Why CMV is a candidate for elimination and then eradication

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Abstract

Cytomegalovirus (CMV) is well-known for the end organ diseases (EODs) it causes following viraemic dissemination in immunocompromised hosts. These are termed the direct effects of CMV, where a diagnosis can be made in an individual patient. In addition, CMV is associated with indirect effects where populations can be seen to be disadvantaged compared to those without CMV. These indirect effects have been described in solid organ transplants, bone marrow transplants, advanced HIV, people admitted to intensive care units, the elderly and the general population.

We summarise the evidence that associates CMV with its direct effects following congenital infection, solid organ transplantation, bone marrow transplantation and advanced HIV as well as its indirect effects in all patient populations. We propose that the greatest worldwide burden of CMV comes from its indirect effects. Control of this infection at the population level is being sought through the development of vaccines to control EODs where cost effectiveness is expected. We propose that the financial case for universal immunisation will be enhanced even further by the potential benefits vaccines may produce against the indirect effects of CMV.

Keywords: CMV, cytomegalovirus, indirect effects, vaccine, immunocompromise

Introduction

In this article, we take a historical perspective to describe how individual diseases caused by CMV were first recognised. We focus on congenital CMV, infections in solid organ transplants, bone marrow transplants and advanced HIV using data from developed countries, because other articles in this issue will consider Africa, HIV-infected or exposed neonates with CMV and the prospects for developing CMV vaccines.

Congenital CMV

This was first recognised about 100 years ago when intranuclear inclusion bodies were seen in histopathological sections from cases of stillbirth [1]. The availability of cell culture [2] diagnosed infection in cases who survived intrauterine infection to be born with features of cytomegalic inclusion disease (Table 1). Prospective natural history studies also showed that apparently normal neonates could have congenital CMV infection. Importantly, those with symptoms at birth had higher levels of CMV in the urine than those without symptoms (Figure 1) in what was the first demonstration in humans of the phenomenon now termed viral load [3]. The implications of this observation from 1975 were profound (Table 2) and have all turned out to be correct. This includes the threshold concept, that disease occurs once viral load exceeds a critical value, so that benefit against disease may be greater than predicted from the activity of a drug against infection.

Prospective studies also showed that those born without symptoms were at risk of developing disease later in childhood, with sensorineural hearing loss and damage to intellectual function predominant [4]. This association of progressive disease in the presence of ongoing virus replication suggested that the former might be limited if the latter could be controlled. Two randomised controlled trials, both conducted by the Collaborative Antiviral Study Group, have addressed this issue. In the first, neonates born with CNS symptoms were randomised to receive ganciclovir intravenously for 6 weeks at a dose of 6 mg/kg twice a day [5]. This dose was chosen because of an earlier comparison of two doses of ganciclovir in sequential cohorts [6]. The results of the randomised controlled trial showed that a lower proportion of ganciclovir recipients developed sensorineural hearing loss and a follow-up report extended this observation to show benefits for intellectual function [5,7]. These results were so important clinically that they were accepted as the standard of care. The second randomised controlled trial [8] gave all neonates born with symptoms of congenital CMV infection (not just CNS symptoms) 6 weeks of valganciclovir at 16 mg/kg twice daily having shown in a previous study [9] that this dose produced equivalent levels of ganciclovir to that found when ganciclovir was given intravenously at 6 mg/kg. After 6 weeks of valganciclovir, the randomised controlled trial continued this drug to complete 6 months of therapy or administered a matching placebo. The results showed that 6 months of treatment was superior to 6 weeks and so became the new standard of care [8]. As predicted by the threshold concept, the four-times longer duration of treatment did not produce four times the clinical benefits; in other words, the initial treatment for 6 weeks had a major effect.

