

Human Cytomegalovirus, Endothelial Function and Atherosclerosis

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KEY WORDS:

■ **ATHEROSCLEROSIS** ■ **HUMAN CYTOMEGALOVIRUS** ■ **ENDOTHELIAL FUNCTION** ■ **PRE-ECLAMPSIA** ■ **TRANSPLANT ARTERIAL DISEASE** ■ **MAREK'S DISEASE VIRUS**

SUMMARY

Human cytomegalovirus (HCMV) serology is linked to several measures of endothelial dysfunction. There is substantial evidence for HCMV having an aetiological role in transplant arterial disease and accumulating evidence for HCMV in the origins of pre-eclampsia. However, whether HCMV is a clinically significant cause of atherosclerosis in the general, immunocompetent population remains to be seen.

Introduction

ATHEROSCLEROSIS IS THE leading cause of death in the developed world and as more people in developing countries adopt more affluent lifestyles it is set to remain a global problem. Conventional risk factors for atherosclerosis, namely smoking, lipids, hypertension, diabetes, obesity, diet, low physical activity and psychosocial factors, account for over 90% of acute myocardial infarction risk factors.¹ Although this would seem to exclude a role for infection in promoting atherogenesis, chronic infections could interact with any of the conventional risk factors, particularly hypercholesterolaemia, and promote the inflammatory processes underlying atherosclerosis.² Such chronic infections are often referred to as the 'pathogen burden', i.e. the aggregate number of chronic infections harboured in any individual as determined by serology, and include human cytomegalovirus (HCMV) as well as *Chlamydia pneumoniae*, herpes simplex virus, Epstein Barr virus (EBV), *Helicobacter pylori* and hepatitis A virus. Despite the wealth of evidence from murine and seroepidemiological studies supporting a role for CMV in atherosclerosis, doubts exist as to whether the human herpesviruses play a significant role in the general, heterogeneous population. Research has focused on investigating whether more homogeneous groups of individuals have evidence of HCMV-induced endothelial dysfunction and enhanced atherosclerosis. This article does not provide an exhaustive survey of animal, epidemiological and cellular studies which have previously been reviewed in *Herpes*;^{3,4} instead, new evidence linking HCMV to endothelial dysfunction and atherosclerosis in the general population and in those with altered immune states is presented.

Background

ANIMAL MODELS

Animal models provide clear evidence that species-specific CMV can accelerate atherogenesis (see references 3 and 4 for review) and indicate that rat CMV can cause endothelial dysfunction.⁵ Further work on animal models, particularly the pro-atherosclerotic

apolipoprotein E-deficient (ApoE^{-/-}) mouse, demonstrates the underlying mechanisms whereby murine CMV infection could cause endothelial dysfunction, be it by local infection of the vessel wall⁶ or by inducing a systemic inflammatory response.^{7,8} The fact that murine CMV can promote atherosclerosis in the ApoE^{-/-} mouse does not necessarily mean that HCMV behaves identically. Thus, supportive evidence for similar processes occurring in humans has been sought.

ANTI-HCMV SEROLOGY

Chronic infections have often been associated with atherosclerosis and its clinical sequelae.^{9,10} Large epidemiological studies, however, have refuted any association between serological evidence of chronic infection and cardiovascular outcome,¹¹⁻¹³ and meta-analyses have shown only a weak association between HCMV serology and the presence of atheroma.¹⁴ However, several smaller studies have shown that antibodies against certain chronic infections correlate with atherosclerotic risk in certain sub-groups including those who already have atherosclerotic disease,¹⁵⁻¹⁸ several risk factors for atheroma,¹⁹ or high inflammatory markers.^{20,21} Raised inflammatory markers are associated with cardiovascular disease but are not a risk factor *per se*.

There are a number of problems with serological studies in humans. The discrepancy between earlier and later studies may be explained by early publication bias and poor selection of end-points. Alternatively, the choice of reference groups may confound results: adjusting for known risk factors can profoundly alter the association between HCMV and atherosclerosis.^{22,23} Another problem is that although a single seropositive result provides evidence of infection, it provides no evidence of how long ago infection occurred or how often HCMV has been re-activated, potentially stimulating inflammation and atherosclerosis. Thus, an association between HCMV serology and atherosclerosis in isolation cannot prove a role for the virus in atherogenesis.

