Myeloid-Specific IκB Kinase β Deficiency Decreases Atherosclerosis in Low-Density Lipoprotein Receptor–Deficient Mice

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Abstract

Objective—Inflammatory responses are the driving force of atherosclerosis development. IκB kinase β (IKKβ), a central coordinator in inflammation through regulation of nuclear factor-κB, has been implicated in the pathogenesis of atherosclerosis. Macrophages play an essential role in the initiation and progression of atherosclerosis, yet the role of macrophage IKKβ in atherosclerosis remains elusive and controversial. This study aims to investigate the impact of IKKβ expression on macrophage functions and to assess the effect of myeloid-specific IKKβ deletion on atherosclerosis development.

Methods and Results—To explore the issue of macrophage IKKβ involvement of atherogenesis, we generated myeloid-specific IKKβ-deficient low-density lipoprotein receptor–deficient mice (IKKβ^ΔMyeLDLR−/−). Deficiency of IKKβ in myeloid cells did not affect plasma lipid levels but significantly decreased diet-induced atherosclerotic lesion areas in the aortic root, brachiocephalic artery, and aortic arch of low-density lipoprotein receptor–deficient mice. Ablation of myeloid IKKβ attenuated macrophage inflammatory responses and decreased

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None.

atherosclerotic lesional inflammation. Furthermore, deficiency of IKKβ decreased adhesion, migration, and lipid uptake in macrophages.

Conclusion—The present study demonstrates a pivotal role for myeloid IKKβ expression in atherosclerosis by modulating macrophage functions involved in atherogenesis. These results suggest that inhibiting nuclear factor-κB activation in macrophages may represent a feasible approach to combat atherosclerosis.

Keywords

atherosclerosis; inflammation; IκB kinase β; macrophage; nuclear factor-κB

Atherosclerosis is a chronic inflammatory disease.[1,2] Many inflammatory pathways that contribute to the initiation and progression of atherosclerosis are regulated by the transcription factor nuclear factor (NF)-κB, a master regulator of innate and adaptive immune responses.[3-5] The NF-κB family consists of 5 members: p65 (RelA), RelB, c-Rel, p100/p52, and p105/p50. NF-κB normally remains in the cytoplasm bound to the inhibitory protein IκB. Activating signals, such as proinflammatory cytokines, reactive oxygen species, and viral products, lead to activation of IκB kinase (IKK), which then phosphorylates IκB and promotes its degradation, allowing NF-κB to translocate to the nucleus where NF-κB can bind to the promoter regions of its target genes.[3,5] IKK consists of 2 kinase subunits, IKKα and IKKβ, and a regulatory subunit NF-κB essential modulator or IKKγ.[3,6] IKKβ is the predominant catalytic subunit of the IKK complex, which is required for canonical activation of NF-κB by inflammatory mediators.[6]

IKKβ-mediated NF-κB activation has been implicated in the pathogenesis of atherosclerosis. Activated NF-κB has been identified in human atherosclerotic plaques and was enhanced in unstable coronary plaques.[7,8] NF-κB activation in human atherosclerosis was IKKβ-dependent and resulted in selective upregulation of major proinflammatory and prothrombotic mediators.[8] Mapping atherosclerosis modifier loci on the apolipoprotein E–deficient (ApoE$^{-/-}$) background had localized the negative regulator of NF-κB, A20 to a locus conferring sensitivity to atherosclerosis in ApoE$^{-/-}$ mice.[9] Indeed, atherosclerosis was increased in A20 haploinsufficient and decreased in A20-overexpressing ApoE$^{-/-}$ mice.[10] In addition, inhibiting NF-κB activity in endothelial cells (ECs) by the deletion of NF-κB essential modulator or expression of dominant-negative IκBα decreased vascular inflammation and atherosclerosis in ApoE$^{-/-}$ mice.[11]

Macrophages are the major inflammatory cells involved in the progression of atherosclerosis, yet the role of macrophage IKKβ in atherosclerosis remains elusive and controversial. NF-κB–mediated inflammatory functions by macrophages have generally been considered to be proatherogenic.[2,4] interestingly, Kanters et al[12] previously reported that low-density lipoprotein–deficient (LDLR$^{-/-}$) mice transplanted with IKKβ-deficient macrophages had increased atherosclerosis. However, macrophage-specific inhibition of NF-κB activation by overexpressing transdominant, nondegradable forms of IκBα decreased foam cell formation,[13] and myeloid-specific IκBα deletion promoted atherogenesis in LDLR$^{-/-}$ mice.[14] Furthermore, LDLR$^{-/-}$ mice with hematopoietic cells deficient in the NF-κB subunit p50 also had decreased atherosclerotic lesion size.[15] The inconsistent conclusions drawn from these studies suggest that further research is needed to define the role of macrophage IKKβ/NF-κB signaling in atherosclerosis.

