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Review

Circulating Immune Complexes as an Important Link between Inflammation and Lipid Alterations in Atherosclerosis

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Abstract: This review article rigorously investigates the emerging facets of atherosclerosis pathogenesis through an exhaustive examination of the existing data on the intricate interplay between lipid metabolism disorders and inflammation. A primary objective of this study is to thoroughly explore the role of Circulating Immune Complexes (CIC) as a pivotal indicator of this multifaceted interaction. The focus of this review centers on the presence of autoantibodies to LDL in the bloodstream and their unequivocal association with atherosclerotic lesions in both patient populations and experimental animal models. Hypothesizing that the modification of lipoproteins incites an immune response resulting in the generation of LDL antibodies, a meticulous experimental design was employed. This entailed meticulous collection and analysis of extensive datasets encompassing LDL-CIC and their intricate relationship with LDL modifications. While ethical considerations were not explicitly discussed in this study, we affirm the indispensability of ethical research practices and urge future investigations to explicitly address these vital aspects. Furthermore, we acknowledge our obligation to clearly articulate the research's contribution to the field. As such, this study imparts invaluable insights into the intricate mechanisms underpinning the progression of atherosclerosis. Notably, it underscores the profound significance of LDL modifications and circulating immune complexes in elucidating the intricate nature of this pernicious disease. In conclusion, this rigorous review article substantiates novel aspects of atherosclerosis pathogenesis, reinforcing the paramount importance of lipid metabolism disorders and inflammation. By elucidating the involvement of circulating immune complexes and their intricate correlations with LDL modifications, this study significantly advances the existing knowledge in the field. The findings presented herein expand our understanding of atherosclerosis and lay a robust foundation for further research endeavors and potential therapeutic interventions.

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Introduction

In fact, atherosclerosis is a proliferative, excessively fibrous fatty inflammatory reaction that occurs as a result of damage to the walls of arteries and includes various types of cells such as individual macrophages, platelets, lymphocytes, and smooth muscle cells. The occurrence and development of atherosclerosis are facilitated by changes in the level of lipoproteins and lipids, blood clotting factors, blood pressure regulation, platelet function, and metabolism of smooth muscle cells of the arteries (Linton *et al*., 2019). It is important to mention the notable impact of immunological elements, such as LDL-containing Circulating Immune Complexes (LDL-CIC) and autoantibodies on LDL. Additionally, a common characteristic in the early progression of atherosclerosis involves the accumulation of intracellular lipids (both esterified and free cholesterol) on the artery wall, leading to the creation of foam cells. According to the results of past studies, it was found that LDL-CIC in particular and modified LDL in general are the primary agents that contribute to the accumulation of surplus cholesterol

within vascular cells resulting in the development of atherosclerotic lesions. Simultaneously, the intracellular storage of lipids triggers the appearance of these lesions (Sobenin *et al*., 2014). Consequently, inflammatory responses, cell proliferation, and the excessive synthesis of connective tissue matrix components take place. This leads to the progression of atherosclerotic lesions, ultimately resulting in the widespread clinical presentation of atherosclerosis (Rafieian-Kopaei *et al*., 2014).

Using a previously known simple method for determining the level of LDL-CIC, it was found that the ratio of apo B/apo A-1 and the level of LDL-CIC determined the difference between patients without stenosis and patients with atherosclerosis. It was also found that the presence and severity of atherosclerosis did not depend on Lp[a], apo B, apo A1, triglycerides, HDL cholesterol, and total cholesterol (Rabizadeh *et al*., 2021). At the same time, it was determined that in comparison with other parameters of the lipid profile, it is the level of LDL-CIC that is considered the key indicator of atherosclerotic lesions. It is important to note that a relationship between LDL-CIC levels and the extent of atherosclerosis was identified. Therefore, it was proposed to use this biochemical parameter to detect atherosclerosis. At the same time, with the progression of atherosclerosis, the prognostic value of LDL-CIC still remains unknown (Summerhill *et al*., 2019).

LDL-CIC is involved in the formation of atherosclerosis. This capability stems from inducing significant cholesterol buildup in vascular cells under cultivation. This aspect may explain the significant role LDL-CICs play in the development of atherosclerosis (Hao and Friedman, 2014). This review delves into the involvement of modified Low-Density Lipoprotein (LDL) within Circulating Immune Complexes (CIC) and their association with atherosclerosis development. It explores the immunological factors, the physicochemical traits of LDL-CIC, the immune response to altered LDL, and the atherogenic and inflammatory aspects of immune complexes with modified LDL.

Study Design

The review article presents an analysis of previous studies conducted on the topic. The research utilizes data from various experiments, including in vivo and in vitro studies, to investigate the relationship between LDL-CIC and atherosclerosis. The study design involves literature review, data synthesis, and interpretation to provide valuable insights into the development of atherosclerosis and the participation of LDL-CIC in this progression. The article also describes the methodology used in previous studies, such as affinity chromatography and affinity testing of human autoantibodies, to determine the reactivity and affinity of antibodies to modified LDL.

