Structures of Human MD-2 Coreceptor and Its Complex with Antiendotoxic Lipid IVa Preventing Endotoxin Shock

Innate immunity is the first line of defense against microbial infections. Defense responses are activated when microbial components are recognized by a variety of pathogen sensors including the Toll family of receptors and Nod-like receptors. Among microbial components, lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria is a potent stimulant of immune responses, and a small difference in LPS structure has a great influence on host responses against the bacteria.

Human MD-2 is a glycoprotein with a 16 amino-acid secretion signal and two *N*-linked glycosylation sites (*1*) and represents a class of MD-2-related lipid recognition (ML) proteins that also include mite allergen proteins. Secreted MD-2 forms a stable receptor complex with Toll-like receptor 4 (TLR4) on the cell surface, and the complex recognizes LPS, reading to activation of innate immune responses (*2*). Excessive responses to the endotoxic LPS frequently cause septic shocks, a rapidly progressing inflammatory disease with high mortality.

Lipid A, the immunostimulatory core of LPS, is diverse in several species, and these variations are discriminated by the TLR4-MD-2 receptor complex as endotoxic or as antiendotoxic. The hexa-acylated form of *Escherichia coli* lipid A acts as a potent agonist in human macrophage cells and in mouse cells. However, its precursor lipid IVa, the tetra-acylated form of lipid A, acts as an antagonist in human cells but as an agonist in mouse cells.

We have determined the crystal structures of human MD-2 itself and of its complex with lipid IVa (*3*). MD-2 was expressed in methyltropic yeast *Pichia pastoris*, and its polysaccharide moieties of MD-2 were trimmed off by endoglycosidase treatment which leaves a single *N*-acetyl-glucosamine (NAG) at each glycosylation site. Crystals of monomeric MD-2 showed severe crystal twinning, but was successfully transformed into single crystals through optimization of cryoprotectant (*4*). The structure of the thus obtained native crystal was determined at 2.0 Å resolution by multiple isomorphous replacement (Protein Data Bank code 2E56). A cocrystal with the lipid IVa complex was also obtained as a twinned form nearly isomorphous to the native crystal. The structure of the complex was refined at 2.2 Å resolution (Protein Data Bank code 2E59). Diffraction datasets were collected at 100 K on beamline **BL38B1**, with a CCD detector.

MD-2 with two *N*-linked NAGs at Asn^{26} and Asn^{114} is folded into a single domain consisting of two β sheets in the immunoglobulin fold conserved among the ML proteins: one sheet consists of three antiparallel β strands, and the other of six anti-parallel strands (Fig. 1). Between these sheets is a large and deep hydrophobic cavity. Both the glycosylation sites are distant from the cavity region, indicating that the glycosylation plays a role, not in ligand binding, but presumably in the secretion and protection of MD-2. In the native structure, unexpected three electron-densities were observed in the cavity; these were attributed to bound myristic acid molecules.

The MD-2 structure of the lipid IVa complex is not significantly altered upon lipid IVa binding. In the complexed structure, electron densities in the cavity were assigned to the parts corresponding to lipid IVa: two glucosamine, two phosphate, and four fatty acid chains (Fig. 1). Residues Phe^{119} to Gly^{123} are important for the LPS recognition (Fig. 2), and these residues, with the exception of Lys^{122}, are conserved in all the species of MD-2. Hydrophobic and electrostatic surface potentials in the vicinities of

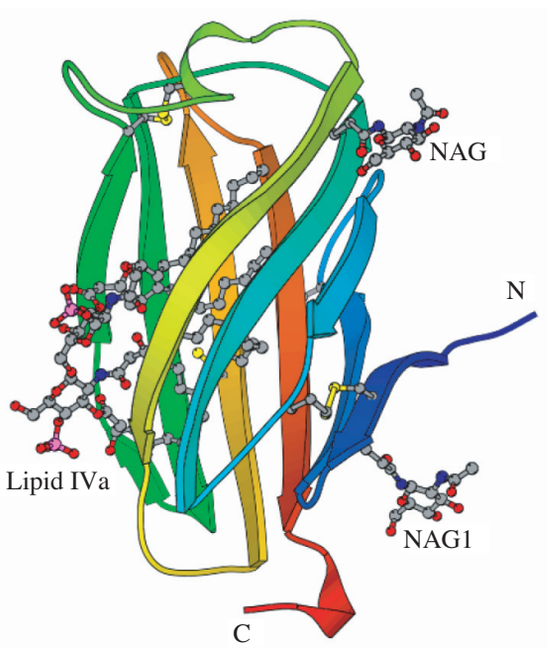

Fig. 1. Ribbon model of human MD-2 in complex with lipid IVa. The lipid IVa moiety is drawn as a ball-and-stick model: O atoms in red, N in blue, C in gray, and P in pink.

