Human cytomegalovirus (CMV), one of the eight herpesviruses that commonly infect humans, is best known for its propensity to cause disease in immunocompromised patients, especially transplant recipients, patients with advanced AIDS, and congenitally infected newborns. Advances in molecular virology coupled with improvements in diagnostic methods and treatment options have vastly improved our understanding of and ability to manage CMV, but many uncertainties remain, including the mechanisms of persistence and pathogenesis and its hypothesized roles in a variety of human illnesses. Here we review recent advances that are reshaping our view and approach to this fascinating virus.

A century of progress
Human CMV infection is extremely common. A recent analysis in the United States revealed an overall 50% seroprevalence among adults (1), but rates in some populations are even higher. For example, approximately 90% of Mexican-Americans in the United States are seropositive by age 50, as are 88% of stem cell transplant patients in Italy and 96% of individuals in southern Brazil (1–4). Despite its high worldwide prevalence, CMV infections are generally inapparent, except in newborns and immunocompromised individuals, for whom they can cause life-threatening disease affecting many organ systems.

The virus was first detected in newborns during the early 20th century, when multiple reports described large cells in the urine of children with an often fatal systemic infection referred to as cytophagic inclusion disease (5). The midcentury development of cell culture methods enabled propagation of CMV, but its detection in clinical specimens often required weeks of cultivation. Rapid diagnostic testing by centrifugation-enhanced inoculation combined with detection of CMV antigens in the 1980s was a transforming advance, enabling the diagnosis of CMV to be made in a clinically useful time frame (6). The emergence of effective antivirals in the 1990s was opportune, as CMV-associated disease was increasing in parallel with the AIDS epidemic and use of solid organ transplantation (SOT) and HSC transplantation (HCT). During the past two decades, further advances in diagnosis and treatment have greatly improved our ability to control CMV disease, but the virus still accounts for substantial morbidity, mortality, and cost.

Along with these clinical advances, remarkable progress has been made in understanding the molecular biology of CMV. Application of the nucleic acid and protein analytic methods led to an appreciation of the extraordinary complexity of CMV, a point solidified by the landmark report of the first complete sequence of a CMV strain in 1990 (7). Refinements to methods for studying CMV gene function have continued to reveal myriad mechanisms underlying CMV’s evolutionary success. However, understanding the pathogenesis of CMV diseases remains an enormous challenge, in large part because the virus only grows in human cells and it differs substantially from even its primate-infecting cousins (8–10).

Here we summarize the current understanding of CMV biology and disease. The topic is too broad to cover completely in this form, so the interested reader may wish to consult more comprehensive reviews (11, 12), as well as new reports that are certain to appear in the months and years ahead.

CMV replication: insights but limitations from the lab
Human CMV is the prototypic member of the β herpesvirus subfamily, which also includes human herpes viruses 6 and 7 and many animal CMVs. Its DNA genome is approximately 230 kb in size, the largest among known human viruses, and consists of unique long (UL) and unique short (US) segments, each of which is flanked by inverted repeats (RL and RS; Figure 1). Most of the approximately 200 genes encode proteins, but some express only noncoding RNAs, including approximately 14 microRNAs (miRNAs; refs. 13–15). The central portion of the UL region contains clusters of core genes that have homologs in other herpesviruses, such as DNA polymerase, glycoprotein B (gB), and glycoprotein H (gH), whereas the remainder of the genome contain genes primarily found only in β herpesviruses or unique to human CMV (16, 17). In fact, considerable variation has been detected even among human CMV isolates (18). By convention, CMV genes are named by their position within the genome, although some also have additional descriptive names. For example, UL54 (the 54th gene in the UL region, according to the original report of the CMV, strain AD169, sequence; ref. 7) is the DNA polymerase gene.

