When, where and how to target vascular inflammation in the post-CANTOS era?

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This editorial refers to 'Stage-Dependent Differential Effects of Interleukin-1 (IL-1) Isoforms on Experimental Atherosclerosis', by A. Vromman et al., on page….

In the last 30 years, basic science data have clearly demonstrated the causal role of immune-inflammatory responses in all phases of atherosclerosis, from atherogenesis to plaque vulnerability.[1,2] Observational epidemiological studies have also highlighted the inflammatory nature of the disease, however, translation of this knowledge in to the clinic is still in its infancy and currently no immunomolulatory drug is routinely used to safely control clinical atherosclerosis.[2]

The CANTOS trial (Canakinumab Anti-inflammatory Thrombosis Outcomes Study), published in September 2017,[3] is the first large, randomised, double-blind, placebo-controlled clinical trial targeting interleukin (IL)-1β for secondary prevention in patients previously affected by myocardial infarction (MI), with residual inflammatory risk defined by high levels of C-reactive protein (hsCRP). Treatment of patients with the human monoclonal antibody canakinumab, given in addition to the standard care, significantly reduced the rate of a composite endpoint major cardiovascular events, however, without reducing all-cause mortality. This large >10000 patient trial provided seminal clinical evidence in support of targeting inflammation in atherosclerosis and opened an important discussion on *how to do it in the most efficient way*.

CANTOS trial results have been followed by the publication of the Cardiovascular Inflammation Reduction Trial (CIRT). Treatment with low-dose methotrexate failed to lower cardiovascular event rates in patients with previous MI or multi-vessel coronary artery disease and additionally affected by type 2 diabetes or metabolic syndrome.[4] In CIRT, patients had hsCRP levels in the normal range and this highlights the importance of patient stratification. The importance of carefully selecting patients with persistent inflammation is highlighted by the fact that in post hoc observations within CANTOS, the largest reduction in cardiovascular mortality was observed in the patients showing the greatest reduction in the circulating inflammatory mediators IL-6 and CRP,[5] whilst, within CIRT, methotrexate administration had no effect on IL-1β, IL-6 or hsCRP.

In summary, we have learned a lot from both trials; however, as expected, several important questions remain to be answered before anti-inflammatory therapies may become a viable approach for the treatment of atherosclerosis-related cardiovascular disease (CVD). The current clinical debate is focused on (i) evaluation of risks, given the strong immunosuppression associated with long-term immunomodulant treatment in chronic inflammation; (ii) how patients should be stratified for future therapies; (iii) at which stage of the pathology targeting inflammation may be beneficial; and, most importantly, (iv) what is the best way to control vascular inflammation.

In this respect, targeting the IL-1 pathway has been the most logical choice in CANTOS. IL-1 is the first discovered pro-inflammatory cytokine. Two isoforms of IL-1 have been identified: IL-1α and IL-

1β. They share the same receptor (IL-1R type I) and are both produced as precursors. While the IL-1α precursor form can bind to its receptor, the precursor form of IL-1β requires activation by either caspase-1 via NLRP3 inflammasome or extracellular neutrophilic proteases.[6] The IL-1 pathway has been shown to play key roles in atherosclerosis. In early experiments, the absence of the IL-1R antagonist IL-1RA (a decoy receptor inhibiting IL-1) led to enhanced foam cell formation and increased plaque development.[7,8] Whilst, vice versa, overexpression of sIL-1RA reduced atherosclerosis formation.[7] Similarly, administration of human recombinant IL-1RA reduced plaque formation in apolipoprotein-E (*apoE*)$^{-/-}$ mice.[9]

Despite similarities, the net contribution of the two IL-1α and β isoforms to atherosclerosis is still under debate. Both isoforms induce the expression of adhesion molecules on endothelial cells, supporting the homing of both innate and adaptive immune cells in target tissues, including the vasculature. Moreover, both isoforms enhance the expression of matrix metalloproteinases.[6] Deletion or inhibition of IL-1β reduced the development of experimental atherosclerosis.[10,11] However, surprisingly, lesion formation is not affected in *apoE*$^{-/-}$ mice lacking inflammasomes[12] and, more importantly, fatty acid-induced mitochondrial uncoupling elicited a response towards selective inflammasome-independent production of IL-1α, leading to vascular inflammation in atherosclerosis.[13] This led to suggestions that IL-1α more than IL-1β may be the right target for CVD prevention.