To explore the issue of macrophage IKKβ involvement of atherogenesis, we generated myeloid-specific IKKβ-deficient LDLR$^{-/-}$ mice (IKKβ$^{\Delta Mye}$LDLR$^{-/-}$). Here, we report that myeloid IKKβ deficiency protected LDLR$^{-/-}$ mice from diet-induced atherosclerosis, most likely because of modulated macrophage functions involved in atherogenesis.

Methods

Animals

Myeloid-specific IKKβ knockout (IKKβ[ΔMye]) mice were generated by crossing mice carrying loxP-flanked IKKβ alleles (IKKβ[F/F]) with LysM-Cre transgenic mice,[16] as previously described.[17,18] IKKβ[F/F] mice were kindly provided by Dr Michael Karin at the University of California, San Diego. The mice used in this study were backcrossed at least 7 additional generations onto the C57BL/6 background (>98% C57BL/6) using the marker-assisted Microsatellite Genotyping technique. To increase susceptibility to atherosclerotic lesion development, the IKKβ[ΔMye] mice were crossed to LDLR^{−/−} mice (Jackson Laboratories) to generate IKKβ[ΔMye]LDLR^{−/−} and IKKβ[F/F]LDLR^{−/−} mice. All mice used in this study had IKKβ[F/F]LDLR^{−/−} double-mutant background, and IKKβ[ΔMye]LDLR^{−/−} mice carried heterozygous knock-in for LysM-Cre. For atherosclerosis study, 4-week-old experimental male IKKβ[F/F]LDLR^{−/−} and IKKβ[ΔMye]LDLR^{−/−} littermates were weaned and fed with a high-fat Western diet (21.2% fat, 0.2% cholesterol; TD 88137, Harlan Teklad) for 12 weeks until euthanization at 16 weeks of age. Body composition was measured by EchoMRI (Echo Medical System). All procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee.

Blood Analysis

Plasma total cholesterol and triglyceride concentrations were determined enzymatically by a colorimetric method.[19] Plasma from multiple mice (n=6) was pooled, and plasma lipoprotein cholesterol distributions were determined by fast-performance liquid chromatography.[20]

Atherosclerotic Lesion Analysis

Optimal cutting temperature compound–embedded hearts or brachiocephalic arteries were sectioned and stained with Oil-red-O, and atherosclerotic lesions were quantified as previously described.[19] Aortas were harvested and fixed with 10% formalin, and en face analysis was performed as previously described.[21]

Macrophage Isolation and Function Assays

Macrophages were isolated as previously described.[20,22] Bone marrow–derived macrophages (BMMs) were isolated from the femurs of mice and cultured in DMEM medium supplemented with 10 ng/mL recombinant mouse macrophage colony–stimulating factor (Invitrogen) for 7 days before the experiment. Elicited peritoneal macrophages were harvested from each genotype by peritoneal lavage with PBS 3 days after intraperitoneal injection of 1 mL of 3% thioglycollate. For adhesion assay, calcein acetoxymethyl–labeled peritoneal macrophages were incubated with primary porcine ECs, and attached cells were fixed and counted.[23] Migration assays were performed using transwells with 8.0-μm pore polycarbonate membrane inserts (Corning).[22] Macrophages were seeded on the transwell filters, and the lower chambers were filled with either control media or media containing 500 ng/mL lipopolysaccharide (LPS). After 5 hours, cells were removed from the upper surface of the insert by scraping using Q-Tips. The membranes were fixed with 1% glutaraldehyde (Sigma), stained with hematoxylin (Leica) and mounted on the slides using glycerol gelatin. Hematoxylin-stained cells were counted under the microscope. For lipid uptake assay, macrophages were incubated with DMEM containing 100 μg/mL of oxidized LDL (Biomedical Technologies) for 24 hours, followed by washing with PBS and staining with Oil-red-O/hematoxylin.[20]

RNA Isolation and Quantitative Real-Time PCR Analysis

Total RNA was isolated from mouse tissues or cells using TRIzol Reagent (Life Technologies), and quantitative real-time polymerase chain reaction (PCR) was performed using gene-specific primers and the SYBR green PCR kit (Life Technologies) as previously described.[19,20] The primer sets used in this study are listed in Table I in the online-only Data Supplement.

Statistical Analysis

All data are expressed as mean±SD. Statistically significant differences between 2 groups were analyzed by 2-tailed Student t test for data normally distributed and by the Mann-Whitney test for data not normally distributed using Prism software. A P value <0.05 was considered significant.

