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Vizantin Inhibits Endotoxin-Mediated Immune Responses via the TLR 4/MD-2 Complex

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Vizantin has immunostimulating properties and anticancer activity. In this study, we investigated the molecular mechanism of immune activation by vizantin. THP-1 cells treated with small interfering RNA for TLR-4 abolished vizantin-induced macrophage activation processes such as chemokine release. In addition, compared with wild-type mice, the release of MIP-1β induced by vizantin in vivo was significantly decreased in TLR-4 knockout mice, but not in TLR-2 knockout mice. Vizantin induced the release of IL-8 when HEK293T cells were transiently cotransfected with TLR-4 and MD-2, but not when they were transfected with TLR-4 or MD-2 alone or with TLR-2 or TLR-2/MD-2. A dipyrromethene boron difluoride–conjugated vizantin colocalized with TLR-4/MD-2, but not with TLR-4 or MD-2 alone. A pull-down assay with vizantin-coated magnetic beads showed that vizantin bound to TLR-4/MD-2 in extracts from HEK293T cells expressing both TLR-4 and MD-2. Furthermore, vizantin blocked the LPS-induced release of TNF-α and IL-1β and inhibited death in mice. We also performed in silico docking simulation analysis of vizantin and MD-2 based on the structure of MD-2 complexed with the LPS antagonist E5564; the results suggested that vizantin could bind to the active pocket of MD-2. Our observations show that vizantin specifically binds to the TLR-4/MD-2 complex and that the vizantin receptor is identical to the LPS receptor. We conclude that vizantin could be an effective adjuvant and a therapeutic agent in the treatment of infectious diseases and the endotoxin shock caused by LPS. The Journal of Immunology, 2014, 193: 4507–4514.

During the past 40 years, compounds extracted from the cell wall of Mycobacterium tuberculosis have been shown to have immunotherapeutic activity in patients with malignancy (1–4). Several groups have reported that trehalose-6,6'-dimycobylate (TDM; known as cord factor), a major constituent of the outer surface membrane of Mycobacterium tuberculosis (5, 6), boosts the potency of these immune responses (7, 8). Furthermore, it was revealed that TDM possesses potent antitumor and anti-infectious disease activities in various in vivo models (9–11). However, TDM is unsuitable for human use because it overactivates the immune response in mice, leading to serious symptoms such as septic shock (12, 13). We are interested in the development of nontoxic immunomodulated compounds (adjuvants), and recently developed vizantin as a safe immune-stimulating compound through a structure-activity relationship study with (2R, 3R)-trehalose-6,6'-dicorynomycolate (TDCM), which is a shorter carbon chain analog of TDM (14, 15).

Recently, adjuvant research has been invigorated by the discovery of pattern recognition receptors for microbial ligands (16–19). TLRs are the most characterized family of pattern recognition receptors and recognize a wide spectrum of pathogen-associated molecular patterns (20–22). Among the microbial pathogen–associated molecular patterns is LPS, a major virulent component of Gram-negative bacterial cell walls, which elicits a potent immune response through TLR-4 (23, 24). TLR-4 is the trans-membrane part and signal transmitter of the LPS receptor (27–29). LPS in the bloodstream binds to the LPS-binding protein, and the complex then binds to CD14 (30). Subsequently, LPS is transferred to the TLR-4/MD-2 complex (31). MD-2 is a soluble protein that interacts with the extracellular domain of TLR-4 (24, 32). When LPS is bound to this complex, TLR-4 oligomerizes and triggers the downstream signaling cascade (33, 34).

In a series of studies on the relationship between the molecular structure and biological activity of TDCM derivatives, the types of fatty acid and sugar moieties present in synthetic TDCM derivatives were shown to affect the toxicity of these compounds (8, 14, 35).
In our previous study, we reported that the TDCM derivative vizantin is an effective adjuvant, has antimetastatic effects, and is much less toxic than TDCM (14, 15).

In this present study, we investigated the relationship between vizantin, TLRs, and its adaptor proteins, and found that vizantin specifically binds to the TLR-4/MD-2 complex. This finding will contribute to the development of safe adjuvant and anti–septic shock therapies targeting TLR-4/MD-2.

Materials and Methods

Reagents and mice

Vizantin, fluorescence-labeled vizantin (fluorescence-vizantin), and magnetic Fc bead–conjugated vizantin (beads-vizantin) were synthesized according to the reported procedure (14, 15, 36). TLR-2, TLR-4, and MyD88−/− mice were purchased from Oriental BioService (Tokyo, Japan). C57BL/6 mice (wild-type) were purchased from Nihon SLC (Shizuoka, Japan). The experimental protocols were approved by the Institute Animal Care and Use Committee and the Institutional Biosafety Committee at Tokushima Bunri University (Tokushima, Japan). The mice were housed in plastic cages under controlled environmental conditions (temperature 22°C, humidity 55%). Food and water were freely available. Vizantin and other compounds were solubilized in DMSO (for in vitro experiments) or 5% BSA (for in vivo experiments). Ultrapure Escherichia coli LPS (serotype O111: B4) was purchased from InvivoGen (San Diego, CA).

