The direct and indirect consequences of cytomegalovirus infection and potential benefits of vaccination.

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Abstract

Active infection with cytomegalovirus (CMV) occurs in patients who are immunocompromised and may produce the high viral loads required to cause end-organ disease. Such patients have complex medical histories and many experienced physicians have speculated that CMV may, additionally, contribute to adverse clinical outcomes. In 1989, Dr Bob Rubin coined the term "indirect effects" to describe this potential relationship between virus and patient. Examples include accelerated atherosclerosis in patients after heart transplant or with underlying HIV infection, the number of days patients require ventilation after admission to intensive care units, the development of immunosenescence in the elderly and mortality in many groups of patients, including the general population.

It is difficult to distinguish between CMV acting as causal contributor to such diverse pathology or simply having a benign bystander effect. However, recruitment of patients into placebo-controlled randomised trials of antiviral drugs with activity against CMV offers such a potential. This article describes the studies that have been conducted to date and emphasises that mortality after stem cell transplant (not attributed to CMV end-organ disease) has recently become the first proven indirect effect of CMV now that letermovir has significantly reduced non-relapse deaths.

The implications for CMV vaccines are then discussed. Vaccines are already predicted to be highly cost-effective if they can reduce CMV end-organ

disease. Health planners should now consider that cost effectiveness is likely to be enhanced further through reduction of the indirect effects of CMV. A prototype scheme for assessing this possibility is provided in order to stimulate discussion within the field.

1. Introduction

I am honoured to have been asked to contribute an article in memory of Dr Mark Prichard who had a long-term interest in cytomegalovirus (CMV). I will take the opportunity to speculate on the possible long-term effects of this virus which I know Mark would have been pleased to discuss and debate.

Cytomegalovirus is a common infection that may cause minor symptoms during primary infection, but more often appears to be clinically benign when first acquired. However, increasing evidence supports the concept that CMV contributes to a variety of serious medical conditions, especially in immunocompromised patients who have difficulty controlling active CMV replication. In this article, I will briefly introduce recent discoveries about the replication of this virus before reviewing its natural history in transplant patients. The clinical consequences of active replication will then be set in the context of published historical and recent randomised controlled trials.

2. Cytomegalovirus infection in the laboratory

Recent research has identified the multiple proteins CMV employs to enter cells and the cellular receptors involved.(Ciferri, Chandramouli et al. 2015)

Glycoprotein H plus glycoprotein L binds glycoprotein O to form a trimer. This engages with platelet derived growth factor alpha (PDGFalpha) on the fibroblast cell surface.(Ciferri, Chandramouli et al. 2015) Glycoprotein H plus glycoprotein L also binds three smaller glycoproteins (termed UL128, UL130 and UL131A) to form a pentamer. This binds to neuropilin 2 (Nrp2) on the surface of endothelial or epithelial cells. (Martinez-Martin, Marcandalli et al. 2018)

In order to trigger internalisation, these initial binding events recruit glycoprotein B which is responsible for fusing the viral envelope with the plasma membrane.(Martinez-Martin, Marcandalli et al. 2018) This involves direct fusion at neutral pH in fibroblasts, but acidification via endosomes in epithelial cells.

3. Natural history of cytomegalovirus infection

Approximately 60% of adults in developed countries and virtually 100% of adults in developing countries have IgG antibodies against CMV.(Zuhair, Smit et al. 2019) The acquisition of infection is more common in people from lower socio-economic groups and those who migrate in childhood tend to adopt the risk profile of their new country.(Pembrey, Raynor et al. 2013) Primary infection is usually asymptomatic, or accompanied by such mild symptoms that medical attention is rarely sought, because the immune system effectively limits extensive virus replication, leading the virus to establish latency.

