Promoting Atherosclerosis in Type 1 Diabetes Through the Selective Activation of Arachidonic Acid and PGE$_2$ Production

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Diabetes Promotes an Inflammatory Macrophage Phenotype and Atherosclerosis Through Acyl-CoA Synthetase 1
Kanter et al

Type 1 diabetics harbor a greatly elevated risk for progressive atherosclerosis and cardiovascular events, but the mechanistic basis for this phenomenon is not entirely clear. Although this link is likely to involve many factors, the specific activation of a lipid-driven inflammatory phenotype in monocytes and macrophages of people with type 1 diabetes is an attractive causal mechanism, due to the ability of inflamed macrophages to exacerbate plaque deposition, expansion, and instability.

In a recent article in PNAS, Kanter et al. recapitulate the dyslipidemic inflammatory phenotype of monocytes and macrophages in 2 mouse models of type 1 diabetes and show that the expression of genes involved in cytokine and eicosanoid synthesis are activated by diabetes in both mice and humans. Further, they demonstrate that the levels of cytokines and prostaglandin E2 (PGE$_2$) produced by monocytes and macrophages under diabetic conditions are significantly increased. These results nicely mirror those observed in macrophages from humans with type 1 diabetes, suggesting an essential connection between type 1 diabetes mellitus and an inflammatory macrophage phenotype. The mechanisms giving rise to this abnormal inflammatory state may derive from either the elevated serum glucose or fatty acids levels present in type 1 diabetes or from other factors yet to be determined. A clue to a potentially important role for fatty acids in either the activation or execution of the inflammatory phenotype in this context came with the intriguing observation that long-chain acyl-CoA synthetase 1 (ACSL1) mRNA expression was elevated in each of the mouse and human macrophage populations in diabetes.

ACSL1 is an important member of the class of enzymes responsible for activating intracellular free fatty acids to their CoA thioesters. Fatty acids are critical components of cell membranes (eg, phospholipids), energy storage, and transportation molecules (eg, triglycerides) and are used for the production of potent inflammatory signals (eg, eicosanoids). Because they are not water-soluble, a large array of regulated proteins has evolved to steward the itinerary of fatty acids throughout the cell. One class of enzymes—the long-chain acyl-CoA synthetases—plays a particularly important role by activating and solubilizing free fatty acids by forming fatty acid-CoA esters. These fatty acyl-CoA molecules are soluble in the cytoplasm and can serve as substrates for fatty acid metabolism, the assembly of complex lipids, and β-oxidation.

Previous reports on the ACSL1 gene suggested a role for the enzyme in activating or directing fatty acids toward β-oxidation.²⁻⁴ Fatty acids can stimulate inflammatory signaling through a variety of mechanisms in multiple cell types by either serving as substrates for inflammatory mediator production (eg, arachidonic acid and eicosanoids) or through other mechanisms related to lipid overload at tissues, lipotoxicity, and metabolic inflammation (eg, palmitate exposure), including potential interactions with pathogen sensors.⁵ Hence, Kantor et al generated a myeloid-specific depletion of ACSL1 to interrogate the role fatty acid activation in type 1 diabetes-induced inflammation in bone marrow-derived populations, particularly in macrophages and monocytes. As it turns out, elevations of ACSL1 were not simply associated with the inflammatory phenotype but fundamentally contributed to it. Myeloid-specific ACSL1 deficiency protected macrophages from the inflammatory effects of type 1 diabetes, suggesting either a primary role for fatty acid activation in inflammation or a secondary rescue of the phenotype by the same mechanism. Interestingly, ACSL1 depletion did not appear to have a significant effect on inflammatory processes in monocytes or macrophages in nondiabetic mice. This finding is an intriguing insight into the link between the lipid handling and immune response and suggests the importance of fatty-acid metabolism and availability in the emergence or alleviation of macrophage inflammation. Because macrophage inflammation is an important element of atherosclerosis, these results raised the possibility that inhibition of ACSL1 may improve atherosclerotic process.