LDL-CIC and Its Physicochemical Characteristics

Anti-LDL autoantibodies were identified in the blood tests of patients for the first time, who had hyperlipidemia, coronary heart disease, or myeloma. In 1965, in one of his studies, Beaumont described the relationship between atherosclerosis, xanthomatosis, hyperlipidemia, and antibodies to β-lipoprotein (Beaumont *et al*., 1970). It is important to mention that antibodies against LDL binding factors and lipoproteins were detected in the blood of both healthy patients and those who suffered from CAD. In the course of their work, Bauer established that the main LDL-binding proteins are immunoglobulins (Bauer, 1983). Based on the appearance of autoantibodies to LDL in the blood, lipoproteins can be considered as antigens. High immunogenicity was determined in modified LDL. Their ability to generate antibodies was also established. As a result, it is common to find autoantibodies to modify LDL. Additionally, patients with diabetes mellitus have been observed to have autoantibodies against glycosylated LDL. Furthermore, autoantibodies specific to modified malondialdehyde LDL were detected in both CAD patients and healthy individuals (Sveen *et al*., 2021). The same results were obtained during the study of animal blood samples. In vascular lesions resulting from atherosclerosis, researchers identified deposits of immune complex components. Patients with angiographically confirmed coronary atherosclerosis were found to have autoantibodies of the immunoglobulin G class. Additionally, individuals with atherosclerosis showed higher levels of autoantibodies to LDL compared to those without the condition (Hong *et al*., 2015). Due to their properties, these autoantibodies are quite close to malondialdehyde-modified LDL and neuraminidase-treated LDL. Based on this, it can be assumed that antibodies are produced in vivo. What occurs is a reaction to the presence of altered LDL in the bloodstream (Orekhov *et al*., 2014).

In individuals and animals with atherosclerotic lesions, antibodies targeting LDL were discovered in the bloodstream, particularly those altered with Malondialdehyde (MDA). Elevated levels of oxidized lipids (F2-isoprostanes; MDA) were also detected in the blood of individuals with Coronary Heart Disease (CHD) (Frostegård, 2013; Van den Berg *et al*., 2018). Nevertheless, there is a basis to argue that there is no significant accumulation of oxidized lipids in human LDL. This is due to the fact that high-density lipoproteins can help eliminate toxins from the body or redirect them to the liver. Desialylated LDL, small/dense LDL, and electronegative LDL were detected in patients with atherosclerosis. Similar LDLs have a lower level of sialic acid compared to the native LDL (Burchardt *et al*., 2013).

It can be said that the presence of antibodies against LDL is a consequence of the immune response that was caused by the modification of lipoproteins. So Tertov *et al*. in their study described the isolation of circulating immune complexes from blood serum (using polyethylene glycol 6000) (Tertov *et al*., 1990). Thanks to this, they were able to establish that LDL-CIC differs in many ways from native LDL. So, due to the fact that this type of LDL has a low level of sialic acid, it becomes clear that LDL-CIC is desialylated. Also, based on the established high electrophoretic mobility of LDL-CIC, it can be said that it is more electronegative, unlike native LDL. Also, LDL-CIC has a lower content of phospholipids and neutral lipids (Millar *et al*., 1999). At the same time, its particles have a higher density and a smaller diameter. And, at the same time, LDL-CIC can cause intracellular accumulation of neutral lipids in the cells of the intact intimate aorta. Due to the established factor, it turned out that LDL-CIC has quite a lot of similarities with desialylated LDL. At the same time, it was possible to establish a relationship between the concentration of desialylated LDL and the content of LDL in circulating immune complexes (Tertov *et al*., 1996). Based on this, it is quite possible to assume that the de-desialylated LDL forms a complex with autoantibodies. It also indicates that the affinity of circulating autoantibodies to LDL is lower for native than for desialylated LDL. It is important to say that autoantibodies against LDL bind much more effectively to the LDL of patients with a high level of desialylated LDL. Desialylated LDL may have certain modifications. These modifications can induce immune response changes in the tertiary structure of apo B and in the composition of carbohydrates, aggregation of lipoprotein particles, and modification of lysine amino groups (Glanz *et al*., 2022).

Immunogenicity of Modified LDL

As in animals and humans, most modified LDLs are immunogenic. In their work, Steinbrecher *et al*. (1990); Palinski *et al*. (1995) described the immunogenic reactions responsible for the formation of antibodies in animals. In the future, many new studies have been conducted to study the autoimmune response in humans.

To determine the corresponding antibodies in humans, human serum was subjected to affinity chromatography in columns with sepharose, which was conjugated with AGE LDL and ox-LDL. IgG 1 subtype and IgG 3 subtype were the predominant isotopes of antibodies to AGE-LDL and oxLDL. They were followed by IgM, as well as IgA in very small concentrations (Virella and Lopes-Virella, 2003).

At the same time, it was found that the average affinity constants for AGE-LDL and oxLDL antibodies in humans are higher than in rabbits. Due to this, it becomes clear that the antibodies that were caused by animal inoculation have a higher affinity than human autoantibodies. Also, a study was conducted on a sample of more than 400 people to compare the affinity constants of antibodies to ox-LDL (from IC or whole serum). The Kd of antibodies from IC is 0.98±0.06×10-8 moL/L and the Kd of antibodies from whole serum is 1.53±0.13×10-8 moL/l. Based on this, it can be concluded that the antibodies that remain in free circulation have a lower affinity than those that participated in the formation of IC (Ylä-Herttuala *et al*., 1994).