Table 1. Cytomegalic inclusion disease

Clinical features
• Microcephaly
• Petechiae
• Jaundice
• Hepatosplenomegaly
• Lymphadenopathy
• Blueberry muffin rash
• Intrauterine growth retardation
• Sensorineural hearing loss
Laboratory abnormalities
• Thrombocytopaenia
• Conjugated hyperbilirubinaemia
• Raised liver transaminases
Ultrasound abnormalities
• Periventricular calcifications
• Ventriculomegaly

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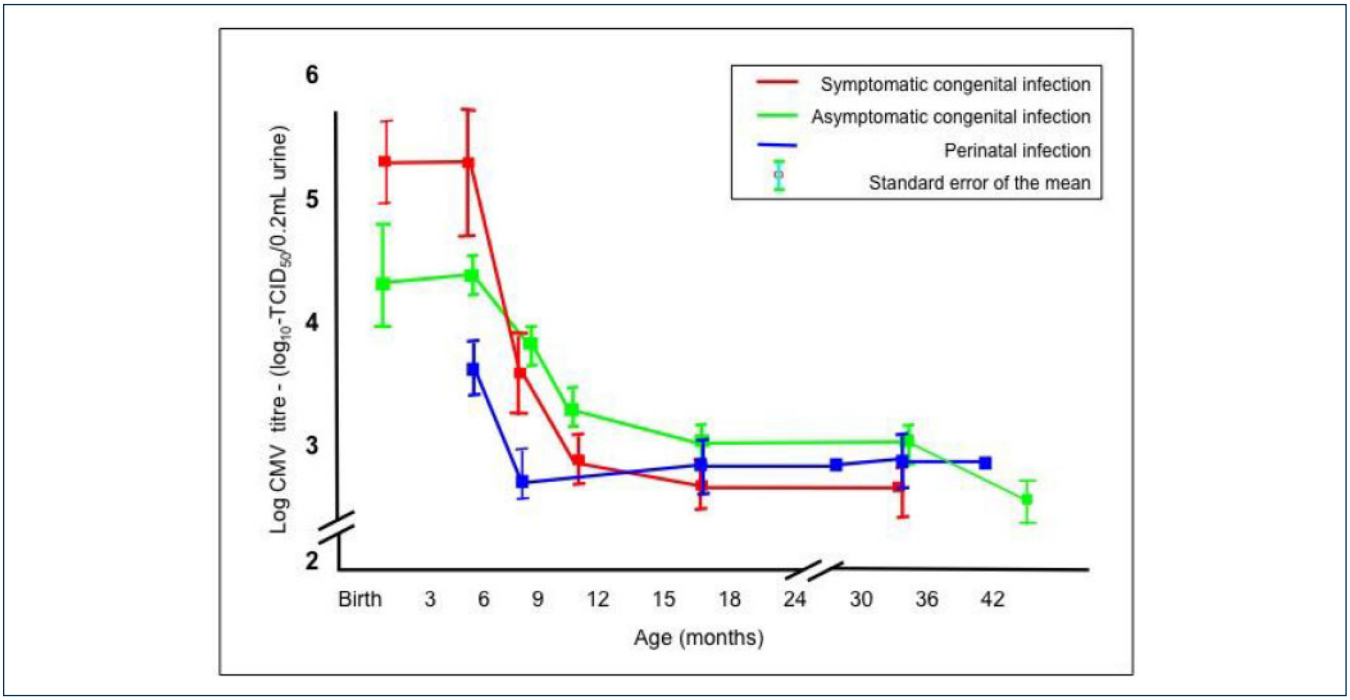

Figure 1. Association between quantity of CMV found in the urine and the severity of disease in neonates. Redrawn from Stagno *et al. J Infect Dis* 1975; **132**: 568–577

Table 2. Implications of results shown in Figure 2

- CMV is a systemic infection
- Sampling urine provides information about clinically inaccessible sites e.g. inner ear
- CMV is a chronic infection in neonates so there may be a CMV-specific immune defect
- CMV may cause disease once a threshold value of viral load is exceeded
- Treatment may be beneficial if viral load is kept below this threshold value
- Postnatal treatment may be beneficial even if short-term

