IκBNS regulates interleukin-6 production and inhibits neointimal formation after vascular injury in mice

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1. Introduction

Nuclear factor-κB (NF-κB) plays a central role in mediating cytokines, cell adhesion molecules, and other proteins of immunity in cell types resident to the plaque microenvironment, i.e. endothelial cells, smooth muscle cells (SMCs), and macrophages. Activation of NF-κB produces many inflammatory immune mediators including interleukin(IL)-6, tumour necrosis factor-α (TNF-α), and monocyte chemotactic protein-1 (MCP-1), and these mediators participate in the development of atherosclerotic lesions. Activated NF-κB induces SMC proliferation and plays a central role in neointimal hyperplasia after vascular injury. Because neointimal hyperplasia is a major contributor to restenosis, understanding the mechanisms of neointimal hyperplasia after vascular injury provides many important implications for treatment of post-angioplasty restenosis. NF-κB activity is controlled by several mechanisms. IκBNS (also known as IκB-d, or Nkbid; nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, delta) is a nuclear IκB protein which regulates NF-κB-dependent IL-6 production in mice. A recent report demonstrated that IκBNS disrupts docking between activated NF-κB and IL-6 promoter site, and inhibits IL-6 production after lipopolysaccharide (LPS) stimulation in murine macrophages in vitro. In addition to

Keywords

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2. Methods

2.1 Animals

The experimental protocol used in the study conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996) and was approved by the National Defense Medical College Board for Studies in Experimental Animals. We used 8- to 12-week-old male IkBNS-deficient (IkBNS−/−; C57BL/6 background) and wild-type (IkBNS+/+) mice. IkBNS−/− mice were provided by Kiyoshi Takeda (Osaka University).

2.2 Blood pressure measurement

Systolic blood pressure was measured by the tail-cuff method (MK-2000; Muromachi Kikai) without anesthesia.

2.3 Femoral artery cuff injury

Mice were anaesthetized by intraperitoneal injection of pentobarbital (dose: 50 mg/kg bodyweight), and we added it if the mice moved to pain at operative time. We, thus, did not detect any movement of mice to pain, and their heart rate and respiration rate were also stable during the procedure. The right femoral artery was dissected from its surrounding and vascular injury was induced by placing a non-occlusive polyethylene cuff (length 2 mm, internal diameter 0.56 mm, Becton Dickinson) around the femoral artery. Furthermore, to evaluate the role of IkBNS in TLR4 signalling pathway, we injected 20% Pluronic (F-127, Wako Pure Chemical Industries) gel containing LPS (1 mg/mL) between femoral artery and cuff. IkBNS−/− mice were described in the previous report. Mice were fed with normal chow diet.

2.4 Tissue preparation, image analysis, and quantification

Two weeks after cuff injury, mice were euthanized by pentobarbital injection and perfused with 0.9% saline followed by 4% paraformaldehyde. The femoral arteries were fixed in 10% formalin for 48 h, embedded in paraffin, and sectioned (5 μm thickness). We used equally spaced (200 μm interval) 10 cross sections to quantify neointimal lesion for each mouse. All samples were routinely stained with elastica van Gieson. Areas of intima and media were measured using NIH Image J 1.42 (National Institutes of Health, public domain software).

2.5 Immunohistochemistry

Primary antibodies are shown in the Supplementary material online, Methods. Sections were visualized using the avidin–biotin complex method with diaminobenzidine as the substrate.

2.6 Immunofluorescence staining for IkBNS

The femoral arteries were fixed in 4% paraformaldehyde overnight, embedded in OCT compound (Sakura Finetek 4583), and sectioned (10 μm thickness). Expression of IkBNS was detected by IkB-α antibody (sc-135483, Santa Cruz Biotechnology) and donkey anti-goat IgG-FITC (sc-2024, Santa Cruz Biotechnology) was used for secondary antibody. Nuclei were stained by UltraCruzTM Mounting Medium which includes DAPI fluorescent dye (sc-24941, Santa Cruz Biotechnology).

