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Murine Norovirus 4 (MNV-4) Infections Trigger Various Effects on Atherosclerosis Development

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ABSTRACT

Murine norovirus (MNV) infection can cause morbidity and mortality to immune compromised mice, especially colonies in research laboratory. MNV also can infect and propagates in macrophages and dendritic cells which trigger atherosclerosis development through the accumulation of these cells followed by the formation of foam cells. Recently, MNV-4 infection was associated with an increase in aortic sinus lesion size in LDLR (low-density lipoprotein receptor) and ApoE (Apolipoprotein E) deficient mice, both are well established mouse models for atherosclerosis research. Therefore, this review is intended to summarize the impacts of MNV infection in these two mouse models of atherosclerosis. The findings from all the related studies are important in understanding the fundamental effect of MNV infection on atherosclerosis development. In addition, this information could provide insight to researchers on the evaluation to eliminate MNV infection in research facility to avoid any unintended effect in their research, particularly in-vivo studies involving mice.

Keywords: Murine norovirus, Atherosclerosis, Low-density lipoprotein receptor-deficient, Apolipoprotein E-deficient

INTRODUCTION

Atherosclerosis is a chronic inflammatory disease which causes death for millions of people worldwide [1,2]. Atherosclerosis is initiated by the accumulation of lipids especially low density lipoprotein (LDL) which then become oxidised LDL (OxLDL) due to oxidative stress in sub-endothelial space [3]. OxLDL induces endothelial cells to express leukocytes adhesion molecules such as vascular adhesion molecule (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) and P- and E-selectin molecules which facilitate the adhesion of leukocytes such as monocytes and T cells to the activated endothelial monolayer which then facilitated the migration of the bound leukocytes into the intima [4]. Upon entry, monocytes differentiate into macrophages under influence of M-CSF5. Consequently, excessive uptakes of oxLDL by macrophages lead to foam cells formation [5,6]. The progression of lesion stimulates the migration of smooth muscle cells (SMCs) from the tunica media to the tunica intima, the proliferation of resident intimal SMCs and media-derived SMCs, and elicits the synthesis of extracellular matrix macromolecules such as collagen, elastin and proteoglycans [4]. Plaque macrophages and SMCs then undergo apoptosis in advanced lesions [4]. Consequently, the extracellular lipid derived from dead cells accumulates in the central region of a plaque known as necrotic core [4]. The advanced plaques consist of cholesterol crystals and micro vessels which undergo thrombosis, the ultimate complication of atherosclerosis which is due to a physical disruption [4]. The rupture of the plaque's fibrous cap stimulates blood coagulation components to burst out and make contact with tissue factors in the plaque's interior, triggering the thrombus that extends into the vessel lumen, where it can obstruct blood flow [4]. The presence of atherosclerosis disease in the heart may lead to myocardial infarction and heart failure, whereas in the major arteries that supply blood and oxygen to the brain, it can cause transient ischemic attack or stroke [7]. It is well documented that innate and adaptive immune responses play a pivotal role in the development of atherosclerosis from the initiation stage to the thrombotic complication. Since infections also stimulate inflammatory immune responses, it has been recognized as one of the contributing factors to the atherosclerosis development either direct or in indirect

