

# Flow beats inflammation, but not always

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In this issue of *Blood*, a report is presented, suggesting that the anti-inflammatory effect of prolonged laminar shear stress on the endothelium is mediated by modulation of the amount of nuclear phospho-ATF2 but not by interfering with NF-κB and is therefore restricted to the nonacute inflammatory state.

It has been known for some time that being exposed to laminar shear stress is good when you are a vascular endothelial cell.<sup>1,2</sup> It keeps you calm and quiescent and overall relaxed. There seem to be several possible mechano-transduction mechanisms<sup>3</sup> mediating flow-activated effects, including up-regulation of Kruppel-like factor 2<sup>4</sup> (KLF2). This transcription factor regulates prominent flow-induced genes such as endothelial nitric oxide synthase (NOS3) and the anticoagulatory endothelial membrane protein thrombomodulin as well as “antiinflammatory” effects.

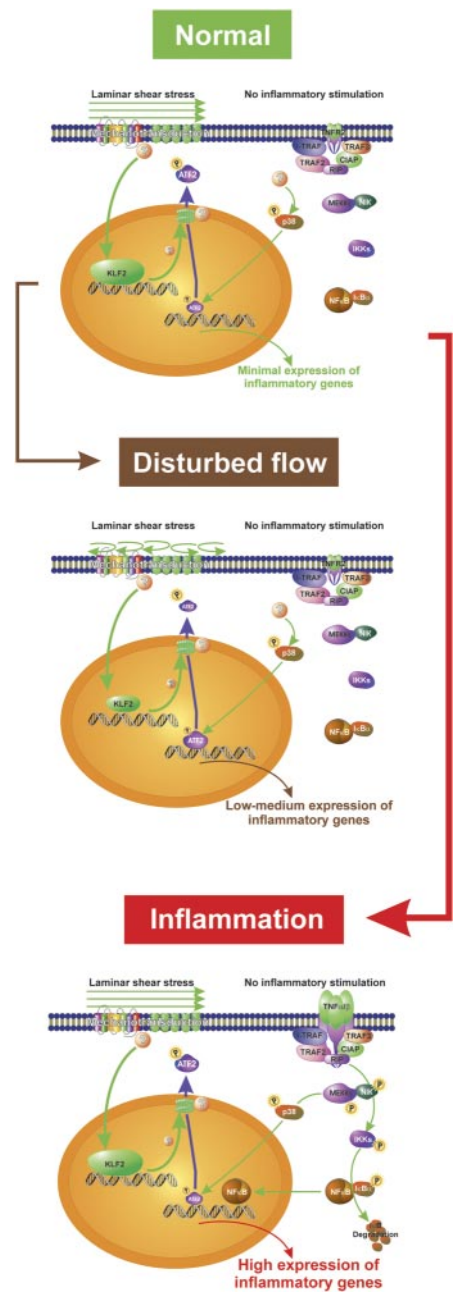
Atherosclerosis is a vascular disease with a major inflammatory component.<sup>5</sup> Sites in the vascular system, predisposing for atherosclerotic vascular lesions are those where laminar flow is disturbed. This suggests that in such areas the “antiinflammatory” effects of laminar flow are absent. How is this flow-dependent anti-inflammatory effect achieved? The major proinflammatory transcription factor is the nuclear factor-κB (NF-κB). This heterodimeric transcription factor is held in the cytosol by its inhibitor (IκB); upon activation by inflammatory cytokines such as TNF-α, IκB is phosphorylated, ubiquitinated, and degraded in the proteasome. In turn NF-κB is set free, translocates to the nucleus, and drives transcription of inflammatory target genes. For full activation of such genes, binding of additional factors such as activator protein 1 (AP-1) complex and coactivators such as CREB-binding protein CBP/p300 and in turn formation of a transcriptional complex is, however, necessary.

In addition to this cytokine-induced inflammatory response, also a basal, “background” or constitutive inflammatory activity seems to be operative in endothelial cells. This state is characterized by the absence of nuclear translocated NF-κB and low transcription of inflammatory target genes and might be re-

flected by the slightly increased CRP levels found in atherosclerotic patients.

