JACC: BASIC TO TRANSLATIONAL SCIENCE
VOL. 2, NO. 4, 2017
ISSN 2452-302X
http://dx.doi.org/10.1016/j.jacbts.2017.06.001

EDITORIAL COMMENT

TILRR Steers Interleukin-1 Signaling

Co-Receptor Provides Context and a Therapeutic Target*

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In signaling, whether in health or disease, context is everything. The spatiotemporal dynamics of ligand cues, the location or cell specificity of receptor expression, the presence or absence of co-receptors, and many more contextual components can change the size and character of the signal and of the cell or tissue-level behavior. The ability to understand this context, which greatly increases the complexity of the system under consideration, requires computational modeling to identify the key mechanisms and the optimal components for therapeutic targeting. Using new knockout mice and agent-based models, in this issue of *JACC: Basic to Translational Science* Smith et al. (1) identified the in vivo consequences of previous findings that the signaling of the key inflammatory cytokine receptor interleukin-1 receptor type I (IL-1RI) is more complex than "on–off." Its strength of activation, regulated by the co-receptor toll-like and IL-1 receptor regulator (TILRR), leads to at least 3 states, each with different consequences for inflammation and inflammatory disease.

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The signaling and cellular behavior in question here goes through nuclear factor (NF)-κB; however, this study looked upstream where context-dependent signaling begins with ligands and co-receptors. For more on the signaling pathways between IL-1RI ligated by IL-1 and NF-κB, see reviews by Mitchell et al. (2) and Verstrepen et al. (3). Here, the focus is at the cell membrane itself. Previous work by Zhang et al. (4) identified a co-receptor for IL-1RI called TILRR (toll-like and IL-1 receptor regulator). This membrane-associated glycoprotein increases IL-1RI expression, ligand binding, recruitment of the MyD88 adapter protein, NF-κB activation, and proinflammatory gene expression (4). Further in vitro studies identified specific TILRR residues involved in the TILRR–IL-1RI interaction and the potentiation of proinflammatory signaling (5).

Previous studies showed that total or near-total loss of IL-1 or of its receptor IL-1RI (i.e., of IL-1RI signaling) led to decreased plaque formation, but with the additional problem of increased vulnerability to rupture of the plaques that did form (**Figure 1**). This creates an "out of the frying pan, into the fire" situation; fewer plaques is not an optimal outcome if those remaining plaques are more likely to have devastating consequences. This is where understanding the system in its entirety has great therapeutic benefit. By going beyond simplistic thinking such as "IL-1RI signaling is pro-atherosclerotic, therefore we should eliminate that signaling," targeting TILRR provides an opportunity to take a middle ground; IL-1RI signaling is diminished but not eliminated. This also leads to a middle ground in the tissue-level effects, with twin therapeutic consequences: eliminate as many plaques as possible, but keep those that remain as safe as possible. Computational models, including those developed by Qwarnstrom and colleagues and described below, enable the identification of these therapeutic "sweet spots."

The investigators used an agent-based model (ABM) to simulate the IL-1/NF-κB system. The model

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

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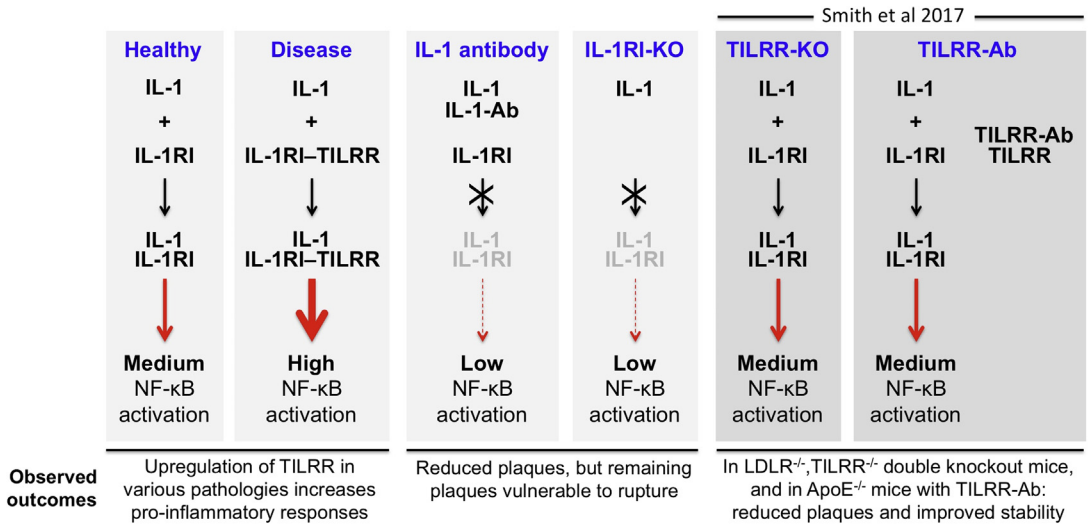
The author attests he is in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 2, NO. 4, 2017
AUGUST 2017:415–7