The specifics of how ACSL1 deficiency protected macrophage from diabetes-induced inflammation were surprising in several ways. First, in contrast to its reported role in other tissues, ACSL1 depletion did not affect the accumulation of lipids or the β-oxidation of fatty acids in macrophages. Thus, it is possible that in monocytes/macrophages, the induction of inflammation may not be due to the obvious stresses of a general oversupply of fatty acids as observed in type 2 diabetes or in other tissues, such as hepatocytes. Alterna-

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tively, activated macrophages favor glycolytic metabolism, and therefore changes in β-oxidation may not have a substantial influence on energy metabolism at this state. Second, the depletion of ACSL1 lead to a decrease in cellular arachidonyl-CoA (AA-CoA) levels that was specific and not generalizable to other fatty acyl-CoAs measured in this study. This is in contrast to the reported more general role of the enzyme in activating many types of fatty acids in other tissues.8,9 ACSL1 appears therefore to mediate an accumulation of AA-CoA that may be a specialized function of myeloid cells. AA is the precursor of proinflammatory eicosanoids including prostaglandins and leukotrienes, and some of the pathways that assemble AA into membrane pools (where AA is liberated for eicosanoid production) require that AA first be activated into its CoA thioester. Thus, a logical explanation of the results is that ACSL1 deficiency in macrophages may specifically deny the cell the arachidonic acid precursor it needs to generate inflammatory mediators such as eicosanoids. How and why ACSL1 mediates this specialized enrichment of AA in macrophage when it appears to have a specificity for different fatty acids in other tissues is an interesting and important question for future research.

Supporting a specific role for AA in the macrophage inflammatory phenotype, Kanter et al showed that ACSL1 deficient macrophage also produced less PGE2, and secreted a decreased level of inflammatory cytokines. Thus, ACSL1 appears to play a central role in the activation of inflammation under type 1 diabetic conditions. Two important results of this study were that treating cultured macrophages with PGE2 replicated the activation of the inflammatory phenotype seen in type 1 diabetes, and the ACSL1-deficient phenotype could be recapitulated macrophages from diabetic animals by the addition of a cyclooxygenase 2 inhibitor. The authors also established the functional significance of the myeloid ACSL1 deficiency in vivo. Mice deficient in myeloid ACSL1 were protected from diabetes-induced increases brachiocephalic artery atherosclerotic lesions and increased levels of macrophage infiltration. Importantly, no differences between ACSL1-deficient and wild-type mice were observed in the absence of the diabetic state. These experiments suggest that fatty acid–derived mediators including PGE2 are upstream of the activation of the inflammatory phenotype in macrophage cells in type 1 diabetes, an important finding and a solid concept on which to explore therapeutic options.

