Abstract
Cytomegalovirus (CMV) is a recognised cause of morbidity and mortality in immunocompromised individuals. This review will concentrate on recent advances in the understanding of the complex interplay between the host and parasite and the pathological consequences of perturbation of the host immune system. The classic view of CMV as a slowly replicating virus is challenged by recent in vivo findings suggesting that active replication occurs dynamically in the human host, with a doubling time of approximately one day. In addition, CMV load plays a major role in viral pathogenesis, such that increased CMV replication is a significant risk factor for disease in all immunocompromised groups studied to date. These studies focus attention on understanding the virological and immunological determinants of enhanced viral replication and its pathological consequences.

Genetics and replication of CMV
The entire DNA sequence of a prototype laboratory strain, Ad169, has been available since 1990. The genome comprises 229 354 bp and has the capacity to encode in excess of 200 genes. However, it has become clear in recent years that this highly passaged virus might not be truly representative of clinical strains of CMV, and a recent study has shown that low passage pathogenic laboratory strains of CMV (Towne and Toledo) have up to 20 kbp of additional DNA within the genome. Most of the genes in this region are unique but have structural motifs reminiscent of cellular proteins—for example, CXC motifs and RGD motifs. Thus, at present, CMV appears to contain the largest genome of any virus infecting humans. Multiple strains of virus circulate both within the population and within an individual, and evidence exists for interstrain recombination. These factors contribute to the diversity of CMV within the population. Table 1 shows a list of key genes encoded by CMV, grouped according to their known or predicted functions.

CMV replication in vitro is relatively slow and frequently cultures of clinical specimens need to be maintained for many weeks before characteristic cytopathic effects are observed. However, until recently, the relevance of these observations to the replication of CMV in vivo was unclear. The most common cell type for the propagation of CMV in vitro is the primary cell type for the propagation of CMV in vivo.
Investigation of CMV disease in immunocompromised patients

B virus (HBV), and hepatitis C virus (HCV) replication occurs dynamically in the human host, and so contrasts with the results obtained from in vitro propagation. These data demonstrate that, during an active CMV infection, the doubling time of CMV load in blood following antiviral intervention is approximately one day; thus, CMV replication occurs dynamically in the human host, and so contrasts with the results obtained from in vitro propagation. The implications of these data for the monitoring and treatment of CMV infection will be discussed later.

The immune system and CMV

Because most immunocompetent individuals do not suffer from CMV disease during either primary or recurrent infections, the immune system must be effective in controlling replication. However, this simplistic assumption masks a level of complexity between the virus and host that is still being unravelled. The B cell response to CMV is largely dominated by an anti-glycoprotein B cell response, and most of the neutralising antibodies are also directed against this protein. In contrast, the cytotoxic T cell (CD8) response is almost exclusively directed against the pp65 (UL83) protein and epitopes have been mapped for human major histocompatibility complex (HLA) types A*0201, B*0701, B*0801, and B*3501 (table 1). The frequency of cytotoxic T lymphocytes (CTLs) against CMV in immunocompetent individuals has been estimated to be 1/5000 by classic endpoint dilution chromium release assays but, using more recently developed HLA class I tetramer technology, this frequency may be much higher.

Pathological consequences of CMV infection

The incidence of CMV disease in different patient groups is not uniform and in most groups has declined as more aggressive management strategies have been used—for example, early treatment of active infection or prophylactic treatment with antivirals. Nevertheless, CMV disease remains a substantial problem in many groups and, with the increased use of potent antiviral drugs, resistant strains are becoming evident. Table 2 summarises the different pathological manifestations observed in solid organ transplant recipients, bone marrow transplant recipients, and patients with AIDS. The predominant disease in patients with AIDS has been retinitis, whereas CMV pneumonitis is a major disease in bone marrow transplant recipients, whereas its incidence is much lower in solid organ transplant recipients. In liver transplant recipients, CMV hepatitis is frequently diagnosed, whereas this is not the case.

