



Complexity in Association of Virus and Immune Regulators on the Development of Atherosclerosis via Formation of Foam Cells

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Abstract

Numerous evidences have associated the relation of bacterial and viral agents with atherosclerosis development. Murine norovirus (MNV) especially MNV-4 has been correlated to the regulation of atherosclerosis development in mice. Different studies have demonstrated variable impacts of MNV-4 infection in murine models of atherosclerosis and *in vitro* studies. Here, we also included the effect of MNV-3 strain on macrophage foam cell formation *in vitro* produced by our laboratory.

Keywords: Atherosclerosis; Foam cells; Macrophage; Murine norovirus

Abbreviations: ABCA1: ATP-Binding Cassette Transporter A1; CD36: Cluster of Differentiation 36; CMV: Cytomegalovirus; HCV: Hepatitis C virus; HIV: Human Immunodeficiency Virus; IFN: Interferons; IL: Interleukins; iNOS: Inducible Nitric Oxide Synthase; MCP-1: Monocyte Chemoattractant Protein-1; MNV: Murine Norovirus; oxLDL: Oxidized Low Density Lipoprotein; TNF: Tumor Necrosis Factors

Introduction

Atherosclerosis is a chronic condition characterized by the deposition of lipid in the artery. The pathophysiological similarities between atherosclerosis and infection spark the impetus for research among clinical practitioners and researchers. Viral and bacterial agents have been associated with the aggravation of atherogenesis via studies on the epidemiology, local identification of pathogens in atherosclerotic plaque, and experimental demonstrations *in vivo* [1-11]. Infectious agents may participate in atherogenesis development by direct or indirect mechanisms. Direct pathogen infection takes place in the intima of blood vessel which results in primary inflammation that leads to the initiation or progression of atherosclerosis plaque [12].

Direct pathogen involvement is indicated by infection of pathogens in atherosclerosis lesion, microbial demonstration within atherosclerosis plaque, and acceleration of plaque progression in atherosclerotic mouse models post-infection [1]. Indirect mechanism refers to an inflammation caused by pathogens at non-vascular sites that contributes to the increase of cytokines and acute phase proteins that results in the progression of atherosclerosis develop

ment [1,12]. Immunoregulators of both the humoral and cellular immunities are involved at all atherogenesis stages [13]. Cytokines are central in the regulation of inflammatory responses and are grouped into several classes including interleukins (IL), interferons (IFN), tumor necrosis factors (TNF), transforming growth factors, chemokines, and colony stimulating factors. All the cellular components involved in atherosclerosis possess the capacity to secrete and respond to cytokines [14].

Many reports have underlined the influences of bacterial and viral pathogens in animal models of atherosclerosis in the progression of atherogenesis [1,15,16]. Multiple viruses including human immunodeficiency virus (HIV), cytomegalovirus (CMV), hepatitis C virus (HCV), human simplex virus, and Epstein-Bar virus have been identified in atherosclerosis plaque by polymerase chain reaction [3,17,18]. HIV protein Nef, which is associated with the pathogenesis of atherosclerosis in patients infected with HIV was found to play a role in dyslipidemia and the formation of foam cells within blood vessel walls of mice [19]. Infection of murine CMV in *ApoE*^{-/-} mice was shown to result in a more advanced atherosclerosis lesion

and an increase in the level of P38 mitogen-activated protein kinase in aortas compared to atherosclerotic mice that are not infected with murine CMV [20]. HCV perturbs the equilibrium between cellular and humoral immunity and increases ratio of proinflammatory to anti-inflammatory cytokines which has been associated with a heightened cardiovascular risk in patients infected with HCV [21,22].

The earliest stage of atherosclerosis development begins with the formation of macrophage foam cells. Modified LDL accumulation in the intima of the artery results in the activation of the endothelium which increases the secretion of chemo attractants that promote monocyte migration into the intima. In the intima, monocytes differentiate into macrophages which have increased expression of scavenger receptors. Under an atherogenic environment where lipid homeostasis is disrupted, engulfment of modified LDL by macrophages results in the retainment of cholesterol as lipid droplets [23,24], transforming them into lipid-laden foam cells [25].

Murine norovirus (MNV) in Atherosclerosis Development

MNV, the surrogate to human norovirus manifests tropism for dendritic cells and macrophages. MNV infection has been shown to be prevalent in laboratory mouse colonies around the world and has been demonstrated to cause biases in studies involving mouse models [26-28]. MNV-4 has been demonstrated to increase the size and accumulation of macrophages in aortic sinus lesion area in *Ldlr*^{-/-} mice suffering from hyperlipidemia [29]. In another study in 2015 [30], the authors reported that MNV-4 infection in *Ldlr*^{-/-} mice did not influence the progression of atherosclerosis at early stage of development, hypothesizing that influence of MNV-4 on atherosclerosis development depends on the timing of infection, and that MNV-4 only exacerbates established lesions [30]. Infection of MNV-4 in *Ldlr*^{-/-} bone marrow derived macrophages (BMDM) *in vitro* caused increased inflammatory cytokines (IL6, IL1 β , IFN β) and a classically activated macrophage marker, inducible nitric oxide synthase (iNOS) in oxLDL treated *Ldlr*^{-/-} BMDM.