Results

Generation of LDLR$^{-/-}$ Mice With Myeloid-Specific IKKβ Deficiency

To investigate the role of macrophage IKKβ in atherosclerosis, we generated IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice by crossing IKKβ$^{\Delta Mye}$ (LysM-Cre/IKKβ$^{F/F}$) mice[17] with LDLR$^{-/-}$ mice. All mice used in this study had IKKβ$^{F/F}$LDLR$^{-/-}$ double-mutant background, and IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice also carried heterozygous knock-in for LysM-Cre. PCR analysis of genomic DNA indicates that the recombination was specific to the BMMs (Figure I in the online-only Data Supplement), consistent with previous reports that LysM-Cre–mediated gene excision almost exclusively occurs in macrophages and neutrophils.[16,24] The mRNA levels of IKKβ were significantly decreased in peritoneal macrophages and BMMs but not in other major tissues of IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice compared with IKKβ$^{F/F}$LDLR$^{-/-}$ mice (Figure 1A). Consistent with the quantitative real-time PCR results, protein levels IKKβ were also substantially reduced in both peritoneal macrophages and BMMs of IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice (Figure 1B). The protein levels of IKKα were not affected in macrophages of IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice, indicating the specific deletion of IKKβ (Figure 1B). To determine whether deficiency of IKKβ inhibits NF-κB activity in macrophages, BMMs isolated from IKKβ$^{F/F}$LDLR$^{-/-}$ and IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice were treated with NF-κB stimulator, tumor necrosis factor-α (TNFα). TNFα-induced NF-κB subunit p65 translocation from cytoplasm to nucleus was inhibited in BMMs of IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice (Figure 1C). Electrophoretic mobility shift assay confirmed that TNFα-induced DNA binding activity of NF-κB in macrophages was attenuated by IKKβ deficiency (Figure 1D). It is worth noting that NF-κB activity was not completely blocked in macrophages of IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice, which may be because of the incomplete gene deletion induced by LysM-Cre/LoxP (Figure 1A and 1B). These results indicate that IKKβ expression levels were significantly decreased in macrophages of IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice, and activation of NF-κB was inhibited in IKKβ-deficient macrophages.

Deficiency of IKKβ in Macrophages Does Not Affect Plasma Lipid Levels But Decreases Atherosclerosis in LDLR$^{-/-}$ Mice

To assess the impact of deficiency of macrophage IKKβ on atherosclerosis, 4-week-old male IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ and IKKβ$^{F/F}$LDLR$^{-/-}$ littermates were fed a high-fat Western diet for 12 weeks. At 16 weeks of age, no significant differences in body weight, lean mass, fat mass, and fasting glucose levels were observed in IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ and IKKβ$^{F/F}$LDLR$^{-/-}$ mice (Figure II in the online-only Data Supplement). Both IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ and IKKβ$^{F/F}$LDLR$^{-/-}$ mice had diet-induced hyperlipidemia, but myeloid IKKβ deficiency did not affect plasma total cholesterol and triglyceride levels (Figure 2A and 2B). Furthermore,

fast-performance liquid chromatography analysis showed that IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ and IKKβ$^{F/F}$LDLR$^{-/-}$ mice had similar plasma cholesterol distribution pattern (Figure 2C).

Quantification of cross-sectional lesion areas at the aortic root revealed that IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice had 25% decreased lesion area (310 900 ± 28 310 μm^2) compared with IKKβ$^{F/F}$LDLR$^{-/-}$ littermates (414 500 ± 30 940 μm^2; Figure 3A). In addition to aorta, deficiency of myeloid IKKβ also inhibited atherosclerosis development in the brachiocephalic artery, an artery prone to developing advanced lesions. Brachiocephalic artery cross-sectional lesion areas were decreased 51% in IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice (38 930 ± 6571 μm^2) compared with IKKβ$^{F/F}$LDLR$^{-/-}$ mice (79 950 ± 15 320 μm^2; Figure 3B). Furthermore, en face analysis of the aortic arch also showed 32% decreased lesions in IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice (9.96 ± 0.62% in IKKβ$^{F/F}$LDLR$^{-/-}$ mice and 6.73 ± 1.01% in IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice; Figure 3C). Taken together, deficiency of myeloid IKKβ significantly decreased diet-induced atherosclerosis in LDLR$^{-/-}$ mice.

Deficiency of IKKβ Attenuates Macrophage Inflammatory Responses and Reduces Lesional Inflammation

In atherosclerosis, macrophages participate in inflammatory responses that contribute to expansion of the subendothelial layer and lesion formation and progression.[2] To determine the role of IKKβ in the regulation of macrophage inflammatory responses, BMMs were isolated from IKKβ$^{F/F}$LDLR$^{-/-}$ and IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice and treated with endotoxin LPS. Gene expression analyses showed that the ability of LPS to induce expression of mRNAs encoding TNFα, monocyte chemotactic protein (MCP)-1, interleukin (IL)-1α, IL-1β, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 was attenuated in macrophages of IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice (Figure 4A). These results suggest that ablation of IKKβ reduces NF-κB–regulated proinflammatory gene expression in macrophages. Indeed, gene profiling of peritoneal macrophages isolated from Western diet–fed IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice showed downregulation of several proinflammatory molecules, including TNFα, IL-1α, IL-1β, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 (Figure 4B). Interestingly, the expression levels of IL-10, an anti-inflammatory cytokine, were also decreased in peritoneal macrophages of IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice. We also noted that the expression levels of another NF-κB target gene, CCR7,[25] were significantly decreased by IKKβ deficiency. Consistent with in vitro gene expression analyses, immunofluorescence staining showed that the expression levels of several key inflammatory cytokines, including MCP-1, TNFα, and IL-1β, were decreased in the atherosclerotic lesions of IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice (Figure 4C). Thus, deficiency of IKKβ attenuated macrophage inflammatory responses and reduced lesional inflammation.

