Abstract: P4141

Lack of IkBNS promotes cholate-containing high-fat diet-induced inflammation and atherogenesis in low-density lipoprotein (LDL) receptor-deficient mice

Authors:
K Kitamura¹, K Isoda¹, K Akita¹, K Miyosawa¹, T Kadoguchi¹, K Shimada¹, H Daida¹, ¹Juntendo University School of Medicine, Cardiology - Tokyo - Japan,

Topic(s):
Basic Science - Vascular Diseases: Leukocytes, Inflammation, Immunity

Citation:
Background-IκBNS is one of the nuclear IκB proteins and regulates a subset of Toll-like receptor (TLR) dependent genes. LPS acts as extremely strong stimulator of innate immunity. We tried to investigate whether stimulation of innate immunity could promote atherosclerosis in the IκBNS-deficient atherogenic mice. However all IκBNS-deficient mice died of LPS challenge at a dose of which almost all wild-type mice survived, because IκBNS-deficient mice are highly sensitive to LPS-induced endotoxin shock. Then, we decided to use a cholate-containing high fat diet (HFD(CA(+))), which has been widely used as an atherogenic diet in mice. Furthermore, HFD(CA(+)) has been shown to induce TLR4 mediated early inflammatory response. The present study aims to clarify the lack of IκBNS promotes atherosclerosis in LDL receptor-deficient (LDLr/-) mice fed HFD(CA(+)) compared with those fed a cholate-free HFD (HFD(CA(-)).

Methods and Results-Mice that lacked IκBNS (IκBNS/-) were crossed with LDLr/- mouse and formation of atherosclerotic lesions was analyzed after 6 weeks consumption of HFD(CA(+)) or HFD(CA(-)). The extent of atherosclerosis in the aorta (en face) was significantly increased in IκBNS/-/LDLr/- (CA(+)) mice compared with others after 6-week consumption of HFD (p<0.01) (Figure). Interestingly, HFD(CA(+)) did not induce significant atherosclerotic lesions in IκBNS/-/LDLr/- compared with LDLr/- mice after 6-week consumption (Figure). Immunostaining of aortic root lesion revealed that HFD(CA(+)) significantly increased positive area of Mac-3 (macrophage) by 1.5-fold (p=0.01) and TLR4, interleukin-6 (IL-6) expression by 1.7-fold (p<0.05) and 1.5-fold (p<0.05) respectively in IκBNS/-/LDLr/- (CA(+)) compared to LDLr/- (CA(+)) mice. Furthermore, active STAT3 (pSTAT3)-positive cells were significantly increased by 1.7-fold in the atherosclerotic lesions of IκBNS/-/LDLr/- (CA(+)) compared with LDLr/- (CA(+)) mice (p<0.01). TLR4 positive areas, IL-6 positive areas, and pSTAT3 positive cells were overlapped with Mac-3, indicating that TLR4-IL-6-STAT3 axis was activated in macrophages in IκBNS/-/LDLr/- (CA(+)) mice. On the other hand, HFD(CA(-)) could not induce any difference in these immunoreactivities of atherosclerotic lesions between IκBNS/-/LDLr/- (CA(-)) compared with LDLr/- (CA(-)) mice. These findings suggest that IκBNS deficiency and HFD(CA(+)) promote atherogenesis in LDLr/- mice via TLR4/IL-6/STAT3 pathway. Finally, we show the monocytes from peripheral blood of IκBNS/-/LDLr/- (CA(+)) mice were found to contain the most mounts of Ly6Chi among four groups, suggesting that lack of IκBNS enhances inflammation in the response HFD(CA(+)) feeding and thereby influence atherogenesis in IκBNS/-/LDLr/- mice.

Conclusions-The present study is the first to demonstrate that the activation of innate immune system using HFD(CA(+)) induced significant inflammation and atherogenesis in IκBNS/-/LDLr/- compared with LDLr/- mice.
Lack of IkBNS promotes cholate-containing high-fat diet-induced inflammation and atherogenesis in low-density lipoprotein (LDL) receptor-deficient mice.

Authors:

Juntendo University School of Medicine, Cardiology - Tokyo - Japan.

Topic(s):
Basic Science - Vascular Diseases: Leukocytes, Inflammation, Immunity

Background-IkBNS is one of the nuclear IkB proteins and regulates a subset of Toll-like receptor (TLR) dependent genes. LPS acts as an extremely strong stimulator of innate immunity. We tried to investigate whether stimulation of innate immunity could promote atherosclerosis in the IkBNS-deficient atherogenic mice. However, all IkBNS-deficient mice died of LPS challenge at a dose of which almost all wild-type mice survived, because IkBNS-deficient mice are highly sensitive to LPS-induced endotoxin shock. Then, we decided to use a cholate-containing high-fat diet (HFD(CA+)), which has been widely used as an atherogenic diet in mice. Furthermore, HFD(CA+) has been shown to induce TLR4 mediated early inflammatory response. The present study aims to clarify the lack of IkBNS promotes atherogenesis in LDL receptor-deficient (LDLr−/−) mice fed HFD(CA+) compared with those fed a cholate-free HFD (HFD(CA−)).

Methods and Results-
Mice that lacked IkBNS (IkBNS−/−) were crossed with LDLr−/− mice and formation of atherosclerotic lesions was analyzed after 6 weeks consumption of HFD(CA+) or HFD(CA−). The extent of atherosclerosis in the aorta (en face) was significantly increased in IkBNS−/−/LDLr−/−(CA+) mice compared with others after 6-week consumption (p<0.01) (Figure). Interestingly, HFD(CA−) did not induce significant atherosclerotic lesions in IkBNS−/−/LDLr−/− compared with LDLr−/− mice after 6-week consumption (Figure). Immunostaining of aortic root lesion revealed that HFD(CA+) significantly increased positive area of Mac-3 (macrophage) by 1.5-fold (p=0.01) and TLR4, interleukin-6 (IL-6) expression by 1.7-fold (P<0.05) and 1.5-fold (p<0.05) respectively in IkBNS−/−/LDLr−/− (CA+) compared to LDLr−/− (CA+) mice. Furthermore, active STAT3 (pSTAT3)-positive cells were significantly increased by 1.7-fold in the atherosclerotic lesions of IkBNS−/−/LDLr−/− (CA+) compared with LDLr−/− (CA+) mice (p<0.01). TLR4 positive areas, IL-6 positive areas, and pSTAT3 positive cells were overlapped with Mac-3, indicating that TLR4-IL-6-STAT3 axis was activated in macrophages in IkBNS−/−/LDLr−/− (CA+) mice. On the other hand, HFD(CA−) could not induce any difference in these immunoreactivities of arteriosclerotic lesions between IkBNS−/−/LDLr−/− (CA−) compared with LDLr−/− (CA−) mice. These findings suggest that IkBNS deficiency and HFD(CA+) promote atherogenesis in LDLr−/− mice via TLR4/IL-6/STAT3 pathway. Finally, we show the monocytes from peripheral blood of IkBNS−/−/LDLr−/− (CA+) mice were found to contain the most mounts of Ly6Chi among four groups, suggesting that lack of IkBNS enhances inflammation in the response HFD(CA+) feeding and thereby influence atherogenesis in IkBNS−/−/LDLr−/− mice.

Conclusions-
The present study is the first to demonstrate that the activation of innate immune system using HFD(CA+) induced significant inflammation and atherogenesis in IkBNS−/−/LDLr−/− compared with LDLr−/− mice.