Experimental Research

ANNALS OF MEDICINE & SURGERY

OPEN

Exploring the link between T-regulatory cells and inflammatory cytokines in atherogenesis: findings from patients with stable angina pectoris

Muriel Tahtouh Zaatar, PhD[b,*], Rima Othman, MD[a], Elias Abou Samra, PhD[a], Marc Karam, PhD[a]

Background: Atherosclerosis, a chronic inflammatory disease impacting arteries, is closely linked to cardiovascular conditions. Dyslipidemia, marked by high low-density lipoprotein (LDL), low high-density lipoprotein (HDL), and increased plasma triglycerides, is a key risk factor. Atherogenesis begins when modified lipoproteins like oxidized LDL (ox-LDL) activate the immune system. This study explores the roles of T-regulatory cells (Tregs) and interleukins 10 (IL-10), 6 (IL-6), and 17 (IL-17) in atherogenesis.

Methods: Samples were collected from the Hospital patients with stable angina pectoris (SAP). Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll density gradient and analyzed via flow cytometry. IL-10, IL-6, and IL-17 levels in cell culture supernatant were measured using ELISA. Data were expressed as mean ± SEM and analyzed with statistical software.

Results: Results indicate that only patients exhibited reduced Treg and IL-10 levels after high-dose ox-LDL treatment. Significant IL-6 reduction was observed in both NCA and SA groups after high-dose n-LDL and low/high ox-LDL treatments compared to untreated PBMCs.

Conclusions and future directions: Future research will explore n-LDL and ox-LDL effects on Th17/Treg immune responses within a specific cytokine environment known for inducing inflammation, assessing their potential role in atherosclerosis progression.

Keywords: atherosclerosis, dyslipidemia, inflammatory cytokines, T-regulatory cells

Introduction

Atherosclerosis is a chronic inflammatory disease that affects the arteries and leads to a group of cardiovascular diseases (CVDs)[1]. In 2017, CVDs were responsible for an estimated 17.8 million deaths worldwide, resulting in 330 million life years lost and 35.6 million years of disability-adjusted life[2]. Recent studies have shown that the incidence rate of CVDs in individuals aged 18–50 has either remained stable or has increased, while the incidence rate in individuals aged above 50 has remained unchanged[3]. Overall, the prevalence of CVDs has increased due to several risk factors, including dyslipidemia, which is characterized by an increase in low-density lipoprotein (LDL) and a decrease in high-density lipoprotein (HDL), as well as an increase in plasma triglycerides. Dyslipidemia is often associated with obesity, metabolic syndrome, insulin resistance, and diabetes mellitus, which collectively can trigger epithelial dysfunction and the accumulation of lipids in vessel walls[4]. In addition to dyslipidemia, hypertension, smoking, obesity, diabetes, race, and other

HIGHLIGHTS

- Limited immune modulation: Native-low-density lipoprotein (LDL) and oxidized LDL alone do not significantly modulate immune responses at peripheral sites, particularly in patients with stable angina pectoris (SAP).
- Tolerance effect: Even high doses of n-LDL and oxidized LDL (ox-LDL) administered to peripheral blood mononuclear cells (PBMCs) are insufficient to overcome the immune tolerance generated by antigen-presenting cells (APCs).
- Future research: Future investigations will focus on assessing the impact of n-LDL and ox-LDL on Th17/Treg immune responses within a specific cytokine environment associated with inflammation. The aim is to determine if this combination of lipoproteins and inflammatory cytokines contributes to atherosclerosis progression.
- Immunization approaches: Other studies explore immunization against ox-LDL, both passively through anti-ox-LDL antibody injections and actively by administering ox-LDL itself. Such approaches hold promise for potential vaccine development to prevent atherosclerosis.
- Emerging research avenues: Ongoing research directions include miRNA targeting for dyslipidemia and endothelial cellular function, as well as investigations into neural guidance cues regulating macrophage migration to inflammation sites. These avenues represent novel areas of study in atherosclerosis research.

[a]Department of Biology, University of Balamand, Balamand, Lebanon and [b]Department of Biological and Physical Sciences, American University in Dubai, Dubai, United Arab Emirates

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

*Corresponding author. Address: Biological and Physical Sciences Department, American University in Dubai, P.O. Box 28282, Dubai, U.A.E. Tel.: +971 431 835 29. E-mail: mzaatar@aud.edu (M. Zaatar).

Annals of Medicine & Surgery (2024) 86:4456–4462

Received 5 November 2023; Accepted 27 April 2024

Published online 4 June 2024

http://dx.doi.org/10.1097/MS9.0000000000002150

environmental factors also play a role in the atherosclerotic inflammation of the vessel wall and the development of atherosclerosis[5]. Cholesterol is transported in the blood by LDL particles, which contain esterified cholesterol and triglycerides surrounded by a shell of phospholipids, free cholesterol, and apolipoprotein B100 (ApoB100). These circulating LDL particles can accumulate in the intima, the innermost layer of the artery, where ApoB100 binds to proteoglycans of the extracellular matrix through ionic interactions[5]. This subendothelial retention of LDL particles makes them prone to oxidative modifications, which can generate reactive aldehydes through modifications of fatty acid residues in phospholipids, cholesteryl esters, and triglycerides[5]. Modified phospholipids can initiate innate inflammatory responses, leading to the release of bioactive lipids and modifications of the remaining LDL particle, including changes in charge, particle size, lipid content, and other features[5]. Atherogenesis is initiated when modified lipoproteins, such as oxidized LDL (ox-LDL), stimulate both the adaptive and the innate immune systems[6].

