Theory

Structure

The Structural Basis for Lipid and Endotoxin Binding in RP105-MD-1, and Consequences for Regulation of Host Lipopolysaccharide Sensitivity

Highlights

- Atomic-resolution simulations of MD-1 co-receptor and RP105/MD-1 receptor complex
- Maintenance of MD-1 experimental structure dependent upon endogenous phospholipids
- Structural basis for interaction with endotoxin analogs revealed
- Potential of mean force calculations explain endotoxin specificity

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In Brief

Ortiz-Suarez and Bond performed extensive atomic-resolution simulations and free-energy calculations of the RP105 receptor complex and its co-receptor MD-1. These provide the structural basis for binding to endogenous ligands, and explain how the complex binds lipopolysaccharide (LPS) to fine-tune innate immune responses.

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The Structural Basis for Lipid and Endotoxin Binding in RP105-MD-1, and Consequences for Regulation of Host Lipopolysaccharide Sensitivity

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SUMMARY

MD-1 is a member of the MD-2-related lipid-recognition (ML) family, and associates with RP105, a cell-surface protein that resembles Toll-like receptor 4 (TLR4). The RP105·MD-1 complex has been proposed to play a role in fine-tuning the innate immune response to endotoxin such as bacterial lipopolysaccharide (LPS) via TLR4·MD-2, but controversy surrounds its mechanism. We have used atomically detailed simulations to reveal the structural basis for ligand binding and consequent functional dynamics of MD-1 and the RP105 complex. We rationalize reports of endogenous phospholipid binding, by showing that they prevent collapse of the malleable MD-1 fold, before refining crystallographic models and uncovering likely binding modes for LPS analogs. Subsequent binding affinity calculations reveal that endotoxin specificity arises from the entropic cost of expanding the MD-1 cavity to accommodate bulky lipid tails, and support the role of MD-1 as a "sink" that sequesters endotoxin from TLR4 and stabilizes RP105/TLR4 interactions.

INTRODUCTION

MD-1 (myeloid differentiation protein 1) belongs to the MD-2-related lipid-recognition (ML) family, comprising single-domain, β-rich proteins (Figure 1) found in both animals and plants. Despite their relatively low sequence identity and diverse physiological functions, including roles in signaling, metabolism, and defense, an emerging feature is the apparent shared ability to interact with lipids (Inohara and Nuñez, 2002), and a corresponding association with lipid-related disease states (Wymann and Schneiter, 2008). MD-1 associates with RP105 (radioprotective 150 kDa), a type I transmembrane protein whose solenoidal ectodomain is composed of multiple leucine-rich repeats (LRRs) (Figures 1A and S1A). MD-1 and RP105 share architecture and cell-surface colocalization similar to that of the well-characterized MD-2/TLR4 (Toll-like receptor 4) system, which is key to mounting an innate immunological response against invading pathogens (Gay and Gangloff, 2007; Park et al., 2009). This suggests a role in sensing endotoxin or related microbial products, since MD-2/TLR4 recognize bacterial lipopolysaccharide (LPS), a large, complex glycolipid found in the outer membranes of Gram-negative bacteria. Consistent with this, it has been reported that RP105 may regulate LPS responses in a cell-dependent manner, apparently fine-tuning TLR4 pathways, and also regulating TLR2-induced macrophage responses to *Mycobacterium tuberculosis* lipoproteins (Blumenthal et al., 2009). Biologically this may be important in preventing overamplification of the TLR4 response, which can lead to endotoxic shock, a principal cause of death in intensive care units (Martin et al., 2003; O'Neill et al., 2009).

MD-1 shares ~20% identity with MD-2, and both protein structures resemble a β cup with an almost exclusively hydrophobic lining, composed of a three-stranded and six-stranded antiparallel β sheet (βC, βD, βG; and βA, βB, βI, βH, βE, and βF; respectively) plus eight intervening loops (Figure 1B). Unlike TLR4, RP105 does not contain the C-terminal Toll/IL-1 (TIR) intracellular signaling domains necessary to transduce extracellular signals into the cytoplasm (Gay et al., 2014). In addition, the "m-shaped" head-to-head organization of the RP105/MD-1 complex (Ohto et al., 2011; Yoon et al., 2011) juxtaposes the RP105 N termini (Figures 1A and S1B), in contrast with the tail-to-tail assemblies that facilitate interaction of the C-terminal cytoplasmic TIR domains in TLR complexes. RP105 may fine-tune endotoxin-induced activities through a direct interaction with TLR complexes (Divanovic et al., 2005, 2007), possibly as a result of the RP105 C-terminal region and MD-1 replacing the equivalent homodimerization interface of the $[TLR4{\cdot}MD\text{-}2]_2$ receptor complex (Ohto et al., 2011; Yoon et al., 2011) (Figure S1C).

In LPS molecules, the hydrophobic lipid anchor component termed "lipid A" (LPA) contains a phosphorylated, β-(1,6)-linked diglucosamine headgroup connected to multiple acyl tails (Alexander and Zahringer, 2002) (Figure 2A), and is responsible for most of the activity of LPS against TLR4. Hexa-acylated LPA from *Escherichia coli* is a TLR4 agonist, and is endotoxic to human macrophage and mouse cells depending upon its structure (Muroi et al., 2002; Bryant et al., 2010; Needham and Trent, 2013). The tetra-acylated precursor lipid IVa (LPIVa) (Kusumoto et al., 2003) (Figure 2A) acts as an antagonist in human cells but as an agonist in mouse cells (Golenbock et al., 1991; Means et al., 2000). The immunomodulatory properties of different lipid

