Liver Kinase B1 Links Macrophage Metabolism Sensing and Atherosclerosis

Vishal Kothari, Karin E. Bornfeldt

Atherosclerosis is a chronic inflammatory disease characterized by the accumulation of cholesterol-laden macrophages at susceptible sites in the artery wall. Altered cellular metabolism plays an important role in the conversion of a macrophage into a lipid-laden foam cell and also in the ability of the macrophage to mount an inflammatory response. In the atherosclerotic lesion environment, the macrophage is exposed to a plethora of extracellular molecules that govern its phenotype, including cytokines, modified lipids, nutrients, and extracellular matrix components. Intracellular metabolic sensors allow the macrophage to adjust to the lesional environment and to alter its cellular functions accordingly. The molecular mechanisms underlying pathophysiological changes in the metabolism sensing machinery are not fully understood. In the current issue of *Circulation Research*,[1] Liu et al enhance our understanding of foam cell formation by reporting that loss of expression of the metabolic sensor LKB1 (liver kinase B1) in macrophages results in increased uptake of modified lipoproteins, increased foam cell formation, and subsequently, increased atherosclerosis in 2 different mouse models. This study provides new links between macrophage metabolic sensing and atherosclerosis.

Article, see p 1047

LKB1, also known as STK11 (serine/threonine kinase 11), is a kinase originally identified as a tumor suppressor in patients with Peutz–Jeghers syndrome—an autosomal dominant genetic disorder associated with increased risk of developing cancer in the gastrointestinal tract and other organs.[2] Later studies revealed that LKB1 acts in part as an upstream activator of AMPK (AMP-activated protein kinase)—an intracellular energy sensor activated by low nutrient status in cells as a mechanism to preserve energy.[3] However, LKB1 also has AMPK-independent targets in cells. Recent studies have shown that the LKB1/AMPK pathway is activated by glucose deprivation and that reduced levels of the glycolytic intermediate fructose-1,6-bisphosphate play a critical role in mediating glucose sensing by this pathway.[4] In bone marrow hematopoietic cells, complete loss of LKB1 results in reduced levels of ATP and reduced mitochondrial function, despite increased glucose uptake and elevated cellular fatty acids,[5] demonstrating that LKB1 activation is critical for maintaining cellular energy needs. In macrophages, loss of LKB1 has been shown to result in an enhanced ability of bacterial lipopolysaccharide to induce nuclear factor κB activation and cytokine production.[6] Thus, the LKB1/AMPK pathway appears to be activated in cells in response to nutrient (eg, glucose) deprivation to preserve cellular energy rather than to perform energy demanding functions, such as mounting an inflammatory attack.

On the contrary, in a nutrient-sufficient environment, inflammatory activation of macrophages by lipopolysaccharide and other inflammatory mediators causes an increase in glucose uptake through glucose transporter 1, increased aerobic glycolysis, and downstream alterations of cellular metabolism—a metabolic cascade required for full inflammatory activation of these cells[7,8] (Figure). In some cases, exposure of macrophages to increased nutrients increases their inflammatory activation.[9–11] Thus, the nutritional state of the cell governs activation of the LKB1/AMPK pathway (in states of nutrient deprivation) and inflammatory activation in response to lipopolysaccharide and other inflammatory mediators (in states of nutrient plenty). LKB1 might, therefore, act as a rheostat, allowing the cell to preserve energy or expend energy as needed.

Consistently, lipopolysaccharide stimulation of macrophages results in loss of LKB1 levels because of S-nitrosylation and subsequent LKB1 proteasomal degradation, likely because of induction of inducible NO synthase by lipopolysaccharide.[12] Liu et al[1] now demonstrate that modified low-density lipoprotein (oxidized low-density lipoprotein) acts through a similar pathway to suppress LKB1 expression in macrophages and further that LKB1 levels are reduced in atherosclerotic lesions. The group observed reduced levels of LKB1 in human carotid atherosclerotic plaques as compared with a healthy artery, suggesting involvement of LKB1 in the development of atherosclerosis. This was further confirmed in *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ mice fed a Western diet for different durations of time. A reduction in the level of LKB1 was associated with accumulation of lesional macrophages after 16 weeks of Western diet feeding. When the authors[1] treated either Raw264.7 macrophage or primary mouse macrophages in vitro with oxidized low-density lipoprotein, differentiated cells showed low LKB1 protein and mRNA expression, similar to that observed in human atherosclerotic carotid artery. Interestingly, the effect of atherosclerosis and oxidized low-density lipoprotein

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the Division of Metabolism, Endocrinology, and Nutrition, Department of Medicine (V.K., K.E.B.) and Department of Pathology (K.E.B.), University of Washington Medicine Diabetes Institute, University of Washington School of Medicine, Seattle.