Fledderus and colleagues in this issue analyzed in an elegant experiment the relative abundance of transcription factor binding sites in the promoter of 3 groups of genes regulated differentially in endothelial cells by prolonged laminar shear stress and TNF-α, respectively: in the promoter of genes down-regulated by laminar shear stress the authors found an enrichment of ATF binding sites. ATF (also cyclic AMP response element, CRE) consensus sites bind homodimers or heterodimers of members of the ATF family and c-jun, while the slightly different AP-1 consensus site is occupied by homodimers or heterodimers of the major members of the AP-1 family, c-jun and c-fos. Of importance, among the possible members forming a complex that binds to the ATF consensus site, only ATF2 is constitutively expressed in endothelial cells and regulated by phosphorylation and not by transcription. In fact, the authors found phosphorylated ATF2 (pATF2) only in those endothelial cells overlaying atherosclerotic lesions. Furthermore, prolonged laminar shear stress or lentiviral-mediated overexpression of KLF2 reduced nuclear levels of pATF2, and a reduction of ATF2 led to reduced expression of proinflammatory genes while their TNF-α-dependent up-regulation was preserved. From these data, one can conclude that laminar shear stress suppresses basal but not inducible proinflammatory gene transcription by decreasing the amount of phosphorylated ATF2 in the nucleus in a KLF2-dependent fashion (see figure).

Several questions remain: Most importantly, how shear stress and in turn KLF2 mediates the decrease of nuclear pATF2. It is generally assumed that ATF2 is phosphorylated in the nucleus by p38-MAPKinase. However, it was shown recently that ATF2 can shuttle between the nucleus and the cytosol and nonphosphorylated ATF2 is found in



**Schematic representation of the effect of flow on the expression of inflammatory genes in endothelial cells.** Laminar shear stress induces KLF2 that in turn changes distribution of pATF2 between the nucleus and the cytosol. Less nuclear pATF2 leads to a decrease in the transcription of inflammatory target genes (normal condition). Under disturbed flow conditions, more pATF2 remains in the nucleus and expression of inflammatory genes is increased. Upon inflammatory stimulation, NF-κB is activated and overrules the flow-dependent decrease in nuclear pATF2 (TNF-α/β, TNFR2, I-TRAF, TRAF2, TRAF3, CIAP, RIP, NIK, IKKs; components of NF-κB signaling).

the cytosol. Furthermore, does KLF2 cause this effect via transcription of another (known or hitherto unknown) protein or is this effect transcription independent? In any case, the fact that the basal inflammatory “tone” of the vasculature can be blunted by modulating nuclear pATF2, thereby mimicking normal laminar shear stress, opens new horizons for developing anti-inflammatory drugs or regimens effective in the vasculature while preserving the normal cytokine-induced inflammatory response.

*Conflict-of-interest disclosure: the author declares no competing financial interests.* ■

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Comment on Geyeregger et al, page 4288

## “Stimulate the phagocytes!” Fascin-ating! Are DCs actin’ up?

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Geyeregger and colleagues demonstrate that liver X receptor agonists can down-regulate human dendritic cell activation of T cells at the level of the immune synapse.

G.B. Shaw’s play, *A Doctor’s Dilemma*, honored the concepts of phagocytosis (large-particle endocytosis) and opsonization (antibody/antigen binding) described by Metchnikoff and Ehrlich, winners of the 1908 Nobel Prize in Medicine.<sup>1</sup> One theme of Shaw’s 1906 play was that the proper use of phagocytosis and opsonization would cure disease. Medicine was focused on the control and eradication of infectious microbes in the preantibiotic era. Nearly 100 years later, the modern-day phagocytes, dendritic cells (DCs), are important regulators of the antigen-specific immune response. There are 2 major types of human DCs: lymphoid and myeloid. DC antigen processing and cell surface expression result in major histocompatibility complex (MHC) class I and class II restricted antigen responses. The liver X receptor  $\alpha$  (LXR $\alpha$ ) and LXR $\beta$  are nuclear receptors that bind oxidized cholesterol (oxysterols) and dimerize with retinoid X receptors (RXRs). The latter have been shown to influence granulocyte/monocyte cell development.<sup>2</sup> LXRs have been implicated in lipid metabolism and inflammatory responses (reviewed in Kalany and Mangelsdorf<sup>3</sup> and Zeller and Tontonoz<sup>4</sup>). After oxysterol association, LXRs bind nuclear DNA LXR-responsive

