DATA SUPPLEMENT FOR: INNATE IMMUNITY AND THE FAILING HEART: THE CYTOKINE HYPOTHESIS REVISITED

TOLL-LIKE RECEPTOR SIGNALING PATHWAYS

A, Toll-like receptors and interleukin-1 receptors have a conserved cytoplasmic domain, that is known as the Toll/IL-1 R domain. The TIR domain is characterized by the presence of three highly homologous regions (known as boxes 1, 2 and 3). Despite the similarity of the cytoplasmic domains of these molecules, their extracellular regions differ markedly: TLRs have tandem repeats of leucine-rich regions (known as leucine rich repeats, LRR), whereas IL-1 Rs have three immunoglobulin (Ig)-like domains. B, Stimulation of TLRs triggers the association of MyD88, which in turn recruits IRAK4, thereby allowing the association of IRAK1. IRAK4 then induces the phosphorylation of IRAK1. TRAF6 is also recruited to the receptor complex, by associating with phosphorylated IRAK1. Phosphorylated IRAK1 and TRAF6 then dissociate from the receptor and form a complex with TAK1, TAB1 and TAB2 at the plasma membrane (not shown), which induces the phosphorylation of TAB2 and TAK1. IRAK1 is degraded at the plasma membrane, and the remaining complex (consisting of TRAF6, TAK1, TAB1 and TAB2) translocates to the cytosol, where it associates with the ubiquitin ligases UBC13 and UEV1A. This leads to the ubiquitylation of TRAF6, which induces the activation of TAK1. TAK1, in turn, phosphorylates both MAP kinases and the IKK complex, which consists of IKK-α, IKK-β and IKK-γ (also known as IKK1, IKK2, NEMO, respectively). The IKK complex then phosphorylates IκB, which leads to its ubiquitylation and subsequent degradation. This allows NF-κB to translocate to the nucleus and induce the expression of its target genes. (Reproduced with permission from Mann DL, The role of innate immunity in heart failure, in Heart Failure: A Companion to Braunwald’s Heart Disease, edited by Mann DL and Felker GM, 2015, pp 109-126, Elsevier/Saunders, Philadelphia, Pennsylvania).
As shown in panel A of the Figure, the signaling pathway that is used by the TLR family of receptors is highly homologous to that of the IL-1 receptor (IL-1R) family (see below). TLRs are type 1 membrane-spanning receptors that have a leucine-rich repeat extracellular motif and an intracellular signaling motif that is similar to interleukin (IL-1). With the exception of TLR3, all TLRs interact with an adaptor protein termed MyD88 (myeloid differentiation factor 88) via their Toll Interleukin Receptor (TIR) domains (panel B). MyD88-dependent signaling through TLR2 and TLR4 requires an adaptor protein termed TIRAP (TIR domain-containing adaptor protein) to initiate signaling. When stimulated, MyD88 sequentially recruits IL-1 receptor associated kinases 4, 1 and 2 (IRAK4, IRAK1 and IRAK2) to the receptor complex. Phosphorylation of IRAK1 on serine/threonine residues by IRAK4 results in recruitment of tumor necrosis receptor associated factor 6 (TRAF6) to the complex, which is responsible for early responses in response to TLR signaling (see reference 1 for more details on TLR signaling).