In order to establish modified LDLs prone to antibody indexing, Researchers conducted a study to assess the response of human autoantibodies. They tested the reactivity of IgG fractions obtained from immune complexes isolated from the sera of different individuals with a range of modified lipoproteins immobilized. These included MPO-LDL, HEL-LDL, MDA-LDL, MGO-LDL, CML-LDL, AGE-LDL and oxLDL. Notably, IgG antibodies primarily identified AGE-LDL, ox-LDL, and MDA-LDL as the modifications of interest. In total, 13 different Ig fractions were tested during the study. Of these, only MGO-LDL showed a weak reaction. Based on this, it can be assumed that this fraction is less immunogenic than others. Subsequent analysis of MLDL found that oxLDL, which was obtained as a result of copper oxidation, mainly contained MDA, followed by carboxyethyllisine and CML (Lopes-Virella and Virella, 2013). At the same time, AGE-LDL, after an eight-week incubation with glucose-6-phosphate, exhibited elevated levels of CML, along with CEL and a reduced amount of MDA. And MDA-LDL, which was obtained by processing LDL MDA, contained exclusively MDA (Virella *et al*., 2005).

The IgG fraction derived from circulating immune complexes contained antibodies targeting MDA-LDL, oxLDL, CML-LDL, and AGE-LDL. Due to this, it becomes clear that the main immunogenic epitope of oxLDL is MDA-lysine. Although CML-lysine serves as the primary epitope of AGE-LDL, purified antibodies against oxLDL displayed limited reactivity with AGE-LDL. Instead, they exhibited strong reactivity towards MDA-LDL and oxLDL and notably heightened reactivity towards MDA-BSA (Lopes-Virella *et al*., 2011). Conversely, antibodies specific to AGE-LDL in humans primarily interacted with AGE-LDL. There is also evidence that they also cross-reacted with CML-LDL and oxLDL. The presence of MDA-lysine is only moderately reliant on the tertiary structure of the protein to which it is linked. It can also be argued that it is highly dominant. This is supported by the observation of antibody cross-reactivity between oxidized LDL (ox-LDL) and MDA-modified Bovine Serum Albumin (MDA-BSA). The interaction of Advanced Glycation End-product (AGE) antibodies with oxLDL stems from the existence of CML-lysine epitopes within oxLDL. Laboratory-modified lipoproteins have many epitopes. At the same time, some of them are

common for different modifications. It is also worth mentioning that the targets of numerous modifications are precisely LDL molecules that have been modified *in vivo* (Li *et al*., 2022).

Atherogenicity and Pro-Inflammatory Properties of Immune Complexes Containing Modified LDL

In 1970, for the first time, there was a suggestion regarding the involvement of immune complex LDL in the development of atherosclerosis. During their study, Beaumont and colleagues (Beaumont *et al*., 1970) discovered that individuals with IgA myeloma, where monoclonal IgA acted as an autoantibody against LDL, exhibited faster progression of atherosclerosis and significant hyperlipidemia. In a separate investigation carried out by Fust and team in 1978, there was documentation of the emergence of antibodies to IC and LDL in patients with CVD. And already in 1981 Klimov *et al*. (1984) said that complexes that were formed with rabbit anti-apoB IgG and with the human LDL that was labeled with radioactivity showed a rate of excretion from the bloodstream up to three times higher compared to unlabelled LDL. It was noted that mouse peritoneal macrophages showed a higher absorption rate compared to soluble LDL complexes. Furthermore, when incubated with LDL, macrophages exhibited a significant increase in cholesterol content by approximately 60 times. In 1988, researchers confirmed the accumulation of cholesterol esters in human macrophages. These macrophages, when treated with rabbit antibodies and normal human LDL and incubated with Immune Complexes (IC), displayed a paradoxical increase in LDL receptor expression, leading to unregulated uptake of LDL. The research also suggested that the primary mechanism of LDL IC absorption involves Fc receptors. Additionally, human mesangial cells and macrophages were found to absorb LDL (Griffith *et al*., 1988). Those LDL were obtained using human LDL and human or rabbit antibodies to LDL mainly due to FcγRI. FcγRI, as well as FcγRII a, involves the uptake of LDL by U937 histiocytes. This was confirmed, in particular, by a study by Morganelli *et al*. (1992). They used bispecific LDL, which was obtained using conjugated mouse anti-FcγRI and anti-LDL and human macrophages. Furthermore, there is a possibility that FcγRIII plays a role in facilitating the internalization of oxLDL immune complexes by mesangial cells.

LDL IC has pro-inflammatory properties. This is due to the involvement of FcγR. The absorption of LDL by macrophages from human monocytes causes the release of TNF and IL-1. It also leads to the activation of respiratory ejection. At the same time, the impact of LDL IR is more significant compared to the effects seen with control IR (KLH IC, IR VLDL, and IR HDL) (Escate *et al*., 2016). In particular, this was confirmed in a study by Kiener *et al*. (1997). In the course of their work, they were able to establish that the release of TNF by THP-1 cells is caused by LDL, which is derived from native or acetylated human LDL and rabbit anti-LDL. At the same time, they do it more efficiently than thermally aggregated gammaglobulin. Their findings additionally indicated that the absence of this effect is noted when utilizing Fab antibody fragments targeting LDL instead of whole antibodies, affirming the significance of interaction with FcγR in the stimulation of macrophages by LDL IC (Li *et al*., 2016).