2.7 Quantitative real-time PCR

Uninjured and injured arteries at 3, 7, and 14 days after cuff placement were pooled (two to four vessels for each sample). RNA was prepared from the pooled samples using TRI reagent (Sigma-Aldrich). Complementary DNA (cDNA) was synthesized using reverse transcriptase from 200 ng total RNA according to the manufacturer’s protocol (High-Capacity cDNA Reverse Transcription Kit; Applied Biosystems). PCR primers are shown in the Supplementary material online, Table S1. We performed real-time PCR as described before.

2.8 Cell proliferation and migration assay in vitro

Mouse vascular SMCs were isolated from the aorta, as described previously Mouse cell proliferation and migration were assessed by the scratch-wound healing assay. Details of in vitro studies are shown in the Supplementary material online, Methods.

2.9 Analysis for serum IL-6 levels and cholesterol profiles after cuff injury

After fasting for 7 h, blood samples were collected from IkBNS−/− and IkBNS+/+ mice 2 weeks after cuff injury. Serum cytokine and chemokine levels were analysed using the BioPlex kits (BioRad) according to the manufacturer’s protocol. Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride were measured by enzymatic assays as described previously.

2.10 Inhibition of IL-6 by anti-mouse IL-6 receptor antibody (MR16-1) in IkBNS−/− mice

To evaluate the effect of IL-6 on neointimal formation, IkBNS−/− mice were injected intraperitoneally with 10 mg/kg of rat anti-mouse IL-6 receptor antibody (MR16-1) or control IgG three times per week after cuff injury, as described. Mouse-derived IgG was purchased from Jackson ImmunoResearch (012-000-003).

2.11 Statistical analysis

Results are shown as the mean ± SEM. The two groups were compared using Student’s t-test. Two-way ANOVA was used to compare time course expression of mRNA levels between two groups after cuff injury. P < 0.05 was regarded as a significant difference.

3. Results

3.1 Phenotypic characteristics

There was no significant difference in baseline phenotypic characteristics between IkBNS−/− and IkBNS+/+ mice before cuff injury (body weight: 22.2 ± 0.4 g (n = 6) vs. 22.4 ± 0.4 g (n = 6); P = NS, systolic blood pressure: 94 ± 4 vs. 95 ± 2 mmHg; P = NS). These parameters also did not differ between groups 2 weeks after cuff injury (body weight: 23.8 ± 0.4 g (n = 6) vs. 24.2 ± 0.5 g (n = 6); P = NS, systolic blood pressure: 98 ± 3 vs. 102 ± 4 mmHg; P = NS).
3.2 Neointimal formation after cuff injury

To evaluate the role of IkBNS in injured arteries, we investigated the vascular change after cuff injury in both IkBNS−/− and IkBNS+/+ mice. The mean intimal area and the intima/media ratio of IkBNS−/− mice increased 89% \([8066 \pm 1141 \mu m^2 (n = 10)]\) vs. \(4267 \pm 1095 \mu m^2 (n = 10); P = 0.027\) and 100% \((0.72 \pm 0.13 \text{ vs. } 0.36 \pm 0.09; P = 0.032)\) compared with IkBNS+/+ mice. However, we observed no significant difference in the medial area \((12 103 \pm 529 \text{ vs. } 11 528 \pm 350 \mu m^2; P = \text{NS})\) between groups (Figure 1).

To examine post-injury cell proliferation in the intima of both groups, we performed α-smooth muscle cell actin (α-SMA), proliferating cell nuclear antigen (PCNA), and phospho-NF-κB p65 (pNF-κB) staining in injured arteries of both groups. Figure 2A shows the serial sections of injured arteries at 7 days. The neointimal lesion of injured arteries showed α-SMA positive cells in both mice. We detected many PCNA positive cells (54 positive cells) and several pNF-κB positive cells (three positive cells) in the intima of IkBNS−/− mice. In contrast, we observed a few PCNA positive cells and no pNF-κB positive cells in the intima of IkBNS+/+ mice. To quantify cell proliferation and NF-κB activity, we calculated the percentage of PCNA positive cells and pNF-κB positive cells to total cells in the intima of injured arteries. The percentage of PCNA positive cells in the intima of IkBNS−/− mice was 5.4-fold higher \([17.3 \pm 4.7% (n = 7)]\) vs. \(3.2 \pm 1.9% (n = 7); P = 0.024\) compared with IkBNS+/+ mice at 14 days post-injury (Figure 2B). The percentage of pNF-κB positive cells in the intima of IkBNS−/− mice was 5.1-fold higher \([7.1 \pm 1.1% (n = 4)]\) vs. \(1.4 \pm 0.8% (n = 4); P = 0.008\) than that of IkBNS+/+ mice at 7 days post-injury (Figure 2C).