Propagation of CMV in cell culture has been an essential research tool but has definite limitations, in part because passage in the lab selects for mutants adapted for growth in this unnatural setting (19). As a result, commonly used lab strains have multiple mutations, deletions, and rearrangements (20). For example, the end of the UL region in isolates of the lab strains Towne and AD169 lacks approximately 13 kb of DNA, encoding 19 genes that are present in the Toledo strain (21, 22). Remarkably, inactivating mutations in the RL13 gene are detectable by sequence analyses of the viral genome immediately upon propagation of virus in cell culture (23), which suggests that this gene is necessary for success of the virus in humans but strongly inhibitory to replication in cell culture. The fact that most, if not all, prior laboratory-based studies have used only RL13 mutant viruses reinforces the importance of confirming results of laboratory studies with observations from the clinic. Advances in sequencing technology are sure to improve our understanding of the genetic variation of CMV without confounding artifacts that arise from propagation in the laboratory.

Among the important recent advances has been the characterization of a previously unrecognized CMV entry pathway. Most labo-
However, in infected humans, CMV is commonly found receptors including integrins and possibly growth factor receptors in endothelial cells, epithelial cells, smooth muscle cells, and some one or more of these genes, resulting in their failure to replicate in virion glycoproteins (gB, gH, and gL; Figure 1) with cell membrane refs. 27–29). Many laboratory-adapted strains have mutations in and requires the UL128, UL130, and UL131A genes (Figure 1 and HSCs in addition to fibroblasts (26). Entry into at least some of these generated by cells of both the innate and the adaptive immune sys-

Complementing responses within the infected cell itself are defenses generated by cells of both the innate and the adaptive immune sys-
tems. Depletion of NK cells in mice results in higher titers of murine CMV in tissues and increased mortality (32). In humans, NK cell deficiency has been linked to severe CMV disease (33). Considerable evidence indicates that adaptive T cell responses are critical for keeping the virus inactive. For example, restoration of CD8+ and CD4+ T cells is a strong correlate of control of the virus after HSC transplant (HSC; ref. 34). Moreover, adaptive transfer of CMV-specific T cells protects against clinical reactivation (35, 36).

In seropositive humans, a strikingly high fraction (10% or more) of circulating T lymphocytes target CMV (37). Use of tetramers has shown that pp65 is recognized by a high fraction T cells, but many other gene products are also recognized (37, 38). Moreover, the fraction of CMV-specific T cells tends to increase with age, which supports the hypothesis that CMV contributes to immune system exhaustion and dysfunction associated with aging (39).

Antibodies in CMV-infected individuals have been useful for establishing serostatus. Although it has long been thought that only antibodies to gB or gH neutralize the virus, the finding that other viral genes, including UL128, UL130, and UL131A, mediate entry into endothelial and epithelial cells raises new possibilities for therapeutic design. In fact, recent data indicate that neutralizing antibody titers in human sera may be two logs higher against these alternative entry pathway mediators compared with those targeting against gB and gH (40–42). Thus, future studies should revisit the possible role of antibody responses in controlling infections, and these genes will be important to consider in designing future vaccines.

**Viral counterattack**

Faced with such a breadth of host defense systems, the success of CMV in human infection has necessitated evolution of myriad viral evasion strategies. Proteins delivered with infecting virion (e.g., pp65 [UL83], pp71 [UL82], pTRS1, and pIRS1) and made very early after infection (UL36, UL37, IE1, and IE2) block intracellular defenses, including induction of apoptosis, production of interferon and interferon-stimulated genes, and shutoff of protein synthesis (31, 43). CMV also interferes with the cellular immune responses (44, 45). At least seven genes are able to modulate, and in many cases inhibit, NK cell function (Figure 1 and ref. 45). For example, the viral miRNA mIR-UL112 acts synergistically with a cellular miRNA to inhibit expression of the NK-activating ligand MICB (46). Several genes clustered in the US2-11 region prevent presentation of CMV peptides to T cells. US3 binds to and sequesters MHC class I in the ER, US6 inhibits loading of peptides onto the MHC complex, and US2 and US11 cause dislocation of class I molecules from the ER to the cytosol, where they are degraded.

