Subject categories: Clonal haemato poiesis and atherosclerosis

News & Views

Title “Expansion of fibroblast-like cells may explain the CANTO meta-analysis findings for patients with clonal haematopoiesis”

Clonal haematopoiesis of indeterminate potential (CHIP) is a risk factor for cardiovascular disease (CVD), while IL-1β neutralisation (i.e. CANTOS clinical trial) resulted in a bigger reduction in CV events in patients with CHIP. New research reveals some of the CV benefit of anti-IL-1β therapy in patients with CHIP could be delivered by improving plaque stability via increased fibroblast-like cells.

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Clonal haematopoiesis of indeterminate potential (CHIP) is defined as the presence of clonally expanded blood cells at a frequency ≥2%. CHIP increases with age and is marked by somatic mutations most commonly seen in DNMT3A, TET2, JAK2, and ASXL1. CHIP has been associated with an increased risk of cardiovascular diseases in humans and confers a near doubling of risk for atherosclerotic cardiovascular disease, and increased atherosclerosis in mouse models. Studies in experimental models of Tet2−/− and Jak2V617F-driven clone growth indicate a role for increased NLRP3 and AIM2 inflammasome activation, respectively, resulting in increased active IL-1β that drives atherosclerosis. In the Canakinumab ANti-inflammatory Thrombosis Outcome Study (CANTOS), patients with previous myocardial infarction (MI) and high CRP who were given an IL-1β neutralizing antibody (Canakinumab) had significantly lower incidences of major adverse CV events (MACE) and mortality than those on placebo. Interestingly, an exploratory genomic subgroup analysis of CANTOS showed that patients with expanded mutant TET2 clones had improved outcomes with Canakinumab treatment compared to those without TET2 CHIP.
IL-1β neutralisation could be a targeted therapy for the most at-risk patients with both atherosclerotic disease and CHIP.

This new research by Fidler et al.\textsuperscript{,} provides some vital knowledge to understand mechanisms of targeted IL-1β neutralisation in the setting of atherosclerosis with CHIP. The findings suggest that distinct types of non-inflammatory fibroblasts are increased in \textit{Jak2\textsuperscript{V617F}} and \textit{Tet2\textsuperscript{−/−}} mouse models of CHIP treated with anti-IL-1β neutralising antibodies, and this associated with features of reduced plaque vulnerability, as assessed by fibrous cap thickness and necrotic core area. The study also shows that the expansion of this fibroblast-like cell upon IL-1β neutralisation is CHIP driver mutation-dependent. To generate these findings the authors utilised single-cell RNA sequencing (scRNAseq), genetic cell fate-mapping, and an elegant Dre-rox/Cre-lox dual orthogonal recombinase system allowing sequential activation/inactivation of \textit{Jak2\textsuperscript{V617F}} to simulate CHIP development and suppression. In addition, adeno-associated virus to restore \textit{Ldlr} expression in conjunction with a switch to a low cholesterol diet was used to model plaque regression. They also utilised a genetic approach to deplete fibroblast-like cells during atherosclerosis progression, which resulted in reduced fibrous cap thickness in mice receiving anti-IL-1β antibody, supporting a role for fibroblasts upon IL-1β neutralisation. Fate mapping and scRNAseq analysis showed that these two distinct types of fibroblast-like cells increased upon IL-1β neutralisation most likely due to migration and/or proliferation of resident fibroblasts. Specifically, one population showed high expression of matrix-associated and cell migration genes, while the other had raised expression of mitochondrial electron transport chain genes and genes typically seen in synthetic vascular smooth muscle cells (VSMCs). Furthermore, administration of anti-IL-1β antibodies resulted in no change in clonal expansion in \textit{Jak2\textsuperscript{V617F}} mice and a modest 5% increase in monocytes and neutrophils in \textit{Tet2\textsuperscript{−/−}} mice, but without altered lesion or necrotic core area, indicating the expansion of fibroblast-like cells and increased fibrous cap was not secondary to a reduction in mutant clones within the blood.