The oxidation of LDL into ox-LDL in the subendothelial space induces an inflammatory response characterized by the expression of many cell surface molecules such as E-selectins, P-selectins, and VCAM-1, as well as the production of chemo-attractants such as monocyte chemoattractant protein-1 (MCP-1)[7]. These molecules facilitate the infiltration of monocytes into the subendothelial space, where they differentiate into macrophages and engulf the ox-LDL, becoming lipid-filled foam cells. The presence of these cells in the vessel wall triggers the migration of smooth muscle cells and T cells into the subendothelial space. The smooth muscle cells (SMCs) exhibit a very high rate of proliferation and secrete numerous extracellular matrix proteins, which, together with the multitude of cells already present, form an atheroma[7]. In patients with advanced atherosclerosis, a plaque with a necrotic center containing foam cells, few SMCs, and some new vasculature is observed. A thin cap separates the plaque from the lumen of the vessel. Rupture of this cap allows the contents to spill into the blood, forming a thrombus that may cause strokes or myocardial infarction (MI)[7].

Studies on atherosclerosis have been conducted within many disciplines. Molecular studies of ATP-binding cassette transporter 1 (ABCA1)[8] and their corresponding receptors, present on macrophages and other innate immune system cells, demonstrate that they are essential for cholesterol efflux from macrophages and for their binding to apolipoprotein A-I, forming HDL molecules[9,10]. This efflux may affect the endothelial barrier and contribute to the positive feedback loop of inflammation, which is maintained by a combination of cytokines, increased ox-LDL levels, and high blood pressure[11].

Ox-LDL and its components have been reported to activate innate immunity by binding to Toll-like receptors (TLR-1, 2, 3, 4, 5, 7, 9), although this is controversial[11]. Innate immune cells such as neutrophils initially infiltrate the site of inflammation and are replaced by monocytes within a few hours, as they have a short life span. Notably, neutrophils play a role in mediating the inflammatory changes in atherosclerosis. Studies show that neutrophil extracellular traps (NETs) mediate IL-1 production, which is a pro-inflammatory cytokine[11]. Moreover, T and B lymphocytes of the adaptive immune system also play an important role in atherosclerosis. While both B1 and B2 cells are present in both healthy and atherosclerotic vessel walls, B1 cells have protective functions against lipid accumulation, while B2 cells are pathogenic. Accordingly, B2 cells are present in higher levels in an atherosclerotic vessel wall[11]. On the other hand, T helper cells change their activation state and, hence, their cytokine production according to the stage of atherosclerotic development. While the key player in the T cells is T-regulatory (Treg) cells because they express the lineage-defining transcription factor FoxP3. This transcription factor is thought to induce CD4 + cells differentiation into Th1, Th2, Th17, or follicular helper cells[12]. Meanwhile, Tregs themselves may become unstable and, hence, lose their FoxP3 production; in this case, they transform into ExTregs that act as a pro-inflammatory mediator, especially in atherosclerosis[12].

Cytokines play a significant role in plaque formation and development through multiple mechanisms. They induce endothelial cell activation, which may result in endothelial dysfunction, and upregulate adhesion molecules that allow monocytes to attach and enter the subendothelial space, providing a contact point with ox-LDL molecules that transform into ox-LDL-filled macrophages. Other cytokines affect smooth muscle cells in the vessel wall, promoting their proliferation and migration. In later stages, pro-inflammatory cytokines can also promote cap rupture and thrombus formation[11]. One of the cytokines involved in atherogenesis is interleukin-17 (IL-17), which induces the expression of the pro-inflammatory cytokines interleukin-6 (IL-6) and TNF-α. In vitro, the activation of the Th17 subset can be achieved by IL-6, a multifunctional protein produced by a multitude of cells, including T cells, monocytes/macrophages[13]. This activation leads to the induction of the orphan nuclear receptor RORγt expression. Working synergistically with STAT3, the RORγt transcription factor promotes the expression of IL-17 and the suppression of Foxp3 transcription factor responsible for the commitment of Treg cells. Hence, IL-6 and IL-17 are tightly correlated with each other[13]. Finally, IL-10, which is mainly produced by Th2 cells, is a major anti-inflammatory cytokine that functions as a key gatekeeper of the fibrotic pathway and scar formation via both autocrine and paracrine mechanisms[14]. Scar formation is the endpoint of any organ or tissue inflammation, and with resulting fibrosis, one can assume that the atherosclerotic plaque is more stable and, hence, less likely to rupture and cause unstable angina[14].