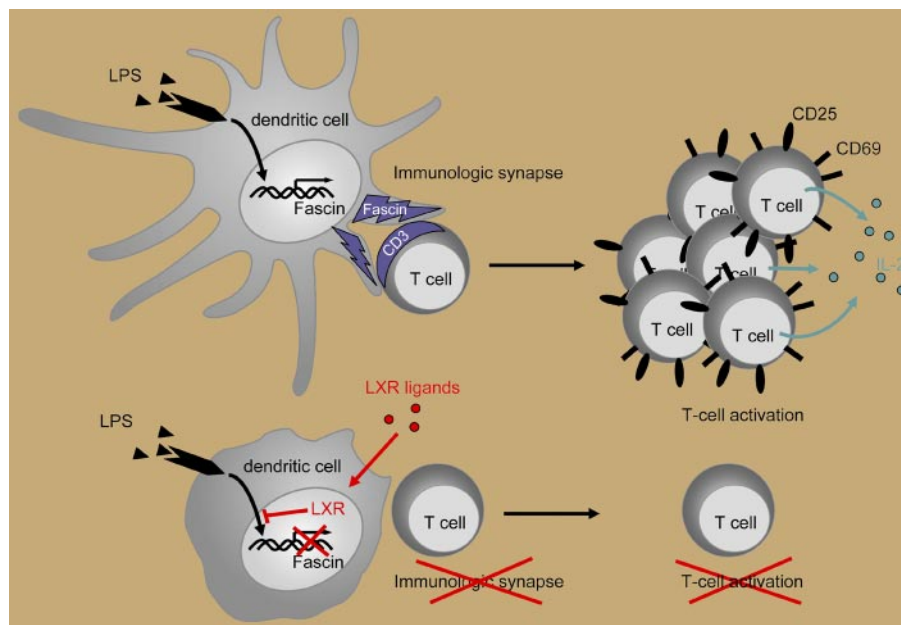
### REFERENCES

- Chien S. Molecular basis of rheological modulation of endothelial functions: importance of stress direction. *Biorheology*. 2006;43:95-116.
- Garin G, Berk BC. Flow-mediated signaling modulates endothelial cell phenotype. *Endothelium*. 2006;13:375-384.
- Tzima E, Irani-Tehrani M, Kiosses WB, et al. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature*. 2005;437:426-431.
- Huddleson JP, Ahmad N, Srinivasan S, Lingrel JB. Induction of KLF2 by fluid shear stress requires a novel promoter element activated by a phosphatidylinositol 3-kinase-dependent chromatin-remodeling pathway. *J Biol Chem*. 2005;280:23371-23379.
- Libby P. Inflammation in atherosclerosis. *Nature*. 2002;420:868-874.

During inflammation, DCs present antigen in the context of the MHC class II molecules to the CD4 helper T-lymphocyte (T-cell) subset Th1, generating a CD4 T-cell Th1 activation response. The T-cell/DC interaction occurs at the immune synapse (IS) that forms during this cellular interaction. The IS depends on an intact cytoskeleton for positioning membrane ligands and receptors. Actin forms cytoskeletal bundles that assist in the formation of cellular projections, in this case, dendrites. A component of the cytoskeleton matrix is fascin, a monomeric actin filament-bundling protein that participates in the DC dendrite formation and cellular interaction (reviewed in Edwards and Bryan<sup>5</sup>).

In a well-designed series of experiments, Geyeregger and colleagues demonstrate a novel approach to the selective control of human myeloid DC maturation and function through the LXR and fascin pathways. The authors first demonstrate that myeloid DCs express high levels of LXR $\alpha$  mRNA. After exposure to LXR agonists, myeloid DCs were poor stimulators of the CD4 T-cell Th1 activation response as measured by decreased CD25 and CD69 expression and decreased IL-2 and IFN- $\gamma$  production. The inhibition of DC stimulation was not mediated by aberrant

elements (LXRLEs) as a dimer, with RXR resulting in gene transcription changes during lipid metabolism and inflammation.



**Lipopolysaccharide (LPS) induces dendritic cell (DC) maturation and fascin production. T-cell activation by DCs depends on formation of an immune synapse (IS). Ligand-induced activation of LXR in DCs inhibits the LPS-induced expression of fascin, an actin-bundling protein that promotes actin bundle formation and is essential for IS formation. By this means, LXR agonists prevent efficient T-cell activation as indicated by diminished proliferation, decreased expression of activation markers (CD25, CD69), and decreased cytokine production (eg, IL-2).**