Dyslipidemia and regulatory T cell migration: an immunometabolic connection?

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The activation of the adaptive immune response plays a key role during atherogenesis (1) with dyslipidemia and cholesterol accumulation in the arterial wall promoting an extensive immune-inflammatory response which also sustains the immunometabolic reprogramming of lymphocyte T cells (2). Hyperlipidemia results in the increase of circulating levels of several lymphocyte T cells subsets including T effector and T regulatory cells (3,4), which is paralleled only by T effector cells increase in the atherosclerotic plaque. On the contrary, a reduced number of T regulatory cells is observed in vulnerable plaques compared to stable plaques (5). Data from experimental models indicate that Treg deficiency promotes atherosclerosis progression and focusses the interest in understanding the mechanisms that might explain why, in spite of increased circulating levels (6,7), plaque Treg migration and perhaps function are affected under hypercholesterolaemic conditions. A simple explanation might imply that hyperlipidemic conditions depotentiate Tregs which partially lose their suppressive function and thus increase in number to compensate this functional deficiency. This however is not enough to control T effector expansion during atherogenesis.

The work by Amersfoort et al. (8) introduces a novel unexpected variable in this equation by showing that dyslipidaemia does not reduce the ability of Treg to migrate in the aorta or in secondary lymph nodes but rather promotes a series of cellular metabolic adaptations which contribute to support their migration into the atherosclerotic plaque.

Indeed, cellular metabolic adaptations play a key role in redirecting the response of T lymphocytes including their ability to proliferate, polarize and migrate to peripheral tissues. How T cells sense the extracellular environment also critically affect their function. Among T cell subsets, Treg are known to rely mainly on oxidative phosphorylation for their basal homeostasis while rapidly upregulate glycolysis to generate a large amount of energy which is required to support their migration (9) as opposed to Th17 cells which appear to depend on de novo fatty acid synthesis for their development (10).

Apart from intracellular metabolism, the availability of extracellular metabolic substrates might also influence their response and potential impact their function. On these premises, a systemic increase in lipids (triglycerides and cholesterol) such as that observed in dyslipidemic conditions should also be sensed by Treg cells and potentially boosts their lipid dependent cellular metabolism. Previously, hyperlipidemic conditions resulting from a high fat diet were shown to prime CD4+ T cells toward an effector phenotype paralleled by a decreased expression of surface receptors implicated in lymph nodes homing such as CD62L or CCR7. At the molecular levels, the increased availability of free fatty acid was shown to impair the PI3Kp110δ-Akt kinase signaling pathway (11). Here, Amersfoort et al (8) aim at testing whether hypercholesterolaemia, such as that observed in LDL-R deficient mice, also impacts the migratory phenotype and cellular metabolism of Treg cells residing in secondary lymphoid organs.

Interestingly the authors observed that elevated LDL-C levels are associated with changes in mTOR signaling and glycolysis, as well as increased PPARδ target gene expression and FA oxidation in splenic Treg cells. Unexpectedly, dyslipidemia increased the capacity of Treg cells to migrate towards sites of inflammation and the PPARδ agonist GW501516 further increased their migration in an FA oxidation-dependent manner. These results imply that under hypercholesteraemic conditions, Treg acquire the ability to migrate in inflamed tissues such as the atherosclerotic plaque. Moreover, some Tregs specific subsets from cholesterol fed mice present increased cellular lipid accumulation as compared to standard diet fed mice. This finding is unexpected; given that LDL-R KO mice mainly transport cholesterol in LDL particles which however cannot be internalized by cells due to the intrinsic deficiency of the receptor. How cholesterol accumulates in LDL-R KO Treg remains to be demonstrated and a detailed lipidomic analysis of Treg from cholesterol fed LDL-R KO mice would help in clarifying these aspects. Data from the authors suggest that CD36 expression is increased and could be involved in the effect observed; however, the possibility that the expression is upregulated as an adaptation to LDL-R deficiency should be considered when discussing these findings.

