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EDITORIAL

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 isoforms on experimental atherosclerosis', by A. Vromman *et al.*, doi:10.1093/eurheartj/ehz008.

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 into the clinic is still in its infancy, and currently no immunomodulatory drug is routinely used to control clinical atherosclerosis safely.[2]

The CANTOS trial (Canakinumab Anti-inflammatory Thrombosis Outcomes Study), published in September 2017,[3] is the first large, randomized, 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 (150 and 300 mg), given in addition to the standard care, significantly reduced the rate of a composite endpoint of non-fatal MI, non-fatal stroke, or cardiovascular death, however without reducing all-cause mortality. This large >10 000 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 multivessel 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 underlines 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] whereas, 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] On the other hand, 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 IL-1β 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

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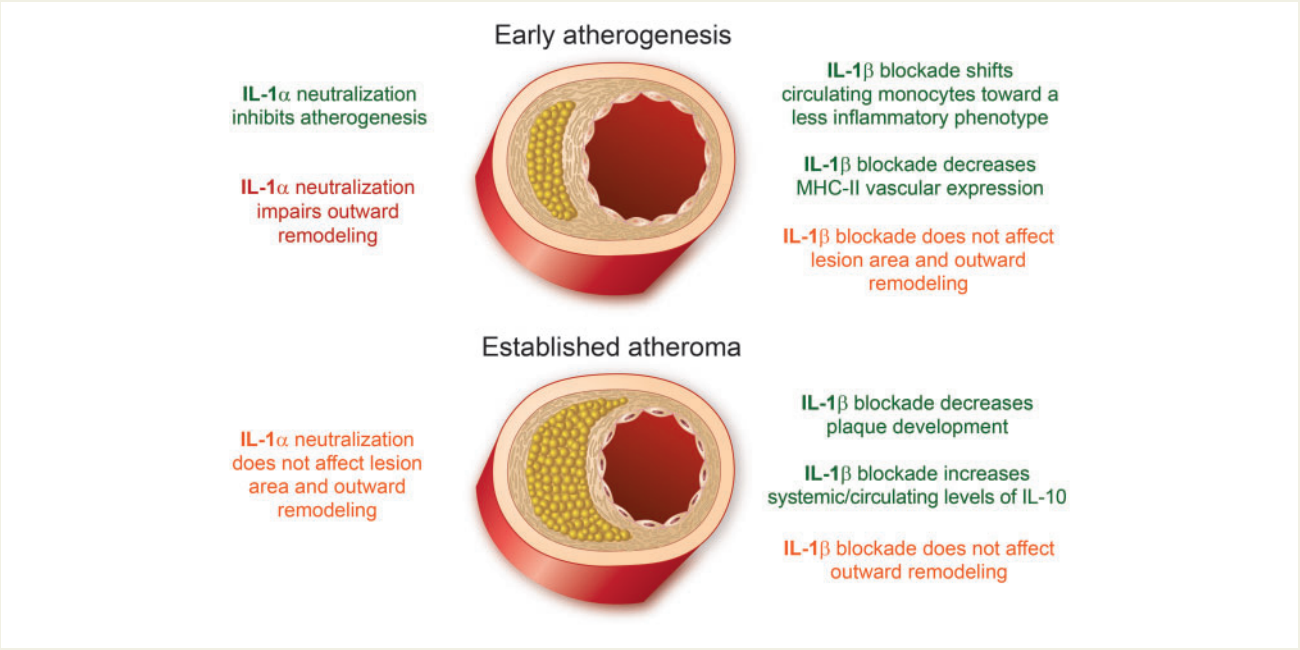

Take home figure Stage-dependent roles of IL-1α and IL-1β in experimental atherosclerosis. Pathological roles for IL-1 isoforms in atherosclerosis are shown in red, with protective roles depicted in green. Lack of effect is shown in amber.

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 remodelling, 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 reconcile these mouse studies with the outcome of the CANTOS trial. As such, in this 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 hyperlipidaemic mice.[17] In a series of *in vivo* experiments, the authors demonstrate a stage-dependent role for 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 the laboratory of G. Owens and the CANTOS trial.[18]

IL-1α neutralization inhibited early atherogenesis but impaired outward remodelling 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. In contrast, 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 remodelling of the aortic roots or brachiocephalic arteries either in the early or in the 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 the 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 the studies of Owen and Libby have used different mouse strains, a different duration of neutralizing antibody treatment, and analysed the pathology at different stages. Therefore, it is difficult to perform direct comparison of the conclusions from both studies.