Ablation of IKKβ Modulates Macrophage Functions Related to Atherosclerosis Development

One of the earliest events in atherogenesis is the entry of monocytes, the precursors of macrophages, into the arterial wall. Several NF-κB–regulated chemokines and adhesion molecules were downregulated in macrophages of IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice (Figure 4). We next investigated the effects of IKKβ deficiency on macrophage adhesion and migration properties. Incubation of freshly isolated macrophages with primary ECs showed that ablation of IKKβ significantly decreased adhesion of macrophage to ECs (Figure 5A). We also examined the effects of IKKβ deficiency on macrophage migration by transwell assay. As shown in Figure 5B, IKKβ deficiency significantly decreased the migration of peritoneal macrophages. Furthermore, LPS stimulated migration of macrophages isolated from IKKβ$^{F/F}$LDLR$^{-/-}$ mice, and this induction was abolished in those from IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice. Therefore, ablation of IKKβ decreases the macrophage adhesion and migration abilities.

Accumulation of lipid-loaded macrophages is a hallmark of atherosclerosis.[26] Interestingly, oxidized LDL uptake and foam cell formation were substantially reduced in macrophages of IKKβ^{ΔMye}LDLR$^{−/−}$ mice compared with that of IKKβ$^{F/F}$LDLR$^{−/−}$ mice (Figure 5C and 5D), which may be attributed to the decreased type A scavenger receptor mRNA levels in IKKβ-deficient macrophages (Figure III in the online-only Data Supplement). Taken together, ablation of IKKβ decreased adhesion, migration, and lipid uptake in macrophages, which may coordinately contribute to decreased atherosclerosis in IKKβ^{ΔMye}LDLR$^{−/−}$ mice.

Discussion

Although the role of IKKβ/NF-κB pathway in inflammation and immune response has been extensively studied in the past 2 decades, the contribution of cell-type–specific NF-κB activation to atherosclerosis development is still poorly understood. In this study, we generated myeloid-specific IKKβ-deficient LDLR$^{−/−}$ mice to investigate the impact of macrophage IKKβ expression on atherosclerosis development. Deficiency of IKKβ in myeloid cells did not affect diet-induced hyperlipidemia but significantly decreased atherosclerosis in LDLR$^{−/−}$ mice (Figure 3). IKKβ deficiency decreased macrophage inflammatory responses and reduced LPS-induced proinflammatory gene expression (Figure 4). Many NF-κB–regulated proinflammatory molecules were downregulated in IKKβ^{ΔMye}LDLR$^{−/−}$ macrophages. Furthermore, the protein levels of several proinflammatory cytokines, including MCP-1, TNFα, and IL-1β, were decreased in the atherosclerotic lesions of IKKβ^{ΔMye}LDLR$^{−/−}$ mice. Those molecules play important roles in atherosclerosis initiation and progression. For example, monocytes are attracted by MCP-1 to lesion-prone areas, which is considered a critical step in atherosclerosis initiation.[1,26] Mice deficient in MCP-1 have significantly reduced atherosclerotic lesions.[27] We also found that IKKβ-deficient macrophages adhered less robustly to ECs and showed impaired migratory responses compared with control macrophages (Figure 5). Therefore, the attenuated macrophage inflammatory responses and impaired adhesion and migration properties may coordinately contribute to the decreased atherosclerosis in IKKβ^{ΔMye}LDLR$^{−/−}$ mice.

In addition to decreased inflammatory responses, lipid uptake and foam cell formation were also substantially reduced in the macrophages of IKKβ^{ΔMye}LDLR$^{−/−}$ mice compared with that of IKKβ$^{F/F}$LDLR$^{−/−}$ mice. The decreased lipid uptake in IKKβ-deficient macrophages may be attributed to the reduced expression levels of type A scavenger receptor that plays an important role in macrophage lipid uptake and foam cell formation.[28,29] Our results are consistent with a previous report that inhibition of NF-κB activity in macrophages reduces foam cell formation.[13] Deficiency of NF-κB subunit p50 was also reported to decrease type A scavenger receptor expression and reduce lipid uptake in activated macrophages.[15] Whereas the in vivo effects of type A scavenger receptor deficiency on atherosclerosis development remain unclear and controversial,[28-30] the decreased atherosclerosis in IKKβ^{ΔMye}LDLR$^{−/−}$ was very likely caused by the combined effects of decreased inflammation, adhesion, migration, and lipid uptake in macrophages.