Proinflammatory and proatherogenic properties are observed in the preparation of IC LDL using native LDL and animal apoB antibodies. Similar observations were noted when employing Immune Complexes (IC) generated with human oxLDL and antibodies targeting human oxLDL. This was elucidated due to the innovative technique for extracting antibodies specific to human oxLDL from serum and IC that had been precipitated. Moreover, it has been discovered that human oxLDL IC, whether attached to human Red Blood Cells (RBC) or reconstituted post-precipitation with 4% Polyethylene Glycol (PEG), elicits a stronger response compared to oxLDL alone in promoting intracellular cholesterol ester accumulation and TNF release in THP-1 cells. In the course of one of the subsequent studies, it was found that IC oxLDL activates the complement system (Di Francesco *et al*., 2020). It was also found that it causes the release of proinflammatory cytokines in significantly higher concentrations, compared to those induced by oxLDL in concentrations that are present in IC oxLDL from macrophages (from MonoMac6 cells and monocytes). The release of higher concentrations of TNF, IL-6, and IL-1ß was facilitated by priming these cells with interferon-γ. It also led to a higher release of IL-10 and IL-12p70. At the same time, it is important to mention that the concentrations of proinflammatory cytokines were lower after incubation with concentrations of KLH IC than after incubation with oxLDL IC (Jundi *et al*., 2020).

The findings from Nagarajan's research indicate that exposing human venous endothelial cells to oxLDL initially and then rabbit anti-MDA subsequently results in the creation of surface-bound immune complexes (Nagarajan, 2007). These immune complexes, aided by FcγRII, play a role in activating and adhering to U397 cells. The adhesion of U937 cells to HUVECS coated with immune complexes is linked to the release of pro-inflammatory chemokines (IL-8 and MCP-1), which serves a significant function in maintaining vascular inflammation. In Oksjoki *et al*. presented findings showing that oxLDL immune complexes support the survival of monocytes cultured in a serum-free environment (Oksjoki *et al*., 2006). Apparently, we can say that this effect is achieved by slowing down spontaneous apoptosis. The apoptosis process is linked to the secretion of Monocyte Colony-Stimulating Factor (M-CSF). It is also noted to occur as a result of the interaction

between FcγRI and oxLDL immune complexes. These findings were validated by analyzing data from a study that examined the impact on overall gene expression by stimulating human monocyte-like cells (U937) with oxLDL IC KLH IC and solely oxLDL. Through this research, it was discovered that oxLDL ICs possess a distinctive capacity to trigger the expression of a specific group of genes involved in promoting cell survival. IC oxLDL also promotes the expression of genes associated with the control of transcription, endocytosis, intracellular lipid transportation, and the inflammatory response, which involves genes coding for TNF and IL-1ß, is documented. There is also evidence that a similar response in U937 cells is caused by prolonged activation of acidic sphingomyelinase (Wang *et al.*, 2022).

The lipid section of oxLDL immune complexes was found in the endosomal region, with apoB and the lipid portion of internal oxLDL coexisting in both the endosomal and lysosomal compartments. Meanwhile, the apolipoprotein part was shifted to the lysosomal area. The slowed breakdown of the lipid component in LDL immune complexes is due to lipid binding in the endosomal area. Most likely, this is due to a decrease in oxidative stress.

Furthermore, the presence of oxLDL Immune Complexes (oxLDL IC) may enhance the survival of U937 cells following incubation (Al Gadban *et al*., 2010).

The pro-survival influence of oxLDL IC is specific to this type of immune complex and is not observed with other modified LDL immune complexes like MDA-LDL IC. Both MDA-LDL IC and oxLDL IC led to similar patterns of gene expression and secretion of proinflammatory mediators such as MCP-1 and IL-6. In comparison to both unmodified oxLDL and oxLDL IC, MDA-LDL IC, and MDA-LDL induce a higher release of matrix metalloproteins. Significantly, the differences between MDA-LDL IC and oxLDL IC are more pronounced. Notably, the secretion levels of the inhibitory molecule TIMP-1 remain unaffected by either oxLDL IC or MDA-LDL IC. Based on these data, it can be assumed that MDL-LDL creates favorable conditions for acute cardiovascular events and plaque instability (Lopes-Virella and Virella, 2019).

Diagnostic and Prognostic Value of LDL-CIC in Atherosclerosis

Orekhov *et al.* found that the distinction between patients with and without atherosclerosis was facilitated solely by the ratio of apo B/apo A-1 and LDL-CIC levels. This was determined by using a simple LDL-CIC measurement method (Orekhov *et al*., 1991). At the same time, it was found that triglycerides, HDL cholesterol, total cholesterol, apo B, apo A1, and Lp[a] were in no way interrelated with the presence of atherosclerosis. Based on this, the researchers concluded that, in comparison with other parameters of the lipid profile, the level of LDL Immune Complexes (LDL-CIC) is considered a highly dependable marker for assessing atherosclerotic lesions. Additionally, it is worth mentioning that the LDL-CIC level has been found to be associated with both the stage and severity of atherosclerosis. Therefore, it is proposed to use this parameter as a specific marker of atherosclerosis (Ference *et al*., 2017).