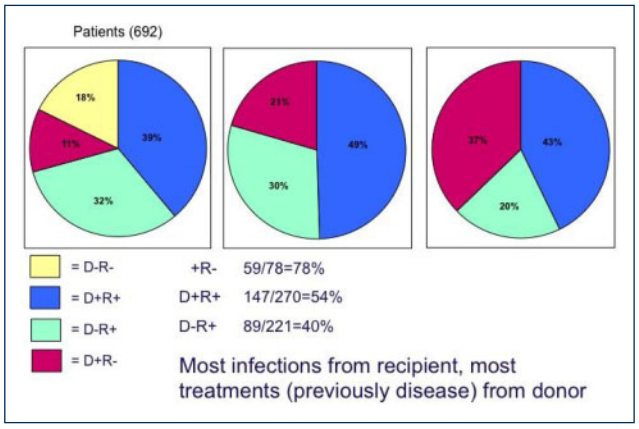

Figure 2. Detection of CMV DNA by real time PCR post-transplant is distinct among four subgroups of patients defined by their donor and recipient serostatus pre-transplant. Redrawn from Atabani *et al.* [14]

Solid organ transplant patients

In the early days of experimental solid organ transplantation, an autopsy series published in the *New England Journal of Medicine* provides sobering reading, because a major limitation was the occurrence of CMV EOD, particularly pneumonitis [10]. With the recognition of this complication of excessive immunosuppression and the availability of safer immunosuppressive drugs, the subject moved forward into the life-saving routine part of medical care that we see today. In contemporary solid organ transplantation, CMV EOD is controlled through the use of valganciclovir either given prophylactically or as part of pre-emptive therapy [11]. In the former, drug is given for a fixed period of time (clinical trials support 100 days or 200 days) from the time of transplant [12,13]. The drug effectively suppresses CMV EOD during this time, but cases occur when the drug is stopped, some of them with strains of CMV resistant to ganciclovir, which then have to be treated with the off-label use of foscarnet. In pre-emptive therapy, patients are monitored regularly for CMV DNA in the blood by real-time PCR and given ganciclovir intravenously or valganciclovir orally if a particular viral load level is detected. In our laboratory, this level is 3000 genomes/mL whole blood (2520 IU/mL) and treatment is continued until that patient has two consecutive samples where CMV DNA is undetectable [14]. Similar protocols with slightly different sampling times and cut-off levels are used in the developed world. This cut-off level was derived from prospective natural history studies aiming to see if the viral load associations seen in neonates (Figure 1) were also found after solid organ transplantation [15,16]. In summary, all of the principles gleaned from that 1975 paper (Table 2) apply to this distinct patient population as well. Specifically, patients can be triaged into four groups depending on the presence of CMV IgG antibodies pre-transplant in donor and recipient (Figure 2). The highest incidence of CMV infection, highest peak viral load and longest duration of viraemia are seen in the D+R- subgroup (where CMV is transmitted from donor to immunologically naive recipient), D+R+ (patients with natural immunity who are at risk of reactivation of latent virus or reinfection from the donor); D-R+ (patients at risk of reactivation of latent virus). The last group (D-R-) have a zero risk of CMV infection except in rare cases where a donor acquires primary infection just before donating an organ.

The term 'indirect effects of CMV' was coined by Dr Bob Rubin in an editorial accompanying a case series of heart transplant patients [17]. Those with CMV EOD had an excess of graft rejection, fungal infections and allograft atherosclerosis as a group, although there were no clinical features to identify which patients had this disease enhanced by CMV [18]. Follow up of heart transplant patients who received ganciclovir prophylaxis reported reduced graft atherosclerosis [19]. A randomised controlled trial in renal transplant patients showed that prophylaxis with high-dose valaciclovir significantly reduced biopsy proven acute graft rejection in D+R- patients experiencing primary CMV infection, but not in those who were seropositive prior to transplant [20].

Bone marrow transplant patients

The same principles of a high viral load being a prerequisite for EOD apply here, but with two differences from solid organ transplant patients. First, the epidemiology is different. Most strains of CMV detected after bone marrow transplant come from reactivation of latent virus in the recipient, with a low risk of transferring CMV from the donor [21]. Second, bone marrow transplant patients get EOD at a lower peak viral load than do solid organ transplant patients [22,23]. Disease is controlled by pre-emptive therapy as described above. The same viral load cut-offs can be used to decide when therapy should be started. Prophylaxis is not used because ganciclovir is toxic to the newly grafting marrow. This situation might change in the future because three new antiviral compounds (maribavir, brincidofovir, letermovir) without bone marrow toxicity are in clinical trial. All three produced encouraging results in Phase II studies in bone marrow transplant patients [24–26]. Two have so far completed Phase III studies, which included a washout period after drug administration, and both failed to reach their primary endpoints [27]. All three drugs can suppress CMV infection, but carefully designed clinical trials will be required to show how they can produce benefits for bone marrow transplant patients [28].

