Regulation of TLR4 Expression Is a Tale About Tail

Zhong-qun Yan

Toll-like receptor (TLR) 4 is the first identified mammalian homolog of the Drosophila Toll protein. TLR4 recognizes bacterial lipopolysaccharide (LPS), and also senses endogenous ligands including hyaluronic acid, oxidized low-density lipoprotein, and heat-shock proteins. Engagement of TLR4 with its ligand triggers a cascade of cellular signals through the intracellular signal transduction domain, known as Toll/interleukin (IL)-1 receptor (TIR), leading to the activation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) signal transduction pathways, consequently inducing expression of inflammatory genes. Although the TLR family of molecules normally contributes to host defense, excess TLR signaling has been implicated in many inflammatory diseases. That TLR4 is implicated in cardiovascular diseases was first suggested by observations of increased expression of TLR4 in failing hearts and in atherosclerotic lesions. The functional importance of TLR4 has subsequently been demonstrated by observations of increased expression of TLR4 in failing hearts and in atherosclerotic lesions. The functional importance of TLR4 has subsequently been demonstrated by the attenuated vascular inflammation and reduced lesion size in TLR4-deficient atherosclerosis prone mice, and by the association of hyporesponsive TLR4 variants with disease development. Furthermore, excessive activation of TLR4 by LPS induces endotoxin shock, a serious systemic disorder with a high mortality rate. Therefore TLR signaling must be tightly controlled to avoid improper activation or suppression. Yet regulation of TLR4 expression in cardiovascular disease remains elusive. In the current issue of Arteriosclerosis, Thrombosis, and Vascular Biology, two sister papers by Feng-Yen Lin and colleagues lend new mechanistic insights that oxidative stress induces posttranscriptional stabilization of TLR4 mRNA in vascular cells.

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Posttranscriptional regulation of mRNA stability is a critical determinant in gene expression, in particular for genes encoding cytokines, transcription factors, growth factors and oncoproteins that have short half-lives. Although it remains poorly understood, recent studies suggest that posttranscriptional control is accomplished by a group of AU-rich element (ARE)-binding proteins that interact in a specific manner with AREs in 3′ untranslated regions (3′-UTR) of mRNA. In most cases, binding of these proteins to AREs causes rapid decay of mRNA. Among ARE-binding proteins, Human antigen R (HuR), also known as ELAV (embryonic lethal abnormal vision)-like protein 1, is unique in that overexpression of HuR has been shown to stabilize and/or to activate translation of a certain subset of genes. It has been postulated that binding of HuR to ARE either competes with or displaces other ARE-binding proteins, thus blocking ARE-containing mRNA from the exosome-mediated degradation (for review see Barreau et al11).

Being motivated by their previous observation that LPS enhances TLR4 expression in human aortic smooth muscle cells (HASMC) by stabilization of mRNA, Lin and colleagues in new studies explored the molecular mechanism for posttranscriptional control of the TLR4 gene. The new study shows that on a low dose of LPS stimulation, HuR rapidly built up in the cytoplasm of HASMCs, as a result of translocation from nucleus to cytoplasm. In contrast, two other ARE-binding proteins, AUf1 and TTP1, did not respond to the stimulation. These data suggest that LPS specifically induces HuR mobilization in HASMCs. To assess the functional relevance to TLR4 expression, HuR was then knocked down by siRNA. This resulted in significant reduction in t1/2 of TLR4 mRNA in the presence of actinomycin D, indicating that HuR functions as a stabilizing factor in the posttranscriptional control of TLR4 mRNA. Using two independent methods, namely cross-linking immunoprecipitation assay and transfection of the cells with a plasmid containing the luciferase reporter linked to the 3′-UTR of TLR4 gene, Lin and colleagues demonstrate that LPS promotes binding of HuR to the 3′-UTR of TLR4 mRNA, and by interacting with cis-acting elements in TLR4 3′-UTR HuR enhances gene expression, although the motifs that interact with HuR in the 3′ UTR of TLR4 mRNA are yet to be defined.

The next question is how LPS induces mobilization of HuR. LPS has been known to activate NADPH oxidase and the MAPK signaling pathway in vascular cells. They found that pretreatment of HASMC with the NADPH oxidase inhibitor diphenylene iodonium, or knockdown of Rac1, a small GTPase which is essentially required for activation of NADPH oxidase, significantly reduces the t1/2 of TLR4 mRNA. This was associated with reduced cytoplasmic levels of HuR. Blockade of the MAPK signaling pathway also led to the same effect on TLR4 mRNA stability and HuR mobilization as inhibition of NADPH oxidase. These findings propose that LPS-induced posttranscriptional stabilization of TLR4 involves activation of NADPH oxidase and the MAPK signaling pathway.

This hypothesis was further tested in the 2nd study by means of pharmacological inhibition of the enzyme activity. Their data depict an intriguing model for the LPS signal in the process of posttranscription regulation. It shows that LPS may initially activate NADPH oxidase through induction of
p47phox translocation to the cell membrane and activation of Rac1, leading to generation of reactive oxygen species (ROS) and further to the activation of the MAPK signaling pathway. Both NADPH oxidase and MAPK signaling are indispensable for the stabilization of TLR4 mRNA. Obviously, the current model is vastly simplified, and does not enlighten whether HuR is the downstream target and phosphorylated by the activated MAPK, and whether NADPH oxidase generated ROS also directly regulates the interaction between HuR and cis-acting elements in 3′-UTR. Nevertheless the study of Lin at all sheds light on the neglected role of NADPH oxidase signaling in posttranscription regulation (Figure).

Finally, results from a study of balloon injured arteries lend further support to the role of LPS signal in upregulation of TLR4. And consistently with the recent experimental study on TLR4 deficient mice, their work suggests that inappropriate TLR4 signaling affects vascular repair in a remarkable way.

The work of Lin and colleagues represents a major advance in the mechanistic understanding of TLR4 expression under oxidative stress and is apt to stimulate future studies of the posttranscriptional regulation of genes implicated in the pathogenesis of atherosclerosis. An incomplete survey of published studies reveals that a number of genes implicated in vascular inflammation bear AUR motifs in mRNA, thus are very likely subjected to oxidative stress induced posttranscriptional regulation in the disease process. These include genes encoding IL-1, IL-2, IL-3, IL-4, IL-6, IL-8, GM-colony stimulating factor (CSF), IFN-γ, tumor necrosis factor (TNF)-α, COX2, and iNOS. Evidently, the list will expand in the near future. More importantly, we also need to assess the importance of enhanced mRNA stability under conditions of chronic inflammation in atherosclerosis and other pathological conditions.

The importance of cytokine mRNA stabilization has been demonstrated in mice with genomic deletion of ARE motifs in TNF-α mRNA or the ARE-binding protein TTP. Both models display elevated levels of circulating TNF-α and markedly prolonged t1/2 of TNF-α mRNA in macrophages. More importantly, the mutant mice spontaneously develop inflammatory and autoimmune pathologies in multiple organs, which can be attributed to excess TNF-α. Hence, alteration in cytokine mRNA stability is a potential mechanism in the pathogenesis of atherosclerosis. Answers to these questions will open a new avenue for modulating inflammatory reactions and thus progression of the disease.

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**References**


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