manner [8]. A wide range of viruses and bacteria have been identified in atherosclerotic plaque by polymerase chain reaction (PCR) [9]. Viral pathogens found in atherosclerosis include cytomegalovirus, human immunodeficiency virus, hepatitis C virus, influenza A, human simplex virus, and Epstein-Bar virus [10-12]. Meanwhile, bacterial pathogens identified in human atherosclerotic plaque are *Chlamydia pneumoniae*, *Mycoplasma pneumonia* as well as periodontal putative bacteria such as *Porphyromonas Gingivalis*, *Aggregatibacter Actinomycetemcomitans*, *Prevotella intermedia*, *Tannerella forsythia*, *Fusobacterium nucleatum*, *Streptococcus sanguis*, and *Streptococcus mutans* [13-15]. Recently, MNV-4 infection in mice which is common in research facilities around the world has shown to invoke immune responses in wild-type (WT) mice [16]. MNV infection also increased deposition of atherosclerotic plaque lesion in aortic sinus of LDLR deficient mice, a well-known mouse model for atherosclerosis research [17]. Furthermore, MNV-4 increased the macrophage content in the atherosclerotic plaque which plays a pivotal role in macrophage derived foam cells, hallmarks of atherosclerosis development [17]. MNV-4-infected macrophages in the intima also may stimulate the secretion of pro-atherogenic cytokines which involve in the atherogenesis. Besides that, the engagement of MNV-4 stimulated macrophages with naive CD4+ T cells in the atherosclerotic plaque may influence the differentiation of Th1, Th2, Treg and Th17 cells. These CD4+ T cell subsets play critical role in either acceleration or suppression of atherogenesis (Figure 1). The purpose of this review was to look and summarize the impacts of MNV-4 infection in atherosclerosis mouse models.

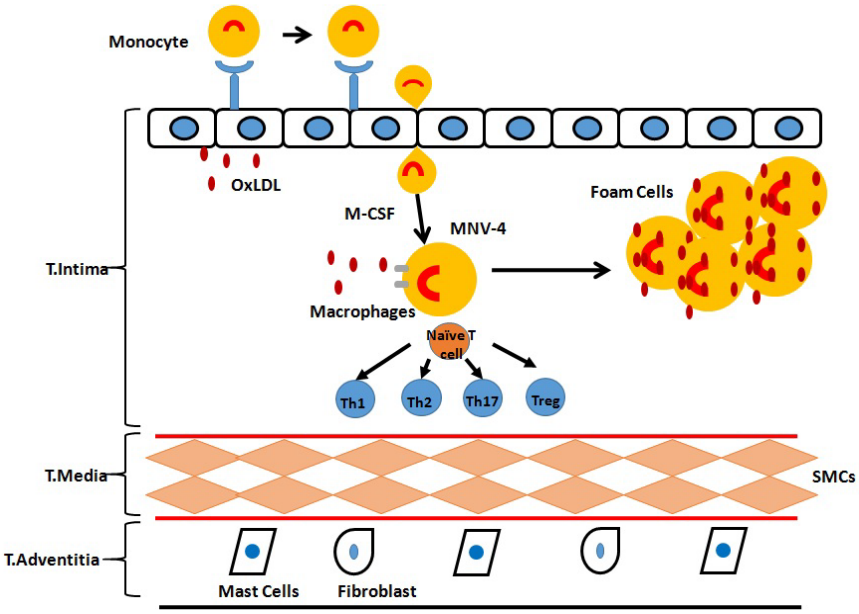

Figure 1 MNV-4 involvement in the development of atherosclerosis

The artery consists of three layers namely tunica adventitia, tunica media and tunica intima. Deposition of LDL inside the sub-endothelial space and the conversion of LDL into the oxLDL due to oxidative stress initiate the atherosclerosis development. OxLDL stimulates endothelial cells to express VCAM-1, P and E-selectin which facilitate the migration of immune cells such as T cells and monocyte into the intima. Monocyte turns into macrophage under influence of M-CSF. Then, excessive engagement of macrophages with oxLDL via scavenger receptor will drive foam cells formation. The accumulation of foam cells will lead to lipid streaks formations which act as an integral in atherogenesis. MNV-4 infections have been found to increase deposition of atherosclerotic plaque in aortic sinus of LDLR mice concomitantly by increasing the macrophage content in the atherosclerotic plaque. MNV-4-infected macrophages in the intima also may induce pro-atherogenic cytokines. Besides that, MNV-4-infected macrophages may interact with naive CD4+ T cells leading to the differentiation of Th1, Th2, Treg and Th17 cells. These CD4+ T cell subsets have a pro-atherogenic and anti-atherogenic role.

