The nuclear receptor superfamilly is a group of transcription factors that regulate diverse physiological processes including development, metabolism, inflammation, and immunity. It contains several groups including steroid receptors, peroxisome-proliferator-activated receptors, liver X receptors, and NR4A receptors that have generated considerable interest in the field of vascular biology because they influence vascular homeostasis and diseases such as atherosclerosis. In this issue of Circulation Research, You et al study the founder member of the NR4A family, a gene called Nur77 (or NR4A1, TR3, NGFI-B), and demonstrate for the first time that it can suppress proinflammatory activation of endothelial cells (ECs).

Nur77 was originally identified by differential hybridization screening as a nerve growth factor–induced gene in pheochromocytoma cells. The domain structure of Nur77 resembles that of other nuclear receptors and contains an N-terminal activating function-1 domain, a zinc finger DNA-binding domain, and a C-terminal ligand-binding domain. A ligand has not been identified for Nur77 (or for any of the NR4A proteins), and it has therefore been classified as an orphan receptor; however, recent structural studies suggest that Nur77 may be activated through ligand-independent mechanisms. This idea is consistent with the observation that Nur77 is regulated at the transcriptional level and can be induced in multiple cell types by a variety of stimuli including growth factors, fatty acids, cytokines, hormones, neurotransmitters, and mechanical forces.

**Nur77 in Atherosclerosis and Angiogenesis**

Emerging data suggest that Nur77 influences vascular homeostasis and disease by controlling the physiology of macrophages, smooth muscle cells (SMCs), and vascular endothelium. Atherosclerosis is a lipid-driven chronic inflammatory disease in which monocytes are recruited to the vessel wall by adhesion to activated ECs. Locally recruited monocytes differentiate into macrophages, which can internalize modified lipoprotein particles and subsequently differentiate into foam cells which influence the disease process by secreting cytokines and growth factors. Recent studies indicate that Nur77 can be induced by proinflammatory stimuli in cultured macrophages and is expressed by macrophages in human atherosclerotic lesions. Furthermore, in vitro studies have revealed that Nur77 reduces macrophage activation by suppressing their ability to internalize oxidized low density lipoproteins (oxLDL) and by inhibiting proinflammatory activation.

SMCs are largely quiescent in healthy vessels where they control vascular tone but can be induced to proliferate by cytokines, growth factors, and mechanical stretch and thus contribute to intimal thickening in atherosclerosis and vein graft restenosis. The first evidence that Nur77 regulates vascular physiology was provided by de Vries and colleagues, who demonstrated that Nur77 can be induced by oxLDL and by mechanical stretch in cultured SMCs. Subsequent studies correlated Nur77 expression with vascular disease by revealing that Nur77 is expressed in neointimal SMCs in both human atherosclerotic lesions and in murine femoral artery lesions but is not expressed in medial SMCs in healthy vessels. The function of Nur77 has been illuminated by a combination of cell culture experiments and studies of transgenic mice which indicated that Nur77 expression can reduce neointima formation in injured arteries by suppressing SMC proliferation.

It has also emerged recently that Nur77 is an important regulator of endothelial proliferation and angiogenesis. Nur77 is expressed at low levels in resting endothelium but can be induced by vascular endothelial growth factor and acts as a positive regulator of angiogenesis in murine models. Endothelial expression of Nur77 has also been documented in human atherosclerotic lesions; however, the effects of Nur77 on arterial inflammation remained uncertain. This question has now been addressed by You et al., who reveal that Nur77 can suppress proinflammatory activation of endothelial cells, suggesting that it may protect the vasculature from inflammation.

**Nur77 Regulates the Nuclear Factor kB Pathway**

The nuclear factor (NF-κB family of transcription factors contains five genes (RelA/p65, NF-κB1/p50, NF-κB2/p52, RelB and c-Rel) that play an important role in numerous physiological processes including cell survival, differentiation, proliferation, immunity and inflammation. Genetic studies in mice demonstrated that NF-κB can exert proatherogenic effects in macrophages by enhancing their ability to internalize modified lipoproteins, and also exert antiathero-
genetic effects by enhancing macrophage expression of the antinflammatory cytokine IL-10. NF-κB can be activated in ECs by several proinflammatory mediators (eg, tumor necrosis factor [TNF]α, oxLDL) and subsequently influences atherosclerosis by inducing cellular adhesion molecules (eg, vascular endothelial adhesion molecule [VCAM]-1, intercellular adhesion molecule [ICAM]-1) and other proteins that promote recruitment of leucocytes to the vessel wall. Indeed, recent genetic studies in atherosusceptible strains of mice have demonstrated that endothelial activation, vascular inflammation, and lesion formation can be suppressed by the inhibition of NF-κB activation in endothelial cells.

In unstimulated cells, NF-κB dimers are sequestered in the cytoplasm through binding to inhibitory IκB molecules. Proinflammatory signaling leads to activation of IκB kinases (IKK), which phosphorylate IκB, which is subsequently ubiquitinated and degradation, thus releasing NF-κB for nuclear translocation and transcriptional activation of target genes. The magnitude and duration of proinflammatory activation in ECs is regulated by the induction of negative regulators including A20 and Cezanne, which inhibit IKK activity, and IκB proteins, which bind to NF-κB complexes and subsequently export them from the nucleus to the cytoplasm (Figure). Our knowledge of the regulation of the NF-κB pathway in ECs has been extended by You et al, who reveal that Nur77 is induced rapidly by TNFα and that it negatively regulates the expression of VCAM-1 and ICAM-1 adhesion molecules by inhibiting NF-κB activation. It is proposed therefore that the induction of Nur77 by proinflammatory signals generates an additional negative feedback loop in the NF-κB pathway (Figure). The mechanism of NF-κB inhibition involves binding of Nur77 to the promoter region of the IκBα gene, which leads to transcriptional activation and enhanced expression of IκBα. Thus, IκBα expression in Nur77-expressing ECs was reduced but not abolished completely by TNFα treatment and residual IκBα was sufficient to suppress NF-κB activation by retaining it in the cytoplasm. This is an interesting mechanism of negative feedback because it suggests that Nur77 does not suppress signaling to IκBα per se but instead alters the threshold of signaling required for NF-κB activation.

The findings of You et al raise several questions that need to be addressed. First, the signaling pathway and transcription factors underlying the induction of Nur77 by TNFα should now be defined, and further work should be carried out to examine the role of endogenous Nur77 in IκBα expression and NF-κB activation in ECs. In addition, the transcriptional targets of Nur77 are largely unknown and should now be investigated at a broader level using chromatin immunoprecipitation followed by microarray analysis or deep sequencing. It would be interesting to extend the study of NF-κB regulation by Nur77 to other cell types and in particular to examine whether the known ability of Nur77 to suppress macrophage activation6 relies on the inhibition of NF-κB. A previous study demonstrated that IκBα expression in ECs is elevated at an atherosusceptible region of the arterial tree that is exposed to relatively low shear stress compared to an atheroprotected region that is exposed to relatively high shear; hence future studies should examine whether shear stress influences IκBα expression levels in ECs by regulating Nur77. Finally, the effects of Nur77 genetic deletion on EC activation, vascular inflammation, and atherosclerosis should now be assessed in murine models of atherosclerosis.

In summary, the article by You et al and previous studies indicate that Nur77 exerts multiple protective effects on the vasculature by suppressing SMC proliferation and by inhibiting proinflammatory activation of macrophages and ECs and suggest that Nur77 may be useful therapeutic target in atherosclerosis.

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