The innate immune system has several large genetically encoded receptor families that interact during the detection and elimination of pathogens. The Toll-like receptor (TLR) family recognizes a diverse group of evolutionarily conserved molecules including lipopolysaccharide (LPS), peptidoglycan, and nucleic acids (16). Engagement of these receptors results in proinflammatory signaling. Another family of innate immune receptors, triggering receptors expressed on myeloid cells (TREM), is capable of altering the downstream signaling potential of the TLR molecules. The TREM family contains both inhibitory and activating receptors that act to fine-tune the inflammatory response mediated by TLR (9). The novel report by Ornatowska and colleagues (15), which appears in this issue of AJP-Lung, sheds light on the intersection between these two families and how molecular cross talk may be functioning to tailor inflammatory responses.

TREM-1 is an activating receptor expressed on neutrophils and monocyte macrophages. The TREM-1 molecule consists of an ectodomain, a transmembrane region, and a short cytoplasmic tail lacking any signaling motifs. The transmembrane domain of TREM-1 has a positively charged residue that mediates the formation of a complex with the signaling adaptor DAP12. DAP12 contains an immunoreceptor tyrosine-based activation motif that upon phosphorylation mediates downstream signaling molecule recruitment (Fig. 1) (10, 11, 12, 17). Studies have shown that TREM-1 is upregulated in infection in vivo and following TLR engagement in vitro (2, 14). Coactivation of TREM-1 and TLR4 results in a 25-fold synergistic increase in TLR4-mediated proinflammatory cytokine and chemokine secretion (1, 2). More recently, colocalization of TREM-1 and TLR4 in lipid rafts was demonstrated following either LPS stimulation or TREM-1 ligation in neutrophils (4). These data suggest that following microbial challenges in vivo, the two pathways may act synergistically to trigger an exuberant immune response. While epithelial cells, endothelial cells, and other cell types express TLR4, coexpression of TLR4 and TREM-1 is limited to macrophage and neutrophils providing these immune cells with a selective advantage in terms of shaping a local inflammatory response through more effective inflammatory signaling.

In animal studies of LPS-induced endotoxemia, the impact of reducing TREM-1 signaling by administration of soluble forms of the TREM-1 molecule, via small molecule blocker (LP17), and silencing RNA was examined (3, 5, 6). All three of these approaches have shown that decreased TREM-1 signaling results in decreased systemic cytokine production and improved survival in these models of endotoxemia. However, in murine fecal peritonitis models, conflicting data on the effect of TREM-1 blockade have been reported. TREM-1 blockade with soluble TREM-1 or the small molecule inhibitor resulted in improved survival, whereas small interfering RNA (siRNA) blockade of TREM-1 resulted in decreased survival. It is worth noting that for these studies, because TREM-1 ligand has remained elusive, blockade of TREM signaling was achieved by surrogate means. Whether these opposing results represent the different experimental conditions or variable levels of TREM-1 blockade is unclear, and identification of TREM-1 ligands remains a critical missing component to understanding the role of this molecule in sepsis.

In their recent article, Ornatowska et al. (15) expand our understanding of the potential mechanism of TREM-1 modulation on TLR4 signaling. Using siRNA silencing of TREM-1 and pathway-specific microarray analysis, they show that TREM-1 silencing in macrophage results in decreased transcription of key proteins in the TLR4 signaling pathway. This downregulation of adaptor proteins during TREM-1 silencing suggests that TREM-1 may be impacting cytokine and chemokine production by increasing the availability of downstream signaling molecules in the setting of acute inflammation.

When TLR4 binds, MD2/LPS Toll/IL-1R homology (TIR) domain containing adaptor molecules are recruited. These molecules can trigger two main signaling pathways: MyD88-dependent and TRIF-dependent (MyD88-independent) pathways. Signal transduction through both of these pathways will result in activation of NF-κB and mitogen-activated protein kinase cascades leading to inflammatory cytokine production. However, only TRIF-dependent pathways result in interferon (INF) and INF-inducible gene expression (16). Because adaptor recruitment dictates downstream cytokine expression, an understanding of the molecular events that control adaptor molecule recruitment is an area of intense investigation. Macrophage can utilize MyD88, TRIF, TRAM, or MAL/TIRAP adaptors following TLR4 ligation. TLR4 recruitment of TRIF results in INF production, whereas MyD88 recruitment leads to NF-κB-mediated responses that include TNF, IL-1, IL-12, IL-8, and MIP-1α production. Clearly, alterations in either selective adaptor recruitment or adaptor availability allow the immune system to tailor the output of the response following TLR ligation.

TREM-1 silencing did not alter TRIF-mediated expression of INF indicating that TREM-1 may not play a significant role in amplifying this TLR signaling pathway. However, the impact of TREM-1 silencing on MyD88, CD14, and other downstream signaling molecules could be the explanation for the decreased proinflammatory signaling observed both in vitro and in vivo following TREM-1 silencing. These authors noted significant decreases in MyD88 and CD14 transcripts as well as decreases in downstream molecules in the NF-κB pathway [IκBα, precursor to p50 (p100), CEBP-B] following LPS stimulation in the setting of TREM-1 silencing. These data

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provide an explanation for how TREM-1 is able to amplify the output of the TLR4 response. It would be interesting to examine whether TREM-1 silencing in the in vivo models results in decreased levels of these signaling molecules as is noted in these in vitro studies.

Another intriguing question is whether different TREM-1 ligands can potentially cause alterations in TLR4 recruitment of adaptor proteins that ultimately control whether MYD88-dependent or -independent pathways are favored. Indeed, it seems that TREM-1 molecules, like many other innate immune receptors, may have the ability to bind multiple ligands. TREM-1 binding to platelets, serum, and viruses has been described, although no ligands have been identified (7, 13, 18). Recent structural data from TLR4/MD-2 complexes awaits discovery.

As functional genomic approaches enable us to identify overlapping signal pathways, the relationship between TREM-1 and TLR downstream signaling molecules will provide a more complete picture of the pathways involved in inflammation and identify potential therapeutic targets to alter these responses.

**REFERENCES**