CMV encodes chemokines, chemokine receptors, and cytokines that likely participate in immune evasion (Figure 1 and ref. 47). For

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**Figure 1**

CMV genome. The genome of CMV clinical isolates, such as the Merlin strain depicted here (GenBank accession no. NC_006273; ref. 118), consists of long (brown) and short (orange) DNA segments, each of which has unique regions (UL and US) flanked by inverted repeats (TRL/TRL and IRL/IRL). These repeats contain segment-specific sequences (b, b1, c, and c′) as well as a variable number of shared sequences repeats in direct orientation at the genomic ends and in an inverted orientation at the junction of the two segments. Laboratory-adapted strains often have deletions of multiple genes at the right end of the UL segment and their replacement with genes duplicated from the left end, resulting in longer TRL and IRL regions (dashed boxes) compared with clinical strains (21, 118). The gene names in this region are not always sequential because of historical precedence in nomenclature assignments and because of rearrangements among strains. The relative position and orientation of transcripts corresponding to several genes are shown, along with grouping by their putative functional classifications (24, 31, 43–45, 47). This diagram is a simplification, since some and possibly many of the genes shown here have more than one function and other genes that are not shown likely contribute to the indicated processes.
The site—or, more likely, sites—of CMV latency or persistence remains in the human are not yet clear. The risk of acquiring CMV by transmission from asymptomatic donors implicates blood as one site. Indeed, many studies of CMV latency have focused on circulating antigenemia, examples of end CMV organ disease commonly occurring in AIDS patients and in transplant recipients are shown. Image credits: antigenemia, pp65+ cell in a leukocyte cytospin preparation (M. Boeckh); retinitis, ophthalmoscopic view of retinal hemorrhage and inflammation (E. Chuang); ependymitis, endoscopic view of shallow esophageal ulcers (G. McDonald); colitis, deep ulcer in a colonic biopsy (G. McDonald); pneumonia, chest CT scan of CMV pneumonia (M. Boeckh).
for research, and these studies have revealed latent infection in immature cells of the myeloid lineage (59). However, these results do not rule out other sites of latency. SOT from seropositive to seronegative donors can transmit CMV (62). Although these organs are undoubtedly contaminated with leukocytes, the fact that the virus has a propensity to reactivate within the transplanted organ suggests that parenchymal cells may harbor latent virus.

At least in cell culture models of latency, CMV expresses only a small number of genes (59), as is true of latency in other herpesvirus systems. A particularly intriguing one of these is an alternatively spliced, latency-associated form of cmvIL-10 (LAcmvIL-10), which retains some but not all of the activities of cmvIL-10. Interestingly, LAcmvIL-10 inhibits MHC class II recognition of infected cells and might thus assist them in avoiding elimination by the immune system (48). Moreover, since LAcmvIL-10 has immunomodulatory properties and might be secreted from latently infected cells for decades, it has the potential to cause chronic immune system dysfunction in otherwise healthy individuals. Clarification of the genes expressed during bona fide latency and elucidating their effects on the host will require much additional research.

**CMV infection and disease**

The common manifestations of CMV infection depend to a large extent on the particular clinical setting. Overt disease is limited primarily to patients with significant immune system dysfunction that can result from other illnesses or iatrogenic causes (Figure 2).