Mouse Model System to Study Atherosclerosis

The availability of small animal models has offered a great platform for researchers in elucidating the basic science of pathophysiological of diseases before it can be translated into more complex studies which involve human subjects.

Factors such as easy-handling, economic, genetic manipulation and ethical issues involving human subjects in research have made the small animal model as an attractive option for researchers to start investigating the various aspects of human diseases. The fundamental mechanism of atherosclerotic plaque development has been widely studied using a mouse model of atherosclerosis. However, genetic manipulations of WT mice which are naturally resistant to atherosclerosis first need to be carried out to increase their susceptibility. Currently, two well-known mouse models of atherosclerosis are commercially available, namely mice deficient in the apolipoprotein E (ApoE-/-) and the LDL receptor (LDLR-/-). ApoE is an apolipoprotein that acts as a ligand for the LDL receptor and chylomicron remnant receptor [18]. ApoE deficiency can cause the defective removal of chylomicron remnant receptors and intermediate density lipoprotein particles from the circulation which can lead to hypercholesterolemia [18]. ApoE-/- mice develop similar atherosclerotic lesions to human plaques which progress from lipid streak to fibro-fatty plaques and advanced lesions [19]. The advantage of the ApoE-/- mouse compared to other mouse models of atherosclerosis is the ability of this mouse spontaneously develops atherosclerosis without feeding them with a high fat diet [20,21]. However, in this model, the lipoprotein profile is dominated by an elevated level of very low density lipoprotein (VLDL) which is different compared to the human case, for which the profile is usually dominated by LDL [19]. LDLR-/- mice are a model that were developed to mimic hypercholesterolemia in humans due to LDLR pathway disruption and widely utilized to study the mechanism of lipid uptake and metabolism [22]. Unlike humans with LDLR defects, LDLR-/- mice only develop severe hypercholesterolemia after consuming an atherogenic, high-cholesterol containing diet [22].

Murine Norovirus (MNV)

MNV are single-stranded, positive-sense RNA viruses that infect mice and are included in the caliciviridae family [23]. These viruses do not induce any disease in mice with a competent immune system but lead to severe illness and fatal disease in mice with specific, genetically modified immunodeficiency [24,25]. Like human noroviruses, transmission of MNV typically follows the fecal-oral route, a common route for gastrointestinal pathogens [26].

MNV infection is common in specific pathogen free (SPF) facilities in the United States, Canada, Asia and Europe [27,28]. Studies have shown that MNV are highly common enteric pathogens in laboratory mice colonies with up to 64% of the population being found positive for MNV in some instances [27,29]. This observation may have an impact on other mouse model research studies especially those involving intestinal pathogens. Recently, disease progression in the mouse model of inflammatory bowel disease caused by bacteria infection has been shown to be affected by MNV infection [30]. Specifically, MNV infection elicited the development of inflammatory bowel disease in Mdrla mice [30]. On the other hand, in MNV-infected C57BL/6 mice, subtle alterations in lymphoid tissue, including increased reactive hyperplasia of mesenteric lymph nodes has been observed [16].

To date, four different strains of MNV have been identified and interestingly, although all these strains are similar genetically, the phenotypes observed during infection varied greatly [31]. MNV-2, MNV-3 and MNV-4 cause persistent infections with prolonged fecal shedding in the immune-competent mice, while the MNV-1 causes a short subclinical infection which is typically shed in the feces of mice for less than one week after experimental inoculation [31]. Unlike human norovirus, MNV replicates efficiently in tissue culture with tropism in macrophages and dendritic cells [32]. This feature makes MNV an excellent model for norovirus studies in *in-vitro* systems. Routine propagation of MNV in RAW264.7 cells (an immortalized mouse macrophage cell line which was established from a lymphocytic lymphoma induced by Abelson murine leukemia) is now common in research laboratories that involve this virus [32]. Furthermore, there are currently three available reverse genetics systems which were established based on the first isolated strain of MNV-1 (CW1). In the firstly introduced system, the viral recovery depends on the transfection of a full-length cDNA construct of MNV-1 under the control of a T7 promoter into cells which initially infected with fowl pox virus expressing T7 RNA polymerase (FPV-T7) [33]. The second system utilizes an RNA polymerase II (Pol-II) promoter-based reverse genetic system [34]. Finally, a RNA-based reverse genetic system for MNV exploiting the *in-vitro* transcribed and enzymatically capped viral RNA which is then transfected into MNV replication permissive cells (BSR-T7 and 293T cells) to produce higher yields of MNV virus [35,36].