Previously, Gomez et al. reported that anti-IL-1β treatment reduced fibrous cap thickness and collagen content within plaques of non-CHIP mice\textsuperscript{,} and that deletion of VSMC \textit{Il1r1} resulted in near complete loss of fibrous caps, suggesting VSMCs are major targets of IL-1. However, in this study, IL-1β blockade increased fibrous cap thickness and collagen content. The authors attribute this discrepancy to different animal models, with the \textit{Jak2\textsuperscript{V617F}} mice providing more
IL-1β over the shorter period of western diet feeding in the Ldlr−/− model, while Gomez used Apoe−/− mice. However, for IL-1β generated during atherogenesis under conditions without CHIP versus with CHIP to have diametrically opposite affects in these models lacks a supportive hypothesis. Future studies to investigate the impact on fibrous cap thickness of specifically blocking IL-1 signalling in fibroblasts or VSMCs in the same atherosclerosis model, with or without CHIP, would provide useful information to explain this discrepancy, and give direct proof that inhibition of fibroblast IL-1R1 indeed alters fibrous cap thickness. Interestingly, Gomez found reduced VSMC-derived ACTA2+ cells in fibrous caps by using Myh11-lineage tracing⁷, whereas this study used scRNAseq and histology to show an increase in ACTA2-positive cells (typically classified as contractile VSMCs) with the overall reduced level of VSMCs traced by Myh11, suggesting ACTA2-positive cells can be derived from a separate reservoir not lineage traced via Myh11.

Moreover, although Jak2V617F is prevalent in CHIP, early studies showed Jak2V617F cells to gradually decrease after transplant⁸, and thus did not effectively model clonal expansion over time, in contrast to what the authors show in a previous study⁹ and this current work. Therefore, whether atherogenesis enables Jak2V617F clonal expansion requires further study.

All research depends on model systems to examine biological phenomena, and thus are always subject to limitations. As the human material from elderly subjects were not individually genotyped for driver mutations, the presence of CHIP is only assumed from the known increase with age at a population level. The effect of anti-inflammatory interventions in CHIP patients will require clinical trials specifically designed to study this, ideally with multi-modality imaging to assess the effect of IL-1β neutralisation on atherosclerotic lesion stability⁹. Furthermore, it’s uncertain if a starting 20% Jak2V617F variant allele fraction is relevant to human patients with atherosclerosis, and most importantly human somatic mutation is heterozygous, unlike homozygous CHIP-driver mutations used in this and other studies.

Furthermore, depleting fibroblasts has emerged as a potential treatment for heart failure, a disease often concomitant with atherosclerosis. Thus, it will be critical to determine if IL-1β neutralisation in heart failure patients with CHIP would also lead to expansion of fibroblast-like cells in hearts, resulting in beneficial effects on plaques but a detrimental increase in
fibrosis in failing hearts. Indeed, as there is a subgroup study of heart failure in CANTOS\textsuperscript{10}, it may be possible to stratify outcome in heart failure patients with or without CHIP.
Figure Legend

**Figure 1. IL-1β neutralisation expanded fibroblast-like cells in atherosclerosis combined with clonal haematopoiesis.** a, CANTOS showed that blocking IL-1β with the neutralising antibody Canakinumab reduced incidence of major adverse cardiovascular events (MACE) by 15% (P=0.02) on patients with previous myocardial infarction (MI) and high level of C-Reactive Protein (CRP> 0.2 mg/dL). b, A meta-analysis of CANTOS showed patients stratified for presence of TET2 clonal haematopoiesis of indeterminate potential (CHIP) had a 62% reduction in MACE (P=0.04), suggesting a greater effect of blocking IL-1β in these patients. c, In preclinical mouse models of atherosclerosis, blocking IL-1β without CHIP had little effect on fibrous caps or fibroblast-like cell accumulation. d, In contrast, blocking IL-1β during atherogenesis in the presence of Tet2−/− or Jak2VF CHIP increased fibrous cap thickness and accumulation of fibroblast-like cells. Thus, the reduced MACE in TET2 CHIP patients receiving Canakinumab is likely mediated via plaque stabilisation.
Competing interests

X.L. is an inventor of the United Kingdom (GB) Patent (application no. 2313514.8) titled “Treatment of Inflammatory Diseases”.

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References