The indirect effects of CMV in this patient population are bacterial or fungal superinfection and death, each of which has been significantly reduced in randomised controlled trials of antiviral drugs [29]. Graft versus host disease has been proposed as an indirect effect, but lacks proof so far. The effect on mortality when CMV seropositives are compared to CMV seronegatives is clear [30]. However, it has not been proven that this effect requires CMV replication.

Advanced HIV

The first cases described in 1981 had CMV retinitis, which would now be termed as an AIDS-defining condition. Active replication with CMV is uncommon until the CD4 cells count has declined below 100 cells/mm^3. CMV replication then becomes common and causes EOD once the viral load reaches a similar value to that seen in solid organ transplant patients [31]. In AIDS patients, most EOD (85%) is retinitis compared to 1% after solid organ transplant; a marked difference in natural history that remains unexplained. We speculate that HIV has an effect on the blood–retinal barrier that facilitates access of CMV to the eye. The remaining EODs are gastrointestinal or neurological, including polyradiculopathy.

The best prevention of CMV EOD involves maintaining the CD4 cell count above 100 cells/mm^3. If this is not possible, prophylaxis with oral ganciclovir (a drug no longer available) has been shown to prevent CMV retinitis, but at high cost and at the risk of developing resistance [32]. Treatment of established CMV retinitis is with intravenous ganciclovir followed by valganciclovir long-term [33]. Because levels of intraocular ganciclovir are low, an implant is available that releases the drug slowly over a period of months. This was useful in the early days of AIDS, but now that HAART is available, CMV EOD is now rare.

The indirect effects of CMV in this patient population are an excess of AIDS-defining conditions and an excess mortality [34,35]. The indirect effects were originally called cofactor effects [36] and were controversial, perhaps because they were first described around the time that some prominent people disputed the fact that HIV causes AIDS. For whatever reason, the possibility of indirect effects has been ignored in advanced HIV infection relative to the other patient groups discussed here. Initially, it was difficult to study cohort effects of CMV, because most HIV-positive men who have sex with men were co-infected with CMV. This problem was overcome by investigating haemophiliacs, who have a seroprevalence of about 60%, but only IgG serology was available [37]. When PCR was deployed, the association of CMV with mortality in the pre-HAART era was greater than that of HIV itself [34]. When HAART was introduced, CMV and the CD4 cell count were identified as major associations with mortality, leading HIV to make a non-significant contribution once these two factors had been accounted for [35]. Clearly, HIV provides the context for increased mortality in AIDS patients, but CMV, in the presence of a low CD4 cell count, provides the coup de grace. The importance of CMV as a cofactor in HIV-positive individuals has recently been revisited in a large Italian cohort study [38]. Results from this analysis demonstrated that patients who were CMV IgG positive at baseline were significantly more likely to develop severe non-AIDS-defining events. In addition, CMV seropositivity was identified as an independent risk factor for both cardiovascular and cerebrovascular disease [38].

As regards the mechanisms for this putative cofactor effect, it was speculated that CMV had 'an immunosuppressive effect' that enhanced the pathogenicity of HIV or that one or more of six specific cellular or molecular interactions between CMV and HIV might be at play [36]. Most of these six mechanisms would be expected to increase the HIV viral load and, in summary, no evidence of this was subsequently found [39]. Meanwhile, the vague description of CMV having an immunosuppressive effect has matured into the concept of CMV-induced immunosenescence (see below) characterised by an excess of CD8 T lymphocytes searching for hidden sites of CMV replication. The abundance of these cells in HIV-positive patients has been reduced by valganciclovir in a placebo-controlled randomised trial [40].

