

Scrutinized role of Cytomegalovirus in the pathogenesis of atherosclerosis from early life

Mohaddeseh Behjati¹, Majid Mohammad Beigi², Roya Kelishadi³

ABSTRACT

Cytomegalovirus (CMV) is a successful ubiquitous virus, its prevalence is more than 60% among adult blood donors, worldwide. Contrary to this old concept, CMV infection is associated with the first leading cause of death among immune-competent hosts. This paradigm is getting stronger by the emergence of growing evidences about the role of CMV in the pathogenesis of various kinds of native and allo- atherosclerosis. Altered gene expression in vascular vessel wall due to the integrated viral genome to the host genetic material superimposes to the inflammatory pool activated by CMV antigenic substrates. Given the latent and persistent nature of CMV infection, modulation of immune system seems beneficial in partly prevention of atherosclerosis in nations. This goal might be achieved by in depth understanding of the joint links between CMV and pathogenesis of atherosclerosis from early life.

KEY WORDS: Cytomegalovirus; Atherosclerosis; Oxidative stress.

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INTRODUCTION

Cytomegalovirus (CMV) is a highly prevalent virus worldwide.¹⁻³ This beta-herpes virus persists at hosts in a latent state.⁴ For many years, it was assumed that CMV is pathogenic only in immune-compromised hosts as transplant recipients and neonates.⁵ Contrary to this old concept, CMV infection is considered as the first leading cause of death among immune-competent hosts.⁶ This paradigm is getting stronger by the emergence of growing evidence regarding the role of CMV in the pathogenesis of various kinds of native and allo- atherosclerosis.^{7,8} The possible association of CMV infection

with atherosclerosis is a new microbial hypothesis suggesting that atherosclerosis may stem from three phases of viral replication with vascular biology. The infectious hypothesis of atherosclerosis is not in contradiction with the multifactorial nature of this disorder and its well-known risk factors.

Interestingly, CMV affects all cells related to atherosclerosis; including smooth muscle cells, macrophages, monocytes and endothelial cells.⁹⁻¹² As CMV induces a subtle subclinical infection in its latent phase, it can be implicated that the virus is not thoroughly dormant.¹³ CMV inoculation into the target cells is associated with the expression of the genes involved in escaping from immune surveillances and in the establishment of viral latency.¹⁴

These immediate early (IE) gene products, mainly IE72 and 84 transactivate their own promoter; whereas Major immediate early protein (MIEP) acts through binding with its multiple NF- κ B binding sites.^{15,16} This is a dual-control pathway, since CMV MIEP also regulates expression of the IE gene products of the virus, which in turn are critical requirements for expression of the early and late gene products of the virus and thereby progression of the viral life cycle in the host cells.¹⁷⁻¹⁹ MIEP

1. Mohaddeseh Behjati, MD,
Isfahan Cardiovascular Research Center,
Isfahan Cardiovascular Research Institute,
Isfahan University of Medical Sciences, Isfahan, Iran.
2. Majid Mohammad Beigi,
Department of Biomedical Engineering, University of Isfahan,
Isfahan, Iran.
3. Roya Kelishadi,
Department of Pediatrics,
Child Growth and Development Research Center,
Isfahan University of Medical Sciences, Isfahan, Iran.
Correspondence:
Mohaddeseh Behjati, MD,
Dept. of Cardiology, Isfahan Cardiovascular Research Center,
Isfahan Cardiovascular Research Institute,
Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: behjati@med.mui.ac.ir

activation is in agreement with the reactivation of the virus from its latency phase.²⁰ The switched through activation of NF- κ B, MIEP controls the expression of various host genes, as those involved in immunity and inflammation pathways.^{21,22} Therefore, cells harbor CMV, are exposed to such a chronic inflammation. It has been demonstrated that contact with CMV particles may lead to the profound modulation and reprogramming of the cellular gene expression, including expression of the inflammatory cytokines, adhesion molecules, and interferon (INF)-stimulate genes.^{23,24}

These initial virus-triggered innate mechanisms are accompanied with the recognition of human CMV (HCMV) virions by the toll-like receptor and its cofactors. The latter are the major components of innate immunity even during the early stages of infection, which would potentiate the cytokine production.²³ Therefore, CMV invades mostly TLR-bearing endothelial cells and smooth muscle cells.²⁵ In this way, CMV invades the vessel walls indirectly from distant sites. Despite the convincing evidences considering CMV as an independent risk factor in the pathogenesis of atherosclerosis and restenosis, it is well-documented that CMV interacts with major risk factors of atherosclerosis.^{26,27}

