

Trained immunity and cardiovascular disease: time for translation in humans?

Giuseppe Danilo Norata¹⁻³

¹Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy.

²Center for the Study of Atherosclerosis, E. Bassini Hospital, Cinisello Balsamo, Milan, Italy.

³Curtin Health Innovation Research Institute, School of Pharmacy and Biomedical Sciences, Curtin University, Perth, Western Australia.

Corresponding Author:

Prof. Giuseppe Danilo Norata

Department of Pharmacological and Biomolecular Sciences, University of Milan

Via Balzaretti, 9 - 20133 Milan ITALY

Phone number: +39250318302

Fax number: +39250318386

e-mail: danilo.norata@unimi.it

One of the major achievement in the last few years in 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 one¹. This results in enhanced cytokine production and provides a more effective protection against re-infection. Epigenetic reprogramming is the key intracellular mechanism supporting the acquisition of trained immunity, with selective changes in histone methylation and acetylation associated to the acquisition of a trained phenotype².

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 β -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 response⁴.

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*, *TNF α* , *MMP2* and *MMP9* that, upon re-challenging, results in an increased production of these cytokines and metalloproteases⁵. Also lipoprotein(a) (Lp(a)) can induce trained immunity, an effect related to the oxidized phospholipids carried by this lipoprotein⁶. 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 arise 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 aspects⁷⁻¹⁰. Bekkering et al. showed that the mevalonate pathway is crucially involved in the training of myeloid cells induced by

several stimuli⁷. 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 immunity⁷. Indeed, they showed that mevalonate enhances IGF-1 receptor signaling, mTOR activation, glycolysis and epigenetic reprogramming under inflammatory conditions⁷. Mitroulis et al. reported that β -glucan induces the expansion of myeloid cell precursors already in the bone marrow, by targeting several pathways associated with cell proliferation, cholesterol biosynthesis and glycolysis⁸. This effect depends on the increased production of IL-1 β and granulocyte-macrophage colony stimulating factor (GM-CSF), which in turn activates a STAT5-dependent pathway⁸. These trained hematopoietic precursors are then more effective in protecting toward chemotherapy-induced DNA damage and cell death⁸. Similar observations were reported by Kaufmann et al., who explored the effect of BCG (Bacillus Calmette-Guérin)-induced training on the protection toward mycobacterium tuberculosis and identified IFN γ signaling as critically involved in this effect⁹. Christ et al. investigated whether and how a cholesterol rich diet may induce trained immunity in a mouse model of hypercholesterolaemia and atherosclerosis (The LDLR KO mouse)¹⁰. 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, there is no indication on whether trained immunity may impact atherosclerotic plaque development¹⁰.

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 phenotype⁷, but the overlap of epigenetic changes with those reported in *in vitro* trained cells is minimal. Moreover, contrary to the expectations, the activating histone methylation marker H3K4me3 was reduced in the promoter of pro-inflammatory genes (TNF α , IL-6, IL-1 β) in monocytes from patients with symptomatic atherosclerosis compared to controls¹¹. These findings support the need for a more comprehensive characterization of the epigenetic signature in the “real world”.

The detailed molecular characterization by Bekkering et al. excluded a direct role for cholesterol but rather pointed to mevalonate as a direct inducer of trained immunity⁷. Mitroulis et al., 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), cholesterol uptake (increased LDLR) and limiting cholesterol efflux (decreased ABCA1)⁸. Surprisingly, Christ et al. showed that trained immunity occurs also in myeloid cells deprived of the LDLR, thus limiting the key role for this pathway in the response¹⁰. 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, the link between statins and inflammation in humans appears to be related to their ability to increase hepatic LDLR expression, thus reducing LDL-C levels and, as a consequence, inflammation¹².

Given the key role of the NLRP3 inflammasome-IL-1 β axis in supporting immune training¹⁰, it is intriguing to speculate that part of the beneficial effects of canakinumab, an antibody targeting IL-1 β , which significantly reduced the incidence of myocardial infarction in patients at very high cardiovascular risk¹³, might depend on the mitigation of trained immunity. The same treatment, however, increased the risk of fatal sepsis with a final neutral effect on total mortality¹³.