Materials and methods

Patient selection

The sample was selected from patients with stable angina pectoris (SAP) who underwent catheterization at the Hospital (N total = 15 (NCA: 5 and SA: 10)). The control group comprised patients with normal coronary artery disease, matched for age (between 55 and 65), sex, and BMI. Exclusions were made for patients with hypertension or diabetes, as well as those receiving lipid-lowering drugs, anti-inflammatory or immune-suppressive agents. Patients with thrombo-aneurism, disseminated intravascular coagulation, advanced liver disease, renal failure, malignant disease, vulvar heart, or atrial fibrillation were also excluded.

Blood collection and PBMC isolation

A total of 30 ml of peripheral blood (PB) was collected from all patients in a fasting state. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient. Cell count and viability were determined using the Trypan Blue exclusion

method. PBMCs were suspended in complete culture medium (RPMI 1640) supplemented with 10% FBS at a density of 5×10^6 cells/ml.

Cell culture and treatment

For cell culture treatment, n-LDL and ox-LDL were added at concentrations of 50 and 100 μg/mL, respectively. The cells were incubated at 37°C with 5% CO_2 for 5 days. The cell culture supernatant was collected and stored at −20°C for cytokine analysis. These methodologies were employed in accordance with established protocols and were consistent with concentrations reported in the existing literature.

T-regulatory cell detection

T-regulatory cells were detected by flow cytometry using 1×10^6 PBMCs stained with Per-CPeFluor710-conjugated anti-human CD4 and the Phycoerythrin (PE)-conjugated anti-human CD25 with their appropriate isotype controls. After a 30-min incubation in the dark, the cells were washed with stain buffer and fixed and permeabilized with the Fix/Perm reagent. Intracellular staining was performed with Alexa Fluor 488-conjugated anti-human Foxp3 and its isotype control antibody for 30 min at 4°C. The frequency of Treg (CD4 + CD25 + Foxp3 +) was expressed as a percentage of CD4 + T cells by sequential gating on lymphocytes and CD4 + T cells.

Cytokine analysis

The levels of interleukin-10 (IL-10), IL-6, and IL-17 in the cell culture supernatant were measured by enzyme-linked immunosorbent assay (ELISA) using commercially available kits and processed according to the manufacturer's instructions. The readings were taken at 450 nm on a Thermo-ELISA microplate reader, with minimal detectable concentrations of 3.125 pg/ml for IL-6, 7.8 pg/mL for IL-10, and 31.2 pg/mL for IL-17. Intra- and inter-assay coefficients of variation were less than 5%.

Data analysis

Data were expressed as mean ± standard error of the mean (SEM) and analyzed using SPSS 11.0 software (LEAD Technologies, Inc.). One-way analysis of variance (ANOVA) with a post-hoc test for multiple comparisons was used to determine statistical significance for variations in T-regulatory cell percentages and cytokine levels between different treatment conditions within the same group. Independent *t*-tests were used to determine significant differences in T-regulatory percentages and cytokine levels between the SA and NCA groups. A p value less than 0.05 was considered statistically significant.

Results

Patients characteristics

Table 1 presents the demographic characteristics of the patient and normal coronary artery groups. Both groups were matched for age, gender, and BMI. The only variable that was not adjusted for is the smoking rate. Blood analysis results showed that levels of blood triglycerides, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), total cholesterol (TC), CRP, and WBCs were within normal ranges for both patient and control groups. CRP is a liver-secreted protein and lipid levels are good plasma markers for inflammation. In our study, both measures indicate the absence of inflammatory stress in the patient and normal coronary artery groups.

Table 1

Patients and healthy groups profile.

Items*	NCA	SA
N total ($n = 15$)	5	10
Age	58	60
Sex (F, M)	2F, 3M	2F, 8M
BMI	27	25.7
Diabetic	None	None
Smoking rate, *n* (%)	0	8 (80)
Total cholesterol (mg/dl)	170	178
Triglyceride (mg/dl)	150	154
HDL-C (mg/dl)	60	54
LDL-C (mg/dl)	100	110
hs-CRP (mg/dl)	< 3	< 3
White blood cells (WBC's)	6120	7130

CRP, C-reactive protein; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; NCA, normal coronary artery; SA, stable angina.
*$P < 0.05$ vs. NCA.

Effect of n-LDL and ox-LDL on the frequency of (CD4 + CD25 + FoxP3 +) T-regulatory Cells

(Fig. 1A & B) display the distribution of individual T-regulatory percentages around the calculated mean value in normal coronary and patient groups. For normal subjects, no significant difference in the frequency of T-regulatory cells was detected between the untreated well (control well) and the four treated conditions, nor was there any significant difference between the wells treated with different doses of n-LDL and ox-LDL (Fig. 1A). In contrast, the patient group exhibited a significant decrease in T-regulatory cells in the well-treated with a high dose of ox-LDL compared to the untreated or control well, while no significance was observed in the remaining treated wells (Fig. 1B). The graph comparing the same conditions between the two study groups, SA and NCA, showed similar percentages of CD4 + CD25 + Foxp3 + T-regulatory cells in both patients and control groups (Fig. 1C).