**Congenital and neonatal infection.** CMV is the most frequent congenital viral infection, occurring in as many as 40,000 cases in the United States each year. A recent review of multiple studies found that of infected infants, 13% had symptoms at birth, and in 0.5%, the infection proved fatal (63). Approximately 20% of the total — primarily, but not exclusively, those symptomatic at birth — suffer from permanent sequelae, commonly sensorineural hearing loss (64). Seronegative mothers who become infected during pregnancy have a much higher risk of transmitting the virus and of bearing an affected infant compared with women who are seropositive at conception, but infants of the latter group can also suffer sequelae (65). It now seems likely that at least some cases result from infection of the pregnant woman with a new virus, rather than from reactivation of a latent virus the women had acquired earlier (66). Antiviral treatment of congenital CMV disease with ganciclovir or valganciclovir has been shown to be beneficial (67). The development of preventative strategies before pregnancy (i.e., immunization, ref. 68), during pregnancy (i.e., CMV-specific immunoglobulin, ref. 69, and valacyclovir), and after birth (i.e., newborn CMV screening, screening for late-onset hearing loss, ref. 70, and preemptive treatment) are high-priority research areas.

CMV can also be acquired in the neonatal period via breast milk (71). Reported transmission rates from mothers to preterm infants vary widely (6%–60%) as do disease rates (0%–35%), so the burden and determinants of breast milk transmission are not yet clear (71).

**Immunocompetent hosts.** Judging from data showing increasing seroprevalence with age, acquisition of CMV may occur at any time during life (1). In early childhood, CMV can be acquired via saliva in family or day care settings. During adulthood, CMV is transmitted sexually and via saliva (e.g., from children), and occasionally via blood transfusions or transplanted organs (11, 72). Primary infection is typically asymptomatic, but may present clinically as a mono- nucleosis-like illness. Very occasionally, CMV seems to cause pneumonia or gastrointestinal disease in immunocompetent hosts (73).

**Hematologic malignancies and HCT.** CMV pneumonia remains one of the most feared infectious complications following HCT. Even with treatment, mortality remains high (74). CMV gastrointestinal disease, which can affect the upper and lower tracts, is presently the most prevalent manifestation of CMV disease in HCT recipients (74). Retinitis, hepatitis, and encephalitis occur infrequently.

The most important pretransplant risk factor for CMV disease is the serological status of the donor and recipient, with seropositive recipients being at highest risk, followed by CMV-seronegative patients receiving stem cells from a CMV-seropositive donor (referred to as D+/R– patients); seronegative recipients of stem cells from seronegative donors have a very low risk of primary infection if CMV-safe blood products are used (75). Other risk factors for CMV infection include the use of high-dose corticosteroids, acute and chronic graft-versus-host disease (GVHD), and use of mismatched or unrelated donors (56). Stem cell source and conditioning regimen only minimally affect the risk of CMV infection and disease, with the exception of umbilical cord blood transplantation, which is associated with reactivation and high disease rates in the absence of antiviral prophylaxis (76). Alemtuzumab, an anti-CD52 monoclonal antibody that results in prolonged CD4+ and CD8+ lymphopenia, may also lead to high reactivation rates in both transplant and nontransplant patients (77). CMV disease is very rare after autologous transplantation, unless CD34+ selected stem cells are used.

Today, the use of preemptive antiviral therapy or prophylaxis is the standard of care in HCT recipients (78). These strategies have reduced the incidence of CMV disease during the first 3 months from approximately 25%–30% to 5% in seropositive recipients; however, late CMV disease may occur and requires continued surveillance in high-risk patients (79).

SOT. CMV can cause a febrile syndrome with leukopenia and/or transaminitis (CMV syndrome) as well as other end-organ disease. There seems to be a predilection of clinical disease manifestations for the transplanted organ, possibly caused by minor HLA mismatches promoting local CMV reactivation and replication (62). Therefore, the end-organ disease manifestations differ according to the type of organ transplantation.

The highest risk for CMV disease occurs in CMV D+/R– patients. In contrast to HCT recipients, reactivation disease (in the seropositive recipients) is less common in SOT recipients. Antiviral prophylaxis and preemptive therapy strategies are widely used in SOT recipients and have led to a reduction of CMV disease during the time they are applied (typically for at least 3 months; refs. 80–82). Despite these advances, late CMV disease continues to be a clinical problem in D+/R– patients receiving antiviral prophylaxis (83). In addition to CMV syndrome and end-organ disease, also called direct effects, CMV causes indirect effects including allograft rejection, decreased graft and patient survival, and predisposition to opportunistic infections and perhaps malignancies (Figure 2 and ref. 55).