Effect of MNV-4 in Mouse Model of Atherosclerosis

LDLR-/- mice: Currently, only the effect of MNV-4 infections has been widely studied in the mouse model of atherosclerosis. MNV-4 infection did not cause any significant changes in weight gain, fasting blood glucose, glucose tolerance, and insulin sensitivity in LDLR-/- mice fed either with high fat, high sucrose (HFHS) (diabetogenic) or high fat, high cholesterol (HFHC) (atherogenic) diets which indicated that MNV infection did not significantly

modify adipose tissue inflammation and insulin resistance [17]. Moreover, MNV infection also led to modest but significant increase in atherosclerotic lesion size and macrophage contents in lesions of LDLR-/- mice fed a HFHC diet but not in those fed a HFHS diet as expected because LDLR-/- mice required high cholesterol diet to develop severe atherosclerosis [17]. Furthermore, MNV infection in mice can take place at any time during the research. Newly purchased young mice may get infected if placed in the same facility with MNV-infected breeding colonies. Therefore, Jsun Paik and colleagues conducted a study to determine whether the timing of MNV infection relative to atherosclerosis development altered the disease phenotype [37]. They found that MNV-4 infection at an early stage of atherogenesis did not significantly change lesion size or macrophage content in the lesion area suggesting that the timing of MNV-4 infection may regulate the development of atherosclerosis in this model [37]. In addition, they also showed that MNV-4 infected bone-marrow derived macrophages (BMDM) induced the cytokine expression (IL-6, IL-1β, IFN-β) at all levels under influenced of oxLDL [37]. Interestingly, oxLDL treatment also suppressed MNV-4-induced cytokine expression in a dose-dependent manner [37]. Moreover, MNV-4 infection also induced the expression of iNOS, a marker of classically activated macrophages whereby oxLDL treatment decreased iNOS expression in a dose-dependent manner [37]. They also demonstrated that anti-inflammatory cytokine, IL-10 expression alters in parallel with pro-inflammatory cytokines [37]. The outcome of the study proposed that the effects of MNV-4 on macrophages are regulated by oxLDL, the auto-antigen of atherosclerosis. Furthermore, the age of the experimental mice also influenced the effect of MNV-4 infection on mouse model of atherosclerosis [37]. The rodents' immune system becomes mature at one month of age [38]. However, the rodents' immunologic memory only established in the first 6 month of age which may influence the quality of immune response against MNV [38]. The difference in infection timing also affects another variable namely obesity as mice fed with high fat diet and infected with MNV-4 at 8 weeks was considered obese prior to infection [37]. In contrast, mice fed with high fat diet and treated with MNV-4 at day 3 did not significantly develop obesity [37]. Obesity can regulate macrophage responses to various infectious agents and promote the influx of macrophage into the atherosclerotic plaques [39,40]. Moreover, any minor changes even single amino acid substitutions in the MNV sequence during virus propagation may affect the biologic responses which may suggest that different strains of MNV may have different responses [41-43]. MNV-4 infection also induced CD36 expression on the macrophage surfaces which accelerating oxLDL uptake while repressing the ABCA1 transporter which facilitate the removal of cholesterol from the macrophages [37].