The inflammatory cytokines and iNOS were reduced by oxidized low density lipoprotein (oxLDL) in a dose-dependent manner suggesting that the effects of MNV-4 infection in atherosclerosis is influenced by the extent of hyperlipidemia. Besides, infection of *Ldlr*^{-/-} BMDM by MNV-4 also demonstrated the potential to enhance the uptake of oxLDL by increased protein expression of cluster of differentiation 36 (CD36) but reduced expression of ATP-binding cassette transporter A1 (ABCA1) [30]. Similar observation on the pattern of expressions of CD36 and ABCA1 was observed in a study using MNV-4 infected *ApoE*^{-/-} BMDM [17]. The authors also reported increased mRNA expressions of IL-6, IL-1 β , IFN- β , TNF- α , monocyte chemoattractant protein-1 (MCP-1) and iNOS in MNV-4 infected *ApoE*^{-/-} BMDM without oxLDL treatment, whereas MNV-4 infection with the presence of oxLDL showed increment in the mRNA expres-

sions of IL-6, MCP-1, and iNOS. IL-6, IL-1 β , IFN- β , TNF- α , MCP-1 cytokines and chemokines have demonstrated to be proinflammatory in atherogenesis. Besides, an *in vivo* study using *ApoE*^{-/-} demonstrated an increased size of atherosclerosis lesion [17].

However, in a second study where the authors used a low-passage MNV-4 preparation, they did not observe the similar outcomes as in the first study. The authors contributed the discrepancy to the different virus passages used which revealed mutations corresponding to two changes of amino acids, where the two preparations resulted in a notably different regulation of cytokines and chemokines in *ApoE*^{-/-} BMDM as well as contrasting effects on atherosclerosis lesion progression [17]. Indeed, different MNV strains, some even with a single substitution of amino acid, may be markedly dissimilar in their replication, virulence, and persistence in infected mice [17,31]. Additionally, [17], MNV-4 infection in *ApoE*^{-/-} mice has shown to result in increased percentages of Ly6C-positive monocytes, which has been linked with proinflammatory responses correlated to the enhancement of monocyte recruitment into atherosclerotic plaques, differentiation into macrophages, and foam cell formation. MNV-4 RNA was also detected locally on the aggravated lesion of the aortic sinus of *ApoE*^{-/-} mice [17].

Effect of MNV-3 on Foam Cell Formation

However, in the mentioned studies [17,30] utilizing *Ldlr*^{-/-} BMDM and *ApoE*^{-/-} BMDM *in vitro*, the authors did not analyze the cholesterol contents in the macrophages post treatment. In our study using MNV-3, we analyzed the *in vitro* effect of MNV-3 infection on RAW 264.7 macrophages treated with oxLDL. We demonstrated the effect of MNV-3 infection on the total cholesterol content of RAW 264.7 after 24 hours incubation period (Figure 1). We found that MNV-3 did not alter the content of intracellular total cholesterol of RAW 264.7 post treatment both with and without the presence of oxLDL (Figure 1). This also corresponds to our previous study with MNV-1 where we did not see alteration on the cholesterol content of RAW 264.7 due to MNV-1 infection [32].

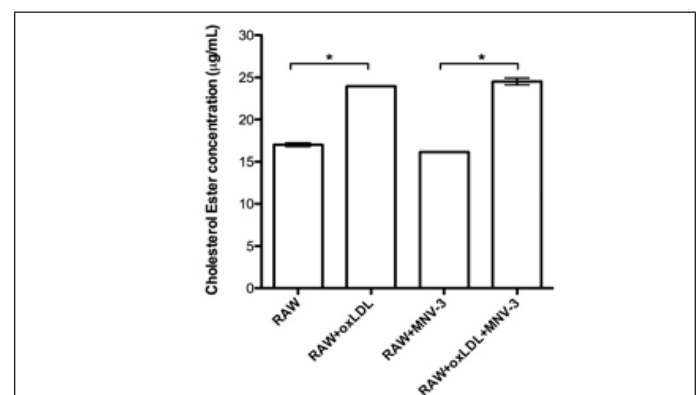


Figure 1: Cholesterol quantification of RAW 264.7 cells. MNV-3 infection does not affect the total cholesterol content of RAW 264.7 with or without the presence of oxLDL. The oxLDL treatment increases the total cholesterol content in RAW 264.7 with and without MNV-3 infection. Statistical analysis was carried out using ANOVA with Bonferroni post hoc $p < 0.05$.

In our study with MNV-3, we infected RAW 264.7 with low-passage MNV-3 (passage 4) and our observation is supported by [17] that did not find lesion development in *ApoE*^{-/-} mice infected with low-passage MNV-4. Furthermore, MNV-4 has shown to only exacerbate established lesion [30] which further supports our finding on MNV-3 did not facilitate the formation of macrophage foam cells *in vitro*. Additionally, despite the upregulated mRNA expressions of interferon stimulated genes following MNV infection, their translation has been shown to be suppressed which was associated to the activities of MNV NS6 protease [33], implying the lack of inflammatory signaling following MNV infection which further supports our observation on MNV-3 in the regulation of foam cell formation.

Conclusion

MNV has shown variable effect in the development of atherosclerosis *in vivo* and *in vitro* [17,29,30,32]. In our own studies on MNV-3 and MNV-1 we have verified that these strains do not assist the formation of macrophage foam cells *in vitro*. Based on our review, the variable effects on atherosclerosis development observed in MNV infection might probably be highly dependent on the stage of atherosclerosis development and virus strain or passage.

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Conflict of Interest

We declare no conflict of interest in our research and in the making of this article.

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