Effect of n-LDL and ox-LDL on IL-10 secretion levels

After ELISA analysis, there was no significant difference in the secretion levels of interleukin-10 in the NCA group between both low (50 μg/ml) and high (100 μg/ml) concentrations of native-LDL and oxidized LDL, as compared to the untreated well (Fig. 2A). However, in the SA group, the secreted interleukin-10 levels were significantly lower in the well-treated with a high dose of oxidized LDL (100 μg/ml) than in the untreated well (Fig. 2B). There was no significant difference in the interleukin-10 secretion levels between the NCA and SA groups for the five different conditions, in line with the T-regulatory results (Fig. 2C).

Effect of n-LDL and ox-LDL on IL-6 secretion levels

In the normal coronary artery group, the interleukin-6 secretion levels were significantly lower in the wells treated with high dose of n-LDL and with both concentrations of ox-LDL as compared to the untreated well (Fig. 3A) *P less than 0.05. Similarly, in the patient group (SA), we observed a significant decrease in

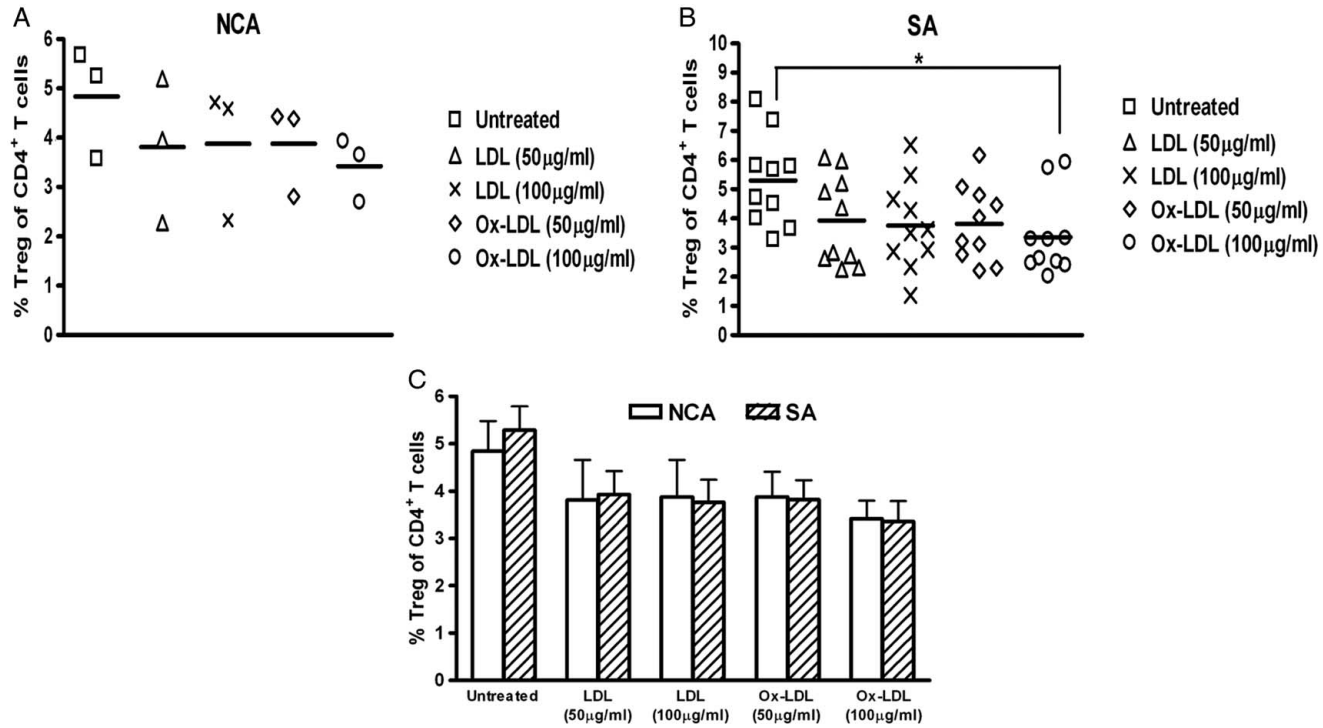

Figure 1. Percentage of T-regulatory cells of CD4 + T cell population after n-LDL and ox-LDL treatment in (A) NCA group ($n = 3$) and (B) SA group ($n = 10$). (A, B) Show the percentage of T-regulatory cells of CD4 + T cell population after treatment with n-LDL and ox-LDL in the NCA and SA groups, respectively. The frequency of cells in each group was compared in reference to the untreated condition and between the different wells of treatment. (C) Represents the comparison between the two groups of study (NCA vs. SA) for the same conditions. Significance was detected for $^*P < 0.05$. LDL, low-density lipoprotein; NCA, normal coronary artery; ox-LDL, oxidized LDL; SA, stable angina ($^*P < 0.05$ vs. NCA).

interleukin-6 cytokine levels while comparing the IL-6 secretion in the last 3 treatments to the control well (Fig. 3B) $^*P$ less than 0.05. Figure 3C compares the IL-6 secretion levels between NCA and SA groups, indicating that SA subjects were more prone to a decrease in IL-6 secretion in the last 3 treatments compared to the normal coronary group ($^*P < 0.05$ vs. NCA).