3.3 Macrophage recruitment and TLR4 expression after cuff injury

To check the recruitment of inflammatory cells, we performed immunohistochemistry for macrophages (MAC-3), neutrophils (Ly-6G), and T cells (CD3). Macrophages were main inflammatory cells recruited in the adventitia after cuff injury (Figure 3A), but neutrophils and T cells were rare (see Supplementary material online, Figure S1). However, we observed no significant difference in the number of macrophage \([35.5 \pm 6.5 (n = 4)]\) vs. \(39.5 \pm 6.9 (n = 4); P = \text{NS}\) between two groups (Figure 3B).

To evaluate the TLR expression after injury, we performed immunohistochemistry for TLR2 and TLR4. TLR2 expression was weak in both mice within 7 days after cuff injury (data not shown). In contrast, we observed strong TLR4 expression in the intima of IkBNS−/− mice at 7 days post-injury (Figure 3C). Real-time PCR analysis also revealed that mRNA expression levels of TLR4 in IkBNS−/− mice were 2.5-fold higher \((P = 0.001)\) than those in IkBNS+/+ mice at 3 days post-injury (Figure 3D).
after cuff injury (Figure 3D). To check the role of IκBNS in TLR4 signalling pathway, we filled the space between femoral artery and cuff with Pluronic gel containing LPS (1 mg/mL). LPS stimulation remarkably accelerated neointimal hyperplasia in IκBNS^{−/−} mice; however, same dose of LPS did not change intimal area in IκBNS^{+/−} mice (Figure 3E and F). Furthermore, the percentage of pNF-κB positive cells in the intima of IκBNS^{−/−} mice was significantly higher [32.1 ± 5.4% (n = 5) vs. 15.6 ± 3.4% (n = 5); *P = 0.032] compared with IκBNS^{+/−} mice at 14 days after LPS stimulation.

### 3.4 Time course of IL-6 and IκBNS expression after cuff injury

We analysed IL-6 and IκBNS expression after cuff injury. IL-6 protein expression was stronger in the intima and the media of IκBNS^{−/−} mice compared with those of IκBNS^{+/−} mice at 7 and 14 days after cuff injury (Figure 4A). To quantify mRNA expression levels of IL-6, we performed real-time PCR analysis using uninjured and injured arteries from both groups. Expression levels of IL-6 mRNA in IκBNS^{−/−} mice were significantly higher compared with IκBNS^{+/−} mice from 3 to 14 days after cuff injury (*P = 0.044 by two-way ANOVA). In particular, at 3 days post-injury, IL-6 mRNA levels in IκBNS^{−/−} mice were 1.8-fold higher (*P = 0.002) compared with IκBNS^{+/−} mice (Figure 4B). We also checked time course of IκBNS expression after cuff injury. We detected IκBNS protein expression in the adventitia at 7 days, and in the media at 14 days after injury in IκBNS^{−/−} mice (Figure 4C). IκBNS mRNA expression was detected in IκBNS^{+/−} mice from 3 to 14 days after injury (Figure 4D).

### 3.5 Cell proliferation and migration assay in vitro

To assess the effect of IκBNS deficiency on cell proliferation, we performed BrdU incorporation assay. We observed PDGF-BB (20 ng/mL)-induced BrdU incorporation in SMCs from both groups, but there was no significant difference in the percentage of BrdU positive cells to total cells between groups [10.0 ± 3.6% (n = 4) vs. 8.8 ± 2.8% (n = 4); *P = NS]. In contrast, we did not detect BrdU incorporation in SMCs from both mice after IL-6 (20 ng/mL) stimulation (see Supplementary material online, Figure S2). To evaluate the effect of IL-6 on cell migration, we performed the scratch-wound healing assay in cultured SMCs. IκBNS^{−/−} SMCs showed significant decrease in wound area [151 ± 14 × 10^4 μm² (n = 4) vs. 227 ± 23 × 10^4 μm² (n = 4); *P = 0.031] compared with IκBNS^{+/−} cells at 12 h after IL-6 (20 ng/mL) stimulation (Figure 5A and B). Furthermore, pretreatment with anti-mouse IL-6 receptor antibody (MR16-1, 100 μg/mL) completely inhibited SMC migration after IL-6 stimulation in both groups (Figure 5C and D).
3.6 Serum IL-6 levels and cholesterol profiles after cuff injury

