Curr Atheroscler Rep (2014) 16:435
DOI 10.1007/s11883-014-0435-z

CARDIOVASCULAR DISEASE AND STROKE (P PERRONE-FILARDI AND S AGEWALL, SECTION EDITORS)

Inflammation and Cardiovascular Disease: From Pathogenesis to Therapeutic Target

Enrica Golia · Giuseppe Limongelli · Francesco Natale · Fabio Fimiani · Valeria Maddaloni · Ivana Pariggiano · Renatomaria Bianchi · Mario Crisci · Ludovica D'Acierno · Roberto Giordano · Gaetano Di Palma · Marianna Conte · Paolo Golino · Maria Giovanna Russo · Raffaele Calabrò · Paolo Calabrò

Published online: 19 July 2014

Abstract Atherosclerosis represents the most common pathological substrate of coronary heart disease (CHD), and the characterization of the disease as a chronic low-grade inflammatory condition is now largely accepted. A number of mediators of inflammation have been widely studied, both as surrogate biomarkers and as causal agents, in the pathophysiological network of atherogenesis and plaque vulnerability. The epidemiological observation that biomarkers of inflammation are associated with clinical cardiovascular risk supports the theory that targeted anti-inflammatory treatment appears to be a promising strategy in reducing residual cardiovascular risk on the background of traditional medical therapy. A large number of randomized controlled trials have shown that drugs commonly used in cardiovascular disease (CVD), such as statins, may be effective in the primary and secondary prevention of cardiovascular events through an anti-inflammatory effect. Moreover, several anti-inflammatory drugs are being tested for their potential to reduce residual cardiovascular risk on the background of validated medical therapy for atherosclerotic disease. In this paper, we review relevant evidence with regard to the relationship between inflammation and CVD, from pathogenesis to therapeutic strategies.

This article is part of the Topical Collection on *Cardiovascular Disease and Stroke*

E. Golia · G. Limongelli · F. Natale · F. Fimiani · V. Maddaloni · I. Pariggiano · R. Bianchi · M. Crisci · L. D'Acierno · R. Giordano · G. Di Palma · M. Conte · M. G. Russo · R. Calabrò · P. Calabrò (✉)
Division of Cardiology, Cardio-thoracic and Respiratory Sciences Department, Second University of Naples, AO Dei Colli-Monaldi Hospital, 80131 Naples, Italy
e-mail: paolo.calabro@unina2.it

P. Golino
Division of Cardiology, Cardio-thoracic and Respiratory Sciences Department, Second University of Naples, AO S. Anna e S. Sebastiano, Caserta, Italy

Keywords Atherosclerosis · Inflammation · Statins · inflammasome · IL-1 beta

Introduction

Atherosclerosis represents the most common pathological substrate of coronary heart disease (CHD). It is largely understood that the initiation, progression, and complications of atherosclerotic plaque are complex phenomena involving the interplay of lipoproteins, vascular wall components, blood cells, and the immune system. A number of mediators involved in the inflammatory process are involved in the regulation of this interaction, defining the prognostic role of chronic low-grade inflammation in atherosclerosis a [1]. Indeed, these molecules have been widely studied as both surrogate biomarkers and causal agents in the pathophysiological network of atherogenesis and plaque vulnerability.

Multiple levels of evidence continue to support the close relationship between inflammation and atherosclerosis. Given the multifactorial nature of this process, studies needed to improve our knowledge are necessarily multifaceted, from experimental models, to the assessment of human tissues and systemic biomarkers, to epidemiologic and clinical observations. In this field, a large number of randomized controlled trials have shown that drugs commonly used in cardiovascular disease (CVD), such as statins, may help in the primary and secondary prevention of cardiovascular events via an anti-inflammatory effect.

As inflammation is a central process in the development of CHD, emerging evidence supports the hypothesis that targeting specific inflammatory proteins or pathways can be effective in reducing the risk of cardiovascular events. Several anti-inflammatory drugs are being tested for their potential to

reduce the residual cardiovascular risk on the background of validated medical therapy for atherosclerotic disease.

In this paper, we review relevant evidence regarding the relationship between inflammation and CVD, from pathogenesis to therapeutic strategies.

Atherosclerosis and Inflammation

The concept of atherosclerosis as an inflammatory process, while seemingly relatively recent, is rooted in very early pathological observations [2, 3]. The role of inflammatory mediators together with the cellular effectors in atherogenesis has been identified in the last few decades.