ApoE-/- mice: The *in-vitro* treatment of MNV-4 on bone marrow-derived macrophages (BMDM) isolated from ApoE-/- mice increased the mRNA expression of iNOS, MCP-1, IL-1β, IL-6, IFN-β, and TNF-α44. Meanwhile, the treatment of oxLDL in MNV-4-infected BMDM induced the mRNA expression of iNOS, MCP-1, and IL-6 which clearly indicated the MNV-4 infection alone or in combination with oxLDL regulates pro-inflammatory cytokines and chemokines involved in atherosclerosis development [44]. In addition, MNV-4 infection alone and in combination with oxLDL increased the cell-surface expression of CD36 protein and suppressed ABCA1 protein, suggesting that MNV4-infected macrophages may increase the uptake and storage of cholesterol [44]. Moreover, the first *in-vivo* study revealed that MNV-4 infection at 12 weeks old of ApoE-/- mice fed with a normal diet had significantly larger aortic sinus lesion size compared to uninfected control mice [44]. A direct effect of MNV-4 infection in exacerbation the formation atherosclerotic plaque was confirmed by detection of MNV-4 genome in the aortic tissue and plaque lesion [44]. Moreover, the induction of Ly6C-positive monocytes percentage in the blood one week after MNV-4 infection also correlated with augmentation of atherosclerosis as the migration of the Ly6C-positive monocyte into the intima region of the artery led to the conversion into macrophages and subsequently formation of foam cells [44]. In contrast, the second *in-vivo* study which involved inoculation of MNV-4 in the ApoE-/- mice at the start of study (4 weeks) showed that the plaque size did not differ compared to the first study and no significant differences were observed in the percentages of total monocytes, macrophages, and dendritic cells, macrophages or neutrophils in the blood [44]. However, when MNV-4 was infected in the ApoE-/- mice at seven weeks of age, the MNV-4 RNA was detected in various location including mesenteric lymph nodes, spleens, aortic arch and in the descending aorta [44]. Interestingly, there were no significant differences in either atherosclerotic lesion size or macrophage content in the lesion between MNV-4 infected mice and uninfected control mice [44]. There are several possible reasons which lead to variable results between the first and second *in-vivo* studies. The first reason may be due to increase numbers of MNV-4 infected macrophages and monocytes which was detected in the lesions, hence accelerating lesion progression [44]. Secondly, the variable effect of MNV-4 infection on atherosclerosis may influence the effect of infection on disease progression which might have been covered by the larger variation in plaque lesion size in uninfected control

mice in the second study compared with those in the first study [44]. Finally, possibly the occurrence of mutation in MNV-4 genome during viral propagation may produce different results between the two studies [44]. Since there were variable effects of MNV-4 infection in hyperlipidemic mice, further study was conducted to evaluate the effect of MNV-4 on *Chlamydia pneumoniae* (Cpn)-accelerated atherosclerosis in ApoE-/- mice [45]. Cpn is a well-known intracellular bacterium involved in the acceleration of atherosclerotic development [13,46-48]. The treatment ApoE-/- derived BMDM with oxLDL, MNV and Cpn significantly increased proatherogenic cytokines and chemokines gene expression such as IL-6, MCP-1, iNOS, and TNF-α compared with Cpn-monoinfected BMDM [45]. On the other hand, *in-vivo* study showed that Cpn-monoinfection increased atherosclerotic plaque development compared with uninfected ApoE-/- mice [45]. However, MNV infection did not had any effect on atherosclerotic plaque formation as MNV-Cpn-coinfected mice reduced 56% of plaque lesion areas compared with Cpn-monoinfected mice [45]. These results suggest that Cpn has proatherogenic role while MNV may has atheroprotective role. Moreover, there was no differences in expression of aortic cytokines (MCP-1, TNF-α, or IL-1β, iNOS) and intracellular adhesion molecule (ICAM)-1 in aortic arch as well as in peritoneal macrophages at one week after infection in MNV-Cpn-coinfected mice compared with Cpn-monoinfected mice [45]. Furthermore, the evaluation of aortic arch and descending aorta tissue from MNV-infected mice at 1 or 8 weeks by RT-PCR indicated no expression of MNV gene detected regardless of Cpn status [45]. These results showed an atheroprotective role of MNV during Cpn-accelerated atherosclerosis in ApoE-/- mice [45].