IKKβ-dependent NF-κB activation has been implicated in vascular pathologies, and many studies have implicated that inhibition of NF-κB activation in macrophages have antiatherogenic effects.[2,13,14] However, Kanters et al[12] previously reported that bone marrow transplantation of macrophages lacking IKKβ increased atherosclerotic lesion sizes in LDLR$^{−/−}$ mice. The discrepancy between our atherosclerosis results and those reported by Kanters et al[12] may be attributed to differences in experimental design. Although the same LysM-Cre transgenic mice were used by both groups, Kanter et al[12] used a different IKKβ$^{F/F}$ mouse model to generate IKKβ^{ΔMye} mice. In their study, 10-week-old irradiated LDLR$^{−/−}$ mice were transplanted with control or IKKβ-deficient macrophages from IKKβ$^{F/F}$

and IKKβ^{ΔMye} mice, respectively. After 4 weeks of recovery and 10 weeks on a high-fat diet (16% fat, 0.15% cholesterol), mice were euthanized, and the authors found that those 24-week-old LDLR$^{−/−}$ mice transplanted with IKKβ-deficient macrophages had increased atherosclerosis in the aortic root,[12] the only location they measured atherosclerotic lesion size. The authors found that IKKβ deficiency inhibited LPS-induced production of IL-10, which has anti-inflammatory and antiatherogenic effects in mice. However, IKKβ-deficient macrophages also exhibited a strong reduction in TNFα production.[12] Therefore, the definitive mechanism for increased lesion area in LDLR$^{−/−}$ mice transplanted with IKKβ-deficient macrophages is unclear.

In this study, we used a different approach to explore the issue of macrophage IKKβ involvement of atherogenesis. Instead of bone marrow transplantation, we brought the well-characterized IKKβ^{ΔMye} mice[17,18] onto a LDLR$^{−/−}$ background. Four-week-old IKKβ^{ΔMye}LDLR$^{−/−}$ mice and their control littermates were fed a standard Western diet (21.2% fat, 0.2% cholesterol) for 12 weeks. We found that IKKβ^{ΔMye}LDLR$^{−/−}$ mice developed smaller lesions in 3 different locations, including aortic root, brachiocephalic artery, and aortic arch, compared with their control littermates. Consistent with previous reports,[17,18] IKKβ deficiency decreased macrophage inflammatory responses. Despite the decreased IL-10 expression levels, the expression levels of many NF-κB–regulated proinflammatory molecules were downregulated in IKKβ^{ΔMye}LDLR$^{−/−}$ macrophages. Furthermore, IKKβ^{ΔMye}LDLR$^{−/−}$ macrophages had impaired adhesion, migration, and lipid uptake properties, which may also contribute to decreased atherosclerosis. Whereas bone marrow transplantation has been used extensively to study macrophage function in atherosclerosis, studies have found that bone marrow cells retain the potential to differentiate into a variety of nonhematopoietic cell lineages, including epithelial cells, hepatocytes, osteoblasts, chondrocytes, adipocytes, and perivascular cells.[31-34] It is plausible that those progenitor cells in bone marrow of animals on different background might affect the atherosclerosis development in LDLR$^{−/−}$ mice. Therefore, the methodological differences may account for the discrepant atherosclerosis outcomes between our and Kanters et al's studies. In addition, the different diets (16% fat versus 21.2% fat), duration of high-fat feeding (10 weeks versus 12 weeks), and ages of animals (24 weeks old versus 16 weeks old) used in these studies may also contribute to the difference in atherosclerotic lesion development.

The role of IKKβ in the regulation of proinflammatory genes has been well documented. Interestingly, IKKβ-mediated NF-κB activation can also transcriptionally regulate anti-inflammatory cytokines such as IL-10 (Figure 4B) and negatively regulate IL-1β secretion in macrophages.[35] For example, Greten et al[35] used a high-dose LPS (30 mg/kg) to treat mice and found that mice with targeted IKKβ deletion in myeloid cells were more susceptible to endotoxin-induced shock. Consistent with our results (Figure 4A), LPS-mediated IL-1β mRNA expression levels were inhibited in IKKβ-deficient macrophages.[35] However, NF-κB also controls genes such as PAI-2 and Bcl-X$_L$, whose products inhibit caspase-1–mediated pro–IL-1β process in macrophages. Thus, NF-κB plays a dual role in the regulation of IL-1β secretion (positively regulate IL-1β mRNA transcription and negatively regulate pro–IL-1β process), and prolonged inhibition of IKKβ may enhance IL-1β secretion on endotoxin challenge. It is currently not clear whether IKKβ can negatively regulate IL-1β secretion in chronic inflammatory diseases such as atherosclerosis and arthritis. Our study showed that deficiency of IKKβ reduced protein levels of IL-1β and other key inflammatory mediators such as MCP-1 in atherosclerotic lesions of IKKβ^{ΔMye}LDLR$^{−/−}$ mice (Figure 4C). Future studies are required to further understand the role of IKKβ in the regulation of IL-1β secretion under chronic inflammatory conditions.