The presence of fatty streaks has been identified as a characteristic of one of the earliest stages of atherosclerosis [4], which can progress to fibrous plaque formation, with subsequent narrowing of the arterial lumen and chronic tissue ischemia. Atherosclerotic plaque may also develop complications (unstable plaque), such as haemorrhage, ulceration, and finally intravascular thrombosis, leading to abrupt arterial occlusion and causing acute coronary syndrome (ACS).

The unique microenvironment of the atherosclerotic plaque and its surrounding tissue is characterized by repeated inflammatory and reparative reaction, which is initiated and amplified by several mediators [4].

Pioneering works by Ross and colleagues described key events in the formation of atherosclerotic plaque, including transendothelial recruitment of macrophage, smooth muscle cell proliferation and migration toward the intimal layer of the vessel wall, and lymphocyte involvement [5]. Subendothelial accumulation of low-density lipoproteins (LDLs) has been identified as a necessary event associated with endothelial injury that initiates atherogenesis [6]. Endothelial dysfunction, which develops at sites where endothelial cell (EC) layers have been injured or placed under metabolic stress – such as in the presence of particular flow patterns, hypertension, diabetes, dyslipidaemia, and metabolic syndrome – is characterized by an imbalance between nitric oxide (NO)- and prostacyclin (PGI2)-mediated vasorelaxation and the increase in endogenous vasoconstrictors such as endothelin-1 (ET-1) [7]. Increased generation of reactive oxygen species (ROS) is also a feature of endothelial dysfunction. In the subendothelial space, LDLs become oxidized (ox-LDL) by ROS, and then exert pro-atherogenic effects. In addition, phospholipase A2 (PLA2) modifies phospholipids in LDL particles to generate atherogenic species. Lipoprotein-associated PLA2 (Lp-PLA2) and secretory PLA2 (sPLA2), in particular, have been linked to atherosclerosis [8]. They are generated by inflammatory cells and modify lipoproteins, leading to more highly oxidized LDL particles. Ox-LDLs induce EC expression of a proinflammatory phenotype, promote the formation of foam cells, and perpetuate endothelial dysfunction [9].

Activated ECs then express adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and selectins. This, together with the secretion of chemoattractant mediators such as complement factors, interleukin (IL)–8, and monocyte chemoattractant protein-1 (MCP-1), determine mononuclear cell recruitment into the vascular wall. Monocytes differentiate into macrophages, which become foam cells via ox-LDL uptake, and then release a variety of proinflammatory cytokines such as soluble CD40 ligand, IL-1, IL-3, IL-8, and IL-18, and tumor necrosis factor (TNF) alpha [10].

Retention of lipoproteins in the vessel wall is associated with the generation of cholesterol crystals, which is another factor exacerbating atherogenesis, inducing cell damage and apoptosis within the plaque. Endogenous products from damaged cells may trigger and amplify the inflammatory response, similar to a pathogen [11]. Cholesterol crystals in particular have been linked to the activation of the so-called inflammasome [12]. Discovered in 2002, the inflammasome is a complex of proteins involved in the maturation and secretion of IL-1 beta [13, 14]. As noted above, IL-1 beta is produced primarily by activated macrophages, ECs, and smooth muscle cells. The nucleotide-binding and oligomerization domain (NOD)-like receptor protein (NLRP) 3 is the most studied among known inflammasomes, and it is involved in the cascade of events leading to the cleavage of IL-1 beta precursor to its functional form. The effects of the IL-1 gene family products are thought to be the result of the balance between levels of active IL-1 beta and IL-1 receptor antagonist (RA), whereas an imbalance of this patterns leads to inflammatory conditions such as arthritis and, most notably, atherosclerosis. Moreover, macrophage autophagy, which is activated under stressful conditions with the aim of promoting cell survival, becomes defective in atherosclerosis. In particular, knockout models of the autophagy gene have demonstrated increased activation of inflammasome in response to cholesterol crystals and subsequent increase in the release of IL-1 beta [15].

The progression to a more fibrous lesion is characterized by the proliferation of vascular smooth muscle cells (VSMCs), which accumulate in the intima and produce extracellular matrix (ECM) [16, 17]. On the other hand, vascular inflammation interferes with fibrous cap formation and induces apoptosis and degradation of the ECM via an upregulation of metalloproteinase (MMP) [17]. The subsequent activation of the coagulation cascade leads to intravascular thrombus formation and the acute clinical events. In this setting, tissue factor (TF), upregulated in the vessel wall by inflammatory stimuli, plays a pivotal role in the pathophysiology of ACS by triggering the formation of intracoronary thrombi following endothelial injury [16, 18].