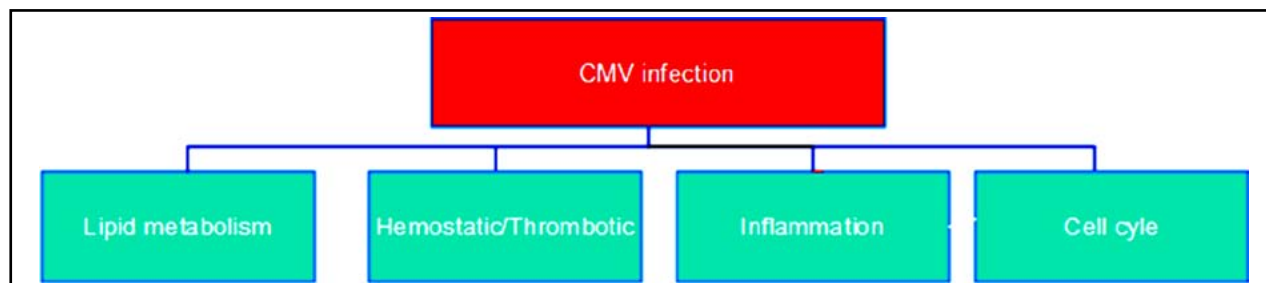
The main venue of interaction occurs in the cases of combination exposure of oxidized-low density lipoprotein (ox-LDL) and CMV exposure, which is associated with the greatest MIEP activity in the effected endothelial cells.²⁸ The interaction of CMV with major risk factors of atherosclerosis is bi-directional. CMV infection is known to be associated with induction of atherogenic lipid profile and accretion of lipids, mainly cholesterol esters in both endothelial and smooth muscle cells.^{29,30} This enhanced lipid accumulation and esterification is due to the increased expression of scavenger receptors (SR), which is critical for cellular uptake of ox-LDL and development of foam cells by further oxidative injury.³¹ The altered lipoprotein profile, namely in terms of increased plasma levels of lipoprotein a Lip(a) levels are considered as an acute phase reactant to infection, and are primarily targeted to protect the host from further injury.³²

These defense mechanisms are accompanied by the changes in the lipid metabolism similar to the atherogenic profile, as increased plasma triglycerides and decreased high density lipoprotein-cholesterol (HDL-C).²⁹ It is worth noting that the presence of infections during childhood may increase synergistically the progression of atherogenesis.³³⁻³⁵ On the other hand the cardiovascular disease risk

factors as altered lipid metabolism exist from early childhood and may have a role in the development of atherosclerosis from early life.^{36,37} However, long period of exposure to the pathogen is needed for such alterations in the process of atherosclerosis and ultimately to clinical disease in later life. Vice versa in hyperlipidemia states, plasma LDL-C enters the sub-intimal space, where it can be oxidized by free radicals produced by endothelial cells and or smooth muscle cells.^{31,32,38,39} The produced ox-LDL, and mainly its peroxide content, is relevant to the activation of MIEP.⁴⁰ The proposed pathway passes through the activation of NF- κ B-mediated processes in all cells of vascular wall. MIEP has been shown to have four NF- κ B binding sites, implies that this *pleiotropic* transcription factor is prerequisite for all stages of viral replication.¹⁷

CMV-induced NF- κ B activity seems to be the likely trigger of the potentially proatherogenic cytokines produced by CMV-infected cells.¹⁶ Therefore, it represents a potential therapeutic target to reduce pro-atherogenic stimuli exerted by the inflammatory responses of the host cells. The precise mechanism of anti-inflammatory effects of statins in atherosclerosis seems to be by a cholesterol-independent mechanism.⁴¹ Statins are shown to down regulate the entire cycle of CMV replication in endothelial cell through limitation of NF- κ B binding activity in CMV-infected cells. It can be suggested that Mevalonate and its metabolite, i.e. Isoprenoids, are implicated in signaling pathways that require prenylated proteins involved in NF- κ B activation.^{42,43} In contrast, addition of mevalonate to the culture media may almost completely abolish these effects on viral kinetic which evince the crucial needs for 3-hydroxy-3-methylglutaryl-CoA lyase deficiency (HMG-CoA activity) for CMV replication in endothelial cells. This may be a plausible explanation for the anti-viral effects of statins besides their anti-inflammatory and lipid lowering mechanisms.⁴⁴

As mentioned above, the dual interplay between atherosclerosis' risk factors and CMV infection is not only related to ox-LDL and its products as lysophosphatidylcholine derivate (LPC), but also related to various risk factors.²⁹ CMV-infection in irradiated rats is shown to be associated with altered heart rate regulation, changes in arterial reactivity, and pronounced reduction of blood pressure, which are more noticeable in small arteries.⁴⁴ This pattern of involvement is contrary to virus predilection for involvement of medium- and large-sized arteries.³¹ These alterations in vascular function are considered



to be mainly due to the modifications in excitation-contraction coupling in blood vessels, and in part due to the cytokine-mediated induction of nitric oxide synthase.⁴⁴ The basic fundamental change is attributed to the sympathetic hyper-arousal⁴⁵, but overall the vascular changes induced by CMV have been considered to be agonist and regionally selective.⁴⁶ It can be suggested that selective alteration of signal transduction may develop in the course of CMV-infection.

