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Interplay between dyslipidemia and inflammation in atherosclerosis: Translating experimental targets into clinical practice.

Short Title: Inflammation, Immunity and Lipids

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ABSTRACT

INTRODUCTION

Lipids have been long recognized to play a pivotal role in atherosclerosis. Inflammation has also been acknowledged as a key biological process in this disorder[1]. Importantly, dyslipidemia and inflammation are closely intertwined in their contribution to atherosclerosis and cardiovascular risk[2]. For example, lipid-lowering drugs that effectively decrease cardiovascular (CV) events also exhibit anti-inflammatory effects[3]. On the other hand, some of the anti-inflammatory effects reported for statins, and also for aspirin and renin-angiotensin modulators[3-5] may result from. lipid-lowering, antithrombotic or anti-proliferative effects, as well as improved endothelial function. In addition, observational studies have suggested beneficial effects of anti-inflammatory drugs used for other indications in terms of reducing CV risk[6]. However, until recently, direct evidence on the efficacy of anti-inflammatory strategies to reduce CV events in humans was lacking. Thus, there was a need for a definitive study to specifically address the specific anti-inflammatory effects on CV events.

The recent Canakinumab Antiinflammatory Thrombosis Outcomes Study (CANTOS) has addressed this question. This trial investigated high-risk patients after acute coronary syndromes (ACS) on statin therapy and increased inflammatory burden (CRP levels >2 mmol/L). It demonstrated that CV outcome was improved after inhibition of interleukin-1β (IL-1β) without affecting lipid levels[7]. These results introduce a new paradigm for the treatment of human atherosclerosis. In this Consensus Paper, we highlight the role of inflammation and dyslipidemia in atherosclerosis and aim to outline the new issues and challenges that are brought up by the interplay between these two CV risk factors in cardiovascular prevention.

LIPID-INDUCED INFLAMMATORY RESPONSES (Figure 1)

A key trigger of atherosclerosis is subintimal retention of low-density lipoproteins (LDL) at regions of complex flow or low shear stress.[8] Modified LDL (mLDL) species are strong inducers of inflammation and have a marked impact on atherosclerosis[9]. They alter vascular physiology by activating pattern recognition receptors, such as toll-like receptors (TLRs), which trigger proinflammatory signals and reactive oxygen species [10] and promote matrix degradation[11]. These TLRs will prime the Nod-like receptor protein 3 (NLRP3) inflammasome for activation by cholesterol crystals leading to IL-1β activation.[12] Pathway activation leads to the increased release of cytokines [13] and activate the endothelium by increasing the expression of adhesion molecules and chemokines, costimulatory molecules, and pro-inflammatory transcription factors, such as CD40 and nuclear factor-κB (NF-κB)[14-16], and trained immunity[17] that promote the recruitment of inflammatory cells into the vascular wall. Macrophages are of key relevance [18] since they can scavenge oxidized LDL leading to their transformation into pro-atherogenic foam cells[1, 6, 19].

Adaptive immune responses play a key role in atherogenesis. Activated T lymphocytes are present in both peripheral blood and coronary plaques in patients with ACS [1, 20], and especially Th1-derived cytokines such as TNFα (tumour necrosis factor α), interferon-gamma and interleukin-12 are associated with atherosclerosis. Although the notion of immunomodulatory effects of lipid-lowering agents emerged from both experimental and clinical studies[21, 22], the causal relation between lipids and immunity with regard to atherogenesis remains incompletely understood.

ANTI-INFLAMMATORY EFFECTS OF LIPID-LOWERING THERAPIES

There is overwhelming evidence showing that statins have anti-inflammatory and immunomodulatory effects. They decrease the activity of the transcription factor NF-κB[3], with subsequent diminution in the expression of adhesion molecules, cytokines and MMPs, interfering also with the arachidonic/cyclooxygenase (COX) pathway[23a-23b]. Also, they reduce plasma levels of inflammatory markers such as C-reactive protein (CRP)[24]. Although most evidence has been obtained with statins, other lipid-lowering approaches show similar inhibitory effects on inflammation. For instance, ezetimibe and fibrates also inhibit the NF-κB pathway and decrease CRP levels[25-28]. Similarly, low fat diet reduces CRP levels[29] and Mediterranean diet also decreases CD40 expression on monocytes and plasma levels of cell adhesion molecules and cytokines[30]. Accordingly, lipid-lowering has been shown to decrease the incidence of cardiovascular events independently of the employed therapy[31].

