Atherosclerosis 220 (2012) 362–368

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Hepatocyte-specific IKKβ expression aggravates atherosclerosis development in *APOE*3-Leiden* mice

Man C. Wong[a,b,*], Janna A. van Diepen[a], Lihui Hu[a], Bruno Guigas[c], Hetty C. de Boer[d,e], Gijs H. van Puijvelde[f], Johan Kuiper[f], Anton J. van Zonneveld[d,e], Steven E. Shoelson[g], Peter J. Voshol[a,1], Johannes A. Romijn[a,2], Louis M. Havekes[a,h,i], Jouke T. Tamsma[a], Patrick C.N. Rensen[a], Pieter S. Hiemstra[b], Jimmy F.P. Berbée[a,j]

[a] *The Dept. of General Internal Medicine, Endocrinology, and Metabolic Diseases, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands*
[b] *The Dept. of Pulmonology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands*
[c] *The Dept. of Molecular Cell Biology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands*
[d] *The Dept. of Nephrology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands*
[e] *The Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands*
[f] *Division of Biopharmaceutics, Leiden/Amsterdam Center for Drug Research, Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden, The Netherlands*
[g] *Joslin Diabetes Center and the Dept. of Medicine, 1 Joslin Place, Boston, MA 02215, USA*
[h] *The Dept. of Cardiology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands*
[i] *Netherlands Organization for Applied Scientific Research – Biosciences, Gaubius Laboratory, P.O. Box 2215, 2301 CE Leiden, The Netherlands*
[j] *The Dept. of Experimental Immunohematology, Sanquin Research Amsterdam, P.O. Box 9190, 1006 AD Amsterdam, The Netherlands*

ARTICLE INFO

Article history:
Received 30 March 2011
Received in revised form 3 June 2011
Accepted 29 June 2011
Available online 12 July 2011

Keywords:
NF-κB
Atherosclerosis
Mouse models
Liver
Hepatocyte
Inflammation
Lipid metabolism

ABSTRACT

Objective: The liver is the key organ involved in systemic inflammation, but the relation between hepatic inflammation and atherogenesis is poorly understood. Since nuclear factor-κB (NF-κB) is a central regulator of inflammatory processes, we hypothesized that chronically enhanced hepatic NF-κB activation, through hepatocyte-specific expression of IκB kinase-β (IKKβ) (*LIKK*), will aggravate atherosclerosis development in *APOE*3-Leiden* (*E3L*) mice.

Methods and results: *E3L.LIKK* and *E3L* control littermates were fed a Western-type diet for 24 weeks. *E3L.LIKK* mice showed a 2.3-fold increased atherosclerotic lesion area and more advanced atherosclerosis in the aortic root with less segments without atherosclerotic lesions (11% *vs.* 42%), and more segments with mild (63% *vs.* 44%) and severe (26% *vs.* 14%) lesions. Expression of *LIKK* did not affect basal levels of inflammatory parameters, but plasma cytokine levels tended to be higher in *E3L.LIKK* mice after lipopolysaccharide (LPS) administration. *E3L.LIKK* mice showed transiently increased plasma cholesterol levels, confined to (V)LDL. This transient character resulted in a mild (+17%) increased cumulative plasma cholesterol exposure.

Conclusion: We conclude that selective activation of NF-κB in hepatocytes considerably promotes atherosclerosis development which is (at least partly) explained by an increased sensitivity to proinflammatory triggers and transiently increased plasma cholesterol levels.

Abbreviations: apoB, apolipoprotein B; CE, cholesteryl ester; Cpt1a, carnitine palmitoyltransferase 1a; cyclo, cyclophilin; *E3L*, *APOE*3-Leiden*; Fas, fatty acid synthase; FC, free cholesterol; FPLC, fast performance liquid chromatography; Gapdh, glyceraldehyde-3-phosphate dehydrogenase; Hmgcr, HMG-CoA reductase; Hprt, hypoxanthine-guanine phosphoribosyl transferase; HPS, hematoxylin–phloxine–saffron; IFNγ, interferon γ; IκB, inhibitor of κB; IKKβ, IκB kinase-β; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-10, interleukin-10; IL-12p70, interleukin-12p70; *LIKK*, liver-specific IKKβ; MCP-1, monocyte chemoattractant protein-1; MTTP, microsomal triglyceride transfer protein; NF-κB, nuclear factor-κB; PL, phospholipid; RT-PCR, real-time PCR; SAA, serum amyloid A; Srebp-1c, sterol-regulatory element binding protein; TC, total cholesterol; TG, triglyceride; TNFα, tumor necrosis factor α; VLDL, very-low-density lipoprotein.