**HIV.** CMV disease occurs in HIV-1–infected persons with advanced immunosuppression (CD4+ counts <50 cells/mm³), HIV load >100,000 copies/ml, and/or prior opportunistic infections. Retinitis is the most common clinical manifestation, followed by gastrointestinal disease and encephalitis. Since the introduction of combination antiretroviral therapy (ART), the incidence of new cases of CMV end-organ disease has declined dramatically, now occurring most commonly in persons who are not receiving ART or who have failed to respond (84).
Other disease associations. In recent years, the ease and sensitivity of diagnostic capabilities have revealed the presence of CMV (or its immunologic correlates) in settings not traditionally known to manifest CMV. Numerous studies have shown that CMV reacts in immunocompetent patients admitted to ICUs, and there appears to be an association with prolonged hospital and ICU stay (53, 85). Moreover, associations of CMV with inflammatory bowel disease, new-onset diabetes, and tumors such as glioblastoma multiforme have been suggested (86–88). Perhaps most intriguing are reports that CMV may play a role in immunosenescence (89) and in the pathogenesis of atherosclerosis (90), possibly through actions of its many immunomodulatory genes (Figure 1). Whether CMV is causative in these diseases and conditions or a bystander is presently the subject of intense research.

Treatments

Current therapies. Ganciclovir, foscarnet, and cidofovir are currently available drugs for CMV treatment and prevention (Table 1). Ganciclovir (and its orally available formulation, valganciclovir) is a guanosine analog that, after phosphorylation by the CMV UL97 kinase, acts as a chain terminator during viral DNA replication. The nucleoside monophosphate analog cidofovir and the pyrophosphate analog foscarnet also inhibit viral DNA polymerase activity, but neither requires prior activation by any other viral protein (91). Ganciclovir products have been tested most widely in randomized controlled trials in both transplant and HIV-infected disease, new-onset diabetes, and tumors such as glioblastoma (53, 85). Moreover, associations of CMV with inflammatory bowel disease, new-onset diabetes, and tumors such as glioblastoma multiforme have been suggested (86–88). Perhaps most intriguing are reports that CMV may play a role in immunosenescence (89) and in the pathogenesis of atherosclerosis (90), possibly through actions of its many immunomodulatory genes (Figure 1). Whether CMV is causative in these diseases and conditions or a bystander is presently the subject of intense research.

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Drug resistance can develop with all available drugs (91). Mutations affecting the viral UL97 kinase or, less often, the viral DNA polymerase can cause ganciclovir resistance. Since foscarnet and cidofovir do not require phosphorylation by UL97, resistance arises only by mutations of the DNA polymerase gene (91). Some DNA polymerase mutations cause resistance to more than one of these agents. Resistance is most frequently seen in D+/R– SOT recipients (probably as a result of prolonged drug exposure and incomplete suppression of CMV); however, it may also occur in other clinical situations when antiviral drug is given for a prolonged period of time at levels that incompletely suppress CMV infection (98). An increase in viral load should trigger molecular testing for mutations that are associated with resistance and empiric switching to another drug.

