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Trained immunity and cardiovascular disease: is it time for translation to humans?

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Commentary on: 'Metabolic induction of trained immunity through the mevalonate pathway' by Bekkering *et al.*, *Cell* 2018[7]; 'Modulation of myelopoiesis progenitors is an integral component of trained immunity' by Mitroulis *et al.*, *Cell* 2018[8]; 'BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis' by Kaufmann *et al.*, *Cell* 2018[9]; 'Western diet triggers NLRP3-dependent innate immune reprogramming' by Christ *et al.*, *Cell* 2018.[10]

One of the major achievements in the last few years in the field of immunology was the discovery that monocytes, macrophages and natural killer cells can retain 'memory' of previous infections (trained cells) and may exhibit an increased responsiveness upon re-challenge with an insult similar or unrelated to the first one.[1] This process results in enhanced cytokine production and provides a more effective protection against re-infection. Epigenetic reprogramming is the key mechanism supporting the acquisition of trained immunity, with selective changes in histone methylation and acetylation associated with the acquisition of a trained phenotype.[2]

How does the first insult induce changes in the epigenetic signature? During trained immunity, monocytes and macrophages undergo a rapid shift in intracellular metabolism following incubation with ß-glucan (a prototypical trained immunity inducing agonist).[3] Resting monocytes and macrophages mainly use oxidative phosphorylation, but during innate immune training, these cells shift toward aerobic glycolysis, a process able to sustain the metabolic needs associated with cell activation/expansion. Immunometabolic reprogramming, therefore, plays a key role in shaping the immune response.[4]

Is trained immunity relevant in atherosclerosis? *In vitro* data indicate that exposure of monocytes to oxidized LDL induces the enrichment of histone methylation (H3K4me3) in the promoter of several pro-inflammatory genes, including IL6, MCP1, IL8, TNFα, MMP2 and MMP9 that, upon re-challenging, results in an increased production of these cytokines and metalloproteases.[5] Lipoprotein (a) [Lp(a)] can also induce trained immunity, an effect related to the oxidized phospholipids carried by this lipoprotein.[6] The trained phenotype of macrophages observed under these conditions supports the existence of a yin/yang effect: trained immunity is protective toward infections but is deleterious in the context of atherosclerosis. A series of additional questions arises from these observations: (i) why do sterile inflammatory factors induce trained immunity?; (ii) is aerobic glycolysis the only metabolic pathway involved in this process?; (iii) given that trained immunity appears to be a long term effect (up to months), how might this fit with the relatively short lifespan of monocytes?

Recent observations from the International Trained Immunity Consortium have contributed to address most of these aspects.[7–10] Bekkering et al.[7] showed that the mevalonate pathway is crucially involved in the training of myeloid cells induced by several stimuli. This effect is prevented by statins but not by a squalene synthase inhibitor, suggesting that an intermediate of cholesterol biosynthetic pathway, rather than cholesterol itself, induces trained immunity.[7] Indeed, they showed that mevalonate enhances IGF-1 receptor signalling, mTOR activation, glycolysis and epigenetic reprogramming under inflammatory conditions.[7] Mitroulis et al.[8] reported that ß-glucan induces the expansion of myeloid cell precursors already in the bone marrow, by targeting several pathways associated with cell proliferation, cholesterol biosynthesis and glycolysis. This effect depends on the increased production of IL-1ß and granulocyte-macrophage colony stimulating factor (GM-CSF), which in turn activates a STAT5-dependent pathway.[8] These trained hematopoietic precursors are then more effective in protecting toward chemotherapy-induced DNA damage and cell death.[8] Similar observations were reported by Kaufmann et al.,[9] who explored the effect of Bacillus Calmette-Guérin (BCG)-induced training on the protection toward mycobacterium tuberculosis and identified IFNγ signalling as critically involved in this effect. Christ et al.[10] investigated whether and how a cholesterol rich diet may induce trained immunity in a mouse model of hypercholesterolaemia and atherosclerosis (the LDLR KO mouse). The activation of the NLRP3 inflammasome was found to play a key role in this process, but, in spite of the extensive characterization of myeloid progenitor cells in this atheroprone mouse model, Christ et al. did not address whether trained immunity may impact atherosclerotic plaque development.[10] Such a study will be crucial to 'close the circle' and causally link trained immunity tuning with atherogenesis.