Discussion

Inflammatory status of SA and NCA groups

Many studies have shown that an increase in peripheral blood levels of ox-LDL may be associated with the pathogenesis of ACS (acute coronary syndrome) and could serve as a potential marker of unstable atherosclerotic plaque[15].

Native-LDL as an immuno-modulator

Animal experiments, epidemiological studies, and clinical investigations have established that high circulating concentrations of cholesterol promote atherosclerotic cardiovascular disease. Malondialdehyde, 4-hydroxynonenal, and other molecular species generated through lipid peroxidation can form adducts on lysyl residues of ApoB100. Proteins with such modified lysyl residues can be immunogenic, as are modified phospholipid species. It is clear that LDL immunogenicity does not depend on one form of oxidation, and it is possible that even minimal or slight modification of native-LDL can lead to the progression of the disease depending on the inflammatory and oxidative milieu surrounding the cells. As discussed above, LDL oxidation generates a range of modifications with various physicochemical properties. Whereas heavily oxidized LDL particles have little similarity to native ones, most oxidative events can cause limited changes to LDL, and the particles maintain most of the features of native particles, including antigenicity. The understanding of LDL immunochemistry is still limited, and further studies will be needed to clarify the role of oxidation in autoimmune responses to LDL. Therefore, in our study, choosing native-LDL as a treatment condition not only serves as a control in comparison to oxidized LDL effects but also ensures that n-LDL could not be immunogenic or recognized by the innate immune system through Toll-like receptors (TLR's) or recognized by antigen-presenting cells (APC's) such as macrophages and dendritic cells or the components of the adaptive immune system[16]. Recent studies have highlighted the potential role of native-LDL in modulating the immune response, with some evidence suggesting it may possess anti-inflammatory properties[17,18]. It was clear to us that the latter yielded the same trend of results with respect to interleukin-6 secretions.

Interleukin-10 and Tregs response to ox-LDL

Interleukin-10 is a major anti-inflammatory cytokine that plays a crucial role in limiting inflammation. It is mainly produced by Treg and Th2 cells and is present on atherosclerotic plaques to counteract the actions of interleukin-12 produced by monocytes[19]. Clinical studies have measured IL-10 levels in patients with stable and unstable angina pectoris and found that it is much lower in the