Endothelial Dysfunction

Endothelial function is the ability of an artery to dilate in response to the release of endothelium-derived nitric oxide. Nitric oxide is a potent, anti-atherosclerotic, anti-inflammatory and anti-coagulant molecule that is synthesized in the endothelium by nitric oxide synthase (NOS). Levels of asymmetric dimethylarginine (ADMA), an endogenously produced NOS inhibitor, are raised in association with various cardiovascular risk factors (most notably hypercholesterolaemia), as well as with endothelial dysfunction, although the exact role of ADMA is unknown.²⁴

Endothelial function can be measured in different vascular beds using various methods, namely coronary reactivity, forearm plethysmography and flow-mediated dilatation. Coronary reactivity and forearm plethysmography involves injecting vasoactive drugs into the coronary or brachial artery and comparing the response to endothelium-dependent vasodilators, such

as bradykinin and acetylcholine, with the response to endothelium-independent vasodilators such as glyceryl trinitrate. Alternatively, endothelial function can be determined by measuring the physiological vasodilatation in the brachial artery seen in response to shear stress (flow-mediated dilatation [FMD]) after a blood pressure cuff on the forearm has been inflated to pressures above systolic for 5 min. FMD has the advantage of being non-invasive and therefore of use in large epidemiological studies.²⁵

Impaired vasodilatation in response to either pharmacological or physiological vasodilators is known as endothelial dysfunction and is found in various disease states associated with atherosclerosis (or risk factors for the disease).^{2,26–28} Endothelial dysfunction can be used as a surrogate for atherosclerosis and to avoid possible confounding factors that influence clinical events in epidemiological studies.²⁹ Therefore, finding a link between herpesviruses and endothelial function in humans could provide evidence for the involvement of these viruses in human atherogenesis.

Clinical Evidence Linking CMV to Endothelial Function

Pathogen burden, determined by serology, is associated with endothelial-dependent dysfunction in the coronary arteries of those undergoing angiography.¹⁸ More specifically, HCMV-seropositivity has been associated with endothelial dysfunction as measured by forearm plethysmography in relatively young men and women.³⁰ This study also found pathogen burden to be associated with impaired vascular responses, although an independent relationship existed only for HCMV.³⁰ Interestingly, although diabetics with impaired immunological responses to infection might have been expected to demonstrate HCMV-induced endothelial dysfunction, no such relationship was found. Another study found no association between HCMV and impaired FMD in young men.³¹ The subjects had few risk factors and therefore had correspondingly high FMD values, and although the numbers were small ($n=65$; only $n=22$ were HCMV-seropositive) this study consistently showed no influence of any infective organisms (*C. pneumoniae*, CMV, EBV or *H. pylori*) and FMD. The discrepancy between these findings from HCMV serology and endothelial function could be due to how endothelial function was tested (coronary vasoreactivity, forearm plethysmography or FMD) or subject selection. Selecting older subjects with coronary disease could account for the difference between the findings of Prasad *et al.*¹⁸ and Khairy *et al.*,³¹ but it is difficult to see why there was such a difference between the latter and Grahame-Clarke *et al.*:³⁰ both studies involved relatively young, low-risk individuals. It is interesting to speculate whether the discrepancy between the two studies could be due to the inclusion of women and whether HCMV-associated endothelial function is a finding particular to women.

Transplant Atherosclerosis

The need for immunosuppression in cardiac transplantation and the resulting HCMV re-activation provides a wealth of evidence for HCMV being involved in the genesis of transplant arterial disease (TAD) and, ultimately, graft rejection. TAD resembles native vessel atherosclerosis and, similarly, endothelial dysfunction has been implicated in its development.^{32,33} HCMV seropositivity has been shown to be a retrospective predictor of cardiac transplant arteriopathy³⁴ and HCMV immunoglobulin (Ig)G/IgM seropositivity was associated with more advanced lesions on intracoronary ultrasound, independent of conventional risk factors.³⁵ Moreover, real-time polymerase chain reaction (PCR) evidence of immediate-early gene transcription

increased the risk of accelerated TAD and graft failure. Interestingly, HCMV-associated transplant vasculopathy appears to be a problem particular to women receiving HCMV-seropositive hearts.³⁶ Retrospective analysis shows that ganciclovir prophylaxis early after transplantation reduces the risk of TAD.³⁷