Latency is one mechanism used by the virus to evade immune recognition.(Dupont and Reeves 2016) Others include expression of viral genes that block the creation, transport into the endoplasmic reticulum, passage through the endoplasmic reticulum and Golgi apparatus and display at the plasma membrane of peptides from viral proteins that can be recognised by T-cells.(Fielding, Weekes et al. 2017) Some of these multiple proteins achieve this objective by decreasing display of Class I and Class II HLA molecules. This renders the cell potentially susceptible to NK cells that recognise the absence of normal HLA display, but the CMV genome encodes further proteins that provide decoy signals to such NK cells.(Tomasec, Braud et al. 2000, Wang, McSharry et al. 2002, Fielding, Aicheler et al. 2014) The resulting cellular disruption is recognised by the unfolded protein response, but attempts to display stress signals at the plasma membrane are blocked by yet further components of the CMV genome, including the “UL binding proteins” and a micro-RNA.(Fielding, Aicheler et al. 2014) The net result is that CMV avoids the hazards presented by antibodies in the extracellular fluid by spreading cell-cell to establish sanctuary sites within the body.(Griffiths, Baraniak et al. 2015)

When infected cells die their antigens are presented to the immune system so that a massive immune response is made against CMV.(Jackson, Sedikides et al. 2017) However, it is difficult for these immune effectors to identify CMV within its sanctuary sites, so a stand-off is established with the immune system fully activated and poised to respond to any attempt of CMV to break out from its sites of persistence. This immune response is so great that it

alters the T-cell and NK cell repertoire within an individual.(Brodin, Jojic et al. 2015)

Changes in the balance of forces in this stand-off can allow CMV to reactivate from latency, produce infectious virions, disseminate in the blood stream of the individual and potentially transmit to others. Examples include exogenous stress (illustrated dramatically in astronauts) or when the immune system is compromised.(Mehta, Laudenslager et al. 2014) Just as the immune system cannot prevent reactivation of latent CMV, exogenous reinfections also occur with different strains of CMV.(Grundy, Lui et al. 1988)

Transmission of CMV from person to person is not efficient so that many exposures do not lead to transmission.(Mayer, Krantz et al. 2017) The reasons for this are not clear, but help to explain why CMV has a relatively low basic reproductive number and why many people live for decades before they become infected.(Griffiths, McLean et al. 2001, Colugnati, Staras et al. 2007) A major source of CMV is the saliva and urine of young children.(Staras, Flanders et al. 2008)

As with primary infection, reactivations and reinfections are usually asymptomatic. However, all 3 types of CMV infection can produce severe disease in patients who are profoundly immunocompromised.(Atabani, Smith et al. 2012)

4. Appearance of overt disease in transplant patients

The very first cases of experimental organ transplantation in humans were complicated by fatal CMV pneumonitis.(Hill, Rowlands et al. 1964) Other organs that may be affected include the gastrointestinal tract (colitis, oesophagitis), the liver (hepatitis) and the eye (retinitis). Cytomegalovirus end-organ disease remained a serious cause of mortality and morbidity to transplant patients until improved diagnosis of active infection coupled with administration of ganciclovir or its oral prodrug valganciclovir became available.(Kotton, Kumar et al. 2018)

The risk of developing end-organ disease varies according to the presence of IgG antibodies pre-transplant in the donor and the recipient.(Atabani, Smith et al. 2012) For recipients of solid organ transplants, the major risk group is those where the donor is seropositive and the recipient seronegative (D+R-) where, typically, 70% of renal transplant patients acquire primary infection.(Atabani, Smith et al. 2012) A low-risk group is where the donor is seronegative and the recipient seropositive (D-R+) where about 40% of renal transplant patients reactivate latent virus after transplant.(Atabani, Smith et al. 2012) The intermediate group where both donor and recipient are seropositive (D+R+) have approximately 54% risk of viraemia, due to either reactivation of recipient virus or reinfection from the donor.(Atabani, Smith et al. 2012) If it is assumed that all seropositive renal transplant recipients have the same risk of reactivation, then the rate of reinfection must approximate to 14% (54%-40%). This compares with the 70% risk of transmission of CMV from a seropositive donor to someone lacking natural immunity. This observation has stimulated

the evaluation of CMV vaccines in recipients to see if they can mimic natural immunity in providing protection against virus transmitted from the donor.