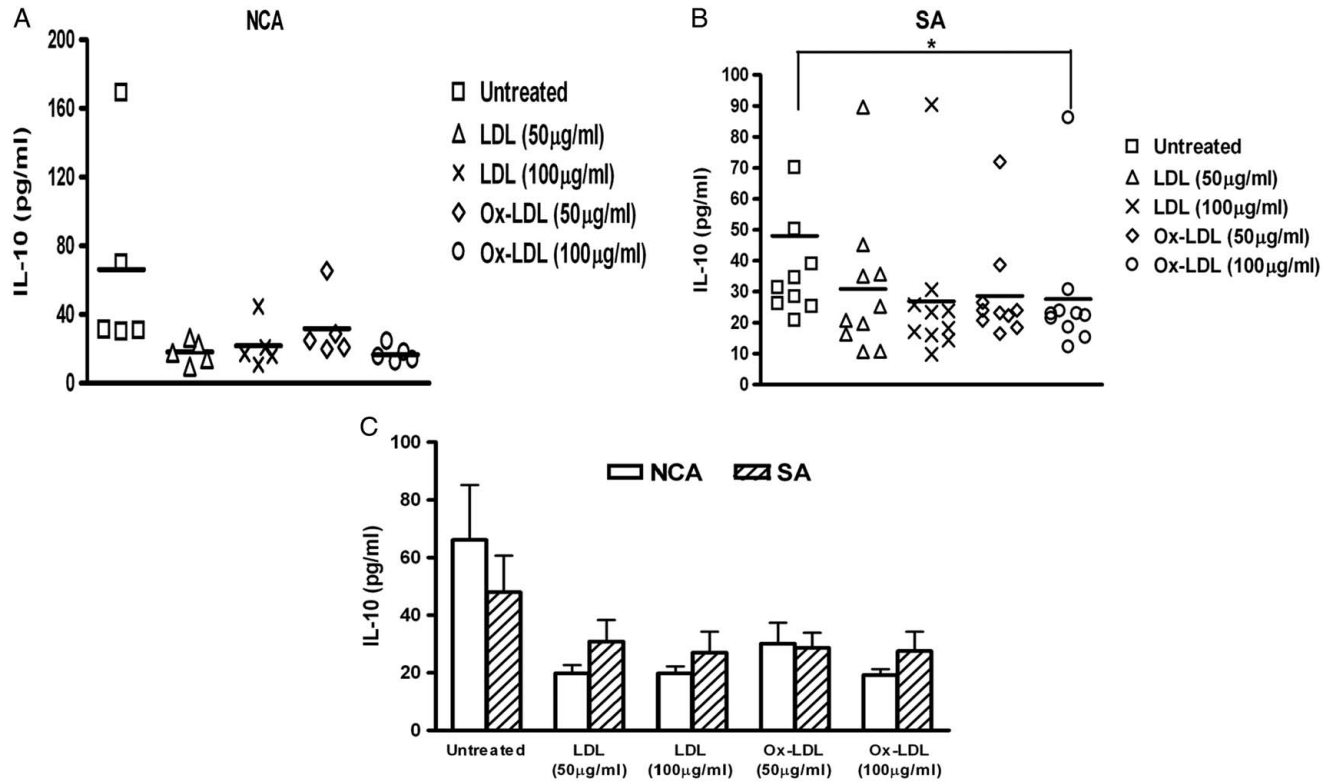

Figure 2. Cell culture supernatant levels of Interleukin-10 in (A) NCA group ($n = 5$) and (B) SA group ($n = 10$). (A, B) The secreted IL-10 in each group was compared in reference to the untreated condition and between the 4 different conditions of treatment. As for the comparison between the 2 groups of study (C) NCA vs. SA, IL-10 levels were compared between the same conditions. Significance was detected for $^*P < 0.05$. LDL, low-density lipoprotein; NCA, normal coronary artery; ox-LDL, oxidized LDL; SA, stable angina ($^*P < 0.05$ vs. NCA).

latter[20]. Our results show that only patients were vulnerable to a decrease in Treg and IL-10 levels after treatment with a high dose of oxidized LDL (Figures 3A and 3B). As there were no differences detected between SA and NCA background profiles (Table 1), our results suggest that SA Treg percentages and IL-10 secretion levels decreased significantly after the addition of 100 µg/ml oxidized LDL, whereas no significant decrease was detected in the NCA group for the same conditions of comparison. This difference in response for SA and NCA groups could be due to low chronic inflammatory stress exerted inside the coronary intimal region of patients, leading to the induction of pro-inflammatory responses by emigrating APCs from arterial intima into the blood circulation. In other words, patient's PBMCs may be more prone than normal coronary artery cells to induce inflammatory immune responses to take over the regulatory anti-inflammatory mechanism. However, the inflammatory priming hypothesis of PBMCs in the patients' group was not reliable for explaining our findings. Both SA and NCA groups showed similar results concerning IL-10 secretions and Treg cells percentages. The association may not be statistically significant due to the small number of subjects in the NCA group.

IL-6 and IL-17 potential role in inflammatory response

Based on the previous information, it is logical to predict that IL-6 and IL-17 secretion levels would increase under atherogenic inflammatory stress. However, our results show a significant decrease in IL-6 secretion levels for both NCA and SA groups in cells treated with a high dose of n-LDL and in those treated with low or high concentrations of ox-LDL compared to the PBMCs in the untreated condition. The ELISA for IL-6 (Figures 3B and 3C) shows that it had even reached lower levels in SA subjects compared to the NCA group. This is controversial since we previously mentioned that the PBMCs of patients are more prone to develop inflammatory immune responses. These contradictory results rendered our interpretation irrelevant and required us to shift our discussion to the peripheral tolerance effect that can be present in both SA and NCA groups. This effect downregulates the co-stimulatory signals on peripheral resident APCs of both patient and control subjects. When APCs lose their co-stimulatory power, they create a tolerant effect against antigens such as LDL and ox-LDL by silencing T cell immune responses, a mechanism known as anergy effect. However, danger signals generated during atherogenesis, such as increased levels of CRP, plasma oxidized LDL, and other inflammatory markers, may activate local macrophages and dendritic cells. This will lead to a switch from tolerance to the activation of adaptive immunity responsible for mediating disease progression. In addition, the LDL particle is a major circulating plasma component; therefore, immunological tolerance to this particle is necessary for survival. Recent studies have found that certain peptides modulate the immune response[21].

Peripheral tolerance effect and reactivity to modified LDL

The inflammatory background profile of both NCA and SA groups (Table 1) shows that the levels of HDL, LDL, total cholesterol (TC), triglycerides (TG), C-reactive proteins (CRP), and white blood cell (WBC) counts are within the normal ranges. The