The mechanism behind this association remains obscure although HCMV can infect endothelial vascular smooth muscle cells and host-derived leucocytes in neointimal lesions with resulting cytokine, chemokine and adhesion molecule expression.³⁸ Yet the failure to identify HCMV in cardiac allografts following even clinically documented infection throws doubt on whether this is actually the mechanism *in vivo*.^{39,40} Reports have shown that HCMV infection can impair endothelial function in those with TAD: in an intravascular ultrasound study of cardiac transplant recipients, HCMV infection was independently associated with impaired ability of the vessel to vasodilate chronically in response to intimal hyperplasia, a phenomenon known as remodelling.³² It is conceivable that impaired remodelling would share some common features with endothelial dysfunction as, although temporally different, both result in an impaired vasodilatory response to the same drugs. Indeed, subsequent coronary vasomotor studies with intracoronary injections of acetylcholine have demonstrated that HCMV-negative recipients of HCMV-positive hearts have impaired distal epicardial endothelial function and an increased incidence of events and death during follow-up.⁴¹

In a separate study, transplant recipients were shown to have abnormally high plasma ADMA levels.⁴² Higher ADMA levels plus HCMV DNA-positive leucocytes were associated with more extensive TAD.⁴² Cell culture experiments demonstrated that HCMV infection of human dermal endothelial cells can enhance ADMA and superoxide production, together with a reduction in dimethylarginine dimethylaminohydrolase (DDAH) activity which metabolizes ADMA.⁴² The same pathophysiological mechanism appears to be responsible for the elevation in ADMA in transplant patients whereby oxidative stress impairs DDAH and leads to elevated ADMA levels.⁴² Thus, transplantation together with HCMV infection can work synergistically with hypercholesterolaemia, hyperglycaemia and other conventional risk factors to promote oxidative stress, impaired DDAH, reduce nitric oxide availability and so contribute to TAD. Further randomized controlled trials are needed to demonstrate that ganciclovir therapy can prevent endothelial dysfunction and TAD.³³

Pre-eclampsia

A successful pregnancy involves an altered immune state of the mother. Consequently, re-activation of HCMV is the most common congenital viral infection. Pre-eclampsia is a common disorder of vascular endothelial cell dysfunction with genetic, as well as inflammatory, aetiologies similar to atherosclerosis. Indeed, having pre-eclampsia is a risk factor for atherosclerosis in later life.^{43–46} Initiating or accelerating the development of endothelial dysfunction which persists after delivery is one mechanism whereby pre-eclampsia might influence later atherosclerosis.⁴⁷

Given the aetiological association between endothelial dysfunction and pre-eclampsia, it is unsurprising that HCMV is implicated in the development of the pre-eclamptic placenta. Studies of uterine microvascular endothelial cells and cytotrophoblasts demonstrate that HCMV-induced interleukin 10 can decrease matrix metalloproteinase 9 (MMP-9) activity and thereby disrupt normal cell function.⁴⁸ MMP-9 has been implicated in native vessel atherosclerosis; soluble levels of MMP-9 can predict mortality in those with coronary disease and are higher in type 2 diabetics, although it is difficult to see a precise mechanism.⁴⁹

