblasts has been used historically to characterize strains, vaccines, and antibodies. However, it has been recognized that during laboratory culture, strains such as AD169 and the Towne vaccine strain readily acquire mutations in the UL128-131 genes that restrict their tropism to fibroblasts, raising concerns over their clinical relevance.

Virus-Immune Détente

Primary infection of immunocompetent CMV individuals rarely leads to active disease but instead leads to a lifelong latent infection, characterized by periodic
reactivation and secondary infection. During latency, multiple copies of the CMV genome appear to reside in a small number of mononuclear cells. Secondary or superinfection with a new CMV strain can be monitored by serum viral titer, a correlate for disease severity. Maintenance of latency requires continual monitoring by the host immune system, such that ∼5 to 10% of all CD8+ and CD4+ T cells recognize CMV peptides, a number which increases to ∼30% in the elderly. To prevent disease in susceptible populations, current treatments include (i) antiviral agents to prevent replication (primarily ganciclovir) in the first 3 months posttransplantation, which are associated with significant toxicity and not approved for use in pregnant women; (ii) polyclonal immunoglobulin preparations enriched in antibodies against CMV (CMV-IVIG); and (iii) adoptive immunotherapy by ex vivo expansion of CMV-specific T cells, which are then reinfused into the patient. Antivirals and CMV-IVIG target viral proteins involved in replication to restrict viral dissemination and limit disease severity in CMV-pregnant women and solid organ recipients, while T-cell therapy has demonstrated efficacy in maintaining latency after stem cell transplantation but has not been standardized for routine use.

While the magnitude of their role has been debated, antibodies appear to limit viral loads by inhibiting aspects of cellular invasion. An early analysis purified antibodies from the serum of seropositive individuals by immunoadsorption onto recombinant vaccinia virus expressing gB. The purified antibodies were then used to neutralize CMV strain AD169 invasion into fibroblasts, concluding that 40 to 70% of neutralizing antibodies target gB (15, 16). Three antigenic domains were later defined: AD-1, a conformational epitope located between residues 552 and 635; AD-2, a linear epitope between residues 69 and 78; and AD-3, another linear epitope at the carboxy terminus, between residues 783 and 906 (17). AD-1 is involved in gB oligomerization and is immunodominant, with antibodies binding this epitope present in all tested individuals. Interestingly, these responses are comprised of neutralizing and nonneutralizing antibodies competing for the same site, which may assist in viral immune evasion (18). Antibodies recognizing AD-2 are only present in about half of individuals but are potently neutralizing. Sequencing has revealed similar variable region usage for antibodies binding AD-2 within a single individual, suggesting that they are derived from a small number of B cells and that the epitope may be difficult to access and/or poorly immunogenic (19). More recently, sequencing of gB-specific memory B cells isolated from seven seropositive individuals showed that >90% of the corresponding antibodies are nonneutralizing. This work also identified two additional, potently neutralizing gB epitopes: AD-4 and AD-5. Antibodies binding these epitopes do not block viral attachment but neutralize a postadsorption fusion event (20). Collectively, neutralizing anti-gB antibodies affect early events of infection and have been shown to prevent viral penetration of fibroblasts and cell-to-cell spread. However, while gB is key for viral entry, it is not essential for viral attachment, assembly, or egress, and thus, anti-gB antibodies may not represent

**FIGURE 2** Human CMV life cycle. (1) The virion binds to cells via the gB and gH/L/UL128-131 glycoproteins and specific cellular receptors, followed by direct membrane fusion (fibroblasts) or endocytosis and low-pH-mediated membrane fusion (endothelial and epithelial cells). (2) The virion contents are released into the cytoplasm, allowing the nucleocapsid to translocate to the nucleus for DNA replication and transcription and packaged viral transcripts to be directly translated. (3) Transcripts are translated in the cytoplasm, followed by processing in the endoplasmic reticulum and Golgi body. (4) Viral DNA and proteins are assembled and enveloped to create new virions, followed by (5) release into the extracellular surroundings or directly into another cell. (6) During this process, fragments of viral proteins are combined with host MHC class I in the endoplasmic reticulum for presentation on the cell surface. Antibodies can directly affect the cellular attachment and internalization steps to prevent primary infection, while T-cell recognition of viral pMHC complexes is crucial for identifying and lysing infected cells. doi:10.1128/microbiolspec.AID-0009-2013.f2
the most critical components of the protective humoral immune response.