To check the serum IL-6 levels and cholesterol profiles after vascular injury, we analysed blood samples from $\text{lkBNS}^{-/-}$ and $\text{lkBNS}^{+/+}$ mice 2 weeks after cuff injury (see Supplementary material online, Table S2). The serum IL-6 level in $\text{lkBNS}^{+/+}$ mice was significantly higher compared with $\text{lkBNS}^{-/-}$ mice ($10.2 \pm 2.8$ pg/mL ($n = 6$) vs. $2.6 \pm 0.6$ pg/mL ($n = 6$); $P = 0.043$). Total cholesterol and HDL cholesterol levels were significantly lower in $\text{lkBNS}^{-/-}$ mice compared with $\text{lkBNS}^{+/+}$ mice [total cholesterol: $81 \pm 4$ mg/dL ($n = 6$) vs. $94 \pm 1$ mg/dL ($n = 6$); $P = 0.031$, HDL cholesterol: $58 \pm 4$ vs. $76 \pm 2$ mg/dL; $P = 0.004$]. However, the triglyceride level was similar in both groups ($22 \pm 3$ vs. $20 \pm 1$ mg/dL; $P = \text{NS}$).

3.7 Inhibition of neointimal formation by anti-mouse IL-6 receptor antibody (MR16-1) in $\text{lkBNS}^{-/-}$ mice

To inhibit the effect of IL-6 on neointimal formation, $\text{lkBNS}^{-/-}$ mice were injected intraperitoneally with 10 mg/kg of MR16-1 or control IgG (purified rat IgG) for 2 weeks after cuff injury. We observed no significant difference in phenotypic characteristics between the MR16-1 and control group [body weight: $26.1 \pm 0.9$ g ($n = 7$) vs. $24.7 \pm 0.3$ g ($n = 7$), systolic blood pressure: $95 \pm 3$ vs. $99 \pm 3$ mmHg] after treatment. However, MR16-1 strongly reduced intimal hyperplasia [1625 ± 417 μm² ($n = 7$) vs. 5379 ± 1345 μm² ($n = 7$); $P = 0.021$] compared with control IgG (Figure 6A and B). Immunohistochemistry showed that MR16-1 did not inhibit IL-6...
protein expression at 14 days after treatment in injured arteries of IkBNS−/− mice (Figure 6C). Real-time PCR analysis also revealed that mRNA expression levels of IL-6 at 3 days after treatment (Figure 6D). However, the percentage of phosphorylated signal transducer and activator of transcription 3 (pStat3) positive cells in the intima of the MR16-1 group was significantly lower than that of the control group \( [31.9\pm4.6\% \text{ (} n=6 \text{) vs. } 64.1\pm4.9\% \text{ (} n=6 \text{); } P=0.001] \) at 7 days after treatment (Figure 6E and F).

4. Discussion

This study is the first to demonstrate the role of IkBNS in injured arteries in vivo and in vitro. IkBNS deficiency caused neointimal formation by increasing TLR4 expression, NF-κB activity and SMC proliferation in the intima of injured arteries. Expression levels of IL-6 mRNA were significantly higher than those of IkBNS+/+ mice. Vascular SMCs from IkBNS−/− mice showed significant increase in cell migration compared with those from IkBNS+/+ mice after IL-6 stimulation in vitro. Furthermore, neointimal formation was inhibited by anti-mouse IL-6 receptor antibody in IkBNS−/− mice. These results suggest that IkBNS plays an important role in arterial inflammation and intimal hyperplasia by inhibiting IL-6 production after injury.