For recipients of haematopoietic stem cell transplants, the risk groups are reversed according to donor and recipient serostatus.(Boeckh and Ljungman 2009, Panagou, Zakout et al. 2016) The major risk comes from seropositive recipients reactivating CMV post-transplant. A seropositive donor makes a minor contribution to transmitting virus to recipients, irrespective of their own serostatus. In fact, there is evidence that seropositive donors may adoptively transfer some natural immunity into the recipient.(Wimperis, Brenner et al. 1986)

5. Link between end-organ disease and high viral load

Natural history studies that collected serial samples from organ transplant patients showed that viraemia, increasing viral load and a high viral load were risk factors for developing end-organ disease.(Cope, Sabin et al. 1997, Cope, Sweny et al. 1997, Emery, Cope et al. 1999, Emery, Sabin et al. 2000) In fact, with frequent monitoring, it can be said that a high viral load is a prerequisite for developing end-organ disease. The only exception to this is occasional cases of gastrointestinal disease that occur early after stem cell transplant where viraemia may not have been detected previously.

The natural history studies showed that immunosuppressive drugs increased the risk of end-organ disease by increasing the viral load. In contrast, administration of steroids increased the risk of end-organ disease by

decreasing the viral load required to cause end-organ disease.(Cope, Sabin et al. 1997) The administration of steroids thus remained an independent risk factor for end-organ disease in multivariate statistical models. This is important because steroids are given for the treatment of graft rejection, so there is a statistical link between rejection and CMV end-organ disease.

Stem cell transplant patients are more susceptible to CMV end-organ disease as measured by incidence and severity. This could be explained either by them having higher viral loads than solid organ transplant patients, or by them being more susceptible to a given viral load. Natural history studies implicated the latter.(Gor, Sabin et al. 1998)

Knowledge of this natural history allowed pre-emptive therapy to be introduced, where patients were monitored to identify those with viraemia and treatment initiated at a defined cut-off with the objective of preventing end-organ disease.(Rubin 1991, Atabani, Smith et al. 2012) This is very effective and is recommended in standard treatment guidelines along with the alternative of giving antiviral drugs prophylactically.(Kotton, Kumar et al. 2018) Valganciclovir can be used for prophylaxis in solid organ transplant patients, but its bone marrow toxicity precludes use in stem cell transplant patients.(Boeckh and Ljungman 2009, Humar, Lebranchu et al. 2010) Recently, letermovir has been shown to be both safe and effective for prophylaxis in stem cell transplant patients.(Marty, Ljungman et al. 2017)

A recent meta-analysis of the published literature showed that the criteria used to associate measures of CMV viral load with end-organ disease are sufficiently robust to be accepted by regulators for use in phase 2 and phase 3 randomised controlled trials of novel antiviral drugs.(Natori, Alghamdi et al. 2018) High viral load in bronchoalveolar samples has also been shown to correlate with CMV pneumonitis after stem cell transplant.(Boeckh, Stevens-Ayers et al. 2017)

6. Association of end-organ disease with other clinical conditions

In general, patients who develop CMV end-organ disease have also been unfortunate enough to experience other complications of transplantation. One example was mentioned above, where the administration of steroids to treat graft rejection facilitated the development of end-organ disease in solid organ transplant patients.(Cope, Sabin et al. 1997) This lesson was re-learned decades later during a phase 3 study of brincidofovir in stem cell transplant patients where administration of steroids for presumed graft versus host disease (some of which was not in fact graft versus host disease) precipitated reactivation of CMV.(Marty, Winston et al. 2019)

Additional complications after organ transplant include atherosclerosis (particularly after heart transplant), immunosuppression facilitating secondary fungal infections, diabetes and death (not attributed to CMV end-organ disease itself).(Rubin 1989) These complications are relatively common, are complex, are multifactorial and, in the case of death, have competing causes which have to be handled statistically. The question is frequently asked

whether CMV has contributed to these causes or whether CMV is a consequence of their development or the treatment required.

In 1989, Dr Bob Rubin wrote an editorial to accompany a manuscript describing CMV infection after heart transplant and coined the phrase "indirect effects" of CMV to explain an excess of atherosclerosis seen in those who had CMV end-organ disease.(Rubin 1989) In contrast to the "direct effects" of CMV, which can be seen histopathologically in individual patients with end-organ disease involving a particular organ, the indirect effects are manifest as a statistical excess of conditions found in a group of patients. These conditions also occur in patients without CMV infection, so must be defined in terms of an excess seen above a baseline figure expected for that population. Given the variability seen in patients between centres, this is difficult to demonstrate in cohort studies.