The elderly

There is no evidence for CMV EOD among the elderly, although the autopsy rate is low.

Several prospective studies of 'free-living' elderly, (i.e. those not receiving institutional care), report that mortality is associated not just with the number of diseases patients have accumulated during life, but with the presence of an immune risk phenotype [41]. Originally defined as an inverted CD4/CD8 ratio, this phenotype has expanded to include detection of excessive levels of cytokines. In 1999, the excess of CD8 lymphocytes, which is key to the immune risk phenotype, was reported to be found predominately in those elderly who were CMV seropositive [42]. In brief, it is speculated that the CD8 cells are themselves aggressively inflammatory, increasing the progression of diseases like atherosclerosis, or that the excess of CD8 cells is a marker for a shortage of CD4 cells required for mounting immune responses to new antigens. Thus, elderly, frail individuals with the immune risk phenotype may have an excess mortality because they have impaired protection against influenza or pneumococcal disease despite being given the appropriate vaccines. There is some evidence of impaired responses to influenza

vaccine among CMV seropositives [43]. No randomised controlled trial of drugs active against CMV has been reported in this population, but in our opinion, one should be conducted to determine if survival, independent life and quality-of-life in the elderly can be enhanced.

General population

A large population-based sample (the National Health and Nutrition Examination Survey) representative of the US population was tested for CMV IgG antibodies. Subjects were over 25 years of age and were followed for a mean of 14 years. Seropositives had an excess mortality [44]. The magnitude of this effect was similar to that of having a raised C-reactive protein and was independent of it. The observed difference due to CMV was attenuated by controlling for age, gender, smoking, diabetes and obesity and yet remained significant. The median reduction in life expectancy compared to a seronegative individual was about 12 months. A similar investigation was performed in a different population in the UK with confirmatory results [45].

Perspective

Cytomegalovirus was originally perceived as a mild, slowly growing virus that could only cause disease in patients with immature or suppressed immune responses. When direct measures of the dynamics of primary CMV replication in blood showed these to be similar to those of primary HIV infection, this view had to be changed; CMV was then seen as an aggressive virus that required extensive commitments from the immune system to stop it causing overt disease. The results summarised here show that the view of CMV needs to change again; it alters the size and shape of the immune system long-term. The ability of CMV to downregulate class I HLA display and interfere with recognition of infected cells by T lymphocytes and NK cells (Figure 3) allows CMV to persist in sanctuary sites and yet changes the proportion of these important immune effectors. Over years, these changes may contribute to inflammatory diseases, decreased immune responses to vaccination and decreased immune surveillance against nascent tumours. In this way, a common, and apparently innocuous virus may be having profound effects on the longevity of our species.

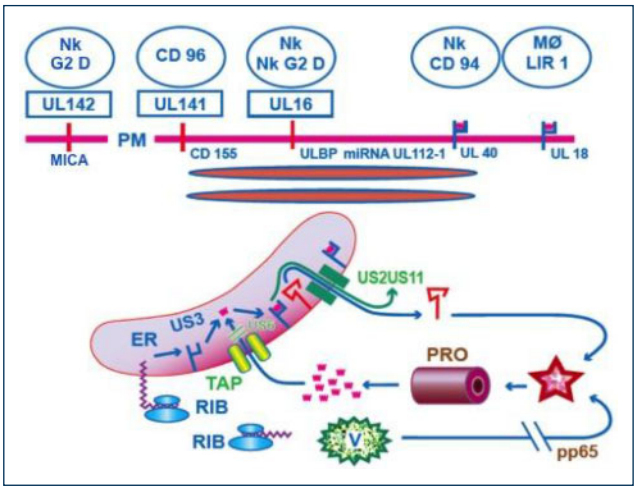

Figure 3. Pathway of biogenesis of class I HLA molecules showing sites where CMV can interfere with their function of immune presentation of virus-encoded peptides. Reprinted with kind permission from: Griffiths. Cytomegalovirus. In: *Principles and Practice of Clinical Virology* (AJ Zuckerman, JE Banatvala, BD Schoub, eds). 6th edn. John Wiley, Chichester, UK. 2009; pp 161–197

Cytomegalovirus deserves to be eliminated from developed countries as a prelude to tackling eradication around the globe, which is more difficult to contemplate because of the early age of acquisition of CMV in developing countries [46].