Nevertheless, the authors performed a series of in vitro experiments with VLDL (highly enriched in triglycerides and cholesterol) which indicated that these lipoproteins can deliver lipids into Treg and redirect cellular metabolism. Testing VLDL and not LDL (which is the lipoprotein class largely increased in LDL-R KO mice) limits the possibility to appreciate whether is cholesterol, TGs/FAs or both that impact the expression of Treg cellular receptors responsible for lipid/lipoprotein uptake. Therefore, how LDL-R or other lipoprotein receptors are modulated in activated Treg, remains unexplored.

An intriguing observation is that the increased availability of cellular lipids, does not translate in increased mitochondrial biogenesis, cellular oxygen consumption or cell proliferation in spite of increased fatty acid oxidation. Moreover, while mTORC1 activity appears to be decreased in Treg from LDL-R KO cholesterol fed mice, compared to Treg from LDLR-KO fed a standard diet, both CPT1 mRNA expression and FA oxidation (both under the control of mTORC1) are not affected or rather increased, thus suggesting that the picture is more complicated and perhaps factors other than mTORC1 play a relevant role in this experimental setting. Unfortunately, authors do not present data from wild type mice fed with a cholesterol rich diet thus limiting the possibility to discriminate between a Treg response dependent solely on hyperlipidemia (hypercholesterolemia) vs that dependent on LDL-R deficiency and related cellular adaptations (increased CD36 expression?).

Do these changes affect Treg biology during atherogenesis?. Data from Amersfoort et al. (8) indicate that Treg ($CD24CD25^{high}$) levels increase in the atherosclerotic plaque, suggesting that the hypercholesterolemic environment improves rather than decrease their ability to reach the inflammatory sites, thus improving their migratory capacity. Whether this represents the direct consequence of hyperlipidemia or marks a mechanism aimed at compensating a decreased functionality remains to be addressed. Indeed, increased

glycolysis appear to be crucial to support Treg migration (10), however data from Amersfoort et al. exclude that Treg from cholesterol fed LDL-R present increased glycolysis (which rather is impaired), thus suggesting that other mechanisms might influence their ability to migrate to the atherosclerotic plaque. Adoptive transfer experiments in the peritoneum homing model showed that Treg from cholesterol fed LDL-R KO mice migrate more efficiently than those from standard fed LDL-R KO controls. However, the comparison with Treg from cholesterol fed WT mice would be more informative to support an increased migratory capacity under hypercholesterolemic conditions or to exclude an increased retention in the plaque.

A final point of discussion is the evolutionary reason for which Treg should home more efficiently to sites of inflammation under hyperlipidemic conditions. Intriguingly plasma cholesterol levels drop after an acute infection or during sepsis and return to basal levels within days when the acute phase is passed. Is therefore the increase in plasma cholesterol levels a condition to instruct Treg to migrate to inflamed site when they need to dampen an effector response? Still this hypothesis does not fit with the observation that circulating Treg levels are increased under several inflammatory conditions including atherosclerosis and rather call for increased levels and/or migratory capacity or tissue retention as feedback mechanisms to potentially balance for an impaired activity. Future studies should be designed to specifically address these aspects.

Conflict of interest.

Nothing to declare

Figure legend

Hypercholetserolemia results in increased levels of lymphocyte T effector cells which infiltrate the atherosclerotic plaque and contribute to the inflammatory response (left part of the panel). Also lymphocyte T regulatory cells increase during hypercholesterolaemia and data from Amesfoort et al. suggest that also Treg cells get engulfed with lipids (increased CD36 expression could be involved in mediating lipid uptake). Increased lipid accumulation contributes to cellular metabolic reprogramming, favoring their migration to the atherosclerotic plaque in a FA oxidation dependent manner. Points of discussion remain the possibility that the increase of Treg cells in the plaque might also result from their

retention and that, in spite of increased content of Treg in the atherosclerotic plaque, they present a compromised immunosuppressive function thus limiting their ability to control the pro-inflammatory response by lymphocyte T effector cells.

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Figure(s)