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The VASP Road to NAFLD: A Macrophage Detour

Diabetes 2015;64:2711–2713 | DOI: 10.2337/db15-0551

Nonalcoholic fatty liver disease (NAFLD), a common clinical disorder, is increasing largely due to increasing consumption of diets high in fructose and fat (1–3). Indeed, NAFLD has been reported to be present in up to 30% of the U.S. population (3), closely mirroring the prevalence of obesity. Its presence carries an increased risk for type 2 diabetes, end-stage liver disease, and cardiovascular disease (2). Obesity and type 2 diabetes are characterized by chronic low-grade inflammation in which maladaptive immune responses contribute to the development of resistance to the metabolic actions of insulin, thus generating a proinflammatory milieu that leads to steatosis and fibrosis of the liver (4).

A critical event in the pathophysiology of tissue inflammation is the shift in the polarization status of macrophages from an anti-inflammatory (M2) to a proinflammatory (M1) phenotype of macrophages (5,6). M1 polarization of hepatic Kupffer cells (KCs) plays an important role in the genesis of hepatic steatosis and insulin resistance in response to chronic overnutrition (6). M1 polarization results in increased expression of proinflammatory mediators, such as CD11c, tumor necrosis factor α, interleukin (IL)-6, and inducible nitric oxide synthase (7). Conversely, the anti-inflammatory M2 phenotype secretes preferentially CD206, arginase1, and IL-10 (8). The role of macrophage M1/M2 polarization status in conditions of insulin resistance has been explored using myeloid-specific knockout and high-fat–feeding paradigms. Several chemokines impact macrophage polarization and subsequent activity. C-C motif chemokine receptor (CCR)2 and its corresponding ligand, monocyte chemoattractant protein 1, are involved in adipose tissue macrophage infiltration, insulin resistance, and hepatic steatosis (9,10). In a rodent model of insulin resistance, deletion of CCR5 is protective against the development of hepatic steatosis via decreased M1 macrophage polarization (11). In addition, hepatocyte-derived Th2 cytokines trigger peroxisome proliferator–activated receptor δ, which in turn mediates M2 polarization (12). Nevertheless, the mechanisms that regulate M1/M2 macrophage polarization are still not completely understood.

Normally insulin signaling though the metabolic phosphatidylinositol-3 kinase/protein kinase B cascade results in increased activation and phosphorylation of endothelial nitric oxide synthase (eNOS) with consequent elevation in bioavailable nitric oxide (NO) (13). In turn, NO activates soluble guanylate cyclase with consequent activation of the cyclic guanosine monophosphate (cGMP) protein kinase (14), which phosphorylates vasodilator-simulated phosphoprotein (VASP) (15). VASP belongs to the enabled (Ena)/VASP family of proteins involved in cytoskeleton assembly and organization (16). In vascular smooth muscle cells, VASP activation modulates proliferation and growth (17). Previously, it has been reported that a high-fat diet results in reductions in liver NO, in parallel with diminished VASP activation, enhanced M1 macrophage polarization, and increased hepatic triglyceride content (15). Both the global deletion of eNOS or VASP recapitulate these findings without need for a high-fat–diet challenge, thus highlighting the role of diminished NO and VASP activation in the pathogenesis of fatty liver (15). The changes in hepatic triglyceride content were further explained by VASP-driven activation of AMP-activated protein kinase and consequent enhanced fatty acid oxidation (18). However, in the setting of insulin resistance and chronic low-grade inflammation, such as obesity, type 2 diabetes, and NAFLD, there is decreased activation of eNOS and reduced bioavailable NO.

In this issue of *Diabetes*, Lee et al. (19) use a model of insulin resistance induced by a high-fat diet to further explore the impact of the novel NO/VASP pathway on macrophage polarization. Transgenic mice overexpressing eNOS were protected from hepatic steatosis, insulin resistance, and inflammation. These changes occurred in concert with increased M2 KC polarization. The authors also investigated the effect of a lack of hematopoietic VASP using sublethally irradiated mice fed a low-fat diet.

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See accompanying article, p. 2836.