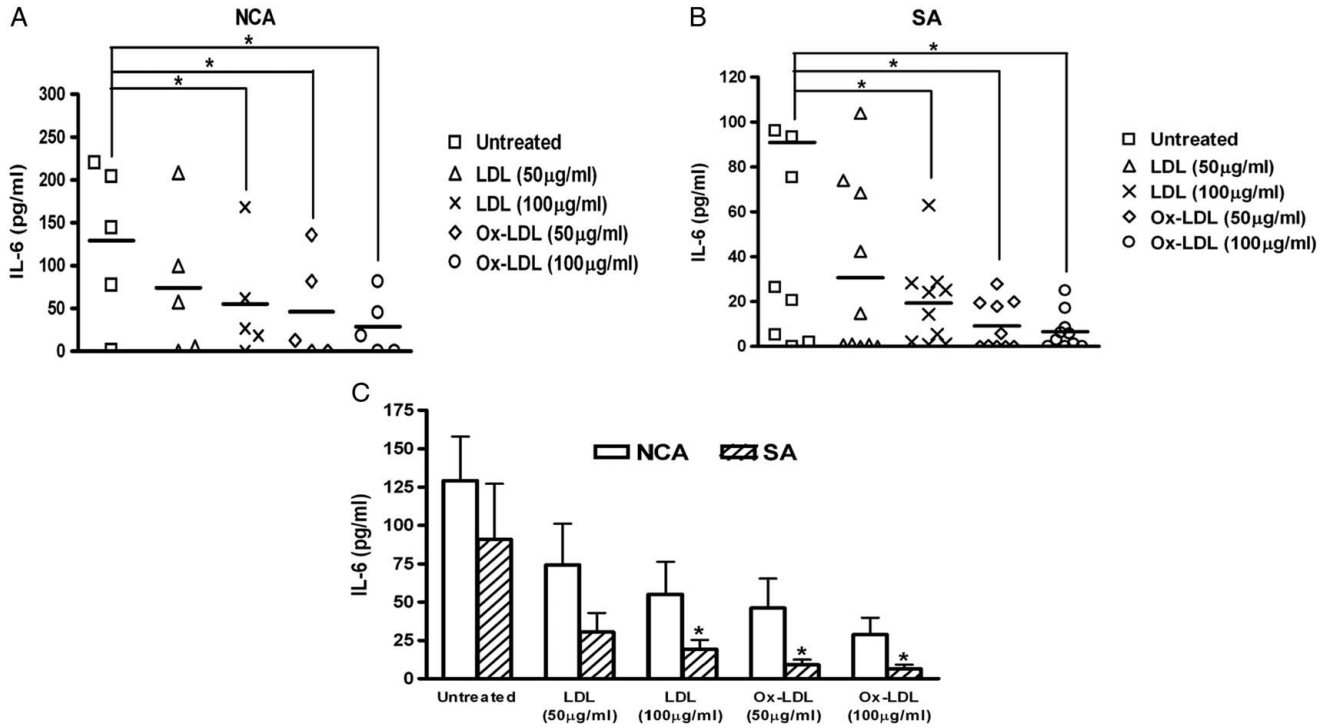

Figure 3. Cell culture supernatant levels of IL-6 cytokine in (A) NCA group ($n = 5$) and (B) SA group ($n = 10$). For (A, B), the IL-6 secretion levels in each group were compared with reference to the untreated condition and between the remaining 4 treatments. As for the comparison between the 2 groups of study (C) NCA versus SA, IL-6 levels were compared between the same conditions. Statistical significance was set at $^*P < 0.05$. LDL, low-density lipoprotein; NCA, normal coronary artery; ox-LDL, oxidized LDL; SA, stable angina ($^*P < 0.05$ vs. NCA).

absence of inflammatory markers in plasma may result in a decrease in pro-inflammatory cytokine secretion like IL-6 by local macrophages.

Navigating within the parameters of our study's constraints, we grappled with several limitations. Inclusion and exclusion factors, time considerations, funding constraints, and the inherent challenges in obtaining patient consent posed intricate hurdles. Yet, within these confines, we strategically maximized the sample size (N), underscoring the careful balance required in such multifaceted investigations.

The robust features of our work pivot on the innovative use of PBMCs sourced from cardiovascular patients undergoing bypass surgery. This distinctive approach, coupled with the treatment involving various combinations of oxidized and native-LDL, constitutes the cornerstone of our study's novelty and strength.

In acknowledging the multifaceted nature of our study, it is imperative to consider potential confounding factors that may influence inflammatory responses. Notably, the impact of smoking on immune modulation warrants attention, and future discussions could explore its implications as a potential confounding factor on our study findings. This consideration aligns with the broader context of understanding the interplay between lifestyle factors and atherosclerosis development.

Conclusion

Our study showed that native-LDL and oxidized LDL, when administered individually, do not autonomously modulate immune responses at peripheral sites in patients with SAP. Even when PBMCs were treated with elevated dosages of n-LDL and ox-LDL, they were unable to overcome the tolerant effect instigated by APCs.

As we look ahead, our research trajectory steers toward investigating the nuanced impact of n-LDL and ox-LDL on Th17/Treg immune responses within a meticulously defined cytokine milieu known for its role in inflammation induction. This exploration seeks to unravel whether the interplay between lipoproteins and inflammatory cytokines can potentially mediate atherosclerosis progression.

Noteworthy are other promising avenues explored in existing studies, such as passive immunization through the injection of anti-ox-LDL antibodies or active immunization by injecting ox-LDL itself[22]. These approaches hold significant promise for the development of a vaccine aimed at preventing atherosclerosis.

Embracing novel research directions, our ongoing investigations include delving into miRNA targeting dyslipidemia and endothelial cellular function. Additionally, we are exploring neural guidance cues regulating macrophage migration to inflammation sites[10].

In conclusion, while our current study underscores the intricate limitations within which it was conducted, the identified avenues for future exploration, coupled with the careful maximization of our sample size, further emphasize the significance and promise of continued research in the intricate landscape of atherosclerosis.

Ethical approval

Hotel Dieu de France Ethics Committee, approve Protocol 310. Ref 2010/23.

Consent

Written informed consent was obtained from the patient for publication and any accompanying images. A copy of the written consent is available for review by the Editorin-Chief of this journal on request.

Source of funding

This research was supported by the University of Balamand—Lebanon.