Our histological findings revealed that deficiency of IkBNS promotes NF-κB activity and SMC proliferation in the intima of injured arteries. Constitutive NF-κB activation is essential for the proliferation of vascular SMCs. Breuss et al. showed post-angioplasty lumen narrowing by enhancing NF-κB activity in rabbit iliac artery. Furthermore, two research groups demonstrated that NF-κB decoy inhibits restenosis after coronary stenting in rabbit and human. These results support that NF-κB plays an important role in the progression of neointimal hyperplasia after angioplasty. NF-κB activation also associates with the development of atherosclerosis. Activated NF-κB is detected in endothelial cells, SMCs, and macrophages of atherosclerotic lesions. Atherosclerotic lesion development includes proliferation of intimal SMCs, therefore examination of neointimal formation in IkBNS−/− mice might provide essential information regarding atherosclerosis progression and restenosis following vascular injury.

We detected many macrophages in the adventitia of injured arteries. Macrophages might induce adventitial inflammation, and may participate in neointimal formation after cuff injury. But we observed no significant difference in the number of macrophage in the adventitia between IkBNS+/+ and IkBNS−/− mice. Although deficiency of IkBNS may not affect macrophage recruitment, IkBNS-deficient macrophages might modify those function, including prolonged NF-κB activation and IL-6 expression. To check the effect of...
IkBNS on fibrotic remodelling, we performed Picrosirius red staining in representative samples from both mice (see Supplementary material online, Figure S3). We observed there was no collagen in the neointima of both mice, suggesting deficiency of IkBNS may not affect collagen reconstruction within 2 weeks after injury.

Activated NF-κB induces various inflammatory cytokines and chemokines such as IL-6, TNF-α and MCP-1. We focused on the IL-6 regulation system in vascular injury. Wang et al. demonstrated that IL-6 promotes rat SMC migration in a dose-dependent manner in vitro. Moreover, the sirolimus-eluting stent reduces neointimal formation and IL-6 production in injured arteries of a porcine coronary model. These results support the importance of IL-6 in neointimal formation after vascular injury. In our study, IL-6 mRNA expression levels were significantly higher in injured arteries of IkBNS-/- mice compared with those of IkBNS+/- mice post-injury. We also evaluated the effect of IkBNS deficiency on vascular SMC in vitro. SMCs from IkBNS-/- mice showed significant increase in cell migration compared with those of IkBNS+/- mice after IL-6 stimulation. Our data suggest that SMCs from IkBNS-/- mice promote cell migration in response to IL-6 itself. Vascular injury induces various immune mediators including IL-1 and PDGF, and these mediators promote IL-6 secretion in vascular SMCs. Furthermore, IL-6 stimulates vascular SMC proliferation via induction of endogenous PDGF. Consequently, elevation in the local IL-6 level might increase neointimal hyperplasia by increasing SMC migration and proliferation after cuff injury in IkBNS-/- mice.

Strong expression of TLR4 was detected in the neointima of IkBNS-/- mice after injury. Furthermore, LPS stimulation significantly accelerated neointimal hyperplasia by increasing NF-κB activity in IkBNS-/- mice. TLR4/MyD88 pathway activates nuclear translocation of NF-κB and IkBNS-deficient macrophages also induce a subset of MyD88-dependent genes including IL-18. Immunostaining revealed that IL-18 protein was detected in the intima of IkBNS-/- mice at 7 days post-injury, but not in IkBNS+/- mice (see Supplementary material online, Figure S4). Previous report showed that IL-18 plays a critical role in neointima formation after vascular injury via NF-κB activation. These findings indicate that TLR4 signalling also participates in the development of intimal hyperplasia in IkBNS-/- mice after vascular injury with or without LPS.

To block the effect of IL-6 on neointimal formation, we treated IkBNS-/- mice by anti-mouse IL-6 receptor antibody (MR16-1). The effect of MR16-1 has been demonstrated in the murine arthritis model, and safety and efficacy of humanized anti IL-6 receptor antibody (tocilizumab) has been established in several clinical trials in rheumatic arthritis patients. Earlier reports show that IL-6 induces phosphorylation of Stat3 in vascular SMC and causes neointimal hyperplasia after balloon injury in rats. We analysed pStat3 positive cells to evaluate the inhibitory effect of MR16-1 in IL-6
signalling pathway. MR16-1 inhibited Stat3 activation and neointimal hyperplasia after cuff injury in IκBNS−/− mice, suggesting IL-6 is a major contributor to neointimal hyperplasia in IκBNS−/− mice, and inhibition of IL-6 might be a new method to prevent neointimal formation after vascular injury.