Figure 1 Distribution of cytomegalovirus (CMV) load in cell types present in the blood (granulocytes/neutrophils, monocytes, B cells, and T cells) during an active CMV infection of a renal transplant recipient. Cell subsets were purified using Dynabeads coated with the appropriate antibody and a combination of positive and negative selection according to the recommendations of the manufacturer.
Table 3 Definitions of cytomegalovirus (CMV) disease

<table>
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<tr>
<th>Disease</th>
<th>Definition</th>
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<tr>
<td>Pneumonia (transplant recipients)</td>
<td>Radiographic changes and/or hypoxia. CMV detected in BAL or lung biopsy</td>
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<tr>
<td>Pneumonia (HIV infected patients)</td>
<td>Symptoms of pneumonia with hypoxemia. CMV detected in the lung.</td>
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<tr>
<td>Gastrointestinal</td>
<td>Absence of other pathogens</td>
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<tr>
<td>Gastrointestinal</td>
<td>Gastrointestinal symptoms with CMV detected by histology</td>
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<tr>
<td>Hepatitis</td>
<td>Abnormal liver function tests coupled with histological changes and CMV detection in a liver biopsy by culture, histology, or DNA hybridisation</td>
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<tr>
<td>Neurological</td>
<td>Symptoms of encephalitis, transverse myelitis, or other CNS symptoms, plus CMV in the CSF by culture or PCR</td>
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<tr>
<td>Retinitis</td>
<td>Typical ophthalmological lesions without virological proof</td>
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It should be noted that some of these manifestations do not reach the criteria for CMV disease outlined in table 3. The relative incidence is indicated by the number of symbols (√).

in other groups. The underlying reasons for these differences are not known, but may reflect a combination of the proinflammatory cytokine cocktail generated following organ transplantation, together with the duration of replication. For example, in patients with AIDS and congenitally infected infants, CMV replication usually occurs at a persistently high level for many months, whereas most transplant recipients have an acute CMV infection, which subsequently results in disease in a relatively short time frame. In addition to viral replication directly causing pathology (for example, hepatitis), there is increasing evidence that the host's immune system might also contribute to the pathology observed. This has been suggested to account for the pathology of CMV pneumonitis, because it is only when patients are able to mount a sufficient immune response that the disease is observed. Thus, patients with AIDS who frequently harbour CMV in their lungs have a low incidence of CMV pneumonitis, whereas the disease has been observed in HIV positive individuals with high CD4+ cell numbers. The recent introduction of highly active antiretroviral therapy (HAART) for HIV infection has resulted in an inflammatory viremia in patients with CMV retinitis which, in some cases, has resulted in the loss of vision. Thus, paradoxically, the CMV replication accompanying sight threatening CMV retinitis can be controlled by the administration of HAART but the regeneration of specific immune responses causes a new pathology, which can be equally destructive to the host tissue and deleterious to the quality of life of the patient.

Diagnosis of CMV disease

As mentioned above, CMV disease can take many forms, depending upon the type of patient group under consideration. A consensus is available from the international CMV workshop for the definition of CMV disease, and is summarised in table 3. Thus, CMV hepatitis can only be proved if the virus is detected by histopathology in the liver biopsy, either by observation of CMV inclusion bodies or by immunohistochemical staining. On the other hand, CMV retinitis can be diagnosed by a qualified ophthalmologist in the absence of any virological evidence because this condition is usually self evident. The guidelines shown in table 3 are purposefully rigorous and have aided the interpretation of clinical trial data and population based studies. However, they are likely to lead to an under appreciation of the contribution of CMV to patient morbidity. This is particularly true in the case of the histopathological diagnosis of CMV. My group has correlated CMV load in tissues obtained at postmortem examination of patients with AIDS with the histological evidence of CMV inclusions and found that a viral load of > 5 000 000 genomes/µg DNA is required before CMV inclusions are observed. Therefore, it seems likely that some cases of CMV hepatitis may be dismissed because they do not reach the definition in table 3, despite the presence of abnormal liver function tests and CMV DNA in the blood. It is important to appreciate that, with the advent of more sensitive molecular based assays, a reappraisal of many of these definitions may be required.