Indeed, the link between inflammation and atherosclerosis may be more complex, as there is ample evidence of the

involvement of specialized immune response at several stages in the progression of plaque formation. The T helper (Th) cell response has been shown to be critical in the exacerbation of atherosclerosis. In particular, T lymphocytes are activated by one or several antigens in the vessel wall, with subsequent inflammatory cascade and the recruitment of both immune cells and endothelial and smooth muscle cells, followed by an amplification of the pathways, ultimately leading to plaque formation and progression [19••]. Moreover, monocytes may also differentiate into dendritic cells, which act as antigen-presenting cells [20]. The presence of Toll-like receptors (TLRs) within atherosclerotic plaques and on infiltrating leukocytes also provides evidence for the involvement of innate immunity in atherogenesis. TLR4, in particular, is expressed more highly in areas of unstable plaque [19••].

Furthermore, there is very recent evidence of the potential role of microparticles (MPs) and MicroRNAs (miRNAs) in the modulation of these pathways. MPs are submicron fragments of the cellular plasma membrane consisting of phospholipids and proteins, resembling the composition of the original cell and dependent upon the stimulus that triggers their release [21]. Interestingly, ECs, erythrocyte, leucocytes, platelets, and smooth muscle cells are potential sources of MPs. They are released in a regulated manner into circulation and function as paracrine and endocrine effectors. Most notably, they may contain miRNAs and messenger RNAs (mRNA). A variety of inflammatory stimuli may trigger the release of MPs from membranes via cellular activation or induction of apoptosis [22]. Included among them are shear stress, angiotensin II, TNF-alpha, and thrombin. Several studies have shown detrimental effects of MPs on atherogenesis, as they contribute to endothelial dysfunction and ROS generation. MPs have also been found in the plaque, where they may exert MMP activity and induce TNF-alpha shedding, ICAM-1, and TF expression. Conversely, some reports indicate that MPs may play a protective role [22].

MiRNAs are single-stranded noncoding RNAs that affect gene expression by binding to mRNA target sequences [23•]. They have been associated with various biological processes that may contribute to atherosclerosis, such as lipid metabolism and vascular inflammatory response, from the production of adhesion molecules to monocytes differentiation and ox-LDL uptake, smooth muscle cell proliferation, and apoptosis [23•, 24]. Moreover, miRNAs are also thought to interfere with oxidative stress and inflammation in adipose tissue in relation to obesity [23•].

Inflammation as a Therapeutic Target

As noted above, several cell types and mediators have been implicated in the initiation and progression of atherosclerosis. These patterns have been recognized in conditions associated with vascular risk, such as central obesity, diabetes, hypertension, and dyslipidaemia. In particular, recent evidence has indicated that inflammation may represent the pathophysiological link between visceral obesity and CVD, and there has been increasing interest in the role of adipose tissue as an active trigger of this systemic inflammatory response through the release of adipocytokines that might act as both immediate effectors and upstream regulators of the inflammatory cascade [25–27].

Epidemiological observations that biomarkers of inflammation are associated with clinical cardiovascular risk support the theory that treatment targeting inflammation may be a promising strategy on the background of traditional medical therapy in reducing residual cardiovascular risk.

C-reactive protein (CRP) has been one of the most studied candidates as a non-traditional biomarker of cardiovascular risk, as it meets most of the criteria of a useful indicator. Beginning with the pioneering work of Ridker et al. in apparently healthy individuals [28], there have been more than 30 epidemiological studies demonstrating an association between CRP and increased cardiovascular risk [29, 30•], both in primary and secondary prevention. Its incremental utility as a clinical biomarker in comparison with traditional indicators of risk, however, has been called into question. This issue was recently addressed in a comprehensive meta-analysis from the Emerging Risk Factors Collaboration (ERFC) [31•] demonstrating the role of high-sensitivity (hs-CRP) in better risk stratification of subjects at intermediate risk for CVD. Specifically, for every 400 to 500 individuals screened for hs-CRP or fibrinogen level, one additional cardiovascular event could be prevented over a period of 10 years. The utility of Hs-CRP for predicting CVD risk was comparable to that of total and high-density lipoprotein cholesterol or blood pressure.