Macrophage migration plays an essential role in atherosclerotic lesion initiation and progression. The down-regulation of NF-κB–regulated chemokines and adhesion molecules such as CCR7, MCP-1, and intercellular adhesion molecule-1 may contribute to decreased adhesion and migration of IKKβ-deficient macrophages (Figure 5). Recent studies also demonstrated the possibility to decrease atherosclerosis by increasing macrophage emigration from atherosclerotic lesions.[36,37] CCR7 has been identified to be an important chemokine for promoting the egress of macrophage from the arterial wall during atherosclerosis regression.[36,37] It is possible that the decreased CCR7 expression in IKKβ-deficient macrophages may affect atherosclerosis regression. However, deficiency of CCR7 have been shown to decrease atherosclerotic plaque development in ApoE$^{-/-}$ mice[38] and lead to suppressed monocyte recruitment and increased levels of circulating leukocytes in ApoE$^{-/-}$ mice during atherosclerosis regression.[39] Very recently, Gils et al[40] reported that other factors such as netrin-1 also regulate macrophage emigration, and targeted deletion of netrin-1 in macrophages promoted the emigration of macrophages from plaques and decreased atherosclerosis. Thus, the mechanisms that regulate atherosclerosis regression are not well understood, and it would be interesting to investigate the potential role of IKKβ in regulating macrophage emigration and atherosclerosis regression in the future.