According to Salonen and colleagues, the concentration of antibodies against MDA-modified LDL in the bloodstream is linked to the advancement of atherosclerosis (Salonen *et al*., 1992). A recent study in the epidemiology of diabetes interventions and complications trial revealed that individuals showing progression in IMT of the internal carotid artery had elevated levels of apolipoprotein B and cholesterol within their immune complexes. At the same time, it was the cholesterol content in immune complexes that was the determining marker of the progression of IMT. The presence of high cholesterol in CIC can be attributed to surrogate markers of modified LDL. Specifically, the LDL particles linked to cardiovascular incidents and heightened intima-media thickness in the carotid arteries were investigated by Lopes-Virella and colleagues. In their study, they quantified oxidized LDL, which was modified by malonic LDL dialdehyde in CIC, and the end products of LDL glycation. The researchers were able to determine the correlation between elevated carotid artery BMI in individuals with type I diabetes and assessed how strong this association was in comparison to traditional risk factors (Lopes-Virella *et al*., 2011). In the course of their study, Sobenin *et al.* evaluated the prognostic and diagnostic role of LDL-CIC and other lipid parameters in the early stages of atherosclerosis (Sobenin *et al*., 2014). The assessment was performed using high-resolution ultrasound examination in mode B as an increase in the IMT of the carotid arteries. People with established high levels of LDL-CIC were characterized by increased average and maximum thickness of intima-media arteries. They also had elevated levels of LDL cholesterol and serum total. It is worth noting that the level of serum LDL cholesterol and LDL-CIC depended on the degree of atherosclerosis. Serum LDL cholesterol P = 0.049, LDL-CIC-P = 0.042). At the same time, in comparison with the usual parameters, LDL-CIC had the highest indicators of specificity and sensitivity. In contrast to other lipid measures, LDL-CIC is directly linked to the advancement of atherosclerosis. It can be argued that it carries the highest odds and relative risk ratios. An LDL-CIC level below 16.0 micrograms/mL is considered normal. This specific parameter was the sole indicator capable of predicting the absence of atherosclerosis progression in the subsequent two years with a prognostic accuracy of 78.3% (95% CI 67.1-87.3). Levels of HDL, LDL, and total cholesterol in the blood serum generally do not hold

significant predictive values. Consequently, elevated LDL cholesterol levels are correlated with an increase in the thickness of the intima-media. Also due to this, it can be considered as a predictor of a high risk of developing and progressing atherosclerosis (Sobenin *et al*., 2014).

In extensive meta-analyses of prospective population studies comprising over 160,000 participants without prior cardiovascular disease in 37 prospective cohorts (1968-2007) with 15,126 cases of fatal or non-fatal CVD outcomes (4,994 strokes and 10,132 coronary heart disease) over a median tracking period of 10.4 years (with an interquartile range of 7.6-14 years), initial LDL and HDL cholesterol levels emerged as robust predictors of both coronary heart disease and stroke. In these studies, the usual parameters of the lipid profile were considered prognostic variables (LDL-CIC was not among them) (Collaboration *et al*., 2012). But, at the same time, based on the results, it can be said that in a healthy population, the biomarkers of the diagnosis of atherosclerosis do not coincide with the prognostic biomarkers. Therefore, in the future, it is especially important to investigate the diagnostic and prognostic role of LDL-CIC both in patients with established atherosclerosis and in those who do not suffer from it.

Conclusion

Based on the analysis of available data, this study has provided valuable insights into the role of lipoprotein-containing immune complexes in the development of atherosclerosis (the formation of fatty deposits in arteries). Findings strongly suggest that IC-LDL (lipoprotein-containing immune complexes) has a significant impact on the initiation and progression of early atherosclerotic lesions.

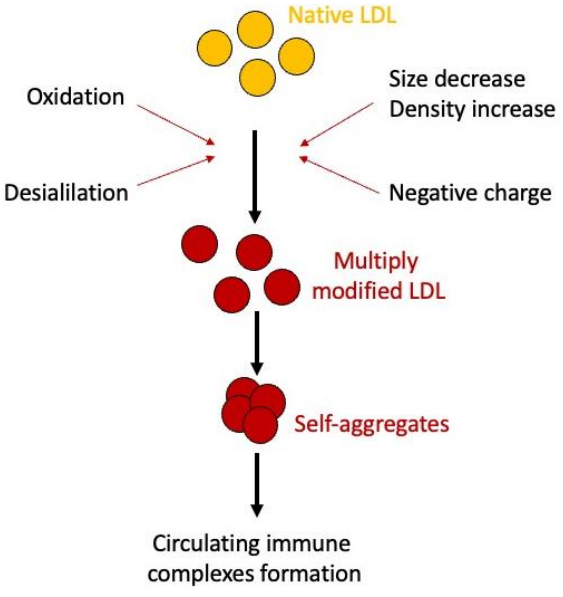

Fig. 1: Circulating immune complexes formation

One of the key mechanisms identified is the association between atherogenically modified LDL (low-density lipoprotein) and the production of anti-LDL autoantibodies. These antibodies interact with LDL, causing the creation of immune complexes that include LDL particles. We schematically represented the formation of CIC in Fig. 1. Importantly, this interaction between the antibodies and modified LDL amplifies the atherogenic potential of LDL, setting the stage for the development of atherosclerosis.