Several guidelines have subsequently taken into consideration the role of hs-CRP as a risk predictor [32, 33]. Moreover, this concept is again confirmed in recent data from Kaptoge et al. of observations of a prospective cohort of initially healthy individuals [34••]. Circulating levels of several different proinflammatory cytokines were associated with CHD risk independently of several conventional and emerging cardiovascular risk factors. In particular, data emerged from the updated meta-analysis that 1-SD higher baseline levels for each of IL-6, IL-18, and TNF-alpha were associated with a 10–25 % increase in the risk of non-fatal myocardial infarction or CHD death. This effect was less than that associated with CRP, and in subjects where a direct comparison was possible, after adjusting for the entire panel of mediators tested, only hs-CRP as a downstream inflammatory marker and TNF-alpha as an upstream inflammatory marker remained significant predictors of cardiovascular risk.

Role of Statins

Hydroxymethylglutaryl coenzyme A (HMG- CoA) reductase inhibitors, which fast became known for their “pleiotropic”

effects, are the lipid-lowering agents most commonly used in clinical practice (Table 1). The lipid-lowering effect of statins associated with a reduction in cardiovascular risk has been well documented in several large randomized trials within recent decades.

However, a number of studies, and more recently, a large meta-analysis, have confirmed that the majority of the beneficial effects of statins in CVD are independent of their lipid-lowering action. From a pathophysiological point of view, statins inhibit the mevalonate pathway, preventing the formation of isoprenoids, and reduce the expression of proinflammatory mediators, with subsequent reduction in asymmetrical dimethylarginine (ADMA) that is involved in endothelial dysfunction [35, 36]. Specifically, the inhibition of the formation of isoprenoid intermediates prevents modification of transcription factors and membrane receptors (isoprenylation) that alter their function and activate inflammatory patterns.

Furthermore, studies have shown that statins reduce the levels of CRP by 25 % to 50 % [37–40]. The Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) included men and women without CHD, who were randomized to receive either lovastatin or placebo. Treatment with lovastatin showed a 37 % reduction in the risk of first acute coronary event over a period of five years. An outcomes analysis with data from the participants (aged 35 to 62 years), stratified by high or low plasma total cholesterol/HDL-C ratio and plasma hs-CRP values, showed that the statin arm demonstrated marked event reduction in the high ratio/high CRP, high ratio/low CRP, and low ratio/high CRP groups compared to the placebo arm. In contrast, statin therapy had little effect on the rate of events in individuals with low ratio/low CRP values. Similar results were noted in subjects stratified by plasma LDL-C and hs-CRP levels above and below baseline median values [39].

Data from the PROVE-IT TIMI 22 (Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22) trial demonstrated that among patients with ACS, those who had low hs-CRP levels after statin therapy had better clinical outcomes than those with higher CRP levels, regardless of the level of LDL cholesterol achieved [40]. In WOSCOPS (West of Scotland Coronary Prevention Study), men with no history of CVD were randomized to receive either pravastatin or placebo. The rate of coronary events and mortality was significantly reduced in the treatment arm [41]. The PRINCE (Pravastatin Inflammation/CRP Evaluation) study demonstrated a significant reduction in serum CRP levels with the administration of pravastatin 40 mg/day in subjects with and without CVD. These effects were independent of any changes in LDL levels [38]. Other, smaller studies have suggested anti-inflammatory properties of statins through reduction in CRP and proinflammatory cytokines levels in patients with metabolic syndrome, diabetes mellitus, and hypercholesterolemia [42, 43].

The more recent results of the JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial reinforced the need to address the question of inflammation-guided therapy for cardiovascular risk prevention [44]. The trial investigated whether statin therapy might affect cardiovascular risk in subjects with LDL cholesterol levels below those qualifying for treatment (<3.36 mmol/L), but considered at increased vascular risk because of high CRP levels (>2 mg/L). Patients were randomized to either placebo or rosuvastatin 20 mg. The study was terminated early due to a striking 44 % reduction in the composite cardiovascular endpoint and a 20 % reduction in all-cause mortality in the treatment arm. This association increased with increasing levels of baseline hs-CRP but not with baseline LDL cholesterol. Due to the pleiotropic effects of statins, however, of both in cholesterol reduction and anti-inflammatory properties, it is impossible to define the proportion of benefit of each of these pathways.