Experimental therapies. Several new anti-CMV compounds are presently in phase II clinical development (Table 1). These include CMX001, a lipid derivative of cidofovir (99), and AIC246, which blocks a late step (possibly CMV terminase activity) in CMV replication (100). The UL97 kinase inhibitor maribavir has little serious toxicity and showed some efficacy in one controlled trial (101, 102), but appeared to be ineffective in an as-yet-unpublished phase III trial, and plans for its further development are currently unclear. In addition, there are licensed drugs that have anti-CMV activity in vitro, including leflunomide (FDA approved for arthritis treatment; ref. 103), which inhibits a late step in virion assembly (104), and imatinib, a tyrosine kinase inhibitor used to treat chronic myelogenous leukemia that blocks CMV entry into cells (25). Leflunomide has been used in salvage situations for CMV disease (105); however, no randomized trials have been conducted to evaluate its efficacy and toxicity as either monotherapy or combination therapy.
does not appear to be active in vivo (106). Donor-derived CMV-specific T cell therapy has been used in selected patients in salvage situations and is a field of active research (107).

CMV-specific and pooled immunoglobulin prophylaxis have had little success in transplant recipients (108, 109), although a meta-analysis of studies performed in the 1990s in SOT recipients suggested a beneficial effect (110). One recent uncontrolled trial suggested it might be useful as a prenatal therapy to prevent infection and disease in infants whose mothers acquired CMV during pregnancy (69). Randomized trials are ongoing to test this potential application more rigorously.

**Prevention**

The transmission patterns of CMV suggest several ways to prevent primary CMV acquisition. Transmission by sexual secretions can be prevented by use of condoms (111). The risk of transmission via saliva (e.g., child-to-mother transmission) can be reduced by handwashing and gloves (112). Transmission via blood transfusion or organ transplantation is almost completely prevented by leukocyte reduction techniques applied to the blood products or by donor selection, respectively (75). The strength of the evidence that these measures are effective and feasible on a population base varies, with the strongest evidence existing for reducing CMV transmission via the blood supply.

The development of vaccines has been a primary goal for controlling CMV. Indeed, an Institute of Medicine report declared that development of a CMV vaccine should be a top priority (113). After decades of development and incremental advances (114), the recent report that a subunit vaccine, consisting of CMV gB with MF59 adjuvant, reduced acquisition of CMV in seronegative mothers who had recently given birth (68) was a major advance. However, this vaccine reduced CMV acquisition by only 50%, possibly because the vaccine could not induce antibodies that prevent entry into endothelial and epithelial cells (40–42). Other approaches to vaccine development are being studied, including chimeric live-attenuated vaccines, DNA vaccines, and alphavirus replicons encoding CMV proteins (115). The recent finding that rhesus macaques mount immune responses to antigens delivered by repeated sequential inoculation of rhesus CMV recombinants highlights a potential role for live CMV vaccine vectors, but also illustrates the challenges in developing a vaccine that can block CMV infection itself (116).

**Future directions**

Decades of CMV research have resulted in remarkable advances in our understanding of basic CMV biology and mechanisms of immunologic control. However, many questions remain about the functions of numerous CMV genes, the mechanism of latency, and the pathogenetic processes that account for differing disease manifestations in various clinical settings. Nonetheless, enormous progress has been made in diagnostics, drug therapy, immunotherapy, and vaccine development. At the same time, it has become apparent that the spectrum of CMV morbidity may be much larger than originally appreciated. One task for the future will be to assess conclusively whether CMV is a pathogen or bystander in critically ill pediatric and adult patients as well as in other diseases such as atherosclerosis, inflammatory bowel disease, and tumors, including glioblastoma multiforme. The possible role of CMV in immunosuppression is particularly interesting and will be a field of active research for years to come. New therapeutics with improved toxicity profiles are urgently needed, not only for transplant recipients, but also for congenital disease and possibly for future new indications in immunocompromised persons. Meanwhile, management strategies with currently available drugs should be optimized. The field of adoptive T cell immunotherapy is also well on its way to overcoming obstacles that have prevented widespread application, including the time needed to generate sufficient numbers of T cells and failure to restore persisting T cell immunity in the presence of high-dose steroids (117). Finally, exciting developments toward a CMV vaccine, arguably the holy grail of prevention, are underway (68). Thus, myriad challenges remain, but judging from recent progress, we suspect that major advances are on the horizon.

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