Author contribution

E.A.S.: data collection, analysis, and writing the paper. R.O.: data analysis and writing the paper. M.K.: data analysis and writing the paper. M.Z.: data analysis, writing the paper, and supervision of the final paper design and submission.

Conflicts of interest disclosure

The authors declare that they have no conflict of interest. We confirm that this research was conducted in compliance with ethical guidelines and regulations, and that all necessary approvals were obtained. We believe that the results reported in this paper are objective, unbiased, and of scientific and practical significance.

Research registration unique identifying number (UIN)

Not applicable.

Guarantor

Elias Abou Samra Rima Othman Marc Karam Muriel Zaatar.

Data availability statement

Data sharing is not applicable to this article.

Provenance and peer review

Not applicable.

Acknowledgements

The authors appreciate UOB and AUD for providing an environment conducive to innovation and research. The collaborative efforts of these entities have significantly enriched this research.

References

[1] Lu H, Daugherty A. Atherosclerosis. Arterioscler Thromb Vasc Biol 2015;35:485–91; Erratum in: Arterioscler Thromb Vasc Biol. 2016 36:e32. PMID: 25717174; PMCID: PMC4511379.

[2] Andersson C, Vasan RS. Epidemiology of cardiovascular disease in young individuals. Nat Rev Cardiol 2018;15:230–40.

[3] Gisterå A, Hansson GK. The immunology of atherosclerosis. Nat Rev Nephrol 2017;13:368–80.

[4] Flora GD, Nayak MK. A brief review of cardiovascular diseases, associated risk factors and current treatment regimes. Curr Pharm Des 2019; 25:4063–84.

[5] Mundi S, Massaro M, Scoditti E, *et al*. Endothelial permeability, LDL deposition, and cardiovascular risk factors—a review. Cardiovasc Res 2018;114:35–52.

[6] Wolf D, Ley K. Immunity and inflammation in atherosclerosis. Circ Res 2019;124:315–27.

[7] Emini Veseli B, Perrotta P, De Meyer GRA, *et al*. Animal models of atherosclerosis. Eur J Pharmacol 2017;816:3–13.

[8] Xia XD, Yu XH, Chen LY, *et al*. Myocardin suppression increases lipid retention and atherosclerosis via downregulation of ABCA1 in vascular smooth muscle cells. Biochim Biophys Acta (BBA)-Mol Cell Biol Lipids 2021;1866:158824.

[9] Jacobo-Albavera L, Domínguez-Pérez M, Medina-Leyte DJ, *et al*. The role of the ATP-binding cassette A1 (ABCA1) in human disease. Int J Mol Sci 2021;22:1593.

[10] Hartley A, Haskard D, Khamis R. Oxidized LDL and anti-oxidized LDL antibodies in atherosclerosis – novel insights and future directions in diagnosis and therapy. Trends Cardiovasc Med 2019;29:22–6.

[11] Fatkhullina AR, Peshkova IO, Koltsova EK. The role of cytokines in the development of atherosclerosis. Biochemistry (Mosc) 2016;81:1358–70.

[12] Mensah GA, Roth GA, Fuster V. The global burden of cardiovascular diseases and risk factors: 2020 and beyond. Journal of the American College of Cardiology 2019;74:2529–32.

[13] Wang T, Liu W, Xu S, *et al*. The role of Th17 cells/IL-17A in AD, PD, ALS and the strategic therapy targeting on IL-17A. J Neuroinflammation. 2022;19:98.

[14] Steen EH, Wang X, Balaji S, *et al*. The role of the anti-inflammatory cytokine interleukin-10 in tissue fibrosis. Adv Wound Care 2020;9:184–98.

[15] Zhou ZX, Qiang H, Ma AQ, *et al*. Measurement peripheral blood index related to inflammation and ox-LDL, ox-LDLAb in patients with coronary heart disease and its clinical significance. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2006;31:258-62.

[16] Sun J, Zhang M, Chen K, *et al*. Suppression of TLR4 activation by resveratrol is associated with STAT3 and Akt inhibition in oxidized low-density lipoprotein-activated platelets. Eur J Pharmacol 2018;836:1–10.

[17] Melzer S, Gister A, Heiss GH. A novel concept regarding the immunomodulatory properties of native LDL. Atherosclerosis 2023;370: 124–32.

[18] Pinto DS, Fernandes J, Pita R, *et al*. Native LDL reduces atherosclerosis in a mouse model by suppressing macrophage activation and promoting plaque stability. Circulation 2022;146:1232–45.

[19] Wei H, Li B, Sun A, *et al*. Interleukin-10 family cytokines immunobiology and structure. Adv Exp Med Biol 2019;1172:79–96.

[20] Justo-Junior AS, Villarejos LM, Lima XTV, *et al*. Monocytes of patients with unstable angina express high levels of chemokine and pattern-recognition receptors. Cytokine 2019;113:61–7.

[21] Thota LN, Ponnusamy T, Lu X, *et al*. Long-term efficacy and safety of immunomodulatory therapy for atherosclerosis. Cardiovasc Drugs Ther. 2019;33:385–98.

[22] Kobiyama K, Ley K, Atherosclerosis A. Chronic inflammatory disease with an autoimmune component. Circulation Res 2018;123:1118–20.