Are we ready to move trained immunity in the clinical setting for the treatment of cardiovascular disease? Patients with mevalonate kinase deficiency, who accumulate mevalonate in monocytes, present a constitutive trained immunity phenotype,[7] but the overlap of epigenetic changes with those reported in *in vitro* trained cells is minimal.

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Epigenetics may mediate the effects of environmental risk factors on cardiovascular disease, and, for instance, a methylome analysis has shown a clear impact of epigenome changes on inflammatory vascular remodeling.[11] Moreover, epigenomic and transcriptomic approaches are now considered crucial to identify novel targets for diagnosis and therapy of the ischaemic heart.[12] However, contrary to the expectations, the activating histone methylation marker of trained immunity (H3K4me3) was reduced in the promoter of pro-inflammatory genes (TNFα, IL-6, IL-1ß) in monocytes from patients with symptomatic atherosclerosis compared with controls.[13] These findings support the need for a more comprehensive characterization of the trained immune cell epigenetic signature in the 'real world'.

The detailed molecular characterization by Bekkering et al. excluded a direct role for cholesterol in inducing epigenetic changes but rather pointed to mevalonate as a direct inducer of trained immunity.[7] Mitroulis et al.,[8] however, showed that the whole cholesterol pathway is upregulated during 'training' of immune cells to support the cholesterol demand, by forcing cellular cholesterol biosynthesis (increased HMGCoAR) and cholesterol uptake (increased LDLR) and limiting cholesterol efflux (decreased ABCA1). Surprisingly, Christ et al.[10] showed that trained immunity occurs also in myeloid cells deprived of the LDLR thus limiting the key role for this pathway in the response. These results, which might depend on the different stimuli used as trained immunity inducers, raise the possibility of targeting the mevalonate pathway with statins to dampen trained immunity and vascular inflammation. Although statins possess many pleiotropic effects *in vitro*, the link between statins and reduced inflammation in humans appears to be related to their ability to increase hepatic LDLR expression, thus reducing LDL-C levels and, as a consequence, inflammation.[14] In line with this, Christ et al. observed increased trained immunity only in mice fed a cholesterol rich diet, but not on control diet, further supporting the relevance of cholesterol-driven systemic inflammation for training.

Thus, a LDL-C-independent effect for statins on trained immunity in humans will be hard to prove, unless epigenetic reprogramming of innate immune cells could be observed in patients who are not responding to statins (no changes in LDL-C upon treatment), but in whom a long-term benefit of the therapy on atherosclerosis and cardiovascular outcomes is achieved.

Nevertheless, the key role of the NLRP3 inflammasome-IL-1ß axis in supporting immune training[10] suggests this pathway as a potential target to achieve cardiovascular benefits. In this context, recent data from the CANTOS trial have shown that canakinumab, an antibody targeting IL-1ß, significantly decreases the incidence of myocardial infarction in patients at very high cardiovascular risk,[15] an effect independent of plasma lipid reduction. The 'toll' of the robust tuning of the innate response, however, is a significant increase in the risk of fatal sepsis.[15] It is intriguing to speculate that blocking IL-1ß with canakinumab might also mitigate the acquisition of a trained phenotype, thus contributing to the anti-inflammatory and cardiovascular protective effects observed.[15]

In summary, we now have a clearer picture of molecular mechanisms controlling trained immunity. The next years of research will have to focus on a successful translation in the clinical setting of approaches aimed at controlling trained immunity with the purpose of improving cardiovascular outcomes; and at the same time, should carefully monitor any increase in the risk of infections.

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