In summary, we demonstrate that myeloid-specific IKKβ deficiency decreases atherosclerosis in LDLR$^{-/-}$ mice. IKKβ plays an important role in modulating macrophage functions related to atherosclerosis. Ablation of IKKβ attenuated macrophage inflammatory responses and decreased macrophage adhesion, migration, and lipid uptake. These findings suggest that inhibition of NF-κB activity in myeloid may represent a feasible therapeutic strategy to combat atherosclerosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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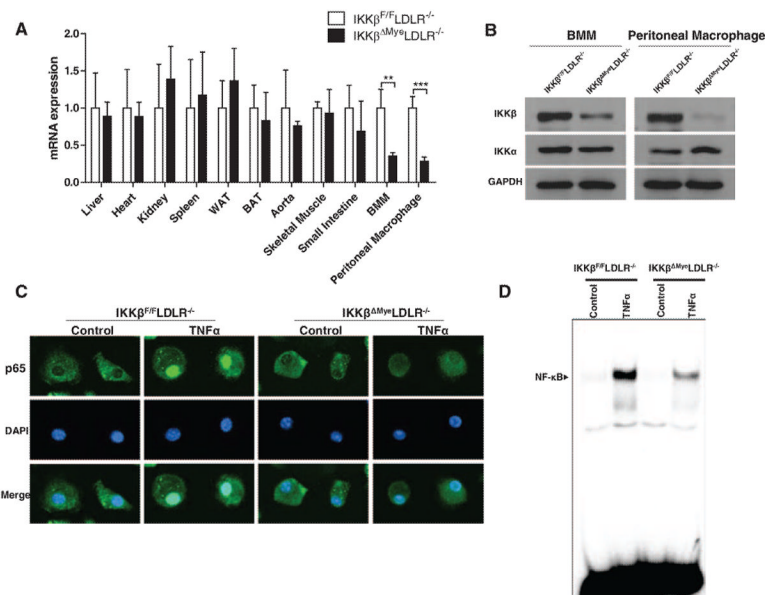
Figure 1.
Generation of low-density lipoprotein–deficient (LDLR$^{-/-}$) mice with myeloid-specific IκB kinase β (IKKβ) deficiency. **A**, IKKβ mRNA expression levels in major tissues and macrophages of IKKβ$^{F/F}$LDLR$^{-/-}$ and IKKβ^{ΔMye}LDLR$^{-/-}$ mice were analyzed by quantitative real-time polymerase chain reaction (**$P<0.01$, ***$P<0.001$, n=5). **B**, Western blot analysis of IKKβ and IKKα protein levels in bone marrow–derived macrophages (BMMs) and peritoneal macrophages of IKKβ$^{F/F}$LDLR$^{-/-}$ and IKKβ^{ΔMye}LDLR$^{-/-}$ mice. **C**, BMMs isolated from IKKβ$^{F/F}$LDLR$^{-/-}$ and IKKβ^{ΔMye}LDLR$^{-/-}$ mice were stimulated with tumor necrosis factor-α (TNFα) (20 ng/mL) or vehicle for 30 minutes. Cells were then fixed and stained with anti-p65 primary antibody, followed by fluorescein-labeled secondary antibody (green). The nuclei were visualized with diamidino-2-phenylindole (blue). A representative figure from 3 independent experiments with similar results is shown (original magnification, ×400). **D**, Macrophages were stimulated with TNFα (20 ng/mL) or vehicle for 30 minutes. Nuclear proteins were extracted, and nuclear factor (NF)-κB binding activity was determined by electrophoretic mobility shift assay. WAT indicates white adipose tissue; BAT, brown adipose tissue.
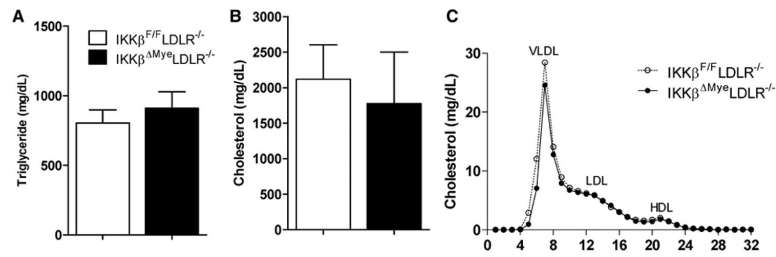
Figure 2.
Myeloid-specific IκB kinase β (IKKβ) deficiency does not affect plasma lipid levels in low-density lipoprotein–deficient (LDLR$^{-/-}$) mice. Four-week-old male IKKβ$^{F/F}$LDLR$^{-/-}$ and IKKβ^{ΔMye}LDLR$^{-/-}$ littermates were fed a Western diet for 12 weeks. The plasma levels of triglyceride (**A**) and cholesterol (**B**) were measured by standard method (n=9–10 per group), and plasma cholesterol distribution (**C**) was analyzed by fast-performance liquid chromatography. HDL indicates high-density lipoprotein; VLDL, very-low-density lipoprotein.
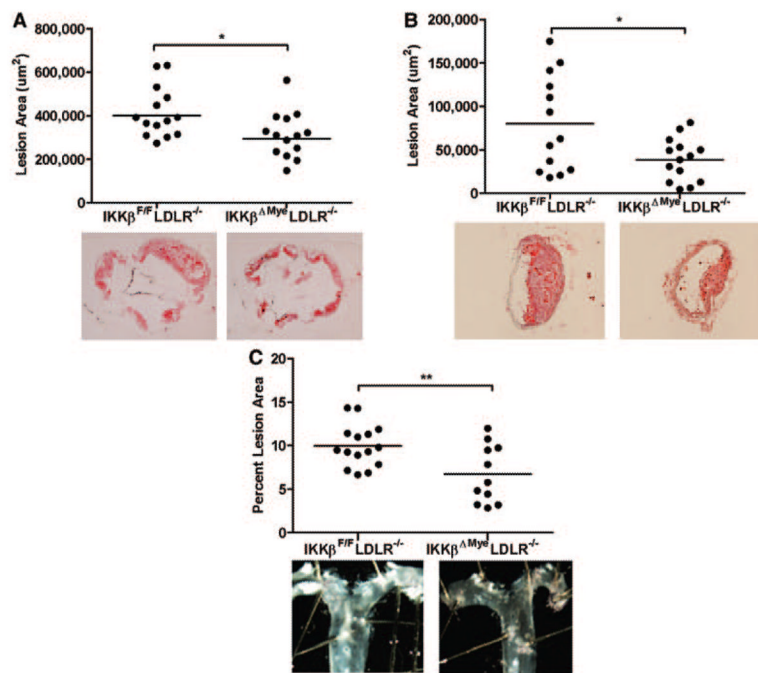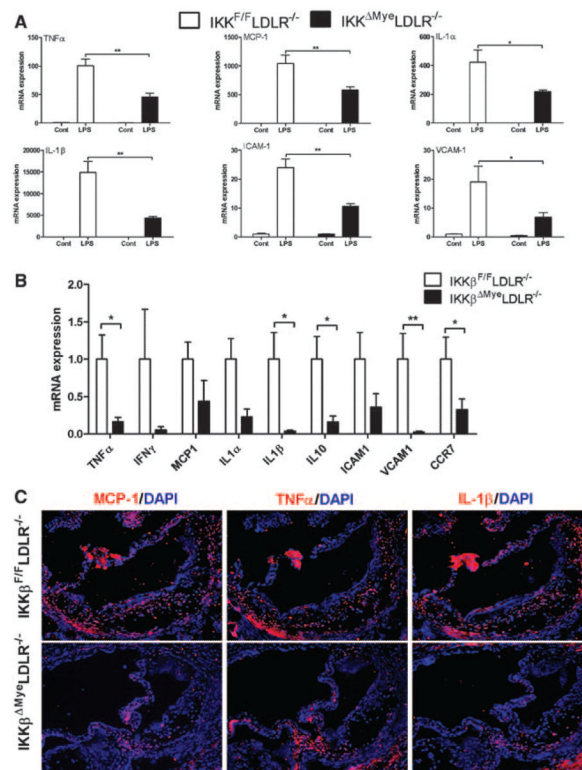
Figure 3.
Deficiency of myeloid IκB kinase β (IKKβ) decreases atherosclerosis in low-density lipoprotein–deficient (LDLR$^{-/-}$) mice. Quantitative analysis of the lesion area in the aortic root (**A**), brachiocephalic artery (**B**), and aortic arch (**C**) of IKKβ$^{F/F}$LDLR$^{-/-}$ and IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice (n=11-15 per group, *P<0.05 and **P<0.01). Representative Oil-red-O–stained sections or en face aortic arches from each genotype are displayed next to the quantification data.