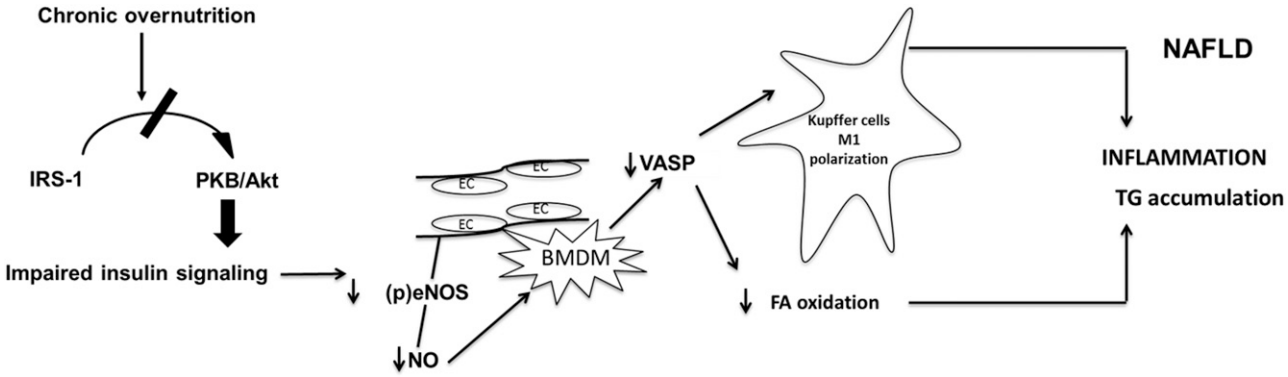

Figure 1—Proposed role of eNOS and signaling through VASP on KC polarization status and contribution to inflammation in the liver and NAFLD. EC, endothelial cell; TG, triglycerides; (p)eNOS, phospho-eNOS; PKB/Akt, protein kinase B/Akt.

Mice reconstituted with VASP-negative bone marrow exhibited hepatic insulin resistance and M1 KC polarization. In vitro studies using cultured bovine aortic endothelial cells and bone marrow–derived macrophages (BMDMs) were also undertaken. The ability of bovine aortic endothelial cells to produce NO was diminished by small interfering RNA. In conditions of NO depletion, BMDMs stimulated with lipopolysaccharide (LPS) and interferon-γ (IFN-γ) demonstrated increased M1 polarization. On the contrary, when NO production was normal, the expression of M2 macrophage polarization markers was increased in response to IL-4. When macrophages were stimulated with an NO donor, the expression of M1 markers was decreased in the presence of LPS/IFN-γ, and M2 polarization was enhanced when stimulated with IL-4. Furthermore, the impact of the NO/VASP pathway on M1/M2 polarization status was explored. Lack of VASP in BMDMs resulted in decreased expression of M2 markers, reduced fatty acid oxidation, and decreased activation of the IL-4 downstream signaling protein phospho-STAT6. Increased expression of VASP decreased M1 polarization in a macrophage line pretreated with LPS/IFN-γ.

Lee et al. (19) logically conclude that M1/M2 macrophage polarization status is modulated by eNOS via downstream signaling involving VASP. M1 KC polarization and subsequent production of proinflammatory mediators are tonically inhibited by NO/VASP signaling and thus are protective against high-fat diet–induced insulin resistance and hepatic inflammation. Thus, NO/VASP signaling favors the anti-inflammatory M2 KC phenotype and is required to prevent inflammation and insulin resistance in the liver (Fig. 1).

These novel findings could potentially translate into therapeutic interventions. Medications that promote NO signaling, such as the cGMP-specific phosphodiesterase-5 sildenafil, have been shown to improve glucose homeostasis and systemic, as well as skeletal muscle, insulin sensitivity in mice fed a high-fat diet (20). One important caveat of this study is that the authors do not address the role of high-fructose feeding. As the high-fructose, high-fat diet is ubiquitously consumed in modern societies and has been consistently linked to NAFLD (1), further studies are warranted. As described, M1/M2 polarization status is controlled by several factors other than eNOS, and the absolute importance of the NO/VASP pathway relative to other potent inflammation mediators, such as nuclear factor-κB and peroxisome proliferator–activated receptor γ, among many others, remains to be fully uncovered. Additionally, eNOS is implicated in several biologic processes in numerous tissues, including oxidative stress, vascular reactivity, and platelet aggregation. Thus, modulation of NO availability is likely to have pleiotropic effects that still require further characterization and could impact the potential of this strategy for treatment and/or prevention of NAFLD.

Acknowledgments. The authors would like to thank Brenda Hunter (Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, University of Missouri) for her editorial assistance.

Funding. This research was supported by the National Institutes of Health (R01 HL73101-01A and R01 HL107910-01) and the Veterans Affairs Merit System (0018) to J.R.S.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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