Recently, another class of lipid-lowering drugs, Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, have been demonstrated to reduce the of cardiovascular events[32, 33]. Although PCSK9 inhibitors do not decrease CRP plasma levels, [34 35] they reduce Lipoprotein (a) levels -a molecule that promotes inflammation, oxidative stress, and coagulation[36]- and decrease monocyte activation and transmigration in patients with familial hypercholesterolemia[34]. Moreover, antibody-based PCSK9 inhibition in atherosclerotic mice diminished plaque macrophage and necrotic core content [37].. Conversely, up-regulation of hepatic LDL receptors (LDLR) by PCSK9 inhibition results in increased lipopolysaccharide clearance, a decreased inflammatory response, and improved survival following sepsis in mice,[38] while patients with PCSK9 loss-of-function variants exhibit improved clinical outcomes during septic shock[39]. Likewise, PCSK9 expression can be experimentally induced by pro-inflammatory molecules, such as Lipopolysaccharide, TNFα, and hepatocyte nuclear factor-1α [40-41].

Also, PCSK9 modulates LDLR expression in macrophages, [46, 47] promoting the expression of pro-inflammatory markers, and inhibiting anti-inflammatory molecules[47]. In humans, plasma PCSK9 concentrations increase in sepsis[48], trauma[49], and in acute coronary syndromes, and they are positively associated with hsCRP[50], white blood cell count, and fibrinogen in coronary patients[51]. In conclusion, this evidence confirms that all LDL-c-lowering therapies employed to date decrease inflammation.

ANTI-INFLAMMATORY THERAPY AND CARDIOVASCULAR RISK

Inflammatory cytokines such as IL-1[52] and TNF[53] have been detected in human coronary atherosclerosis. Observational studies have revealed an association of different anti-inflammatory treatments, when used for their indications, with reduced cardiovascular risk, providing support for the concept of anti-inflammation in cardiovascular prevention[6]. It is the case of anti-TNF therapy in rheumatoid arthritis[54, 55] and anti-leukotrienes in asthmatics[56] that apparently decreased the incidence of cardiovascular events.

On the other hand, other anti-inflammatory drugs failed to decrease the cardiovascular risk, as was observed for steroids in patients with unstable angina[57]. With the exception of aspirin, non-steroidal anti-inflammatory (NSAIDs) drugs, especially COX-2 inhibitors, have in general increased cardiovascular risk[58], [59, 60], indicating that this class of anti-inflammatory drugs should be limited to patients without other alternatives[59]. Interestingly, the PRECISION (Prospective Randomized Evaluation of Celecoxib Integrated Safety versus Ibuprofen or Naproxen) trial showed non-inferiority of celecoxib versus ibuprofen and naproxen regarding cardiovascular events[61] highlighting some hypertensive effects of these drugs. Interestingly, these effects were minimal for celecoxib.

The recent CANTOS trial sets a new paradigm in the relationship of inflammation with atherosclerosis and lipids[7]. In this randomized, double-blind trial, 10,061 high-risk patients with a previous myocardial infarction, majority using moderate to high intensity statin therapy and hsCRP>2 mg/L received canakinumab, a monoclonal antibody that blocks IL1β, or placebo. After a median follow-up of 3.7 years there was a ~15% decrease in the incidence of the primary end point composed of nonfatal myocardial infarction, nonfatal stroke with the highest dose investigated. Interestingly, an even higher risk reduction of ~27% was observed in patients characterized by above median reduction of CRP following canakinumab administration (ESC presentation Ridker). While there was an increase in the incidence of fatal infections, neutropenia or thrombocytopenia in patients on canakinumab, there were also non-CV benefits, comprising a reduction in the incidence of lung cancer, cancer mortality, arthritis, and gout [7, 62].

IMPACT OF THE CANTOS TRIAL ON OUR UNDERSTANDING OF INTERPLAY BETWEEN LIPIDS AND INFLAMMATION

The results from the CANTOS trial provide a first proof-of-principle about the link between lipids and inflammation. In this trial, canakinumab did not decrease lipid levels[62]. Thus, the CANTOS data excludes the possibility that canakinumab reduces CV-risk through lipid-dependent mechanisms linked to IL-1β.