More recently, analysis with cell surface-expressed and purified CMV antigens suggested that the major neutralizing antibody response blocking entry into epithelial cells is directed against the gH/gL complex and not gB (21). Anti-gH/L neutralizing antibodies isolated from patient memory B cells either neutralized viral infection on all cell types tested with moderate 50% inhibitory concentration (IC_{50}) values or potently protected epithelial, endothelial, and myeloid cells with low IC_{50} values, between 20 and 200 pM, but did not protect fibroblasts. Competition assays showed that antibodies in the first group recognized at least three distinct neutralizing sites on gB and two on gH, while antibodies in the second group recognized at least seven distinct sites on the gH/L/UL128-131 complex, some of which span the interfaces between adjacent proteins (21, 22). Even short peptides from UL128 and UL130 elicit high neutralizing titers in rabbits which are able to block viral entry into epithelial cells from the mucosa (23). This is consistent with other reports that anti-gH antibodies, which are present at high levels during acute infection, block cell-to-cell spread (24) while those against UL128 and UL130 specifically inhibit endothelial cell invasion (14, 23, 25). Less potently neutralizing human monoclonal antibodies recognizing other viral glycoproteins (gB, gH, or gM/gN complex) have been isolated which neutralized infection of epithelial/endothelial cells as well as fibroblasts (22), although their epitopes are less well characterized. It has been suggested that the extensive glycosylation on gN may serve to shield these glycoproteins from neutralizing antibodies (26).

Cellular immune responses to CMV are mediated primarily by the cytolytic activity of CD8+ cells, but these require CD4+ help to persist (27). After a primary infection, latency is maintained by an array of immune evasion tactics, including down-regulation and degradation of antigen-presenting major histocompatibility complex (MHC) molecules to avoid recognition by cytotoxic T cells (28). The role of cellular immunity has been demonstrated convincingly by adoptive transfer studies in which donor-derived, CMV-specific CD8+-T-cell lines or clones were first isolated by coculture with virus or antigen-pulsed dendritic cells, expanded in vitro, and finally, infused into HLA-matched hematopoietic stem cell transfer patients. This procedure restored antigen-specific immunity and prevented CMV-associated clinical outcomes (29, 30); however, it is time-intensive and laborious, requiring a total of 4 to 6 weeks for cell line expansion (31). Recent efforts to streamline the process have included direct isolation of CMV-specific T cells via HLA tetramers prior to in vitro expansion and T-cell transfection with genes encoding CMV-specific TCRs (32). Several T-cell lines recognizing pp65 peptides in complex with multiple HLA restrictions have been shown to be effective in vitro, in terms of cytokine secretion and target cell lysis (33), but it remains to be seen whether these can be transferred to the clinic.

As these procedures evolve, a key question becomes “what T-cell specificities are required for protection?” Careful analysis of the molecular details of this recognition event requires identification of the peptides and corresponding TCRs involved in protection. Several immunodominant T-cell epitopes have been identified, including the tegument phosphoproteins 65 (pp65, the most abundant protein in the tegument) and 150 (pp150) and the immediate-early protein 1 (IE-1) which are conserved across strains (34, 35). Transcripts of pp65 are packaged within virions and translated directly after cellular invasion; thus their display provides an early marker for infection. The dominant effector cells in adoptive immunotherapy respond to these three peptides (29, 30). One clinical study adoptively transferred pp65 or IE-1-specific CD8+ T cells, observing a dramatic reduction of viral load in all 9 patients (36). In contrast, in vitro experiments using peptides derived from 213 open reading frames demonstrated T-cell responses to more than 70% of expressed genes (37). This raises the question “are the dominant epitopes we observe in vivo actually critical for CMV suppression or an artifact of viral immune evasion tactics?”

Looking at the other half of the interaction, analyses of the αβ CD8+ TCR sequence diversity has identified a large number of public TCRs, comprised of the same germ line variable regions and used by different individuals to recognize these dominant CMV peptides when presented by HLA (38). The cells displaying public TCRs recognize virally infected cells with greater avidity and exhibit a more terminally differentiated phenotype (39). Recently, tetramers comprised of the pp65_495-503 peptide in complex with the human HLA A*0201 were used to isolate human CD8+ T cells, which were then subjected to single-cell sequencing of the TCR alpha and beta chains. Public and nonpublic TCR sequences were observed, and TCR sequence diversity was correlated with serum antibody titers as an indirect measure of viral titer and disease propensity. The primary conclusion of this study was that the diversity, but not the magnitude, of the cellular response inversely correlated with the serum anti-CMV titer, which is itself a marker for increased viral load (40). These data suggest that immunothe-
Vaccine Development
A vaccine to prevent CMV disease has been a top priority since 1999, and despite considerable effort, none are likely to receive regulatory approval soon (5, 6). Challenges have included the lack of a clear serological correlate of protection, appropriate animal models, and target population. To maximize the clinical benefit, it has been suggested that all seronegative young women, CMV- stem cell donors, and solid organ transplant recipients be immunized to protect future children and transplant recipients, respectively. Over the last 50 years, two vaccine approaches have been extensively evaluated in clinical trials: live attenuated vaccine and gB-based subunit vaccines, with numerous other DNA, vectored virus, and peptide formulations in development (for a review, see reference 41).