7. Validation of the concept of indirect effects through randomised controlled trials

Dr Hannah Valantine followed up, for a mean of 4.7 years, heart transplant patients in the original randomised controlled trial (RCT) comparing intravenous ganciclovir with placebo for its ability to control CMV and reported less atherosclerosis among those who had received the drug.(Merigan, Renlund et al. 1992, Valantine, Gao et al. 1999)

For the indirect effect of mortality after stem cell transplant, three RCTs are relevant. Meyers reported reduced mortality among patients who received

prophylaxis with acyclovir.(Meyers, Reed et al. 1988) These results were not widely accepted at the time, because the trial was an unusual example of a placebo-controlled study that was not randomised. In order not to deny patients the benefits of keeping HSV reactivation suppressed, those who had IgG antibodies to both HSV and CMV were given acyclovir while those who had antibodies to CMV alone were given placebo. A randomised double-blind, double-dummy placebo-controlled trial of intravenous acyclovir followed by oral acyclovir was subsequently conducted by Prentice who reported reduced mortality among those allocated the highest dose of acyclovir.(Prentice, Gluckman et al. 1994) Again, these results were not widely accepted, because acyclovir did not significantly reduce CMV end-organ disease and the relationship between high viral load and end-organ disease had not been defined. Furthermore, there was scepticism because CMV was known not to contain a thymidine kinase, the enzyme in HSV required to begin the first step in the anabolism of acyclovir towards its active triphosphate form. We now know that the protein kinase from CMV gene UL97 performs this function.(Talarico, Burnette et al. 1999, Prichard 2009) Finally, letermovir significantly reduced non-relapse mortality (i.e. deaths that could not be attributed to recurrence of the underlying haematological malignancy) in a pre-specified secondary endpoint in a phase 3 RCT.(Marty, Ljungman et al. 2017) A recent detailed analysis by Ljungman shows that the survival deficit was most evident in recipients of placebo who developed viraemia whereas letermovir removed the link between death and viraemia.(Ljungman, Schmitt et al. 2019) This directly links the pharmacological effect of letermovir, which suppresses virus replication by inhibiting the enzymic activity of the terminase

complex, with viraemia and the clinical outcome of death. Taking all of this evidence into account, it is clear that active CMV infection after stem cell transplant contributes to overall mortality in a way that cannot be captured by recording the clinical symptoms of individual patients.(Boeckh and Nichols 2004) Overall mortality following stem cell transplant is thus the first formally proven indirect effect of CMV. Although the results are consistent with those reported previously with acyclovir, the letermovir results are definitive because this drug, unlike acyclovir, has activity only against CMV and so implicates this herpesvirus alone in the pathogenesis of excess death after stem cell transplant.

In contrast, despite the suspicion of many experienced clinicians, no study has yet shown in a double-blind, randomised placebo-controlled trial that a drug with activity against CMV can reduce the incidence of graft rejection following solid organ transplantation.

An analysis by the Cochrane Consortium of the small number of RCTs that have compared antiviral prophylaxis with pre-emptive therapy failed to show any difference in mortality or the incidence of graft rejection or the need for patients to return to haemodialysis after irreversible graft failure.(Owers, Webster et al. 2013) It follows that, at present, there is no evidence that the low levels of viraemia found in patients before initiation of pre-emptive therapy are sufficient to trigger graft rejection or other putative indirect effects. Future larger RCTs with the statistical power to address this question are required.