While there are 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 the 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. Immune system complexity indicates that we may need different immunomodulatory therapies to affect atherosclerosis onset, progression and plaque rupture (*Take home figure*). 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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References

1. Libby P, Hansson GK. Adaptive immunity in acute coronary syndromes: chicken or egg? *Eur Heart J* 2018;**39**:1098–1099.
2. Welsh P, Grassia G, Botha S, Sattar N, Maffia P. Targeting inflammation to reduce cardiovascular disease risk: a realistic clinical prospect? *Br J Pharmacol* 2017;**174**:3898–3913.
3. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, Kastelein JJP, Cornel JH, Pais P, Pella D, Genest J, Cifkova R, Lorenzatti A, Forster T, Kobalava Z, Vida-Simiti L, Flather M, Shimokawa H, Ogawa H, Dellborg M, Rossi PRF, Troquay RPT, Libby P, Glynn RJ; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017;**377**:1119–1131.
4. Ridker PM, Everett BM, Pradhan A, MacFadyen JG, Solomon DH, Zaharris E, Mam V, Hasan A, Rosenberg Y, Iturriaga E, Gupta M, Tsigoulis M, Verma S, Clearfield M, Libby P, Goldhaber SZ, Seagle R, Ofori C, Saklayen M, Butman S, Singh N, Le May M, Bertrand O, Johnston J, Paynter NP, Glynn RJ; CIRT Investigators. Low-dose methotrexate for the prevention of atherosclerotic events. *N Engl J Med* 2019;**380**:752–762.
5. Ridker PM, MacFadyen JG, Everett BM, Libby P, Thuren T, Glynn RJ; CANTOS Trial Group. Relationship of C-reactive protein reduction to cardiovascular event reduction following treatment with canakinumab: a secondary analysis from the CANTOS randomised controlled trial. *Lancet* 2018;**391**:319–328.
6. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* 2011;**117**:3720–3732.
7. Devlin CM, Kuriakose G, Hirsch E, Tabas I. Genetic alterations of IL-1 receptor antagonist in mice affect plasma cholesterol level and foam cell lesion size. *Proc Natl Acad Sci USA* 2002;**99**:6280–6285.
8. Isoda K, Sawada S, Ishigami N, Matsuki T, Miyazaki K, Kusuhara M, Iwakura Y, Ohsuzu F. Lack of interleukin-1 receptor antagonist modulates plaque composition in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol* 2004;**24**:1068–1073.
9. Elhage R, Maret A, Pieraggi MT, Thiers JC, Arnal JF, Bayard F. Differential effects of interleukin-1 receptor antagonist and tumor necrosis factor binding protein on fatty-streak formation in apolipoprotein E-deficient mice. *Circulation* 1998;**97**:242–244.
10. Kirii H, Niwa T, Yamada Y, Wada H, Saito K, Iwakura Y, Asano M, Moriwaki H, Seishima M. Lack of interleukin-1beta decreases the severity of atherosclerosis in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol* 2003;**23**:656–660.
11. Bhaskar V, Yin J, Mirza AM, Phan D, Vanegas S, Issafras H, Michelson K, Hunter JJ, Kantak SS. Monoclonal antibodies targeting IL-1 beta reduce biomarkers of atherosclerosis *in vitro* and inhibit atherosclerotic plaque formation in Apolipoprotein E-deficient mice. *Atherosclerosis* 2011;**216**:313–320.
12. Menu P, Pellegrin M, Aubert JF, Bouzourene K, Tardivel A, Mazzolai L, Tschopp J. Atherosclerosis in ApoE-deficient mice progresses independently of the NLRP3 inflammasome. *Cell Death Dis* 2011;**2**:e137.
13. Freigang S, Ampenberger F, Weiss A, Kanneganti TD, Iwakura Y, Hersberger M, Kopf M. Fatty acid-induced mitochondrial uncoupling elicits inflammasome-independent IL-1α and sterile vascular inflammation in atherosclerosis. *Nat Immunol* 2013;**14**:1045–1053.
14. Gomez D, Baylis RA, Durgin BG, Newman AAC, Alencar GF, Mahan S, St Hilaire C, Müller W, Waisman A, Francis SE, Pinteaux E, Randolph GJ, Gram H, Owens GK. Interleukin-1β has atheroprotective effects in advanced atherosclerotic lesions of mice. *Nat Med* 2018;**24**:1418–1429.
15. Alexander MR, Moehle CW, Johnson JL, Yang Z, Lee JK, Jackson CL, Owens GK. Genetic inactivation of IL-1 signaling enhances atherosclerotic plaque instability and reduces outward vessel remodeling in advanced atherosclerosis in mice. *J Clin Invest* 2012;**122**:70–79.
16. Interleukin 1 Genetics Consortium. Cardiometabolic effects of genetic upregulation of the interleukin 1 receptor antagonist: a Mendelian randomisation analysis. *Lancet Diabetes Endocrinol* 2015;**3**:243–253.
17. Vromman A, Ruvkun V, Shvartz E, Wojtkiewicz G, Santos Masson G, Tesmenitsky Y, Folco E, Gram H, Nahrendorf M, Swirski FK, Sukhova GK, Libby P. Stage-dependent differential effects of interleukin-1 isoforms on experimental atherosclerosis. *Eur Heart J* 2019;doi:10.1093/eurheartj/ehz008.
18. Hansson GK. Inflammation, protection, and the problems of translation. *Nat Rev Cardiol* 2018;**15**:729–730.
19. Cicha I, Chauvierre C, Texier I, Cabella C, Metselaar JM, Szebeni J, Dézsi L, Alexiou C, Rouzet F, Storm G, Stroes E, Bruce D, MacRitchie N, Maffia P, Letourneur D. From design to the clinic: practical guidelines for translating cardiovascular nanomedicine. *Cardiovasc Res* 2018;**114**:1714–1727.