Two live attenuated strains have been developed and evaluated in vaccine trials, the laboratory-adapted AD169 and Towne’s strains. The first clinical trials with Towne’s, originally a neonatal clinical isolate, demonstrated that it could elicit both neutralizing antibodies and T-cell responses lasting up to 10 years, with a good safety profile. It was subsequently shown to reduce viral load and mitigate CMV disease, but not to prevent primary or secondary infection, when administered to kidney transplant patients at least 8 weeks prior to transplantation (42, 43, 44). When administered to seronegative mothers, Towne’s showed no efficacy in reducing transmission from children shedding CMV virions (45). In a challenge study with healthy volunteers, vaccinated individuals were protected more than seronegative individuals but were still 5 to 10 times more likely to become ill than naturally seropositive individuals. This may be a result of Towne’s deletions in the UL128-131 region, meaning that the vaccine can induce antibodies blocking fibroblast but not epithelial cell invasion. More-recent efforts to improve live attenuated vaccines include coadministration with interleukin-12 as an adjuvant (46) and chimerization with the unattenuated Toledo strain. The moderate protective effects observed in live attenuated CMV vaccine trials, coupled with concerns that heavily attenuated strains are less relevant (47) and pose regulatory concerns, have slowed development of these vaccines.

The second most developed vaccine strategy is a subunit vaccine, based on the observation that gB dominates the humoral immune response. While prior trials focused on safety and immunogenicity, in 2009, a subunit vaccine was shown for the first time to be effective against primary maternal CMV infection and, later, in transplant recipients. This vaccine is comprised of a squalene-water emulsion of an engineered gB with the novel proprietary adjuvant MF59. The gB protein is modified to remove the internal cleavage site and introduce a stop codon before the transmembrane region, resulting in a soluble product expressed in CHO cells. In a phase II placebo-controlled efficacy trial, 18 of 225 women receiving the vaccine versus 31 of 216 receiving placebo acquired primary CMV infection during a 1-year period, for overall vaccine efficacy of ≈50%. Peak levels of anti-gB were about fivefold higher than in naturally seropositive individuals but did not induce antibodies preventing endothelial/epithelial cell invasion (48). A subsequent phase II randomized study with kidney and liver transplant patients showed that anti-gB titers correlated inversely with the duration of viremia (49). This adjuvanted gB subunit vaccine remains the most promising candidate, but additional trials are required for licensure. A variety of additional vaccine designs have been evaluated, including recombinant canary pox and peptide vaccines designed to elicit strong cellular responses against known immunodominant peptides (50).

While still in early stages, new approaches based on the pentameric gH/L/128-131 complex—notably absent from the AD169 and Towne’s vaccine strains—appear promising. This complex is essential for viral invasion of endothelial/epithelial cells, and clinical studies have determined that antibodies recognizing the UL128-131 complex appear within 2 to 4 weeks after infection, last for at least a year, and are stronger than responses to gH (25). Moreover, the bulk of the neutralizing response in CMV-IVIG is now thought to be mediated by antibodies binding gH/L/UL128-131 (21), and these have been proposed as a correlate of protection (51, 52). When the complex is restored to the AD169 vaccine strain, it induced 10-fold-higher neutralizing antibody titers than AD169 or the gB subunit vaccine in rabbits and monkeys (53). This effect has been shown consistently across several animal models, as a modified vaccinia Ankara virus expressing all five rhesus homologs developed neutralizing antibody titers similar to natural infection and exhibited reduced plasma loads after postvaccination challenge in monkeys (54). Finally, an alphavirus replicon particle coexpressing only gHgL produced broadly cross-reactive complement-independent neutralizing antibodies in mice at higher titers than those with gB (55).
THERAPEUTIC ANTIBODIES