The independent association of increased blood pressure and murine (MCMV), and not dependent to other risk factors, is a simultaneous event with increased plasma cytokines as renin and angiotensin through activation of NF- κ B II from renal epithelial cells of CMV-infected cases.⁴⁷ These factors in addition with the secreted growth factors as platelet derived growth factor (PDGF-B) chain, either in auto- or paracrine fashion, exert an important role in enhanced proliferation and migration of arterial smooth muscle cells and matrix synthesis.^{48,49}

In addition to the above mentioned cytokines, increased expression of tumor suppressor TP53 gene is seen during CMV infection by its activation via IE72 and 84, and may lead to the arterial smooth muscle cell proliferation.⁵⁰ CMV infection is associated with vWF depletion and elevated plasma levels of fibrinogen, factor VIII and protein C.⁵⁰⁻⁵² These procoagulant properties of CMV stem mainly from inflammatory activations of endothelial cells lining the vascular wall, which makes a chronic hypercoagulable state and pro-thrombotic milieu.^{51,52} Thrombin has been shown to upregulate immediate early (IE) promoter activity.⁵³ These raised pro-thrombotic activity is in accordance with decreased fibrinolytic activity of infected endothelial cells.⁵⁴ Atherosclerotic plaques with greater contents of thrombosis may harbor more CMV insides; this would implicate that the pro-thrombotic conditions are because of the virus.⁵¹

Despite well-documented concepts about atherosclerosis as an age-related vascular disease, there is a growing body of evidence about the role of inflammation and immunity in this process. The

conjunction between these overlapping processes is considered as "endothelial cell injury", which is the initiating step in the atherosclerosis. In addition to the major causes of endothelial cell injury as hypertension, diabetes mellitus, smoking, and hyperlipidemia, infectious agents as CMV infection may have a role.^{55,56} The association of CMV and atherosclerosis has been established by several animal/experimental, pathological and cross-sectional seroepidemiologic studies conducted among middle-age population group.⁵⁷ Such correlation between CMV, as an independent risk factor for genesis, progression and complication of the atherosclerotic process has been found by the high prevalence of atherosclerosis among patients with high antibody titers against CMV.^{58,59}

This correlation is strongest among elderly patients, which have higher antibody titers against CMV, and are more susceptible to atherosclerosis.⁶⁰ This correlation has also been shown between CMV infection, atherosclerosis and serum levels of markers of systemic inflammation.⁶¹ Another confirmatory evidence of this concept comes from reports on the presence of the complete and conserved viral genome in the atherosclerotic arterial blood vessels.⁶² It was first described by Melnick et al at nearly two decades ago.⁶³ It is well-documented that this intracellular complex virus, with a large genome and more than 200 open reading frames has many interaction sites with vessel walls.⁶⁴⁻⁶⁷ CMV has a peculiar tropism to endothelial cells⁶⁸, with a predilection of medium and large-sized arteries⁶⁹⁻⁷¹; vessels more prone to atherosclerosis.⁷² This distribution pattern is somewhat different from those induced by cholesterol over feeding in experimental studies.⁷³

Detection of viral particles in the vascular wall cells of atherosclerotic vessels, in both reproductive and latent viral infection^{3,62}, is in favor of a local and ongoing inflammatory process, as well as direct viral invasion via cell-to-cell contact. This correlation is considered to be more consistent with severe atherosclerosis rather than with its first stages or with normal blood vessels.⁶² Although some

investigators did not detect such correlation, this point is thoroughly explainable by the sensitivity of the applied methods. Polymerase chain reaction method is documented to be superior to the In Situ Hybridization (ISH) techniques for the cases with severe atherosclerosis.⁷⁴ The low sensitivity of the ISH method is because of the localization of CMV in the smooth muscle cells of the media, accompanied by massive degeneration with increased numbers of inflammatory infiltrates and few numbers of smooth muscle cells.⁷⁵ In spite of the primary interaction of the virion particles with endothelial cells, the distribution pattern and localization of CMV is different. The primary localization of this virus is demonstrated to be within smooth muscle cells in the media and adventitia layer.⁷⁶