There are certainly some questions raised by this study that challenge a standard view of fatty acid handling and lipid metabolism. The authors correctly point out that source of the increased AA-CoA in macrophages in type 1 diabetes is not likely to relate to increased intracellular synthesis because macrophages are generally thought to acquire their AA from plasma and because it is unlikely that AA would be uniquely affected among fatty acids by a change in the overall synthesis rates. It is less clear, however, whether the increased AA-CoA in diabetic macrophages is a result of increased activation of newly acquired AA by ACSL1 (eg, uptake from plasma) or an increase in the activation of AA that is liberated intracellularly to meet the demands of a proinflammatory phenotype (Figure). Kantor et al argue that it is the former, and that ACSL1 deficiency in the macrophage leads to a reduction in the accumulation of AA into membrane phospholipids that would serve as pools for the generation of PGE2 and other eicosanoids through the canonical AA cascade. It should be noted that this hypothesis, while not a central posit of the report, remains to be tested—and it is unclear whether ACSL1 deficiency in fact leads to a reduction of AA in membrane phospholipids, or for that matter what impact ACSL1-deficiency had on free AA levels. Perhaps even more curious is the fact that AA-CoA is explicitly not a substrate for cyclooxygenase, as COX1 and COX2 use free AA to produce PGE2 and other prostaglandins. Thus, in one view, increased ACSL1 activity should actually deplete the AA pool available for PGE2 production by competing with COX enzymes for the free AA substrate. So the critical mechanistic question really is, where in the long string of reactions between serum AA and PGE2 does ACSL1 act to increase the inflammatory phenotype and why is this effect only seen in the presence of diabetes? A classic perspective on lipids would suggest that the most likely cause for AA-CoA levels to rise uniquely among fatty acids in response to inflammation is as a result of increased PLA2–liberated membrane AA and a subsequent conversion of some of these AA molecules to AA-CoA by ACSL1. This view would explain both the specific increase in AA-CoA with inflammation and the decrease in AA-CoA in ACSL1 deficiency, but it most certainly does not explain the profoundly anti-inflammatory effects of depleting ACSL1. One unorthodox but plausible alternative may be that AA-CoA is made at some point as an intermediate in the pathway between diabetes-associated PLA2 cleavage of AA and COX activity. Clearly, there is lipid biology left to understand here, and important biology at that. An informative next step would be to profile in detail the free fatty acid and phospholipid composition of ACSL1-deficient macrophages in the context of diabetes and compare to control cells.

Although it is unexpected for ACSL1 to have AA-specific activity in macrophages, it is a parsimonious interpretation of the data and it raises a new and interesting therapeutic strategy that the authors nicely describe. Namely, cutting cells off from their inflammatory substrate is a potentially powerful strategy for modulating inflammation and inflammation-driven phenotypes. There are two intriguing possibilities for this approach—the first is to generally deny a cell access to exogenous fatty acids by diminishing the ability of the cell to activate transported fatty acids into acyl-CoAs. This could affect cellular metabolism in many ways, including relieving an overload of fat and carbon or by forcing a switch in fuel type used by the cell. Another way inhibiting ACSL enzymes could affect cell metabolism is by denying specific fatty acids entry into the intracellular metabolic pool. This second strategy would provide more intricate control of metabolic effect—but raises the complexity considerably.

Cutting cells off from their inflammatory substrate, or at least interrupting the intracellular distribution of substrate, is an intriguing and largely unexplored strategy for modulating inflammation and inflammation-driven phenotypes. This is particularly possible for lipid-mediated inflammation because the substrate for lipid mediators are exogenous, non–water-soluble, and can be prevented from arriving at key pools by
inhibiting related enzyme activities. With this in mind, it would have been interesting to explore the incorporation and utilization of eicosapentaenoic acid (EPA), arachidonic acid’s omega-3 counterpart. EPA is processed by the same pathways as AA (liberation from membrane phospholipids by phospholipases, formation of active oxygenated metabolites by cyclooxygenases and lipoxygenases) but yields anti-inflammatory and antithrombotic series-3 prostaglandins and series-5 leukotrienes as well as proresolving mediators such as resolvins. An interesting question for future work is whether ACSL1 depletion or inhibition might also inhibit an anti-inflammatory effect of EPA-loading macrophages, or whether monocytes and macrophage can retain their EPA-associated eicosanoid production while blocking their AA-associated eicosanoid production with this strategy. Ultimately, for therapeutic purposes, it is not clear that the depletion of ACSL1 would achieve a different result than COX inhibition and what would be the consequences for other cell types that may rely on ACSL1 for other purposes (as the existing literature on ACSL1 implies). Nevertheless, there is much promise in the concepts and results presented in this study, and perhaps a lot contributed to our knowledge of the diabetes-associated increase in cardiovascular risk.

Principally, ACSL1 inhibition has the promise to affect inflammation only under diabetes-activated conditions and perhaps to add a new dimension of control over the use of fatty acid substrates for the production of inflammatory and anti-inflammatory products.

References
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