Direct Anti-inflammatory Therapy

As noted above, upstream mediators of inflammation such as TNF-alpha and IL-6 lead to hepatic, vascular, and adipose

Table 1 Pleiotropic effects of statins [19••]

Inhibition of protein isoprenylation	↓endothelial dysfunction via ↓ in ADMA and ↑in NO bioavailability
	↓pro-inflammatory cytokine via ↓ in Ras and Rho pathways
	↓oxidative stress
	↓ in angiotensin (AT) II effects on vascular wall and ↓ in AT1 receptor expression
	↓ ATII stimulation of production of NADPH-derived ROS
Anti-inflammatory effects	↓ NFkB, AP-1 and HIF-1 activation in VSMC
Effects on proinflammatory cytokines	↓IL-6, IL-18, MCP-1,
	↓MMPs
Effects on adhesion molecules	↓expression of adhesion molecules via downregulation of Rho pathways
Effects on adaptive immune response	Inhibition of differentiation of T cells in Th17 lymphocytes and induction of differentiation of T cells in Treg lymphocytes

production of downstream mediators, which include CRP [45]. Robust epidemiologic evidence exists of the role of both upstream and downstream mediators of inflammation in the prediction of cardiovascular risk [34••, 31•]. Together, these studies provide significant support for the need to investigate whether anti-inflammatory drugs may affect cardiovascular risk. Of particular interest are agents that inhibit IL-1, TNF-alpha, and IL-6 regulatory pathways.

As noted above, IL-1 beta is produced by innate immune cells, including monocytes, as an inactive precursor (pro-IL-1 beta) that requires proteolytic cleavage to attain biological activity [14]. This is typically mediated by a complex of intracellular proteins known as the NLRP3 inflammasome. Cholesterol crystals are perhaps the most important triggers of the activation of the inflammasome and therefore the IL-1 beta pathway [12, 13, 11]. IL-1 is implicated in smooth muscle cell proliferation, recruitment of inflammatory cells into the atherosclerotic plaque, and regulation of IL-6 and CRP production.

The Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) is the first large randomized controlled trial testing the use of canakinumab in secondary cardiovascular prevention in subjects with stable coronary artery disease considered at high inflammatory risk due to persistent elevation of CRP (>2 mg/L) despite usual therapy, including statins [46]. Canakinumab is a human anti- IL-1 beta monoclonal antibody that has already been associated with a reduction in IL-6, CRP, and fibrinogen in patients at high vascular risk [47]. Until now, its indication has been for the treatment of several rare IL-1 beta overexpression disorders.

It has been demonstrated that patients with systemic inflammatory disorders such as rheumatoid arthritis have a TNF-alpha-induced endothelial dysfunction [48]. Patients treated with the TNF-alpha-specific monoclonal antibody infliximab have shown an improvement of endothelial function and a reduction in vascular event rates. Similar results have come from studies conducted among patients taking the IL-6 inhibitor tocilizumab or low-dose methotrexate [49]. On this basis, the National Heart, Lung, and Blood Institute initiated the Cardiovascular Inflammation Reduction Trial (CIRT), in which patients with chronic atherosclerosis and either diabetes or metabolic syndrome will be randomized to usual care plus placebo or usual care plus low-dose methotrexate (15–20 mg/week) [50]. This is the treatment of choice for rheumatoid and psoriatic arthritis. Among subjects suffering from these conditions, methotrexate reduces IL-6 and CRP levels. Moreover, recent evidence has demonstrated that the antiatherogenic effect of methotrexate may be due to the stimulation of the adenosine A2A receptor, which is associated with reverse cholesterol transport. This effect may then prevent the formation of foam cell [8, 51]. Furthermore, methotrexate may also have direct anti-atherosclerotic effects, as demonstrated in animal models [52].

The ENTRACTE investigators, on the other hand, are conducting a randomized trial (ClinicalTrials.gov Identifier: NCT01331837) to assess the effects on CVD of tocilizumab compared to TNF-alpha blocking therapy with etanercept. The study will include patients with advanced rheumatoid arthritis, aged 50 years or older, with a history of CHD or multiple cardiovascular risk factors. The population enrolled and the absence of a placebo arm, however, will likely limit the generalizability of the results.

