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MINI-FOCUS: INFLAMMATION IN CARDIAC INJURY

The IL-1RI Co-Receptor TILRR (*FREM1* Isoform 2) Controls Aberrant Inflammatory Responses and Development of Vascular Disease

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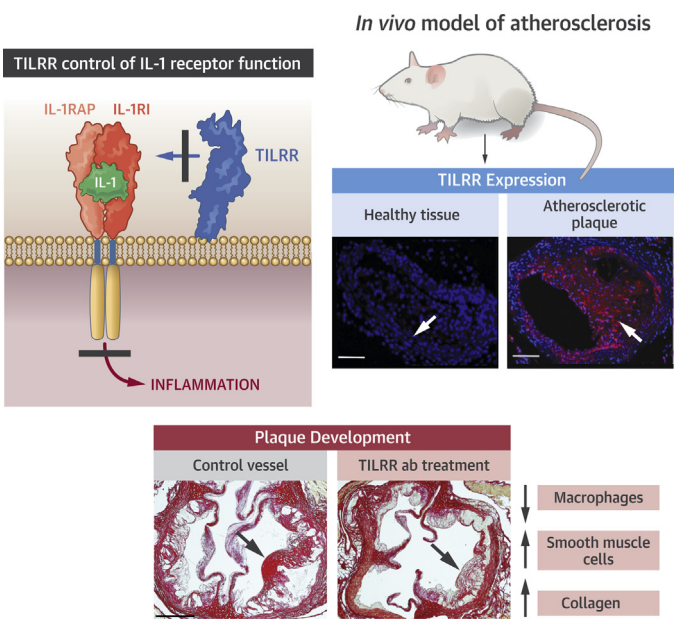
VISUAL ABSTRACT



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HIGHLIGHTS

- **The IL-1RI co-receptor, TILRR, is a potent amplifier of IL-1–induced responses.**
- **Blocking TILRR inhibits IL-1 receptor function and activation of inflammatory genes.**
- **TILRR expression is high in atherosclerotic lesions but low in healthy tissue, allowing distinct inhibition at sites of inflammation.**
- **Genetic deletion of TILRR and antibody blocking of TILRR function reduce plaque development and progression of atherosclerosis. Lesions exhibit low levels of macrophages and increased levels of smooth muscle cells and collagen, characteristics of stable plaques.**

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SUMMARY

Expression of the interleukin-1 receptor type I (IL-1RI) co-receptor Toll-like and interleukin-1 receptor regulator (TILRR) is significantly increased in blood monocytes following myocardial infarction and in the atherosclerotic plaque, whereas levels in healthy tissue are low. TILRR association with IL-1RI at these sites causes aberrant activation of inflammatory genes, which underlie progression of cardiovascular disease. The authors show that genetic deletion of TILRR or antibody blocking of TILRR function reduces development of atherosclerotic plaques. Lesions exhibit decreased levels of monocytes, with increases in collagen and smooth muscle cells, characteristic features of stable plaques. The results suggest that TILRR may constitute a rational target for site- and signal-specific inhibition of vascular disease. (J Am Coll Cardiol Basic Trans Science 2017;2:398–414) © 2017 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY 4.0 license (http://creativecommons.org/licenses/by/4.0/).

ABBREVIATIONS AND ACRONYMS

ApoE = apolipoprotein E

DK = double knockout

GAPDH = glyceraldehyde 3-phosphate dehydrogenase

iBALT = inducible bronchus-associated lymphoid tissue

IgG = immunoglobulin G

IκBα = inhibitor kappa B alpha

IL = interleukin

IL-1RI = interleukin-1 receptor type I

KO = knockout

LDLR$^{-/-}$ = low-density lipoprotein receptor$^{-/-}$

LPS = lipopolysaccharide

NF-κB = nuclear factor-kappa B

NSTEMI = non-ST-segment elevation myocardial infarction

PBS = phosphate-buffered saline

PCR = polymerase chain reaction

qPCR = quantitative polymerase chain reaction

SDS = sodium dodecyl sulfate

STEMI = ST-segment elevation myocardial infarction

TILRR = toll-like and interleukin-1 receptor regulator

Receptors of the toll-like and interleukin (IL)-1 family are central to control of immunity and inflammation, and their importance in disease is well documented (1–4). Activation is induced by ligand binding and by association of system-specific co-receptors, which regulate signal amplification and transcriptional activity (1,4,5). Changes in co-receptor expression and causative mutations impact responses to infection, tissue damage and stress, and affect development of disease (5).

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Proteoglycans and glycosylated proteins act as co-receptors in a number of regulatory systems. Recruited to the receptor complex, they control receptor function, ligand binding, and extracellular interactions associated with aberrant signal activation and disease (6–8). The IL-1 receptor type I (IL-1RI), and its ligand, the cytokine IL-1, are potent activators of nuclear factor-kappa B (NF-κB) and intrinsically linked with acute and chronic inflammation (9). Dysregulation of NF-κB and IL-1-induced gene activity underlie development and progression of conditions such as atherosclerosis (10,11). Our earlier studies identified Toll-like and IL-1 receptor regulator (TILRR) (*FREM1* isoform 2) (12), a cell surface proteoglycan, as an IL-1RI co-receptor (13,14). We have demonstrated that TILRR association with IL-1RI causes enhanced expression of IL-1RI and increased recruitment of the MyD88 adapter to the Toll/interleukin-1 receptor homology domain of IL-1RI (13). Further, we have shown that the resulting increase in signal amplification at the level of the receptor complex directs TILRR control of aberrant activation of NF-κB and inflammatory genes.

The present study investigates the role of TILRR in host defense and disease and demonstrates that TILRR is highly expressed in areas of vascular inflammation and lung fibrosis. Characterization of the inflammatory phenotype of our TILRR knockout (KO) mouse shows that changes in IL-1 receptor levels, signal transduction, and inflammatory gene activity, caused by genetic deletion, are consistent with molecular mechanisms of TILRR function identified in our published in vitro studies (13,14). Using well-established models of vascular disease we demonstrate that TILRR KO and antibody blocking lead to reductions in monocyte activation, inflammatory gene activity, and disease progression, without causing development of vulnerable plaques. Taken together our results suggest that TILRR is a central regulator of inflammatory responses related to development of vascular disease, and that it may constitute a highly specific therapeutic target.

METHODS

MOUSE STRAINS. TILRR$^{-/-}$ mice. Mice were derived by the Center for Mouse Genome Modification (University of Connecticut, Farmington, Connecticut). The mouse TILRR transcript is encoded within a genomic region spanning across exons 24 to 36 of the *Frem1* gene, and the amino terminus of the TILRR protein from aa1-17 is encoded in the intron preceding exon 24 of *Frem1*. To create a null allele for TILRR, LoxP sites were inserted flanking exons 24 and 25 (Supplemental Figure 1). The targeting vector was prepared by recombineering according to Lee et al. (15). Briefly, we first retrieved approximately 12.8 kb of *Frem1* genomic sequence spanning 4 kb upstream of exon 24 to 3 kb downstream of exon 26 from the BAC, RP23-365E9, into PL253 containing the herpes simplex virus thymidine kinase negative