The atherosclerosis process roots from childhood⁷⁷, and interestingly as a price paid for protection of pathogens invading from the blood stream. These evolved unique defense mechanisms are effectively used by CMV to accomplish its own life cycle. CMV applies these primary signal transduction pathways used by the cell for multiple purposes to facilitate expression of its genetic program. Among these programs, inflammation and oxidative bursts are the main points. Overproduction of reactive oxygen intermediates (ROI) has been implicated in the vascular dysfunction, which provides attractive mechanism to link CMV infection with atherogenesis.⁷⁸ This implies that atherosclerotic blood vessels are more conducive to CMV than normal healthy vessels⁷⁹, which is in concordance with the findings that MIEP activity is greater in atherosclerotic arteries rather than in normal blood vessels.⁸⁰ The increased prevalence of atherosclerosis in some countries with low rates of CMV infection^{5,81-83}, implicates the different genetic background of populations⁸⁴, which determine the dominant type of immune response to the pathogen (humoral or cellular).¹³ Also particular nature of host- pathogen interaction is detrimental factor in the contribution of CMV to the process of atherosclerosis.⁸⁵ The capacity of host to suppress CMV-induced inflammation determines the host susceptibility to atherogenic effects of CMV.¹³

Besides not all infected cases manifests atherogenic effects of CMV and just a subset of CMV infections has the risk of atherosclerosis. This risk is elevated in the diabetic patients with impaired immune responses to viral antigens⁸⁶; a clue toward the impact of host conditions in interplaying with infection. Not only different hosts, but also different cells respond variously to the CVM infection.

Endothelial cell-passaged CMVs are highly infective, in contrast with fibroblast-passaged laboratory strains of CMV which manifest limited infection without cytopathic effects of CMV.⁸⁷ Heterogeneous response to CMV is also observed within endothelial cells of the single vessel wall, which leads into abortive infection in some cells and a permissive phenotype in a productive infective phase in other cells.⁶⁰ The interaction of CMV and host-raised immune responses is complex and many aspects of this interaction are yet to be identified. One basic problem which is the main cause of controversies is due to the simple speculation that high CMV antibody titers may be a reflection of more recent infection, reactivation or possibly a harmful infection.

CMV infection leads to the activation of monocytes/macrophages down to the sites of endothelial cell injury by MCP-1 secretion. Myelomonocytic precursor cells are CMV-reservoirs; leads to a small sub-population of released circulating monocytes which act as a vector for CMV which facilitates delivery of the virus to the arterial lesion. These cells are recruited to the sub-intimal space, as part of the inflammatory response to injury. Then the virus is activated by the constituents of blood vessel; endothelial and smooth muscle cells and their oxLDL content. The other proposed mechanism for CMV activation residing in monocytes is monocyte differentiation upon adherence and entry to the vessel wall, with the consequent secretion of differentiation-dependent factors. In fact, circulating monocytes are non-permissive to CMV infection, but the more differentiated the monocytes; the more permissive to the viral gene expression. In the other way, monocytes are highly trafficked to the sites of the arterial injury with increased expression of MCP1.⁸⁸

This endothelial tropism and viral delivery seems to be mediated through the redox-sensitive mechanisms regulated by the generation of the free radicals and ROI; the primary defense mechanisms.²⁸ Accumulated ROIs in the vascular wall cells will ultimately lead to endothelial cell injury and apoptosis⁸⁹, which results in the disintegration of endothelial cell barrier. It seems that the induced apoptosis induced by ROI, is mechanism for destruction of virally-infected cell to prevent viral transmission to the neighboring cells. But CMV has survival mechanisms evolved millions of years, which pass through these defense mechanisms.⁹⁰ Transmitted virus to endothelial and smooth muscle cells lead to the escalating cycle of cell proliferation⁸³, since IE72 IE86 bind to TP53 and

inhibit its action.^{52,91} Active CMV infection manifests as a patchy involvement of intima and adventitia⁴⁵, occlusive fibro-muscular intimal thickness⁹² and focal inflammation, edema and necrosis similar to atherosclerotic degeneration.⁸⁷

CONCLUSION

Given the latent and persistent nature of CMV infection, modulation of immune system seems beneficial in partly prevention of atherosclerosis in nations. This goal might be achieved by in depth understanding of the joint links between atherosclerosis and CMV pathogenesis.

Conflict of interest: Authors have no conflict of interest.

Abbreviations:

HCMV: Human cytomegalovirus. **oxLDL:** Oxidized LDL. **LDL:** Low-density lipoprotein. **HDL:** High-density lipoprotein. **IE:** Immediate-Early. **ROI:** reactive oxygen intermediates. **MCP:** Monocyte chemotactic protein. **vWF:** Von Willebrand factor. **TLR:** Toll-like receptor. **NF- κ B:** NF-kappaB. **MIEP:** Major immediate early protein.

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