8. Indirect effects of CMV in non-transplant populations

When the first cases of what became known as AIDS were reported in 1981, they were complicated by the presence of CMV retinitis.(Centers for Disease and Prevention 1981) From those early days, there was suspicion that CMV was interacting with what became known as HIV to accelerate the rate at which AIDS developed, or the rate at which patients died.(Webster, Lee et al. 1989) There were several possible ways in which HIV and CMV could interact at the cellular level in vitro, but studies failed to find evidence for this in vivo.(Griffiths 1998) However, it remained clear in a prospective cohort study that the major risk factors for developing AIDS defining conditions and for death were the CD4 count and the presence of CMV viraemia; indeed, once these two factors were accounted for, the presence of HIV was no longer significant in a multivariate model.(Deayton, Sabin et al. 2004) A cohort study reported lower mortality among patients with CMV retinitis given systemic ganciclovir and a meta-analysis of randomised controlled trials of acyclovir reported reduced mortality.(Ioannidis, Collier et al. 1998, Kempen, Jabs et al. 2003) The analogy with the indirect effects of CMV in transplant patients was striking, but the absence of a defined mechanism in AIDS patients led to scepticism. A likely candidate mechanism is now thought to be the excess of T lymphocytes specific for CMV that contribute to inflammatory diseases such as atherosclerosis.(Jackson, Sedikides et al. 2017) A small randomised placebo-controlled trial of valganciclovir showed that the abundance of these T-cells was reduced by an 8 week course and remained suppressed during a wash-out period.(Hunt, Martin et al. 2011) This seminal work identifies a biomarker that could be used in future studies of larger numbers of patients

followed for a sufficient time to detect potential changes in the progression of atherosclerosis after reduction of this risk factor.

The immune system of elderly patients commits more resources to controlling CMV than it does to controlling all the other viruses combined that these patients have experienced in their long lives.(Pawelec, Derhovanessian et al. 2009) The same abundance of T-cells specific for CMV (termed TEMRA because they are T effector memory cells expressing the RA antigen) seen in AIDS patients has been identified in elderly patients and associated with immunosenescence.(Jackson, Sedikides et al. 2017) Detailed investigation shows that these cells are actively contributing to the control of CMV.(Jackson, Sedikides et al. 2017) An RCT of valganciclovir, similar to the one described by Hunt and colleagues, is required to determine if the abundance of these cells can be reduced in the elderly and if this translates into a better clinical outcome.

Another group of patients with complicated medical histories who reactivate CMV without clinical signs is those admitted to intensive care because of a heart attack, extensive burns or septic shock.(Limaye and Boeckh 2010) These patients are not immunocompromised in the conventional sense, but approximately one third of them reactivate CMV to give detectable levels of viraemia.(Limaye and Boeckh 2010) The detection of CMV was associated with a longer duration of ventilation, with release of cytokines that are toxic to the lung as a proposed explanation.(Cook, Yenchar et al. 1998) Stimulated by these natural history studies, Limaye and colleagues conducted a placebo-

controlled phase 2 proof of concept RCT of ganciclovir/valganciclovir prophylaxis.(Limaye, Stapleton et al. 2017) The drug was shown to be safe and able to significantly suppress CMV viraemia. There was also a significant reduction in the number of ventilator-free days which identified a clinical parameter that could be used in a future phase 3 study.(Limaye, Stapleton et al. 2017) The potential financial benefits of successfully reducing use of ICU facilities are enormous.

Simanec and colleagues studied 14,000 adults from a large population (NHANES cohort) which is representative of the whole USA population. They reported that those who were CMV seropositive had, after a mean of 13.7 years follow-up, a statistical excess mortality which was attenuated, but remained statistically significant, once common causes of death such as diabetes, smoking and obesity had been controlled for.(Simanek, Dowd et al. 2011) The overall hazard ratio was 1.19. A second study from the UK confirmed these conclusions using a large population of 13,000 adults, reporting an overall hazard ratio of 1.16 after a mean 14.3 years follow-up.(Gkrania-Klotsas, Langenberg et al. 2013) In both studies, some of the excess deaths were attributed to cardiovascular disease or cancer.

9. Clinical evaluation of prototype CMV vaccines

Six phase 2 or 3 RCTs have so far been published (Table 1). The live attenuated Towne strain CMV vaccine given to seronegative patients on the waiting list for renal transplant reduced the severity of CMV end-organ disease post-transplant, probably by reducing viral load, although the

necessary assays were not available at that time.(Plotkin, Smiley et al. 1984, Baraniak, Reeves et al. 2018, McBride, Sheinson et al. 2019)

Recombinant soluble glycoprotein B (gB) vaccine with MF59 adjuvant provided 50% protection against primary infection in seronegative post-partum women and 43% protection to seronegative teenagers.(Pass, Zhang et al. 2009, Bernstein, Munoz et al. 2016) The gB/MF59 vaccine also reduced viral load parameters post-transplant when given to patients on the waiting list for transplantation of a kidney or liver.(Griffiths, Stanton et al. 2011)