One has to consider how this phenomenon can have been missed for so long. It may be that, as in the Indian proverb of blind men encountering different parts of an elephant, specialised physicians have seen only one aspect of CMV and classified it accordingly (Figure 4). With this broader vision, the case is now clear for controlling CMV at the population level by universal immunisation. At present, the costs of vaccine development and evaluation are being justified financially by prevention of EOD in congenital infection and in transplant recipients [47]. Based on the evidence presented here, we suggest that the gains achieved in terms of prevention of the indirect effects of CMV will greatly enhance the cost effectiveness of CMV vaccines.

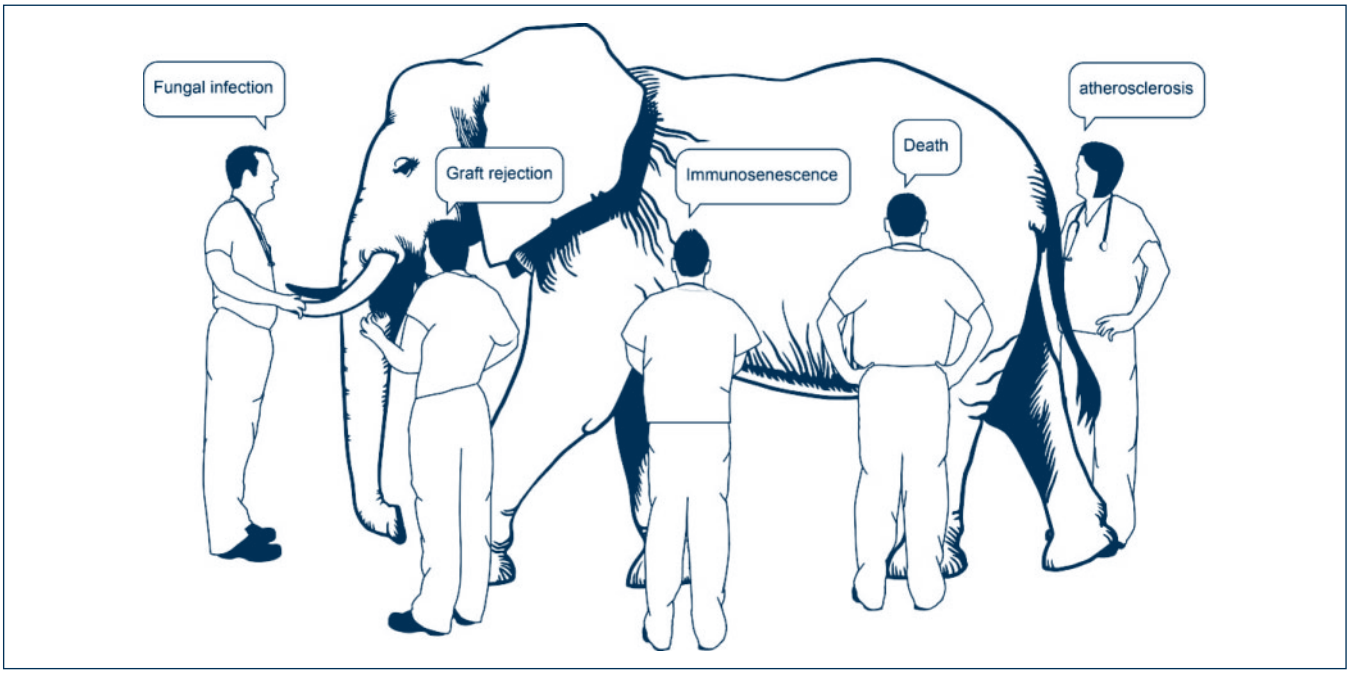

Figure 4. Schematic representation of specialist physicians who are blind to holistic medicine describing the indirect effects of CMV that present in their patients. It is based on an Indian proverb of blind men examining an elephant

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