Conclusions

As was recently claimed by Ridker and colleagues, it is now clear that atherosclerosis is a systemic disease, and in light of its inflammatory nature, it requires systemic therapies that can no longer be restricted to the reversal of arterial stenosis. Statins have clearly demonstrated their role as anti-atherosclerotic drugs, with their pleiotropic effects, and particularly their anti-inflammatory and immune-modulating properties, which have established them as a cornerstone in the therapy of atherosclerosis and atherothrombosis. The persistent cardiovascular risk in patients optimally treated with traditional medications and revascularization, however, highlighted the need for novel therapeutic strategies. As noted above, drugs targeting proinflammatory pathways are now being investigated for their potential antiatherosclerotic effects. Moreover, several other possible therapeutic targets are being tested, including phospholipase A2, adhesion molecules, and even immune system pathways, with the goal of developing a vaccine to prevent atherosclerosis.

The next decade will likely be highly informative for the scientific community, as the results of large trials such as CIRT and CANTOS will finally be published, shedding light on these novel therapeutic approaches to atherosclerosis.

Acknowledgments Supported by a grant from the Italian Ministry for Education, University and Research (FIRB RBFR12W5V5) to Paolo Calabrò

Compliance with Ethics Guidelines

Conflict of Interest Enrica Golia, Giuseppe Limongelli, Francesco Natale, Fabio Fimiani, Valeria Maddaloni, Ivana Pariggiano, Renatomaria Bianchi, Mario Crisci, Ludovica D'Acierno, Roberto Giordano, Gaetano Di Palma, Marianna Conte, Paolo Golino, Maria Giovanna Russo, Raffaele Calabrò, and Paolo Calabrò each declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Libby P, Ridker P, Maseri A. Inflammation and atherosclerosis. Circulation. 2002;105(9):1135–43.
2. Ross R. Atherosclerosis – an inflammatory disease. N Engl J Med. 1999;340:115–26.
3. Wong BW, Meredith A, Lin D, McManus BM. The biological role of inflammation in atherosclerosis. Can J Cardiol. 2012;28(6):631–41. doi:10.1016/j.cjca.2012.06.023. *A recent comprehensive review on the biological basis of the involvement of inflammation in atherosclerosis*.
4. Holman R, McGill HJ, Strong J, Geer J. The natural history of atherosclerosis: the early aortic lesions as seen in New Orleans in the middle of the of the 20th century. Am J Pathol. 1958;34:209–35.
5. Ross R. The pathogenesis of atherosclerosis–an update. N Engl J Med. 1986;314(8):488–500. doi:10.1056/NEJM198602203140806.
6. Tabas I, Williams K, Boren J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. Circulation. 2007;116:1832–44.
7. Chatzizisis Y, Coskun A, Jonas M, Edelman E, Feldman C, Stone P. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. J Am Coll Cardiol. 2007;49:2379–93.
8. Zalewski A, Macphee C. Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology, epidemiology, and possible therapeutic target. Arterioscler Thromb Vasc Biol. 2005;25(5):923–31. doi:10.1161/01.ATV.0000160551.21962.a7.
9. Berliner JA, Leitinger N, Tsimikas S. The role of oxidized phospholipids in atherosclerosis. J Lipid Res. 2009;50(Suppl):S207–12. doi:10.1194/jlr.R800074-JLR200.
10. Libby P, Ridker P. Inflammation and Atherothrombosis: From Population Biology and Bench Research to Clinical Practice. J Am Coll Cardiol. 2006;48:A33–46.
11. Abela G. Cholesterol crystals piercing the arterial plaque and intima trigger local and systemic inflammation. Clin Lipidol. 2010;4:156–64.
12. Rajamäki K, Lappalainen J, Oörni K, Välimäki E, Matikainen S, Kovanen PT, et al. Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation. PLoS One. 2010;5:e1175.
13. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Nature. 2010;464(7293):1357–61. doi:10.1038/nature08938.
14. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell. 2002;10(2):417–26.
15. Razani B, Feng C, Coleman T, Emanuel R, Wen H, Hwang S, et al. Autophagy links inflammasomes to atherosclerotic progression. Cell Metab. 2012;15(4):534–44. doi:10.1016/j.cmet.2012.02.011.