CONCLUSION

Taken together, the effect of MNV-4 infection in a mouse model of atherosclerosis was variable depending on many factors such as types of diet, time of MNV-4 infection in mouse, genetic of mouse model, age of mouse model and mutation incidence during MNV-4 propagation. In addition, under a certain condition such as during atherosclerosis accelerating Cpn-infected ApoE-/- mice, MNV-4 may have atheroprotective role. Besides that, the impact of other MNV strains, MNV-1, MNV-2 and MNV-3 on the atherosclerosis development also warrant further studies. The effects of MNV infection on the CD4+ T cells differentiation which populate in the atherosclerotic plaque also need to be determined. Finally, MNV infection in laboratory mice needs to eliminate as it can lead to a variable and unpredictable effect on the development of certain inflammatory diseases.

DECLARATIONS

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Conflict of Interest

The authors have disclosed no conflict of interest, financial or otherwise.

REFERENCES

1] Legein, Bart, et al. “Inflammation and immune system interactions in atherosclerosis.” *Cellular and Molecular Life Sciences,* Vol. 70, No. 20, 2013, pp. 3847-69.

2] World Health Organization. Management of Substance Abuse Unit. *Global status report on alcohol and health.* World Health Organization, 2014.

3] Levitan, Irena, Suncica Volkov, and Papasani V. Subbaiah. “Oxidized LDL: diversity, patterns of recognition, and pathophysiology.” *Antioxidants & Redox Signalling*, Vol. 13, No. 1, 2010, pp. 39-75.

4] Libby, Peter, Paul M. Ridker, and Göran K. Hansson. “Progress and challenges in translating the biology of atherosclerosis.” *Nature*, Vol. 473, No. 7347, 2011, p. 317.

5] Smith, Jonathan D., et al. “Decreased atherosclerosis in mice deficient in both macrophage colony-stimulating factor (op) and apolipoprotein E.” *Proceedings of the National Academy of Sciences*, Vol. 92, No. 18, 1995, pp. 8264-68.

6] Lindemann, S., et al. “Platelets, inflammation and atherosclerosis.” *Journal of Thrombosis and Haemostasis,* Vol. 5, No. 1, 2007, pp. 203-11.

7] Hettema, M. E., H. Bootsma, and C. G. M. Kallenberg. "Macrovascular disease and atherosclerosis in SSc." *Rheumatology,* Vol. 47, No. 5, 2008, pp. 578-83.

8] Rosenfeld, Michael E., and Lee Ann Campbell. "Pathogens and atherosclerosis: update on the potential contribution of multiple infectious organisms to the pathogenesis of atherosclerosis." *Thrombosis and Haemostasis,* Vol. 106, No. 5, 2011, pp. 858-67.

9] Reszka, Edyta, et al. "Detection of infectious agents by polymerase chain reaction in human aortic wall." *Cardiovascular Pathology,* Vol. 17, No. 5, 2008, pp. 297-302.

10] Latsios, George, et al. "Detection of cytomegalovirus, Helicobacter pylori and Chlamydia pneumoniae DNA in carotid atherosclerotic plaques by the polymerase chain reaction." *Acta Cardiologica*, Vol. 59, No. 6, 2004, pp. 652-57.

11] Boddi, Maria, et al. "Hepatitis C virus RNA localization in human carotid plaques." *Journal of Clinical Virology,* Vol. 47, No. 1, 2010, pp. 72-75.

12] Eugenin, Eliseo A., et al. "Human immunodeficiency virus (HIV) infects human arterial smooth muscle cells in vivo and in vitro: implications for the pathogenesis of HIV-mediated vascular disease." *The American Journal of Pathology,* Vol. 172, No. 4, 2008, pp. 1100-11.