The role of CMV load in pathogenesis

As the sensitivity and reproducibility of quantitative laboratory methods has improved, it has become increasingly evident that the viral load is a central factor in the pathogenesis of many viral infections. In the case of CMV, the viral titre in the urine of congenitally infected infants (who excrete large amounts of virus at birth) was first shown to be associated with concurrent disease and the likelihood of developing disease in 1975. However, because most immunocompromised hosts have much lower amounts of virus circulating in the blood during an active infection, measurement using classic cell culture methods proved to be unsatisfactory. The advent of molecular methods circumvented these problems and data from a range of studies using DNA hybridisation and semiquantitative polymerase chain reaction (PCR) showed that symptomatic immunocompromised patients usually had significantly higher CMV loads than patients with active CMV infection who remained asymptomatic. My group has substantially extended these studies by using quantitative competitive PCR in longitudinal analyses of different patient groups at risk of CMV disease.

In bone marrow transplant, renal transplant, and liver transplant recipients, peak CMV load is the major risk factor associated with CMV disease (table 4). Other established risk factors for CMV disease, such as donor/recipient serostatus, are entirely explained by raised CMV...
The reader should consult the reference list for a comprehensive set of analyses. In these analyses, raised viral load explained established risk factors for disease, such as immunodeficiency virus (HIV) infected individuals enrolled in ACTG204.

The presence of CMV DNA in the blood of HIV positive patients identifies a group of patients who are almost 20 times more likely to progress to CMV disease than those who remain negative for CMV DNA in the blood. In addition, an increasing CMV load in the blood was associated with an increased risk for disease progression. Subsequently, the availability of data from large cohorts participating in prophylactic trials of anti-CMV drugs, such as ganciclovir and valaciclovir, has verified these initial studies.

A typical Kaplan–Meier survival curve for progression to death according to baseline CMV load is shown in fig 2. Indeed, recent analysis of the ganciclovir 1654 study has shown that the effect of CMV load at the initiation of drug treatment (some considerable time period before patients exhibit CMV disease) has more effect on survival than does HIV RNA load.

Conclusions
The availability of modern molecular methods has facilitated the study of CMV in vivo. The results demonstrate that CMV load is crucial in the pathogenic process and provide new insights into optimal drug regimens and duration of treatment. Obviously, the pathological processes that are responsible for the risk of CMV disease, and the variation between different immunocompromised hosts, require delineation. However, our improved understanding of CMV replication in vivo is likely to impact on patient management and the deployment of existing and newer therapeutic agents in the near future.

I am grateful to my colleagues and collaborators for allowing me to cite their data in this review. Work in my laboratory is supported financially by the Medical Research Council, the National Institutes of Health USA, and the Wellcome Trust.


### Table 4

<table>
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<tr>
<th>Patient group</th>
<th>OR for CMV disease (95% CI)</th>
<th>p Value</th>
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<tr>
<td>Renal transplant recipients</td>
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<tr>
<td>CMV load</td>
<td>1.65 (1.14 to 2.39)</td>
<td>0.008</td>
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<tr>
<td>Liver transplant recipients</td>
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<tr>
<td>CMV load</td>
<td>2.70 (1.41 to 5.17)</td>
<td>0.003</td>
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<tr>
<td>Methylprednisolone/1 g</td>
<td>1.61 (1.04 to 2.51)</td>
<td>0.03</td>
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<tr>
<td>Bone marrow transplant recipients</td>
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<tr>
<td>CMV load</td>
<td>1.43 (1.12 to 1.82)</td>
<td>0.004</td>
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All ORs refer to an increase in CMV load of 0.25 log10 genomes/ml of blood.

Figure 2 Kaplan–Meier survival curves showing the influence of baseline cytomegalovirus (CMV) load in the blood on (A) time to disease and (B) death in a cohort of human immunodeficiency virus (HIV) infected individuals enrolled in ACTG204.
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