IL-6 is known as a representative inflammatory biomarker because this cytokine induces acute phase protein such as C-reactive protein and fibrinogen. The serum IL-6 level is strongly associated with severity of ankle-brachial index in peripheral arterial disease patients. Furthermore, IL-6 is associated with restenosis after coronary stenting and a strong predictor of cardiovascular disease. In our study, the serum IL-6 level was significantly higher in IκBNS−/− mice compared with IκBNS+/− mice, although there was no significant difference in hepatic IL-6 mRNA expression levels between two groups [1.0 ± 0.1 (n = 6) vs. 1.0 ± 0.1 (n = 6); P = NS] at 2 weeks after cuff injury. Furthermore, we observed no significant difference in heart weight to the body weight ratio [6.7 ± 0.3 mg/g (n = 6) vs. 6.9 ± 0.3 mg/g (n = 6); P = NS] and left ventricular wall thickness (1.3 ± 0.1 vs. 1.3 ± 0.1 mm; P = NS) between groups at 2 weeks after injury. These findings suggest that the high serum IL-6 level in IκBNS−/− mice might be induced by local injury and may not affect cardiac hypertrophy within 2 weeks after injury. With regard to cholesterol metabolism, we observed lower HDL cholesterol in IκBNS−/− mice after vascular injury. A previous study demonstrated that recombinant IL-6 injection with high-fat diet accelerates atherosclerosis in wild-type mice. Moreover, high serum levels of IL-6 are independently associated with low HDL cholesterol levels in elderly people. Taken together, there is a possibility that

Figure 6 Anti-mouse interleukin-6 receptor antibody (MR16-1) inhibits neointimal formation after vascular injury in IκBNS−/− mice. (A) Femoral artery specimens stained with elastica van Gieson from IκBNS−/− mice treated with MR16-1 or control IgG at 2 weeks after cuff injury. Arrow heads indicate the IEL (scale bars = 100 μm). (B) Results of morphological measurement (bars = mean ± SEM, *P < 0.05, n = 7 for each group). (C) Immunohistochemical analysis for interleukin-6 (IL-6) at 14 days after treatment. Arrow heads indicate the IEL (scale bars = 50 μm). (D) Real-time PCR analysis for mRNA expression levels of IL-6 at 3 days after treatment (bars = mean ± SEM, n = 6 for control, n = 5 for MR16-1). (E) Immunohistochemical staining for phosphorylated signal transducer and activator of transcription 3 (pStat3) at 7 days after treatment. Arrow heads indicate the IEL (scale bars = 50 μm). (F) Results of the percentage of pStat3 positive cells to total cells in the intima of injured arteries (scale bars = 50 μm, bars = mean ± SEM, **P < 0.01, n = 6 for each group).
IκBNS inhibits neointimal formation

IκBNS $^{1,2}$ mice exacerbate atherosclerosis by cholesterol-rich diet. But further investigation is needed to evaluate the role of IκBNS in hyperlipidaemia because we used normal chow diet in this study. NF-κB and its regulator IκB proteins participate importantly in the regulation of host immune responses. In this study, we elucidated the role of IL-6 that participates in neointimal formation after vascular injury in IκBNS $^{1,2}$ mice. It has been also reported that IL-6 expression is detected in human atherosclerotic lesions. $^{3,9}$ Therefore, further investigation for IκBNS in human endothelial cells, SMCs, and macrophages may aid development of new therapies for cardiovascular diseases. In conclusion, IκBNS down-regulates TLR4 expression and NF-κB activity, and inhibits induction of TLR4-dependent genes including IL-6 after cuff injury. IκBNS might suppress intimal hyperplasia caused by vascular inflammation such as atherosclerosis, and restenosis after angioplasty.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

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References