Polyclonal CMV-IVIG

The available antibody-based anti-CMV therapies are polyvalent immunoglobulin preparations enriched in antibodies against CMV (CMV-IVIG) (Table 2) and were of particular interest before antivirals became widely available. The CMV-IG is made by pooling serum from ~1,000 donors screened for high anti-CMV serum titers and purifying the antibodies by cold ethanol precipitation, followed by a low-pH or solvent-detergent virus inactivation step. This process results in a product with ~50 mg of immunoglobulin G (IgG)/ml and anti-gB titers of ~1:400,000, two- to fourfold higher than in seropositive individuals, although lot-to-lot variability is observed (56). While the enzyme-linked immunosorbent assay is a primary characteristic, it does not always track with the neutralization titer and in some cases, standard IVIG can have a higher neutralizing titer than CMV-IVIG (57). This activity typically protects endothelial and epithelial cells more potently than fibroblasts. A recent report suggested that the bulk of the neutralizing response in CMV-IVIG (Cytogam) is due to antibodies recognizing the gH/L/128-131 pentamer with little role for anti-gB antibodies (21). Antibody isotype may also be a key criterion, as IgG3 antibodies have been found to be 10-fold more potently neutralizing than the IgG1 isotype (58). Collectively, the antibodies in CMV-IVIG are thought to act by neutralizing the virus’ ability to infect both fibroblast and endothelial/epithelial cells (59), reducing inflammation, and/or reducing cytokine-mediated immune responses, although these latter mechanisms have not been well characterized (60).

CMV-IVIG was first licensed in 1991 and is well tolerated with no severe side effects. It has been used for prophylaxis of CMV disease associated with at-risk patients receiving solid organ and stem cell transplants, typically, CMV− recipients with CMV+ donors. To improve efficacy, it is often combined with antiviral ther-

### Table 2 Antibody-based CMV therapeutics

<table>
<thead>
<tr>
<th>Therapeutic</th>
<th>Target</th>
<th>Indication</th>
<th>Development stage</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human immune sera; polyvalent (Cytogam; CSL/Behring, MedImmune)</td>
<td>CMV envelope glycoproteins (block virus entry)</td>
<td>Transplant recipients, congenital CMV</td>
<td>Approved</td>
<td><a href="http://www.cytogam.com/">www.cytogam.com/</a></td>
</tr>
<tr>
<td>Human immune sera; polyvalent (Cytotect; Biotests AG)</td>
<td>CMV envelope glycoproteins (block virus entry)</td>
<td>Transplant recipients, congenital CMV</td>
<td>Approved</td>
<td><a href="http://paviour.org/cytotect.htm">http://paviour.org/cytotect.htm</a></td>
</tr>
<tr>
<td>Human monoclonal antibody (MSL109; PDL)</td>
<td>CMV envelope glycoprotein H (block virus entry)</td>
<td>CMV retinitis; stem cell transplant recipients</td>
<td>Phase I/II trials completed; no further progress</td>
<td>85, 87</td>
</tr>
<tr>
<td>Human monoclonal antibody MSL109 in combination with ganciclovir</td>
<td>CMV envelope glycoprotein H (block virus entry)</td>
<td>CMV retinitis</td>
<td>Phase I/II</td>
<td>83, 92</td>
</tr>
<tr>
<td>Human IgG1 monoclonal antibody C23/regnivirumab (Teijin Ltd.)</td>
<td>gB epitope</td>
<td>CMV retinitis in HIV− individuals, bone marrow recipients</td>
<td>Phase II</td>
<td>81; US5043281 (1990)</td>
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<td>Human neutralizing monoclonal antibodies (Humabs, LLC)</td>
<td>CMV gUL131A-128 trimer</td>
<td>Preclinical</td>
<td>22, 88; US7947274 (2011)</td>
<td></td>
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<tr>
<td>TCR-like antibody</td>
<td>CMV peptide p64 or p65 displayed by MHC</td>
<td>Preclinical</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>TCR anti CD3-bispecific antibody</td>
<td>CMV-infected cells</td>
<td>Preclinical</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>Humanized antibodies (Scotgen Biopharm)</td>
<td>gB, gH epitopes</td>
<td>CMV infection in immunocompromised patients</td>
<td>Preclinical</td>
<td>24, 80</td>
</tr>
<tr>
<td>Combination of monoclonal antibodies (Children’s Hospital, Inc.)</td>
<td>Human CMV glycoprotein A</td>
<td>Preclinical</td>
<td>US5126130 (1987)</td>
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therapy. However, the results of clinical trials evaluating its use have been variable, most likely due to lot-to-lot variability, various doses and schedules, and mixed patient populations with different risk profiles. While early studies suggested that prophylactic CMV-IVIG could reduce the severity of CMV infection in liver transplant patients (61), others have suggested that it is less effective than combinations of antivirals (62) and that IVIG-antiviral combination therapy could actually increase both survival and rejection risk (63). With high-risk kidney transplant recipients, IVIG is associated with an excellent 1-year survival rate (64) and combination therapy with low-dose valaciclovir was efficacious in preventing CMV disease (65). A meta-analysis pooling 11 trials with almost 700 solid organ transplant recipients found a positive effect of prophylactic CMV-IVIG on recipients: improved total survival and moderately reduced CMV disease and CMV-associated deaths (66). CMV-IVIG use in stem cell transplant patients is less promising. A multicenter, randomized, double-blind, dose-effect, placebo-controlled study of 200 stem cell transplants concluded that the use of immunoglobulin therapy did not significantly reduce CMV infection and disease (67). A second meta-analysis combining 30 trials with 4,223 stem cell transplant patients observed no differences in mortality, infections of any kind, CMV infections, or graft-versus-host disease (68). As a result of this variability, IVIG therapy is approved, but is not standard treatment, for transplant patients.