Correspondence to Karin E. Bornfeldt, PhD, Department of Medicine, UW Medicine Diabetes Institute, University of Washington, 850 Republican St, Seattle, WA. E-mail bornf@u.washington.edu

(*Circ Res*. 2017;121:1024-1026.
DOI: 10.1161/CIRCRESAHA.117.311923.)

***Circulation Research* is available at http://circres.ahajournals.org**
DOI: 10.1161/CIRCRESAHA.117.311923

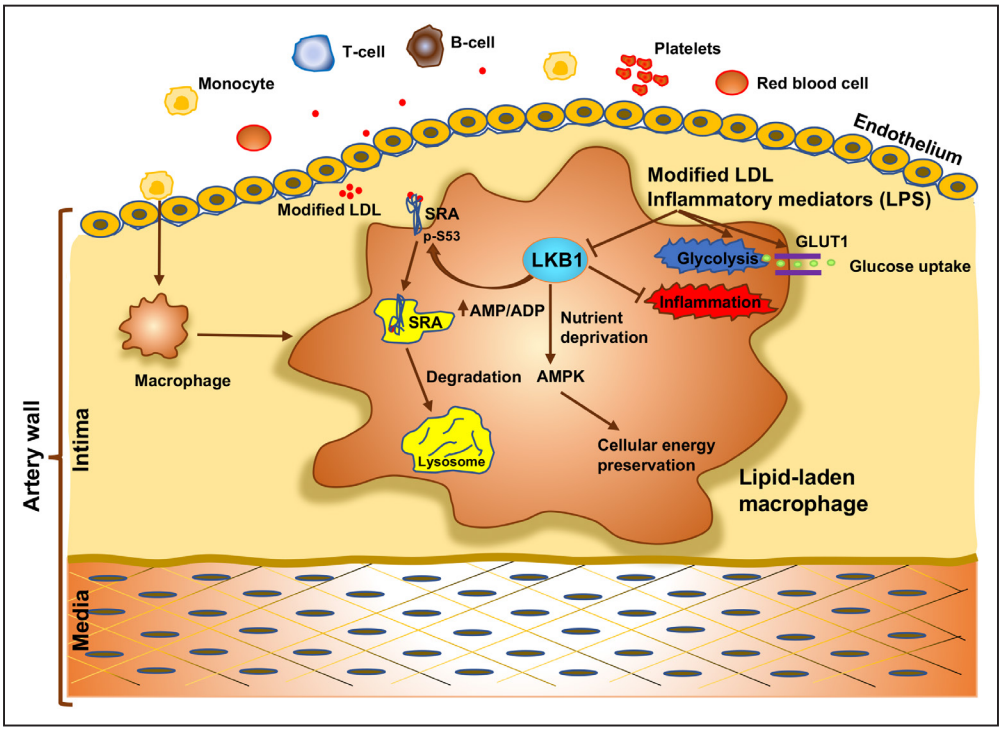

Figure. Schematic representation of some of the effects of the metabolic sensor LKB1 (liver kinase B1) in macrophages. In the atherosclerotic lesion, in areas where nutrients are sufficient, increased inflammatory mediators and modified low-density lipoprotein can cause reduced LKB1 levels through LKB1 degradation in macrophages. Loss of LKB1 in turn reduces serine phosphorylation (p-S53) and subsequent lysosomal degradation of SRA (scavenger receptor A), which associates with increased uptake of modified lipoproteins, increased foam cell formation, and increased inflammatory activation of macrophages in response to inflammatory mediators. Inflammatory mediators also increase glycolysis and downstream metabolic processes required for the macrophage to mount a full immune response. In a nutrient-deprived state, on the contrary, glycolysis and levels of glycolytic intermediated are low, and the LKB1 pathway is activated to preserve cellular energy. The cell is impaired in its ability to respond to inflammatory stimuli. LKB1, thus, may act as a rheostat to allow the lesional macrophage to respond appropriately depending on its nutritional state. LPS indicates lipopolysaccharide.