* Corresponding author at: Leiden University Medical Center, Department of Pulmonology, Postzone: C3-P, P.O. Box 9600, 2300 RC Leiden, The Netherlands. Tel.: +31 071 5262082; fax: +31 071 5266927.
E-mail address: M.C.Wong@lumc.nl (M.C. Wong).
[1] Present address: Metabolic Research Laboratories, Level 4, Institute of Metabolic Science, Box 289, Addenbrooke's Hospital Cambridge, CB2 0QQ, United Kingdom.
[2] Present address: The Dept. of Internal Medicine, University of Amsterdam, P.O. Box 22660, 1100 DD Amsterdam, The Netherlands.

0021-9150/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2011.06.055

1. Introduction

Increased inflammation, in addition to disturbances in lipid metabolism, is the other main contributor to the development of atherosclerosis [1]. Nuclear factor-κB (NF-κB) has been identified as the most important transcription factor in the regulation of inflammatory processes during atherosclerosis development [2]. In unstimulated cells, NF-κB p65/p50 dimer is kept inactive by its inhibitory protein: inhibitor of κB (IκB). A wide range of extracellular stimuli, including cytokines, microbial components, and also free fatty acids, induce activation of the IκB kinase complex, which consists of two kinases (IKKα and -β) and a regulatory subunit, NEMO/IKKγ. This complex mediates the phosphorylation of IκB, resulting in its ubiquitination and degradation, leaving the NF-κB dimer free to translocate to the nucleus and activate its target genes [2].

While general inhibition of the NF-κB pathway by pharmacological agents reduces atherosclerosis development in mice [3,4], the relative contribution of NF-κB may differ at cellular- or tissue-specific level. Suppression of the NF-κB pathway in endothelial cells by ablation of NEMO/IKKγ has been shown to decrease atherosclerosis development [5]. In murine bone marrow transplantation models, inhibition of the NF-κB pathway at distinct levels in hematopoietic cells can have different outcomes, *i.e.* deficiency of the NF-κB p50 subunit resulted in smaller atherosclerotic lesions [6], whereas deletion of IKKβ increased atherosclerosis development [7]. Surprisingly, the role of the NF-κB pathway in hepatocytes on atherosclerosis development has not been investigated thus far.

The liver plays a central role in both lipid metabolism [8] and inflammation [9]. Disturbances in lipid metabolism and increased inflammation are the two main risk factors for atherogenesis [1]. Hepatocytes form the largest population of cells in the liver and execute most of its important functions. During inflammation, acute phase proteins are mainly synthesized by the hepatocytes [10]. Interestingly, hepatocyte-specific deficiency of gp130, a receptor component of IL-6 signaling which signals independent of the NF-κB pathway, decreases atherosclerosis in *apoe*$^{-/-}$ mice [11], suggesting that reduced hepatic inflammation is associated with less atherosclerosis development.

Despite ample evidence implicating the involvement of NF-κB in atherogenesis, the hepatocyte-specific role of NF-κB in atherosclerosis has not been investigated directly. Therefore, in this study we aimed to investigate whether chronic activation of hepatocyte-specific NF-κB aggravates atherosclerosis development. We used transgenic mice with hepatocyte-specific expression of human IKKβ (liver-specific IKKβ or *LIKK* mice), resulting in an increase of active NF-κB [12], crossbred with atherosclerosis-prone *APOE*3-Leiden* (*E3L*) mice. *E3L* mice exhibit a human-like lipoprotein distribution on a cholesterol-rich diet due to transgenic expression of a human mutant of the *APOE3* gene, and are therefore susceptible to atherosclerosis development [13]. Collectively, our results show that hepatocyte-specific NF-κB activation markedly aggravates atherosclerosis development in *E3L* mice.