The presence of maternal antibodies to CMV reduces the risk of fetal infection (3), indicating the potential for antibodies to prevent and/or treat congenital CMV infection. Passive immunization studies with pregnant guinea pigs have demonstrated that antibodies to the virus or gB reduce the fetal infection rate and enhance fetal survival (69, 70). Furthermore, adoptive transfer of B memory cells was protective in T- and B-cell-deficient mice (71). Subsequently, several nonrandomized human trials using CMV-IVIG to prevent CMV infection in utero have been performed. In one, 181 pregnant women with confirmed primary CMV infection were divided into a treatment group (women who had amniocentesis to confirm fetal infection) and a prevention group (those who did not undergo amniocentesis) (72). The treatment group included 55 women, 31 who received Cytotect IVIG, resulting in one infected newborn (3%), and 14 who received no treatment, resulting in seven infected newborns (50%). The prevention group included 37 women receiving Cytotect IVIG, resulting in 6 infected newborns (16%), and 47 who did not receive treatment, resulting in 19 infected newborns (40%). More recently, a case-controlled study compared 32 children with hearing and/or psychomotor deficits with 32 healthy, age- and gestation-matched control children. The mothers of both groups had confirmed or probable primary CMV infection at <20 weeks of gestation. The only risk factor for these children was whether the mother had received CMV-IVIG after infection: of the case patients, 4/32 received IVIG, while 27/32 control mothers received IVIG (73). While promising, larger studies are needed to draw a clear conclusion, especially for an expensive intervention that requires screening for serologic confirmation of infection followed by monthly CMV-IVIG administration (74).

Monoclonal Antibodies

The apparent protective role of antibodies against congenital CMV suggests that these polyclonal immune responses could be deconvoluted to a discrete number of highly protective epitopes. These could, in principle, be targeted with high efficacy by a monoclonal or oligoclonal antibody preparation (75, 76). This perspective is supported by the early characterization of human monoclonal antibody C23 which neutralized the ability of AD169 and clinical CMV isolates to infect lung fibroblasts 1,000 times more potently than human IVIG (77). Monoclonal antibodies have the additional benefits of being very well characterized, with epitopes, binding affinities, and neutralizing mechanisms described; their biochemical and biophysical properties engineered to meet specific design targets; and since they are purified from monoclonal cultures, minimal lot-to-lot variability (78).