Moreover, our findings highlight the transformation of initially non-atherogenic native LDL into atherogenic particles after their binding to anti-LDL antibodies. This transformation is associated with intracellular lipid accumulation and other pathological changes commonly observed in atherosclerosis.

The practical implications of this study are noteworthy. Firstly, it extends our understanding of the underlying mechanisms driving atherosclerosis and provides a potential target for therapeutic interventions. By targeting the formation and activity of IC-LDL, there is a potential to disrupt the onset and advancement of atherosclerotic lesions, ultimately lessening the impact of cardiovascular disease. Furthermore, the identification of the role of anti-LDL autoantibodies in rendering LDL particles atherogenic opens up possibilities for diagnostic and prognostic tools. Detecting the presence and levels of these autoantibodies in individuals could serve as a biomarker for assessing their predisposition to atherosclerotic cardiovascular events, enabling early intervention and personalized treatment strategies.

Overall, this study deepens our understanding of the intricate connections between lipoprotein-containing immune complexes, LDL particles, and atherosclerosis. The knowledge gained not only sheds light on fundamental disease mechanisms but also holds promise for the development of novel therapeutic approaches and diagnostic strategies in the fight against cardiovascular disease.

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Author's Contributions

Anastasia Vladimirovna Poznyak: Written original drafted and prepared.

Mikhail Alexandrovich Popov: Written reviewed and edited.

Nikolay Alexandrovich Orekhov, Vasily Nikolaevich Sukhorukov, Elizaveta Mikhailovna Pleshko and Alexander Nikolaevich Orekhov: Have read and agreed to the published version of the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

References

Al Gadban, M. M., Smith, K. J., Soodavar, F., Piansay, C., Chassereau, C., Twal, W. O., ... & Hammad, S. M. (2010). Differential trafficking of oxidized LDL and oxidized LDL immune complexes in macrophages: Impact on oxidative stress. *PloS One*, *5*(9), e12534. https://doi.org/10.1371/journal.pone.0012534

Bauer, J. E. (1983). Plasma lipids and lipoproteins of fasted ponies. https://pubmed.ncbi.nlm.nih.gov/6838034/

Beaumont, J. L., Beaumont, V., Antonnucci, M., & Lemort, N. (1970). Les auto-anticorps antilipoproteines de myelome. Etude comparée de deux types. L'IgA anti-Lp Pg et L'IgG anti-Lp AS. In *Annales de Biologie Clinique* (Vol. *28*, p. 387).

Burchardt, P., Żurawski, J., Zuchowski, B., Kubacki, T., Murawa, D., Wiktorowicz, K., & Wysocki, H. (2013). State of the art paper Low-density lipoprotein, its susceptibility to oxidation and the role of lipoprotein-associated phospholipase A2 and carboxyl ester lipase lipases in atherosclerotic plaque formation. *Archives of Medical Science*, *9*(1), 151-158. https://doi.org/10.5114/aoms.2013.33176

Di Francesco, V., Gurgone, D., Palomba, R., Ferreira, M. F. M. M., Catelani, T., Cervadoro, A., ... & Decuzzi, P. (2020). Modulating Lipoprotein Transcellular Transport and Atherosclerotic Plaque Formation in ApoE–/–Mice via Nanoformulated Lipid–Methotrexate Conjugates. *ACS Applied Materials and Interfaces*, *12*(34), 37943-37956. https://doi.org/10.1021/acsami.0c12202

Collaboration, E. R. F., Di Angelantonio E, Gao P, Pennells L, Kaptoge S, Caslake M, *et al.* (2012). Lipid-related markers and cardiovascular disease prediction. *JAMA*. https://doi.org/10.1001/jama.2012.6571

Escate, R., Padro, T., & Badimon, L. (2016). LDL accelerates monocyte to macrophage differentiation: Effects on adhesion and anoikis. *Atherosclerosis*, *246*, 177-186. https://doi.org/10.1016/j.atherosclerosis.2016.01.002

Ference, B. A., Ginsberg, H. N., Graham, I., Ray, K. K., Packard, C. J., Bruckert, E., ... & Catapano, A. L. (2017). Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *European Heart Journal*, *38*(32), 2459-2472. https://doi.org/10.1093/eurheartj/ehx144

Frostegård, J. (2013). Immunity, atherosclerosis and cardiovascular disease. *BMC Medicine*, *11*(1), 1-13. https://doi.org/10.1186/1741-7015-11-117

Glanz, V., Bezsonov, E. E., Soldatov, V., & Orekhov, A. N. (2022). Thirty-five-year history of desialylated lipoproteins discovered by Vladimir Tertov. *Biomedicines*, *10*(5), 1174. https://doi.org/10.3390/biomedicines10051174

Griffith, R. L., Virella, G. T., Stevenson, H. C., & Lopes-Virella, M. F. (1988). Low density lipoprotein metabolism by human macrophages activated with low density lipoprotein immune complexes. A possible mechanism of foam cell formation. *The Journal of Experimental Medicine*, *168*(3), 1041-1059. https://doi.org/10.1084/jem.168.3.1041

Hao, W., & Friedman, A. (2014). The LDL-HDL profile determines the risk of atherosclerosis: A mathematical model. *PloS One*, *9*(3), e90497. https://doi.org/10.1371/journal.pone.0090497