2. Methods

Brief descriptions of the most important procedures of this study are provided in this section. An expanded description is available in the supplemental data (available online at http://atherosclerosis-journal.com).

2.1. Animals

Transgenic *LIKK* mice expressing constitutively active human IKKβ in hepatocytes under the control of an albumin promoter [12] were crossbred with *E3L* mice [13] to generate heterozygous *E3L.LIKK* and control *E3L* littermates, as described before [14]. Ten-12 weeks old female mice were fed a Western-type diet for 24 weeks. Blood was drawn every 4 weeks after a 4-h fast.

2.2. Plasma analysis

Plasma levels of serum amyloid A (SAA), inflammatory cytokines, total cholesterol (TC), triglycerides (TG) and phospholipids (PL) levels were determined.

2.3. Lipopolysaccharide stimulation

Mice were injected i.v. with *Salmonella minnesota* Re595 lipopolysaccharide (LPS) (50 mg/kg body weight). Blood was collected 90 min after injection and plasma was assayed for cytokines.

2.4. Atherosclerosis quantification

The extent of atherosclerosis was assessed in the aortic root area. After staining with hematoxylin–phloxine–saffron (HPS), atherosclerotic lesion severity and area were determined.

3. Results

3.1. LIKK causes low-grade inflammation

The overall appearance of *E3L* and *E3L.LIKK* mice during the study was similar. To assess whether expression of *LIKK* affects body weight gain, we measured food intake and body weight weekly. Both were not different between *E3L.LIKK* and *E3L* control mice (Supplemental Fig. 1A and B). The liver- and spleen weight and histological morphology of the liver were also comparable between *E3L.LIKK* and *E3L* mice (data not shown). To gain more insight in the effects of *LIKK* on inflammation, we determined whether *LIKK* expression increased the inflammatory state of the liver and systemic inflammatory markers in *E3L.LIKK* mice on a Western-type diet. We confirmed previous findings [14] showing that the enhanced expression of hepatocyte-specific human IKKβ (Supplemental Fig. 2A) resulted in a 1.4-fold increased hepatic NF-κB activation, as shown by an increase in the phosphorylated p65 subunit (pNF-κB^{Ser536}) (Supplemental Fig. 2B). IKKβ kinase phosphorylates subunit p65 of NF-κB at the position Ser536, which activates the transcriptional activity of NF-κB [15]. The transgenic expression of human IKKβ mRNA was present only in *E3L.LIKK* mice and did not alter murine IKKβ mRNA expression (Supplemental Fig. 2C and D). The enhanced hepatic NF-κB activation in *E3L.LIKK* mice did not result in increased IL-6 expression in whole liver, but did result in a tendency towards increased IL-1β expression ($P = 0.085$) and a significant increase in MCP-1 expression (Supplemental Table 2).

To evaluate whether the increased hepatocyte-specific NF-κB activation in *E3L.LIKK* mice enhanced the systemic inflammatory state, we determined the plasma inflammation marker SAA and plasma cytokines under basal conditions. *LIKK* expression did not affect SAA before (3.1 ± 0.17 μg/mL *vs.* 3.4 ± 0.15 μg/mL) and after 8 weeks (4.4 ± 0.28 μg/mL *vs.* 4.2 ± 0.31 μg/mL) and 24 weeks (4.9 ± 0.51 μg/mL *vs.* 5.4 ± 0.78 μg/mL) of Western-type diet feeding (Fig. 1), and neither the determined plasma cytokine levels (Supplemental Fig. 3A–F). SAA levels increased with Western-type diet feeding in both *E3L* and *E3L.LIKK* mice, but this difference only reached statistical significance in *E3L.LIKK* mice (Fig. 1).

Since we did not observe a clear increased systemic proinflammatory state under basal conditions, we challenged the mice with LPS to boost the inflammatory response. Interestingly, after injection of LPS, proinflammatory cytokines (*e.g.* IL-1β, IFNγ) showed