Most efforts in developing CMV-neutralizing monoclonal antibodies have targeted the gH viral glycoprotein, as low anti-gH titer is a risk factor for CMV disease (79) (Table 2). Phase I clinical trials of several anti-gB and anti-gH human monoclonal antibodies have demonstrated a pharmacokinetic profile similar to that of human IgG1 and lack of immunogenicity, supporting their safe use in passive immunotherapy applications (80, 81, 82). One anti-gH IgG1 human monoclonal antibody isolated from the spleen of a CMV-seropositive individual was able to block in vitro infection of multiple cell types by laboratory and clinical strains of CMV, with IC₅₀ values between 0.03 and 1.02 μg/ml (83, 84). As this antibody also effectively treated CMV retinitis when combined with ganciclovir in rat models, it was evaluated in a series of clinical trials as MSL-109 (Sevirumab/ Protovir; Protein Design Labs), but unfortunately, it has not shown sufficient efficacy.
A randomized placebo-controlled human trial to assess the efficacy of MSL-109 to delay progression of CMV retinitis in AIDS patients was halted after it failed to slow progression of the blinding eye disease (85). Indeed, the MSL-109 treatment group had a higher mortality rate than the placebo group. Subsequently, serum CMV titers were associated with a higher risk of mortality in AIDS patients with CMV retinitis, implying that MSL-109 was ineffective in clearing plasma CMV (86). Similarly, stem cell transplant recipients with CMV+ donors or recipients received MSL-109 biweekly in a randomized, double-blind study. The MSL-109 treatment group showed no difference in survival or pp65 antigenemia versus the placebo groups (87). Recently, it has been suggested that the MSL-109 failure may have been due to the rapid generation of nongenetic escape variants. It appears that infected cells present gH on their surface during viral replication. The gH is available to bind and endocytose circulating MSL-109, and the gH/MSL-109 complex is then incorporated into the viral envelope with the MSL-109 Fc domain exposed. The gH/L complex is no longer available to bind epithelial/endothelial receptors, but the Fc domain of MSL-109 is available to bind Fc receptors, altering the cellular tropism of the virus (84).

Recently, a novel method was used to characterize the repertoire of CMV-reactive antibodies in CMV-immune donors. Human B lymphocytes from CMV-seropositive donors were immortalized with Epstein-Barr virus, followed by screening for CMV neutralization. Of the 1,664 clones screened, 29 neutralized CMV infectivity. Of the four further characterized, two bound gB AD-2, two bound gH, and all were able to neutralize lab strain AD169 or a clinical isolate in both fibroblast and endothelial invasion assays. Importantly, these monoclonal antibodies were ~20-fold more potent than the Cytotect IVIG, supporting development of antibody cocktails over IVIG (88). As described above, it appears that additional potently neutralizing epitopes are present on the pentameric gH/gL/UL128-131 complex and may present promising targets for passive immunotherapy (21, 22). Other less potently neutralizing human monoclonal antibodies isolated from memory B cells recognized viral glycoprotein gB, gH, or gM/gN and were shown to neutralize infection of both epithelial/endothelial cells and fibroblasts (22).

Apart from traditional passive immunotherapies relying on a single antibody to sequester a single epitope, new paradigms are emerging which could be applied to prevent CMV replication or lyse CMV-infected cells. For instance, adoptive immunotherapy has also shown that T cells recognizing the pp65, pp150, and I-E1 peptides form the dominant population of effector cells and uniquely identify CMV+ cells early in infection (34, 35). Antibodies mimicking the specificity of public TCRs recognizing these peptides could deliver a cytotoxic payload or direct cytotoxic lymphocytes to these cells, approaches which have been explored for HIV and cancer treatment (82). An antibody that could be used for this approach has been isolated from a large human Fab phage display library that recognizes HLA A2/pp65495-503 with a 300-nM affinity and is able to specifically detect peptide on the surface of fibroblasts in vitro (90). A similar approach formed a bispecific antibody by chemically linking OKT3 (an FDA-approved anti-CD3 antibody which binds and activates T cells) to CMV-IVIG polyclonal anti-CMV antibodies. After incubation with CMV-infected fibroblasts, specific lysis of CMV-infected cells was demonstrated in vitro (80% for the test versus <20% for the control) (91). Additional reports documenting CMV-neutralizing human monoclonal antibodies (Table 2) reflect the breadth of efforts to development of human/humanized monoclonal antibodies to neutralization CMV.

**FUTURE CHALLENGES**

Based on the high rates of CMV infection in adults and the large percentage of CMV-specific T cells in seropositive individuals, preventing infection appears to be an unattainable goal. In the absence of a vaccine, antibody therapies to prevent or control disease in susceptible populations may be the best solution. Here, there is strong evidence that adoptive transfer of cytotoxic T cells with CMV specificity is able to control secondary disease in stem cell transplant patients, while administration of hyperimmune IVIG appears effective in some solid organ transplant patients and is promising in seronegative pregnant women. In conjunction with recent insights into the function of CMV glycoprotein complexes, further elucidation of protective B- and T-cell epitopes present in the UL128-131 trimer and associated gH/gL pentamer is expected to contribute to design of immunotherapeutics with greater specificity and potency. Ultimately, potent cocktails of high-affinity monoclonal antibodies binding neutralizing epitopes on the virus and TCR-like antibodies to target infected cells may prove effective at suppressing CMV replication.

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Conflicts of interest: We declare no conflict.
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