Hong, J., Maron, D. J., Shirai, T., & Weyand, C. M. (2015). Accelerated atherosclerosis in patients with chronic inflammatory rheumatologic conditions. *International Journal of Clinical Rheumatology*, *10*(5), 365. https://doi.org/10.2217/ijr.15.33

Jundi, D., Krayem, I., Bazzi, S., & Karam, M. (2020). *In vitro* effects of azide-containing human CRP isoforms and oxLDL on U937-derived macrophage production of atherosclerosis-related cytokines. *Experimental and Therapeutic Medicine*, *20*(5), 1-1. https://doi.org/10.3892/etm.2020.9185

Kiener, P. A., Davis, P. M., Starling, G. C., Mehlin, C., Klebanoff, S. J., Ledbetter, J. A., & Liles, W. C. (1997). Differential induction of apoptosis by Fas–Fas ligand interactions in human monocytes and macrophages. *The Journal of Experimental Medicine*, *185*(8), 1511-1516. https://doi.org/10.1084/jem.185.8.1511

Klimov, A. N., Popov, A. V., Nagornev, V. A., & Pleskov, V. M. (1984). Effect of high density lipoproteins on permeability of the rabbit aorta for low density lipoproteins. *Voprosy Meditsinskoi Khimii*, *30*(6), 9-12. https://doi.org/10.1016/0021-9150(85)90100-5

Li, M. S., Li, Y., Liu, Y., Zhou, X. J., & Zhang, H. (2022). An updated review and meta-analysis of lipoprotein glomerulopathy. *Frontiers in Medicine*, *9*, 905007. https://doi.org/10.2337/db10-0915

Li, Y., Lu, Z., Huang, Y., Lopes-Virella, M. F., & Virella, G. (2016). F (ab′) 2 fragments of anti-oxidized LDL IgG attenuate vascular inflammation and atherogenesis in diabetic LDL receptor-deficient mice. *Clinical Immunology*, *173*, 50-56. https://doi.org/10.1016/j.clim.2016.07.020

Linton, M. F., Yancey, P. G., Davies, S. S., Jerome, W. G., Linton, E. F., Song, W. L., ... & Vickers, K. C. (2019). The role of lipids and lipoproteins in atherosclerosis. *Endotext [Internet]*. https://www.ncbi.nlm.nih.gov/books/NBK343489/

Lopes-Virella, M. F., & Virella, G. (2013). Pathogenic role of modified LDL antibodies and immune complexes in atherosclerosis. *Journal of Atherosclerosis and Thrombosis*, *20*(10), 743-754. https://doi.org/10.5551/jat.19281

Lopes-Virella, M. F., & Virella, G. (2019). Modified LDL immune complexes and cardiovascular disease. *Current Medicinal Chemistry*, *26*(9), 1680-1692. https://doi.org/10.2174/0929867325666180524114429

Lopes-Virella, M. F., Hunt, K. J., Baker, N. L., Lachin, J., Nathan, D. M., Virella, G., & Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. (2011). Levels of oxidized LDL and advanced glycation end products–modified LDL in circulating immune complexes are strongly associated with increased levels of carotid intima-media thickness and its progression in type 1 diabetes. *Diabetes*, *60*(2), 582-589. https://doi.org/10.2337/db10-0915

Millar, J. S., Anber, V., Shepherd, J., & Packard, C. J. (1999). Sialic acid-containing components of lipoproteins influence lipoprotein-proteoglycan interactions. *Atherosclerosis*, *145*(2), 253-260. https://doi.org/10.1016/S0021-9150(99)00071-4

Morganelli, P. M., Kitzmiller, T. J., Hemmer, R., & Fanger, M. W. (1992). Redirected targeting of LDL to human monocyte Fc gamma receptors with bispecific antibodies. *Arteriosclerosis and Thrombosis: A Journal of Vascular Biology*, *12*(10), 1131-1138. https://doi.org/10.1161/01.ATV.12.10.1131

Nagarajan, S. (2007). Anti-oxLDL IgG blocks oxLDL interaction with CD36, but promotes FcγR, CD32A-dependent inflammatory cell adhesion. *Immunology letters*, *108*(1), 52-61. https://doi.org/10.1016/j.imlet.2006.09.008

Oksjoki, R., Kovanen, P. T., Lindstedt, K. A., Jansson, B., & Pentikäinen, M. O. (2006). OxLDL–IgG immune complexes induce survival of human monocytes. *Arteriosclerosis, Thrombosis and Vascular Biology*, *26*(3), 576-583. https://doi.org/10.1161/01.ATV.0000201041.14438.8d

Orekhov, A. N., Bobryshev, Y. V., Sobenin, I. A., Melnichenko, A. A., & Chistiakov, D. A. (2014). Modified low density lipoprotein and lipoprotein-containing circulating immune complexes as diagnostic and prognostic biomarkers of atherosclerosis and type 1 diabetes macrovascular disease. *International Journal of Molecular Sciences*, *15*(7), 12807-12841. https://doi.org/10.3390/ijms150712807

Orekhov, A. N., Kalenich, O. S., Tertov, V. V., & Novikov, I. D. (1991). Lipoprotein immune complexes as markers of atherosclerosis. *International Journal of Tissue Reactions*, *13*(5), 233-236. https://pubmed.ncbi.nlm.nih.gov/1806545/