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 limiting inflammation and improving atherosclerotic disease, but at the same time should carefully monitor any increase in the risk of infections.

Acknowledgments

The help of Angela Pirillo in proofreading the text is kindly acknowledged. The work of the author is supported by: Fondazione Cariplo 2016-0852, Ministero della Salute GR-2011-02346974 and Aspire Cardiovascular Grant 2016-WI218287.

1. Netea, M.G., Joosten, L.A., Latz, E., Mills, K.H., Natoli, G., Stunnenberg, H.G., *et al.* (2016). Trained immunity: A program of innate immune memory in health and disease. *Science* 352, aaf1098.
2. Saeed, S., Quintin, J., Kerstens, H.H., Rao, N.A., Aghajani-refah, A., Matarese, F., *et al.* (2014). Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science* 345, 1251086.
3. Cheng, S.C., Quintin, J., Cramer, R.A., Shepardson, K.M., Saeed, S., Kumar, V., *et al.* (2014). mTOR- and HIF-1 α -mediated aerobic glycolysis as metabolic basis for trained immunity. *Science* 345, 1250684.
4. Norata, G.D., Caligiuri, G., Chavakis, T., Matarese, G., Netea, M.G., Nicoletti, A., *et al.* (2015). The cellular and molecular basis of translational immunometabolism. *Immunity* 43, 421-434.
5. Bekkering, S., Quintin, J., Joosten, L.A., van der Meer, J.W., Netea, M.G., and Riksen, N.P. (2014). Oxidized low-density lipoprotein induces long-term proinflammatory cytokine production and foam cell formation via epigenetic reprogramming of monocytes. *Arterioscler Thromb Vasc Biol* 34, 1731-1738.
6. van der Valk, F.M., Bekkering, S., Kroon, J., Yeang, C., Van den Bossche, J., van Buul, J.D., *et al.* (2016). Oxidized Phospholipids on Lipoprotein(a) Elicit Arterial Wall Inflammation and an Inflammatory Monocyte Response in Humans. *Circulation* 134, 611-624.
7. Bekkering, S., Arts, R.J.W., Novakovic, B., Kourtzelis, I., van der Heijden, C., Li, Y., *et al.* (2018). Metabolic Induction of Trained Immunity through the Mevalonate Pathway. *Cell* 172, 135-146 e139.
8. Mitroulis, I., Ruppova, K., Wang, B., Chen, L.S., Grzybek, M., Grinenko, T., *et al.* (2018). Modulation of Myelopoiesis Progenitors Is an Integral Component of Trained Immunity. *Cell* 172, 147-161 e112.
9. Kaufmann, E., Sanz, J., Dunn, J.L., Khan, N., Mendonca, L.E., Pacis, A., *et al.* (2018). BCG Educates Hematopoietic Stem Cells to Generate Protective Innate Immunity against Tuberculosis. *Cell* 172, 176-190 e119.
10. Christ, A., Gunther, P., Lauterbach, M.A.R., Duewell, P., Biswas, D., Pelka, K., *et al.* (2018). Western Diet Triggers NLRP3-Dependent Innate Immune Reprogramming. *Cell* 172, 162-175 e114.
11. Bekkering, S., van den Munckhof, I., Nielen, T., Lamfers, E., Dinarello, C., Rutten, J., *et al.* (2016). Innate immune cell activation and epigenetic remodeling in symptomatic and asymptomatic atherosclerosis in humans in vivo. *Atherosclerosis* 254, 228-236.
12. Catapano, A.L., Pirillo, A., and Norata, G.D. (2017). Vascular inflammation and low-density lipoproteins: is cholesterol the link? A lesson from the clinical trials. *Br J Pharmacol* 174, 3973-3985.
13. Ridker, P.M., Everett, B.M., Thuren, T., MacFadyen, J.G., Chang, W.H., Ballantyne, C., *et al.* (2017). Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med* 377, 1119-1131.