13] Kuo, Cho-chou, et al. "Demonstration of Chlamydia pneumoniae in atherosclerotic lesions of coronary arteries." *Journal of Infectious Diseases,* Vol. 167, No. 4, 1993, pp. 841-49.

14] Chiu, Brian. "Multiple infections in carotid atherosclerotic plaques." *American Heart Journal,* Vol. 138, No. 5, 1999, pp. 534-36.

15] Ford, P. J., et al. "Inflammation, heat shock proteins and periodontal pathogens in atherosclerosis: an immunohistologic study." *Molecular Oral Microbiology,* Vol. 21, No. 4, 2006, pp. 206-11.

16] Paik, Jisun, et al. "Effects of murine norovirus infection on a mouse model of diet-induced obesity and insulin resistance." *Comparative Medicine,* Vol. 60, No. 3, 2010, pp. 189-95.

17] Paik, Jisun, et al. "Murine norovirus increases atherosclerotic lesion size and macrophages in LDLR-/- mice." *Comparative Medicine,* Vol. 61, No. 4, 2011, pp. 330-38.

18] Zhang, Sunny H., et al. "Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E." *Science,* Vol. 258, No. 5081, 1992, pp. 468-71.

19] Jawien, Jacek, Pawel Nastalek, and Richard Korbut. "Mouse models of experimental atherosclerosis." *Journal of Physiology and Pharmacology,* Vol. 55, No. 3, 2004, pp. 503-17.

20] Breslow, Jan L. "Transgenic mouse models of lipoprotein metabolism and atherosclerosis." *Proceedings of the National Academy of Sciences,* Vol. 90, No. 18, 1993, pp. 8314-18.

21] Nakashima, Yutaka, et al. "ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree." *Arteriosclerosis, Thrombosis, and Vascular Biology,* Vol. 14, No. 1, 1994, pp. 133-40.

22] Ishibashi, Shun, et al. "Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery." *The Journal of Clinical Investigation,* Vol. 92, No. 2, 1993, pp. 883-93.

23] Cox, Courtney, Shengbo Cao, and Yuanan Lu. "Enhanced detection and study of murine norovirus-1 using a more efficient microglial cell line." *Virology Journal,* Vol. 6, No. 1, 2009, p. 196.

24] Karst, Stephanie M., et al. "STAT1-dependent innate immunity to a Norwalk-like virus." *Science,* Vol. 299, No. 5612, 2003, pp. 1575-78.

25] Ward, Jerrold M., et al. "Pathology of immunodeficient mice with naturally occurring murine norovirus infection." *Toxicologic Pathology,* Vol. 34, No. 6, 2006, pp. 708-15.

26] Wobus, Christiane E., Larissa B. Thackray, and Herbert W. Virgin. "Murine norovirus: a model system to study norovirus biology and pathogenesis." *Journal of Virology,* Vol. 80, No. 11, 2006, pp. 5104-12.

27] Henderson, Kenneth S. "Murine norovirus, a recently discovered and highly prevalent viral agent of mice." *Lab Animal,* Vol. 37, No. 7, 2008, p. 314.

28] Hsu, Charlie C., et al. "Persistent infection with and serologic crossreactivity of three novel murine noroviruses." *Comparative Medicine,* Vol. 56, No. 4, 2006, pp. 247-51.

29] Müller, B., et al. "Genetic diversity and recombination of murine noroviruses in immunocompromised mice." *Archives of Virology,* Vol. 152, No. 9, 2007, pp. 1709-19.

30] Chase Lencioni, Karen, et al. "Murine norovirus: an intercurrent variable in a mouse model of bacteria-induced inflammatory bowel disease." *Comparative Medicine,* Vol. 58, No. 6, 2008, pp. 522-33.

31] Hsu, Charlie C., Lela K. Riley, and Robert S. Livingston. "Molecular characterization of three novel murine noroviruses." *Virus Genes,* Vol. 34, No. 2, 2007, pp. 147-55.