PROINFLAMMATORY CYTOKINES

Tumor Necrosis Factor Superfamily

The tumor necrosis factor (TNF) superfamily consists of 19 well-characterized ligands (TNFSF) and 34 TNF superfamily receptors (TNFRSF). Members of the TNF superfamily of ligands and receptors are expressed in a broad variety of cell types, including myocardial cells. Notably, all members of the TNF superfamily exhibit pro-inflammatory activity. Of note, recent studies have identified a potential role for TNF superfamily ligands/receptors in terms of mediating inflammatory responses in the heart, including TNF/TNFFR1,TNFFR2 (TNFSF2/TNFRSF1A, TNFRSF1B), FasL/Fas (TNFSF6/TNFRSF6), TWEAK (tumor necrosis factor-like weak inducer of apoptosis) /TWEAKR (TNFSF12/TNFRSF12), and RANKL (Receptor activator of NF-kB ligand)/ RANK (TNFSF11/TNFRSF11A). In contrast to FasL, TNF, TWEAK and RANKL signal through TNFSF receptors that engage a common scaffolding protein
termed TNF receptor associated factor 2 (TRAF2), that is recruited to the TNFSF receptors following engagement of their cognate ligands. In contrast to TNF, TWEAK and RANKL, cardiac-restricted overexpression of FasL (TNFSF6) does not lead to dilated cardiomyopathy. Notably, TNF, TWEAK and RANKL signal through TNFSF receptors that engage a common scaffolding protein termed TNF receptor associated factor 2 (TRAF2), that is recruited to the TNFSF receptors following engagement of their cognate ligands. Cardiac restricted expression of TRAF2 provokes a dilated cardiac phenotype, that phenocopies cardiac restricted overexpression of TNF, and is mediated, at least in part, though NF-κB.5

**Interleukin-1 Family**

Although the original IL-1 family (IL-1F) consisted of IL-1α (IL-1F1) and IL-1β (IL-1F2), the IL-1 family has now expanded to include seven ligands with agonist activity (IL-1α and IL-1β, IL-18 (IL-1F4), IL-33 (IL-1F11), IL-36α (IL-1F6), IL-36β (IL-1F7), IL-36γ (IL-1F8), three receptor antagonists (IL-1Ra [IL-F3], IL-36Ra [IL-F5], IL-38 [IL-F10]), and an anti-inflammatory cytokine (IL-37 [IL-10]). Members of the IL-1 Receptor (IL-1R) family include six receptor chains forming four signaling receptor complexes, two decoy receptors (IL-1R2 and IL-18BP), and two negative regulators (TIR8 or SIGIRR [IL-1R8], IL-1RACPb).6 Similar to TNF, IL-1β and IL-18 appears to synthesized within the myocardium in response to stressful environmental stimuli, and both IL-1β mRNA and protein have been detected in the hearts of patients with dilated cardiomyopathy.7 Moreover, in vivo studies have shown specific blockade of IL-18 using IL-18 binding protein improves contractile function in human atrial tissue following ischemia reperfusion injury,8 as well as lipopolysaccharide-induced LV dysfunction in experimental animals.8

**Interleukin-6 (IL-6) Family**

Based on their functional redundancy, structural similarity, and use of a common signaling receptor, interleukin-6 (IL-6), leukemia inhibitory factor (LIF), cardiotrophin-1 (CT-1),
ciliary neurotrophic factor (CNTF), interleukin-11 (IL-11), and oncostatin M (OSM) are considered to represent the "IL-6 family" of cytokines. Inclusion in the IL-6 family is based on a helical cytokine structure and receptor subunit makeup. The IL-6 family of cytokines triggers downstream signaling pathways in multiple cell types, including cardiac myocytes, either through the homodimerization of the gp130 receptor or through the heterodimerization of gp130 with a related transmembrane receptor. All IL-6 type cytokines potentially activate STAT3, and to a lesser extent STAT1 through their common gp130 subunits. The specificity of cytokine signaling within the IL-6 family is determined by the composition of the cytoplasmic domains associated with the signal-competent receptor complex.\(^9\) The suppressor of cytokine signaling (SOCS) (also referred to as cytokine-inducible SH2 proteins [CIS]) are a family of specific negative regulatory feedback elements of JAK/STAT signaling. Expression of some SOCS family members is regulated transcriptionally by STATs, thereby acting as a negative feedback loop for JAK-STAT signaling. Both SOCS-1 and SOCS-3 interact with the kinase domain of various JAK proteins, thereby preventing STAT phosphorylation. Previous clinical studies showed that the plasma level of IL-6, CT-1, LIF and gp130 are elevated in patients with advanced heart failure and that high levels are associated with a poor prognosis for heart failure patients.\(^{10,11}\)

**REFERENCES**


(4) Ueland T, Yndestad A, Oie E, Florholmen G, Halvorsen B, Froland SS, Simonsen S,