the entrance indicate that the entrance is positively charged and the inside of the cavity is highly hydrophobic (Fig. 3). Lysine and arginine residues located in the vicinities of the entrance mainly contribute to the attraction of negatively charged lipid IVa. None of the phosphate groups of lipid IVa are involved in direct hydrogen bonds to MD-2 atoms. Four fatty-acid chains are all deeply confined in the cavity and are packed next to each other through van der Waals contacts.

The complexed structure that confines most of lipid IVa suggests that MD-2 plays a principal role in recognizing LPS. Moreover, it provides a basis for structure-based development of antiseptic drugs that might be effective in preventing endotoxin shock.

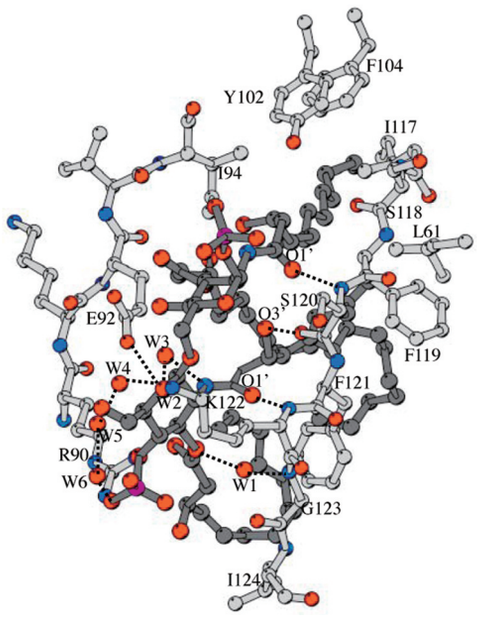

Fig. 2. Close-up view of the binding interface to lipid IVa. Amino acid residues are drawn as ball-and-stick models and the lipid IVa moiety is similarly drawn in darker gray. Water O atoms involved in hydrogen bonds are also depicted.

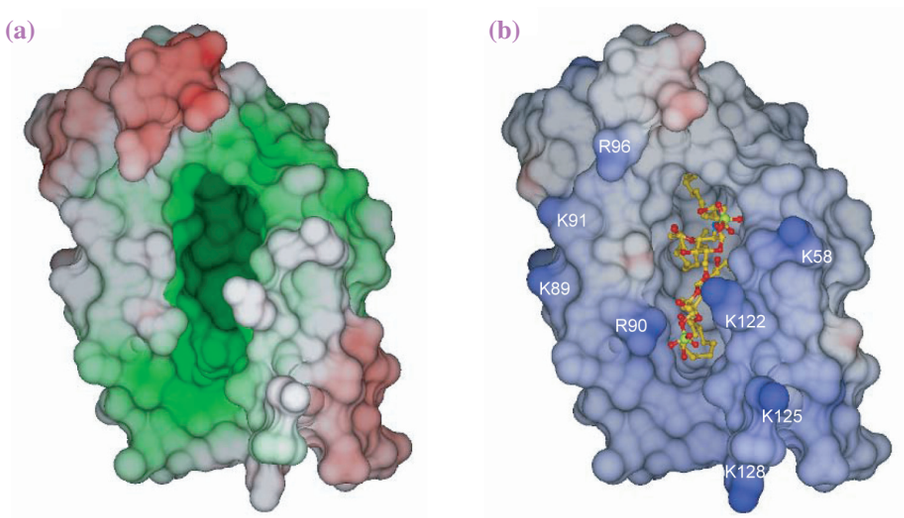

Fig. 3. Binding pocket and surface properties of MD-2. **(a)** Protein surface representing hydrophobicity (green) and hydrophilicity (red), and their extents are indicated by color darkness. **(b)** Electrostatic potential surface. Positive and negative potentials are shown in blue and red, respectively. Bound lipid IVa is drawn as a ball-and-stick representation.

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