16. Cirillo P, Golino P, Calabro P, Cali G, Ragni M, De Rosa S, et al. C-reactive protein induces tissue factor expression and promotes smooth muscle and endothelial cell proliferation. Cardiovasc Res. 2005;68(1):47–55. doi:10.1016/j.cardiores.2005.05.010.
17. Bisoendial RJ, Kastelein JJ, Stroes ES. C-reactive protein and atherogenesis: from fatty streak to clinical event. Atherosclerosis. 2007;195(2):e10–8. doi:10.1016/j.atherosclerosis.2007.04.053.
18. Calabrò P, Cirillo P, Limongelli G, Maddaloni V, Riegler L, Palmieri R, et al. Tissue factor is induced by resistin in human coronary artery endothelial cells by the NF-κB-dependent pathway. J Vasc Res. 2011;48:59–66.
19. •• Tousoulis D, Psarros C, Demosthenous M, Patel R, Antoniades C, Stefanadis C. Innate and adaptiveinflammation as a therapeutic target in vascular disease: The emerging role of statins. J Am Coll Cardiol. 2014. doi:10.1016/j.jacc.2014.01.054. *Very recent and comprehensive review on the role of the immune system in atherosclerosis and the role of statins in the modulation of the atherosclerotic process*.
20. Paulson KE, Zhu SN, Chen M, Nurmohamed S, Jongstra-Bilen J, Cybulsky MI. Resident intimal dendritic cells accumulate lipid and contribute to the initiation of atherosclerosis. Circ Res. 2010;106(2):383–90. doi:10.1161/CIRCRESAHA.109.210781.
21. Zwaal RF, Comfurius P, Bevers EM. Surface exposure of phosphatidylserine in pathological cells. Cellular Mol Life Sci CMLS. 2005;62(9):971–88. doi:10.1007/s00018-005-4527-3.
22. Schiro A, Wilkinson F, Weston R, Smyth J, Serracino-Inglott F, Alexander M. Endothelial microparticles as conveyors of information in atherosclerotic disease. Atherosclerosis. 2014;234:295–302.
23. • Hulsmans M, De Keyzer D, Holvoet P. MicroRNAs regulating oxidative stress and inflammation in relation to obesity and atherosclerosis. FASEB J. 2011;25(8):2515–27. doi:10.1096/fj.11-181149. *Interesting review on microRNAs, novel candidates as regulators of the complex signaling that regulates gene expression during inflammatory response and atherogenesis*.
24. Chen LJ, Lim SH, Yeh YT, Lien SC, Chiu JJ. Roles of microRNAs in atherosclerosis and restenosis. J Biomed Sci. 2012;19(1):79. doi:10.1186/1423-0127-19-79.
25. Memoli B, Procino A, Calabrò P, Esposito P, Grandaliano G, Pertosa G, et al. Inflammation may modulate IL-6 and C-reactive protein gene expression in the adipose tissue: the role of IL-6 cell membrane receptor. Am J Physiol Endocrinol Metab. 2007;293:E1030–5.
26. Calabrò P, Golia E, Riegler L, Limongelli G, Golino P, Russo M, et al. Inflammation: The Link Between Obesity and Cardiovascular Risk. Current Cardiovascular Risk Reports. 2010;4:101–11.
27. Calabro' P, Chang DW, Willerson JT, Yeh ET. Release of C-reactive protein in response to inflammatory cytokines by human adipocytes: linking obesity to vascular inflammation. J Am Coll Cardiol. 2005;46(6):1112–3. doi:10.1016/j.jacc.2005.06.017.
28. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med. 1997;336(14):973–9. doi:10.1056/NEJM199704033361401.
29. Calabro' P, Golia E, Yeh ET. CRP and the risk of atherosclerotic events. Semin Immunopathol. 2009;31(1):79–94. doi:10.1007/s00281-009-0149-4.
30. • Calabro P, Golia E, Yeh ET. Role of C-reactive protein in acute myocardial infarction and stroke: possible therapeutic approaches. Curr Pharm Biotechnol. 2012;13(1):4–16. *A comprehensive review of the predictive role of CRP and therapeutic strategies in cardiovscular disease*.
31. • Emerging Risk Factors Collaboration, Kaptoge S, Di Angelantonio E, Pennells L, Wood AM, White IR, et al. C-reactive protein, fibrinogen, and cardiovascular disease prediction. N Engl J Med. 2012;367(14):1310–20. doi:10.1056/NEJMoa1107477. *This recent study support the predictive role of CRP in cardiovascular disease*.
32. American College of Cardiology Foundation, American Heart Association. ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2010;56(25):e50–103.
33. Anderson TJ, Gregoire J, Hegele RA, Couture P, Mancini GB, McPherson R, et al. 2012 update of the Canadian Cardiovascular Society guidelines for the diagnosis and treatment of dyslipidemia for the prevention of cardiovascular disease in the adult. Can J Cardiol. 2013;29(2):151–67. doi:10.1016/j.cjca.2012.11.032.