32] Wobus, Christiane E., et al. "Replication of Norovirus in cell culture reveals a tropism for dendritic cells and macrophages." *PLoS Biology,* Vol. 2, No. 12, 2004, p. e432.

33] Chaudhry, Yasmin, Michael A. Skinner, and Ian G. Goodfellow. "Recovery of genetically defined murine norovirus in tissue culture by using a fowlpox virus expressing T7 RNA polymerase." *Journal of General Virology,* Vol 88, No. 8, 2007, pp. 2091-2100.

34] Ward, Vernon K., et al. "Recovery of infectious murine norovirus using pol II-driven expression of full-length cDNA." *Proceedings of the National Academy of Sciences,* Vol. 104, No. 26, 2007, pp. 11050-5.

35] Yunus, Muhammad Amir, et al. "Development of an optimized RNA-based murine norovirus reverse genetics system." *Journal of Virological Methods,* Vol. 169, No. 1, 2010, pp. 112-18.

36] Arias, Armando, et al. "Reverse genetics mediated recovery of infectious murine norovirus." *Journal of Visualized Experiments,* Vol. 64, 2012.

37] Paik, Jisun, et al. "Effects of murine norovirus on atherosclerosis in LDLR-/- mice depends on the timing of infection." *Comparative Medicine,* Vol. 65, No. 2, 2015, pp. 114-22.

38] Landreth, K. S. "Critical windows in development of the rodent immune system." *Human & Experimental Toxicology,* Vol. 21, No. 9-10, 2002, pp. 493-98.

39] Amar, Salomon, et al. "Diet-induced obesity in mice causes changes in immune responses and bone loss manifested by bacterial challenge." *Proceedings of the National Academy of Sciences,* Vol. 104, No. 51, 2007, pp. 20466-71.

40] Zhang, Anna JX, et al. "Leptin mediates the pathogenesis of severe 2009 pandemic influenza A (H1N1) infection associated with cytokine dysregulation in mice with diet-induced obesity." *The Journal of Infectious Diseases,* Vol. 207, No. 8, 2013, pp. 1270-80.

41] Bailey, D., L. B. Thackray, and I. G. Goodfellow. "A single amino acid substitution in the murine norovirus capsid protein is sufficient for attenuation in vivo." *Journal of Virology,* Vol. 82, No. 15, 2008, pp. 7725-28.

42] Nice, Timothy J., et al. "A single-amino-acid change in murine norovirus NS1/2 is sufficient for colonic tropism and persistence." *Journal of Virology,* Vol. 87, No. 1, 2013, pp. 327-34.

43] Thackray, Larissa B., et al. "Murine noroviruses comprising a single genogroup exhibit biological diversity despite limited sequence divergence." *Journal of Virology,* Vol. 81, No. 19, 2007, pp. 10460-73.

44] Hsu, Charlie C., et al. "Murine norovirus infection variably alters atherosclerosis in mice lacking apolipoprotein E." *Comparative Medicine,* Vol. 65, No. 5, 2015, pp. 369-81.

45] Patil, Karuna, et al. "Effects of murine norovirus on chlamydia pneumonia-accelerated atherosclerosis in ApoE-/- mice." *Comparative Medicine,* Vol. 66, No. 3, 2016, pp. 188-96.

46] Blessing, Erwin, et al. "Chlamydia pneumoniae infection accelerates hyperlipidemia induced atherosclerotic lesion development in C57BL/6J mice." *Atherosclerosis,* Vol. 158, No. 1, 2001, pp. 13-17.

47] Campbell, Lee Ann, Cho-Chou Kuo, and J. Thomas Grayston. "Chlamydia pneumoniae and cardiovascular disease." *Emerging Infectious Diseases,* Vol. 4, No. 4, 1998, pp. 571.

48] Campbell, Lee Ann, and Cho-cho Kuo. "Chlamydia pneumonia-an infectious risk factor for atherosclerosis?" *Nature Reviews Microbiology,* Vol. 2, No. 1, 2004, pp. 23.