FIGURE 1 3 Levels of IL-1RI Signaling



Three levels of IL-1RI signaling: medium in healthy, high in disease, and low in response to overly aggressive therapy leads to the demonstration in Smith et al. (1) that a more subtle therapy, targeting the co-receptor toll-like and IL-1 receptor regulator (TILRR), restores signaling to a more beneficial level. **Darker gray background** shows the new work contributed by Smith et al. (1). Ab = antibody; ApoE = apolipoprotein E; IL = interleukin; IL-1RI = interleukin 1 receptor type I; KO = knockout; LDLR = low-density lipoprotein receptor; NF = nuclear factor.

is 3-dimensional, and the agents are the molecules in the signaling pathway. Each individual molecule is represented as its own agent; for example, there are up to 3,000 individual IL-1RI-TILRR complexes expressed on the cell surface as it associates with or dissociates from the ligand IL-1 and intracellular scaffold proteins. Molecules can diffuse, and they can interact locally; transcription factors, for example, must enter the nucleus at sites of import receptors. Because all the molecules are being tracked, they can also be summed across the cell space to give a total level that can be validated against comparable experimental measurements. Unlike some ABM approaches, the molecular level that the investigators used enabled them to incorporate detailed biophysics, making these models highly mechanistic, not phenomenological.

Why an ABM rather than a deterministic ordinary differential equation (ODE) model? The key is that the cell is not a well-mixed system. It is structurally heterogeneous, and the local density of signaling molecules can lead to deviations from the "mean concentration" assumptions used in typical ODE systems. Used correctly, ABMs and other stochastic approaches can also give estimates for the cell-to-cell variability in responses, which is of increasing interest as single-cell measurements become more feasible and our understanding of the importance of cell heterogeneity in physiology, pathology, and response to therapy increases. Variation between people, between cells, and between subcompartments is a vital part of biology, and these methods help to quantify and understand it.

Pogson et al. (6) first published an ABM for NF-κB in 2008, and Rhodes et al. (7) extended it in 2015 to include TILRR and identify the mechanism by which it regulates IκBα and NF-κB. In the current paper (1), the investigators applied the model to predict the effects of 2 methods of targeting TILRR—a gene knockout and an antibody. On the basis of their simulations, knockout of TILRR is predicted to substantially reduce the rate of degradation of IκBα, keeping levels of that protein high, decreasing NF-κB activation. The investigators experimentally validated the direction and approximate magnitude of these predictions in cell culture, and even came close to matching the time-course dynamics (compare Smith et al. [1] Figure 1G to Supplemental Figure 3A). The model also predicted that TILRR inhibition would decrease inflammatory gene expression, and this was validated in mice (compare experimental results in Figures 1H and 1I and Online Figure 4 to the model results of Online Figure 3B).

Note that although the mechanistic focus of the paper is on the TILRR, understanding the system requires a model that incorporates the detailed and complex NF-κB intracellular pathway and cytoskeletal elements (7). NF-κB signaling itself, with multiple upstream kinases, inhibitors, and 15 possible NF-κB dimers, is a great example of how mathematical and computational models are needed to understand, probe, and leverage the system complexity (8). It is quite common in models that, to explain 1 phenomenon, we need to include additional networks that provide the mechanistic path to the outcome. Also, by building the model in this way, Smith et al. (1) could predict the outcome on downstream signaling of a molecular addition (e.g., an antibody) or genetic change (e.g., TILRR knockout) or a mechanistic change (e.g., a point mutation eliminating a key interaction). This mechanistic predictability is what makes computational models so promising in the search for better therapeutics.

Experimentally, Smith et al. (1) showed that TILRR exhibited some classic characteristics of a therapeutic target; its expression is locally increased in disease states (myocardial infarction, monocyte activation, carotid ligation, and in apolipoprotein-E [$ApoE^{-/-}$] and low-density lipoprotein receptor [$LDLR^{-/-}$] mice on a high-fat diet) compared with healthy mice. In TILRR knockout mice (including those with $ApoE^{-/-}$ and $LDLR^{-/-}$ background), IL1RI levels were reduced, proinflammatory regulators were less expressed, and neointimal thickening in carotid ligation was reduced. In TILRR knockout cells, or cells treated with an antibody against TILRR, NF-κB activity in response to IL-1 stimulation was down, as was inflammatory gene expression. Thus, clearly, TILRR has a role in inflammation and its inhibition or deletion can inhibit (at least partially) inflammatory responses.

However, the real insight here is that by targeting TILRR, and thus using a reduction in IL-1RI signaling rather than an elimination of that signaling, the inflammatory response is returned to a more intermediate state (**Figure 1**), perhaps closer in character to the absence of injury (recall that in the absence of injury, TILRR expression is lower). In this scenario, plaques are reduced but not eliminated; however, the increased collagen and smooth muscle cell content suggests the plaques are more stable than when IL-1RI is eliminated (**Figure 1**).

In closing, although much research into signaling has focused on regulation downstream, Smith et al. provided a timely reminder that upstream, at the ligand-receptor binding and signaling-initiation stage, there is also considerable complexity and context-dependent regulation with a significant impact on cell and tissue behavior. The combination of computational models and careful experimentation can lead to improved mechanistic understanding of context-dependent signaling in many ligand-receptor systems (9) and, ultimately, to improved therapeutics.

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KEY WORDS context-dependent signaling, co-receptors, immune signaling, inflammation, NF-κB