34.•• Kaptoge S, Seshasai SR, Gao P, Freitag DF, Butterworth AS, Borglykke A, et al. Inflammatory cytokines and risk of coronary heart disease: new prospective study and updated meta-analysis. Eur Heart J. 2014;35(9):578–89. doi:10.1093/eurheartj/eht367. *An interesting prospective study, and the associated updated meta-analysis confirms the role of inflammatory cytokines in cardiovascular risk prediction*.

35. Tousoulis D, Antoniades C, Vasiliadou C, Kourtellaris P, Koniari K, Marinou K, et al. Effects of atorvastatin and vitamin C on forearm hyperaemic blood flow, asymmentrical dimethylarginine levels and the inflammatory process in patients with type 2 diabetes mellitus. Heart. 2007;93(2):244–6. doi:10.1136/hrt.2006.093112.

36. Wolfrum S, Jensen KS, Liao JK. Endothelium-dependent effects of statins. Arterioscler Thromb Vasc Biol. 2003;23(5):729–36. doi:10.1161/01.ATV.0000063385.12476.A7.

37. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. Circulation. 1999;100(3):230–5.

38. Albert MA, Danielson E, Rifai N, Ridker PM, Investigators P. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. JAMA. 2001;286(1):64–70.

39. Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med. 2001;344(26):1959–65. doi:10.1056/NEJM200106283442601.

40. Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, et al. C-reactive protein levels and outcomes after statin therapy. N Engl J Med. 2005;352(1):20–8. doi:10.1056/NEJMoa042378.

41. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. N Engl J Med. 1995;333(20):1301–7. doi:10.1056/NEJM199511163332001.

42. Ascer E, Bertolami MC, Venturinelli ML, Buccheri V, Souza J, Nicolau JC, et al. Atorvastatin reduces proinflammatory markers in hypercholesterolemic patients. Atherosclerosis. 2004;177(1):161–6. doi:10.1016/j.atherosclerosis.2004.07.003.

43. Bulcao C, Ribeiro-Filho FF, Sanudo A, Roberta Ferreira SG. Effects of simvastatin and metformin on inflammation and insulin resistance in individuals with mild metabolic syndrome. Am J Cardiovasc Drugs. 2007;7(3):219–24.

44. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto Jr AM, Kastelein JJ, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med. 2008;359(21):2195–207. doi:10.1056/NEJMoa0807646.

45. Ridker P. Targeting inflammatory pathways for the treatment of cardiovascular disease. Eur Heart J. 2014;35:540–3.

46. Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1beta inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). Am Heart J. 2011;162(4):597–605. doi:10.1016/j.ahj.2011.06.012.

47. Ridker P, Howard C, Walter V, Everett B, Libby P, Hensen J, et al. Effects of interleukin-1beta inhibition with canakinumab on hemoglobin A1c, lipids, C-reactive protein, interleukin-6, and fibrinogen: a phase IIb randomized, placebo-controlled trial. Circulation. 2012;126:2739–48.

48. Hurlimann D, Forster A, Noll G, Enseleit F, Chenevard R, Distler O, et al. Antitumor necrosis factor-alpha treatment improves endothelial function in patients with rheumatoid arthritis. Circulation. 2002;106:2184–7.

49.•• Ridker P, Lüscher T. Anti-inflammatory therapies for cardiovascular disease. Eur Heart J. 2014 *(A complete review of the ongoing evaluation of direct antinflmmatory strategies in cardiovascular disease).*

50. Everett BM, Pradhan AD, Solomon DH, Paynter N, Macfadyen J, Zaharris E, et al. Rationale and design of the Cardiovascular Inflammation Reduction Trial: a test of the inflammatory hypothesis of atherothrombosis. Am Heart J. 2013;166(2):199–207. doi:10.1016/j.ahj.2013.03.018.

51. Reiss AB, Rahman MM, Chan ES, Montesinos MC, Awadallah NW, Cronstein BN. Adenosine A2A receptor occupancy stimulates expression of proteins involved in reverse cholesterol transport and inhibits foam cell formation in macrophages. J Leukoc Biol. 2004;76(3):727–34. doi:10.1189/jlb.0204107.

52. Bulgarelli A, Martins Dias AA, Caramelli B, Maranhao RC. Treatment with methotrexate inhibits atherogenesis in cholesterol-fed rabbits. J Cardiovasc Pharmacol. 2012;59(4):308–14. doi:10.1097/FJC.0b013e318241c385.