The debate has recently been reinvigorated by a study published in *Nature Medicine*,[14] where the authors used smooth muscle cell (SMC) lineage-tracing *apoE*$^{-/-}$ mice to demonstrate that IL-1β neutralization in advanced atherosclerosis, despite inhibiting vascular and systemic inflammation, reduced SMCs and collagen content while increasing macrophages in the fibrous cap area, leading to a more vulnerable plaque phenotype. Moreover, IL-1β neutralization inhibited beneficial outward remodeling, leading to reduced lumen size. Importantly, the conditional knockout of *Il1r1* in SMCs led to the formation of smaller lesions lacking a fully developed fibrous cap. The authors conclude that IL-1β is atheroprotective. These results are in line with previous findings showing that *apoE*$^{-/-}$ lacking IL-1R type 1 developed a vulnerable plaque phenotype[15] and perhaps may, at least in part, explain why genetic variants associated with higher levels of IL-1RA had lower concentrations of inflammatory CRP and IL-6, but were also associated with increased coronary artery disease.[16]

While mechanistically important it is not possible to clearly reconciliate these mouse studies with the outcome of the CANTOS trial. As such, in the present issue of the *European Heart Journal*, the Libby group has reassessed this important topic by directly comparing the effect of neutralizing IL-1α, IL-1β or both isoforms on early atherogenesis and established atherosclerosis in hyperlipidemic mice.[17] In a series of *in vivo* experiments, the authors demonstrate a stage-dependent role of IL-1α and IL-1β in experimental atherosclerosis. Vromann et al.[17] provide new data to address some of the disparities between the work of G. Owens laboratory and the CANTOS trial.[18]

IL-1α neutralization inhibited early atherogenesis but impaired outward remodeling in both the aortic root and the brachiocephalic artery, evaluated via conventional histology and microCT imaging following perfusion fixation and injection of a contrast agent. On the contrary, IL-1β blockade shifted circulating monocytes toward a less inflammatory phenotype and decreased major histocompatibility complex class II (MHC-II) vascular expression, although without affecting lesion area during atherogenesis. In the advanced stages of the pathology, selective neutralization of IL-1β but not IL-1α decreased plaque development in the aortic sinus and increased circulating levels of the anti-inflammatory cytokine IL-10. Importantly, IL-1β neutralization did not alter Glagovian remodeling of the aortic roots or brachiocephalic arteries either in the early or late stages of the pathology. Vromann et al.[17] did not assess classical markers of plaque stability and vulnerability. This issue cannot be easily resolved by animal studies and its importance is also likely to change in the forthcoming years, considering effectiveness of lipid lowering therapies in this respect.

The identified differential role(s) of the two IL-1 isoforms may explain some of the controversial findings discussed above. However, it should be noted that Owen and Libby manuscripts have used different mouse strains, a different length of neutralizing antibody treatment and analyzed the pathology at different stages. Therefore, it is difficult to perform direct comparison of the conclusions from both studies.

While there is a number of concerns regarding how well mouse models of atherosclerosis represent human disease, the study by Vromann et al.[17] is an excellent example of how mechanistic studies, when put in the context of results of clinical trials such as CANTOS or CIRT, inform each other in understanding immunopathology of atherosclerosis.

The debate will continue, and rightly so, simply because the immune system is complex and different pathways may play different roles depending on the cellular, anatomical and environmental context and the different stages of the pathology. However, these disparities indicate that we may need different immunomodulatory therapies to affect disease onset, progression and plaque rupture. Basic and clinical studies should move in parallel and inform each other with the aim to identify novel combinational therapies, new therapeutic targets, better biomarkers for patient stratification and new molecular imaging modalities as well as drug-delivery systems for targeting local and systemic immune mechanisms.[19] Such clinical-translational approaches, from bench to bedside and back again, will be essential for the development of clinically acceptable strategies to reliably assess and therapeutically target vascular inflammation.

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Figure 1: Stage-dependent roles of IL-1α and IL-1β in experimental atherosclerosis. Pathological roles for IL-1 isoforms in atherosclerosis are shown in red, with protective roles depicted in green. Lack of effect is shown in amber.