on LKB1 in arterial smooth muscle cells was negligible, indicating the relatively greater importance of macrophage LKB1 in atherosclerosis. To interrogate the role of macrophage LKB1, the group then silenced LKB1 or used bone marrow-derived macrophages from LKB1-deficient mice. They observed a higher capacity to form foam cells, as indicated by higher level of oil red O staining, by LKB1-deficient macrophages, as compared with control cells. This increased ability of LKB1-deficient cells to form foam cells was mainly because of an increased uptake of modified low-density lipoprotein mediated by upregulation of scavenger receptor A (SRA), rather than reduced cholesterol efflux. The authors demonstrated that LKB1 can directly phosphorylate SRA at serine 53 in the cytoplasmic tail and promote its degradation through a lysosome-dependent pathway (Figure). Importantly, the authors then confirmed these findings in *Ldlr*$^{-/-}$ mice transplanted with bone marrow from LKB1-deficient mice and by using myeloid cell-targeted knockout of LKB1 in *Apoe*$^{-/-}$ mice. The myeloid cell-targeted LKB1 deficiency was observed to promote atherosclerosis, and this proatherogenic effect was associated with increased levels of SRA and macrophage content within lesions. Notably, these effects of LKB1 deficiency were present in the absence of increased plasma lipids. Therefore, LKB1 seems to serve as master regulator in macrophages that link cellular metabolic sensing with atherosclerosis.

This study raises multiple interesting questions and complex challenges. (1) To what extent are the proatherogenic effects of hematopoietic- and myeloid cell-targeted LKB1 deficiency mediated by changes in levels of circulating hematopoietic cells—an issue not addressed by the present study. This is interesting because LKB1 is known to be critically required for hematopoiesis.[5,13,14] (2) To what extent are the effects of LKB1 on foam cell formation and SRA mediated by AMPK? The authors suggest that LKB1 phosphorylates SRA directly and have previously shown that AMPK α1-deficiency does not alter macrophage foam cell formation.[15] SRA might, therefore, be an AMPK-independent target of LKB1 (Figure). (3) Given the role of LKB1 as a regulator of proliferation and cell survival, would loss of LKB1 in lesional macrophages result in increased proliferation and altered necrotic core formation in more advanced lesions? (4) What is the human relevance of the findings by Liu et al?[1] Although the authors demonstrate that LKB1 is reduced in atherosclerotic lesions from human subjects, as compared with normal artery samples, the study population was small (6 patients), and different arteries were compared (atherosclerotic plaques in carotid arteries and healthy internal mammary arteries). Furthermore, an increased risk of cardiovascular disease in patients with Peutz–Jeghers syndrome has not yet been reported. A possible role for LKB1 in cardiovascular disease in humans, therefore, needs additional studies. (5) Could restored activation

of LKB1 be used as a possible strategy for preventing atherosclerosis and inflammation in conditions where LKB1 levels are reduced, and would activation of LKB1 in macrophages reduce foam cell formation, alter cellular metabolism, and increase resolution of inflammation? The fact that LKB1 is involved in a multitude of vital processes in many tissues makes it a challenging prospect as a drug target. Nevertheless, these questions are interesting avenues for future studies. We look forward to future studies addressing the emerging concept of cellular metabolic sensing as an important process in atherogenesis.

Sources of Funding

Research in the authors' laboratory is supported by the National Institutes of Health (grants R01HL126028, R01HL062887, P01HL092969, and P30DK017047).

Disclosures

None.