A vaccine (ASP0113) based on two DNA plasmids, one encoding pp65 (a major target of cell-mediated immunity) and the other gB, was evaluated in stem cell transplant patients.(Kharfan-Dabaja, Boeckh et al. 2012) One pre-transplant dose was given followed by four post-transplant doses between days 30 and 180. The results of this phase 2 study showed a reduced incidence of viraemia, encouraging a phase 3 study which has apparently had disappointing results. ASP0113 also underwent phase 2 evaluation in D+R- renal transplant patients. No vaccine was given pre-transplant, but 5 doses were given at post-transplant days 30, 60, 90,120 and 180. There was no reduction in the incidence of viraemia and no evidence of a strong immune response to the vaccine, although these patients also received antiviral prophylaxis.(Vincenti, Budde et al. 2018)

In future study designs, giving vaccine pre-transplant before patients receive immunosuppressive drugs and recruiting patients managed by pre-emptive

423 therapy may give a clearer picture. It is hoped that one or more of the
424 vaccines currently in clinical trial will be sufficiently safe and potent to allow
425 licensure. If so, it would likely be recommended for universal immunisation.
426
427 **10. Potential benefits of a CMV vaccine**
428 It is widely accepted that a vaccine able to reduce the direct effects of CMV is
429 a high priority and would be cost-effective.(Stratton, Durch et al. 2001, Arvin,
430 Fast et al. 2004, Krause, Bialek et al. 2013) This is largely driven by the
431 benefit of controlling congenital CMV (which has not been discussed here)
432 together with the benefit of controlling CMV end-organ disease post-transplant.
433
434 It should be clear from the evidence presented here that reduction of the
435 indirect effects of CMV could also produce substantial clinical benefits. This
436 potential for reduced indirect effects would also justify accelerated
437 development of CMV vaccines and would likely encourage uptake of a
438 licensed vaccine among the general population once they became aware of
439 these benefits. However, how should policymakers assess the likelihood that
440 a CMV vaccine might theoretically be able to deliver such benefits, given that
441 the indirect effects may be coincidental with CMV infection rather than being
442 caused by the virus?
443
444 I propose in Table 2 a scheme for addressing this conundrum which is not
445 definitive, but is offered as a way of stimulating discussion. My default
446 suggestion is that we should assume that each potential indirect effect is
447 simply an association, rather than being caused by CMV, so that zero benefit

should be allocated initially. However, once consistent and reproducible evidence has been provided from natural history and then from interventional studies for a given putative indirect effect, increasing probabilities should be allocated to the possibility of generating clinical benefits from a CMV vaccine. Application of these principles leads to the results outlined in the table when figures of 10%, 25% and 50%, chosen arbitrarily by the author, are applied to situations with increasing evidence that the CMV association might be causal.

Thus, it would be reasonable to include in analyses of potential cost-effectiveness a 25% reduction in the excess incidence of atherosclerosis attributed to CMV in patients living with HIV and 25% reduction in the excess number of ventilator-free days attributed to CMV among patients admitted to intensive care. Studies are less well advanced for the elderly and the general population, but a reduction of 10% in the excess of immunosenescence and excess mortality found respectively in these two populations seems reasonable.

I suggest that translation of these potential benefits into healthcare dollars would provide a substantial boost to the current predicted cost-effectiveness of a CMV vaccine, which is already high.(Stratton, Durch et al. 2001) However, the long expected time delay between immunisation to prevent primary CMV infection (in toddlers and adolescents) and observation of benefits (many decades later) would reduce the net present value of these improvements.(Griffiths 2012) This should stimulate the desire to find additional types of immunotherapeutic CMV vaccines able to rebalance the

immune system so that the indirect effects of CMV can be brought under control in those who are already naturally infected; that is, the majority of the world's population.(Zuhair, Smit et al. 2019)

11. Table legends

Table 1. Overview of vaccine candidates studied in phase 2 or phase 3 randomised controlled trials

Table 2. Probability of public health benefit from universal vaccination

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