Palinski, W., Miller, E., & Witztum, J. L. (1995). Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis. *Proceedings of the National Academy of Sciences*, *92*(3), 821-825. https://doi.org/10.1073/pnas.92.3.821

Rabizadeh, S., Rajab, A., Mechanick, J. I., Moosaie, F., Rahimi, Y., Nakhjavani, M., & Esteghamati, A. (2021). LDL/apo B ratio predict coronary heart disease in Type 2 diabetes independent of ASCVD risk score: A case-cohort study. *Nutrition, Metabolism and Cardiovascular Diseases*, *31*(5), 1477-1485. https://doi.org/10.1016/j.numecd.2021.01.013

Rafieian-Kopaei, M., Setorki, M., Doudi, M., Baradaran, A., & Nasri, H. (2014). Atherosclerosis: Process, indicators, risk factors and new hopes. *International Journal of Preventive Medicine*, *5*(8), 927. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4258672/

Salonen, J. T., Korpela, H., Salonen, R., Nyyssonen, K., Yla-Herttuala, S., Yamamoto, R., ... & Witztum, J. L. (1992). Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *The Lancet*, *339*(8798), 883-887. https://doi.org/10.1016/0140-6736(92)90926-T

Sobenin, I. A., Salonen, J. T., Zhelankin, A. V., Melnichenko, A. A., Kaikkonen, J., Bobryshev, Y. V., & Orekhov, A. N. (2014). Low density lipoprotein-containing circulating immune complexes: Role in atherosclerosis and diagnostic value. *BioMed Research International*, *2014*. https://doi.org/10.1155/2014/205697

Steinbrecher, U. P., Zhang, H., & Lougheed, M. (1990). Role of oxidatively modified LDL in atherosclerosis. *Free Radical Biology and Medicine*, *9*(2), 155-168. https://doi.org/10.1016/0891-5849(90)90119-4

Summerhill, V. I., Grechko, A. V., Yet, S. F., Sobenin, I. A., & Orekhov, A. N. (2019). The atherogenic role of circulating modified lipids in atherosclerosis. *International Journal of Molecular Sciences*, *20*(14), 3561. https://doi.org/10.3390/ijms20143561

Sveen, K. A., Bech Holte, K., Svanteson, M., Hanssen, K. F., Nilsson, J., Bengtsson, E., & Julsrud Berg, T. (2021). Autoantibodies against methylglyoxal-modified apolipoprotein B100 and apob100 peptide are associated with less coronary artery atherosclerosis and retinopathy in long-term type 1 diabetes. *Diabetes Care*, *44*(6), 1402-1409. https://doi.org/10.2337/dc20-2089

Tertov, V. V., Orekhov, A. N., Kacharava, A. G., Sobenin, I. A., Perova, N. V., & Smirnov, V. N. (1990). Low density lipoprotein-containing circulating immune complexes and coronary atherosclerosis. *Experimental and Molecular Pathology*, *52*(3), 300-308. https://doi.org/10.1016/0014-4800(90)90071-K

Tertov, V. V., Sobenin, I. A., Orekhov, A. N., Jaakkola, O., Solakivi, T., & Nikkari, T. (1996). Characteristics of low density lipoprotein isolated from circulating immune complexes. *Atherosclerosis*, *122*(2), 191-199. https://doi.org/10.1016/0021-9150(95)05737-4

Virella, G., & Lopes-Virella, M. F. (2003). Lipoprotein autoantibodies: Measurement and significance. *Clinical and Vaccine Immunology*, *10*(4), 499-505. https://doi.org/10.1128/CDLI.10.4.499-505.2003

Van den Berg, V. J., Haskard, D. O., Fedorowski, A., Hartley, A., Kardys, I., Caga-Anan, M., ... & Khamis, R. Y. (2018). IgM anti-malondialdehyde low density lipoprotein antibody levels indicate coronary heart disease and necrotic core characteristics in the Nordic Diltiazem (NORDIL) study and the Integrated Imaging and Biomarker Study 3 (IBIS-3). *EBioMedicine*, *36*, 63-72. https://doi.org/10.1016/j.ebiom.2018.08.023

Virella, G., Derrick, M. B., Pate, V., Chassereau, C., Thorpe, S. R., & Lopes-Virella, M. F. (2005). Development of capture assays for different modifications of human low-density lipoprotein. *Clinical and Vaccine Immunology*, *12*(1), 68-75. https://doi.org/10.1128/CDLI.12.1.68-75.2005

Wang, Y., Liu, X., Xia, P., Li, Z., FuChen, X., Shen, Y., ... & Zhang, J. (2022). The Regulatory Role of MicroRNAs on Phagocytes: A Potential Therapeutic Target for Chronic Diseases. *Frontiers in Immunology*, *13*, 901166. https://doi.org/10.3389/fimmu.2022.901166

Ylä-Herttuala, S., Palinski, W., Butler, S. W., Picard, S., Steinberg, D., & Witztum, J. L. (1994). Rabbit and human atherosclerotic lesions contain IgG that recognizes epitopes of oxidized LDL. *Arteriosclerosis and thrombosis: A Journal of Vascular Biology*, *14*(1), 32-40. https://doi.org/10.1161/01.ATV.14.1.32