References

1. Liu Z, Zhu H, Dai X, Wang C, Ding Y, Song P, Zou MH. Macrophage liver kinase B1 inhibits foam cell formation and atherosclerosis. *Circ Res*. 2017;121:1047–1057. doi: 10.1161/CIRCRESAHA.117.311546.
2. Shorning BY, Clarke AR. Energy sensing and cancer: LKB1 function and lessons learnt from Peutz-Jeghers syndrome. *Semin Cell Dev Biol*. 2016;52:21–29. doi: 10.1016/j.semcdb.2016.02.015.
3. Carling D. The AMP-activated protein kinase cascade–a unifying system for energy control. *Trends Biochem Sci*. 2004;29:18–24. doi: 10.1016/j.tibs.2003.11.005.
4. Zhang CS, Hawley SA, Zong Y, et al. Fructose-1,6-bisphosphate and aldolase mediate glucose sensing by AMPK. *Nature*. 2017;548:112–116. doi: 10.1038/nature23275.
5. Gurumurthy S, Xie SZ, Alagesan B, Kim J, Yusuf RZ, Saez B, Tzatsos A, Ozsolak F, Milos P, Ferrari F, Park PJ, Shirihai OS, Scadden DT, Bardeesy N. The Lkb1 metabolic sensor maintains haematopoietic stem cell survival. *Nature*. 2010;468:659–663. doi: 10.1038/nature09572.
6. Liu Z, Zhang W, Zhang M, Zhu H, Moriasi C, Zou MH. Liver kinase B1 suppresses lipopolysaccharide-induced nuclear factor κB (NF-κB) activation in macrophages. *J Biol Chem*. 2015;290:2312–2320. doi: 10.1074/jbc.M114.616441.
7. Nishizawa T, Kanter JE, Kramer F, Barnhart S, Shen X, Vivekanandan-Giri A, Wall VZ, Kowitz J, Devaraj S, O'Brien KD, Pennathur S, Tang J, Miyaoka RS, Raines EW, Bornfeldt KE. Testing the role of myeloid cell glucose flux in inflammation and atherosclerosis. *Cell Rep*. 2014;7:356–365. doi: 10.1016/j.celrep.2014.03.028.
8. Van den Bossche J, O'Neill LA, Menon D. Macrophage immunometabolism: where are we (going)? *Trends Immunol*. 2017;38:395–406. doi: 10.1016/j.it.2017.03.001.
9. Kim HS, Tavakoli S, Piefer LA, Nguyen HN, Asmis R. Monocytic MKP-1 is a sensor of the metabolic environment and regulates function and phenotypic fate of monocyte-derived macrophages in atherosclerosis. *Sci Rep*. 2016;6:34223. doi: 10.1038/srep34223.
10. Kratz M, Coats BR, Hisert KB, et al. Metabolic dysfunction drives a mechanistically distinct proinflammatory phenotype in adipose tissue macrophages. *Cell Metab*. 2014;20:614–625. doi: 10.1016/j.cmet.2014.08.010.
11. Kanter JE, Kramer F, Barnhart S, et al. Diabetes promotes an inflammatory macrophage phenotype and atherosclerosis through acyl-CoA synthetase 1. *Proc Natl Acad Sci USA*. 2012;109:E715–E724. doi: 10.1073/pnas.1111600109.
12. Liu Z, Dai X, Zhu H, Zhang M, Zou MH. Lipopolysaccharides promote S-nitrosylation and proteasomal degradation of liver kinase B1 (LKB1) in macrophages in vivo. *J Biol Chem*. 2015;290:19011–19017. doi: 10.1074/jbc.M115.649210.
13. Gan B, Hu J, Jiang S, Liu Y, Sahin E, Zhuang L, Fletcher-Sananikone E, Colla S, Wang YA, Chin L, Depinho RA. Lkb1 regulates quiescence and metabolic homeostasis of haematopoietic stem cells. *Nature*. 2010;468:701–704. doi: 10.1038/nature09595.
14. Nakada D, Saunders TL, Morrison SJ. Lkb1 regulates cell cycle and energy metabolism in haematopoietic stem cells. *Nature*. 2010;468:653–658. doi: 10.1038/nature09571.
15. Zhang M, Zhu H, Ding Y, Liu Z, Cai Z, Zou MH. AMP-activated protein kinase α1 promotes atherogenesis by increasing monocyte-to-macrophage differentiation. *J Biol Chem*. 2017;292:7888–7903. doi: 10.1074/jbc.M117.779447.

Key Words: Editorials ■ atherosclerosis ■ foam cells ■ glycolysis ■ lipid metabolism ■ macrophages ■ metabolism

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

Liver Kinase B1 Links Macrophage Metabolism Sensing and Atherosclerosis

Vishal Kothari and Karin E. Bornfeldt

Circ Res. 2017;121:1024-1026
doi: 10.1161/CIRCRESAHA.117.311923
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571