Patients in the CANTOS trial had mean LDL-C levels of approximately 80 mg/dl and CRP > 2 mg/L. Efforts to further reduce residual CV-risk now have multiple options. Following the post-hoc analysis in the FOURIER study, Giugliano and collegues showed that CV-benefit by LDL-c lowering is extended to values even below 20 mg/dl (Giugliano, Fourier, Lancet 2017). Conversely, CANTOS shows that a persistent CRP reduction following canakinumab may also convey a 25% CV-risk

reduction (ref Ridker, ESC presentation). Suggested biomarkers to guide personalized medicine in an effort to further reduce residual burden in high CV-risk patients comprise absolute LDL-c levels, CRP levels[70] or IL-1β genotype[71]. However, the role of other inflammatory biomarkers or even imaging strategies (PET/CT ref Tawakol/Fayad/Rudd; MRI-lipid by Choudhury?) to better select high-responders to therapeutic moieties targeting either residual lipid or inflammatory pathways remains to be established[72]. Last but not least, despite the landmark character of CANTOS, the high costs of canakinumab preclude its broader use in cardiovascular prevention.

CONCLUSIONS AND PERSPECTIVES

1) **Interplay between lipids and inflammation:** The reduction in cardiovascular events observed with IL1β-blockade confirms the link between lipids and inflammation. The mechanism involves cholesterol crystals (and possibly other lipid species) which activate non-canonical pathways (TLRs or the NLRP3) to induce maturation of IL-1β which is blocked by canakinumab.

2) Other pro-inflammatory targets appear worth testing. Although IL1β blockade is today the only anti-inflammatory approach shown to reduce cardiovascular risk in a randomized clinical trial, the wealth of evidence linking inflammation with atherosclerosis indicates that other potential targets exist to be evaluated in future trials.

3) Anti-inflammatory therapy is complementary to lipid-lowering and risk factor control. Canakinumab is not a competitor of lipid-lowering therapies given, among other reasons, that the large evidence supporting these therapies cannot be compared with the results of only one clinical trial.

4) CANTOS – a proof-of-principle study for causal role of inflammation in human atherosclerosis. Although the results of CANTOS trial may be considered a milestone in cardiovascular medicine, given all the above considerations it is improbable that

canakinumab is prescribed to patients with cardiovascular risk to improve their prognosis. Alternatively, an attractive idea would be to systematically and randomly explore its effect in patients with rheumatologic disorders or in other context of chronic inflammation using trials with a prespecified cardiovascular endpoint.

5) Would highlight anti-cancer effects. → likely to create impact in the cancer field.

6) Other cheaper tools than antibodies may also do the job of lowering IL1b (e.g. RNA interference – as in ORION).

7) Anti-inflammatory therapy has a narrow therapeutic window as compared to LDL-C lowering therapy. → increased infections, sepsis after anti-IL1b therapy, whereas lowering LDL-C to very very low levels reveals no side effects. → caution with dosing of anti-inflammatory agents.

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FIGURE LEGENDS

Figure 1. Lipid dysregulation triggers inflammatory and immune responses. AP-1: Activator protein-1; **CCR2:** Chemokine receptor type-2; **COX-2:** Cyclooxygenase-2; **DC:** Dendritic cells; **IKK:** IκB kinase; **IL:** Interleukin IL: Interleukin; **INFγ:** Interferon-γ; **JAK-STAT:** Janus kinase and Signal Transducer Activator of Transcription Proteins; **JNK:** Jun kinase; **LDL:** low-density lipoprotein; **mLDL:** modified LDL; **oxLDL:** oxidizwed LDL; **Mø:** macrophages; **MCP-1:** Monocyte chemoattractant protein-1; **NF-κB:** Nuclear factor-κB; **NLR:** NOD-like receptors; **PMN:** Polymorphonuclear; **PRR:** Pattern recognition receptors; **ROS:** Rective Oxygen species; **Scav-R:** Scavenger receptors; **SRA** and **SRB:** Scavenger receptor class A and B; **TGFβ:** Transforming growth factor-β; **TLR:** Toll-like receptor; **TNFα:** Tumor necrosis factor-α; **TNF-R:** Tumor necrosis factor-receptor; **VCAM:** Vascular cell adhesion molecule.

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