Figure 4.
Deficiency of IκB kinase β (IKKβ) attenuates macrophage inflammatory responses and reduces lesional inflammation. **A**, Bone marrow–derived macrophages isolated from IKKβ$^{F/F}$LDLR$^{-/-}$ and IKKβ^{ΔMye}LDLR$^{-/-}$ mice were treated with lipopolysaccharide (LPS) (500 ng/mL) or vehicle control for 4 hours. Expression of proinflammatory cytokines was analyzed by quantitative real-time polymerase chain reaction (QPCR; *$P<0.05$, **$P<0.01$, n=3). **B**, Gene profile by QPCR of peritoneal macrophages from Western diet (WD)–fed IKKβ$^{F/F}$LDLR$^{-/-}$ and IKKβ^{ΔMye}LDLR$^{-/-}$ mice (*$P<0.05$, **$P<0.01$, n=4–6 per group). **C**, Sections of atherosclerotic lesions in the aortic root of WD-fed IKKβ$^{F/F}$LDLR$^{-/-}$ and IKKβ^{ΔMye}LDLR$^{-/-}$ mice were stained with antibodies against mouse monocyte chemotactic protein (MCP)-1, tumor necrosis factor-α (TNFα), or interleukin (IL)-1β followed by fluorescein-labeled secondary antibody (red). The nuclei were stained with diamidino-2-phenylindole (DAPI; blue). A representative figure from 3 mice per group and the similar result is shown (original magnification, ×100). ICAM indicates intercellular adhesion molecule; LDLR$^{-/-}$, low-density lipoprotein receptor–deficient mice.
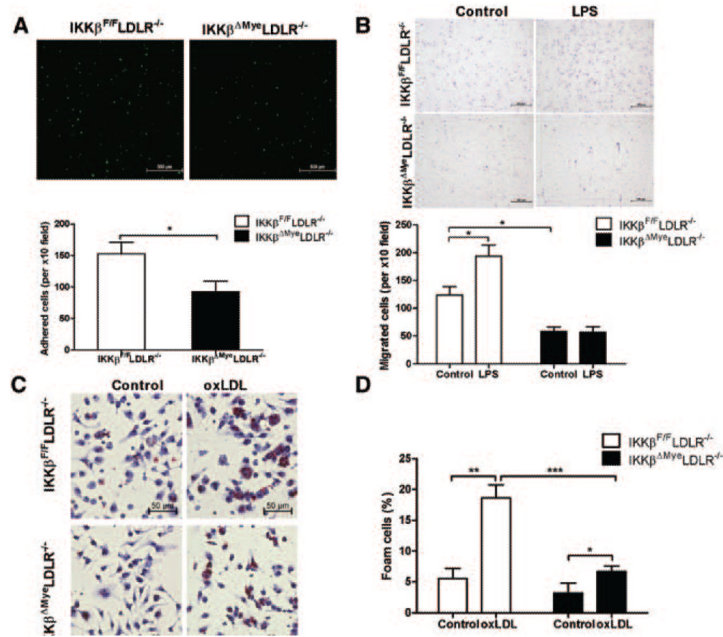
Figure 5.
Ablation of IκB kinase β (IKKβ) modulates macrophage function related to atherosclerosis development. **A**, Peritoneal macrophages isolated from IKKβ$^{F/F}$LDLR$^{-/-}$ and IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice were labeled with calcein acetoxymethyl (AM) and cultured with primary porcine endothelial cell monolayer for 30 minutes. Adhered cells were then counted under a fluorescence microscope using a ×10 objective, and quantification of 3 independent experiments is presented in the **bottom panel** (*P<0.05). **B**, Peritoneal macrophages were seeded on the transwell filters and stimulated with control media or media containing 500ng/mL LPS (**lower chambers**) for 6 hours. Cells that migrated to the underside of transwell were stained with hematoxylin and counted under the microscope (*P<0.05, n=3). **C**, Fresh isolated peritoneal macrophages from IKKβ$^{F/F}$LDLR$^{-/-}$ and IKKβ$^{\Delta Mye}$LDLR$^{-/-}$ mice were incubated with oxidized LDL (oxLDL; 100 μg/mL) for 24 hours and stained with Oil-red-O and hematoxylin. **D**, Foam cell quantification from peritoneal macrophages in studies described in **C** (*P<0.05, *P<0.01, ***P<0.001, n=3). LDLR$^{-/-}$ indicates low-density lipoprotein receptor–deficient mice.