Human CMV may also induce endothelial dysfunction by down-regulating a certain human leucocyte antigen (HLA) class I (HLA-G) expression: reduced HLA-G expression is a feature of pre-eclamptic placentae. During pregnancy MHC I HLA-G is expressed in trophoblastic cells at the materno-fetal interface and may be involved in the local immune response to viral infection. HCMV has been shown to down-regulate HLA-G expression *in vitro* and thus infected cells may evade the innate immune response.⁵⁰ By interfering with HLA-G expression, HCMV infection could alter the critical materno-fetal immuno-interface and lead to pre-eclampsia. Epidemiological studies have shown that the relative risk of developing pre-eclampsia is substantially increased if replicating HCMV sequences are detected by PCR and there is fetal inheritance of a maternal HLA-G*0104 allele.⁵¹ Hence, women appear to be at a greater risk of developing pre-eclampsia if they have a genetic predisposition to the condition as well as reactivated HCMV. Studies looking at uterine and systemic artery function indicate a possible link between pre-eclampsia and later atherosclerosis. ADMA levels are higher in women with pre-eclampsia compared with those having a normal pregnancy,⁵² and raised ADMA levels are associated with uterine artery Doppler evidence of impaired placental perfusion.⁵³ When systemic endothelial function was tested using FMD at 25–35 weeks of pregnancy, dysfunction was associated with raised ADMA levels in women who subsequently developed pre-eclampsia.⁵³ Those with early-onset pre-eclampsia (<34 weeks) have higher IgG HCMV titres than women with late onset pre-eclampsia, normotensive intrauterine growth restriction or normal pregnancy.⁵⁴ Although studies measuring ADMA, HCMV replication and HLA haplotype have not been performed, it would seem reasonable to hypothesize that HCMV infection could lead to raised ADMA levels in genetically susceptible women who develop altered endothelial function in pregnancy, thereby initiating processes underlying atherosclerosis that then manifests itself later in life (Figure 1).

Nitric Oxide and Viral Replication

In the field of virology, nitric oxide is considered a potent antiviral agent rather than a mediator of endothelial function. Marek's disease virus (MDV), a γ -herpesvirus, is an important cause of disease in commercial fowl and was the first animal model in which herpesviruses were conclusively shown to cause atherosclerosis.⁵⁵ Chickens genetically resistant to the development of Marek's disease can produce more nitric oxide than chickens susceptible to the disease,⁵⁶ although whether MDV-susceptible chickens develop endothelial dysfunction and more atherosclerosis is unclear. Moreover, whether such a mechanism exists for HCMV is similarly unknown. This model does illustrate, however, how a herpesvirus challenge could influence nitric oxide production and, as a consequence, influence endothelial function and later atherosclerosis.

Conclusions

The finding that HCMV serology is linked to several measures of endothelial dysfunction in humans provides a mechanistic link between the fields of virology, animal models, seroepidemiology and

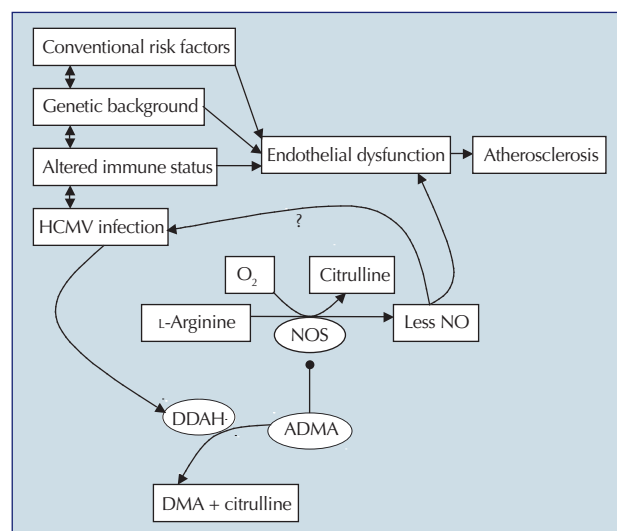


Figure 1: Schematic representation of the potential interactions between conventional risk factors, genetic background, immune status and human cytomegalovirus (HCMV) leading to endothelial dysfunction and thereby promoting atherosclerosis. One mechanism by which HCMV could influence endothelial function is by altering the biochemistry of nitric oxide (NO) via the dimethylarginine dimethylaminohydrolase (DDAH)/ asymmetric dimethylarginine (ADMA) pathway with potential feedback through NO onto viral replication. NOS, nitric oxide synthases; DMA, dimethylarginine.

atherosclerosis research. Although there is substantial evidence for HCMV having an aetiological role in TAD, and accumulating evidence for HCMV in the origins of pre-eclampsia, whether it accounts for atherosclerosis in the general, immunocompetent population to a significant degree remains to be seen. Further research into HCMV and the many measures of endothelial function is warranted.

Acknowledgement

The author thanks Professor Patrick Vallance for his comments on the manuscript.

Conflicts of Interest

No conflicts of interest were declared in relation to this article.

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Received for publication: 4 October 2004

Accepted for publication: 11 May 2005

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