Human WBCs

Heparinized blood (10 ml) was obtained from healthy donors. The blood was layered times PolymorPrep (Alere Technologies AS). After centrifugation at 450 × g for 30 min, layers containing RBCs and WBCs were collected. Residual RBCs were lysed by hypotonic shock; then the cells were suspended in RPMI 1640. Cell viability was monitored using the Trypan blue exclusion technique, and the cells were counted in a hemocytometer.

ELISA

THP-1 cells (2 × 10⁵ cells/ml) were incubated with 100 μM vizantin for 3 h at 37°C; mice were administered vizantin i.v. (5.0 mg/kg); and macrophages were incubated with 100 μM vizantin at 37°C for the times indicated in the figures. The concentrations of cytokines and chemokines were determined by ELISA kits (R&D Systems, Minneapolis, MN).

Small interfering RNA assays

The small interfering RNAs (siRNAs) were purchased from QIAGEN. THP-1 cells were transfected with siRNA (100 nM) for TLR-1, TLR-2, TLR-4, TLR-6, TLR-10, MD-2, CD14, or a negative control siRNA via electroporation by a standard protocol. Transfection efficiency was assessed by RT-PCR using specific primer pairs (Takara, Japan). After transfection by RT-PCR using specific primer pairs (Takara, Japan). After transfection by RT-PCR using specific primer pairs (Takara, Japan). After transfection by RT-PCR using specific primer pairs (Takara, Japan). After transfection by RT-PCR using specific primer pairs (Takara, Japan). After transfection by RT-PCR using specific primer pairs (Takara, Japan). After transfection by RT-PCR using specific primer pairs (Takara, Japan). After transfection by RT-PCR using specific primer pairs (Takara, Japan). After transfection by RT-PCR using specific primer pairs (Takara, Japan).

Culture of mouse macrophages

To elicit peritoneal macrophages, mice were injected with 3–4 ml sterile 3% thioglycolate, and cells were harvested after 5 d by flushing the peritoneal cavity with 10 ml ice-cold PBS four times. Isolated cells were then subjected to density gradient centrifugation (Histopaque 1.083) to remove dead cells and RBC contamination. Subsequently, the cells were washed three times with PBS, resuspended at a concentration of 10⁶/ml in phenol red–free RPMI 1640 medium (Wako Pure Chemical Industries, Japan) supplemented with 5% FBS (BioWest, Kansas City, MO).

Transfection of plasmids into HEK293T cells

HEK293T cells were transfected with pUNO plasmids containing various genes via electroporation. The pUNO plasmids containing TLR-2, TLR-4, and MD-2 were purchased from Life Technologies (Grand Island, NY). The expression of these proteins was confirmed by Western blotting and immunofluorescence staining using anti–TLR-2 (#2229; Cell Signaling Technology, Beverly, MA), anti–TLR-4 (48-2300; Life Technologies), and anti–MD-2 (ab24182; Abcam, Cambridge, MA) Abs.

Western blotting

HEK293T cells transfected with various plasmids were heated in 2% SDS sample buffer at 99°C for 3 min. The samples were separated by SDS-PAGE and then transferred to polyvinylidene difluoride membranes. The membranes were blocked with TBS buffer (20 mM Tris-HCl [pH 7.5], 0.9% NaCl) containing 2% Tween 20 and 5% BSA. The membranes were incubated first with primary Ab in TBS containing 1% BSA, and then with an HRP-conjugated secondary Ab. Proteins were detected using reagents from an ECL analysis kit (Immobilon Western Chemiluminescent HRP Substrate; Millipore, Billerica, MA).

Immunochemistry and confocal microscopy analysis

HEK293T cells were seeded on a poly-t-lysine glass-bottom dish (MatTek, Ashland, MA). The cells were then treated with fluorescence-vizantin at 37°C for 30 min, and fixed with 4% paraformaldehyde in PBS at room temperature for 15 min, followed by three washes with PBS. For Ab labeling, the dishes were then incubated at room temperature for 20 min in 50 mM NH4Cl in PBS containing 0.1% Triton X-100. After washing with PBS containing 0.02% Triton X-100, the dishes were incubated at room temperature for 1 h with PBS containing 4% BSA, followed by incubation with primary Ab (rabbit) in PBS containing 4% BSA at room temperature for 1 h. The washed dishes were then incubated with Alexa Fluor 546–conjugated anti-rabbit IgG in PBS containing 4% BSA at room temperature for 1 h. After washing with PBS containing 0.02% Triton X-100, the dishes were analyzed on a confocal microscope (A1R; Nikon, Japan). All images represent a single section through the focal plane.

Pull-down assay

HEK293T cells expressing both TLR-4 and MD-2 were lysed with lysis buffer (0.2 M Tris-HCl [pH 7.5], 40 μM MgCl₂, 2.5 mg/ml sodium deoxycholate, 50 mM EDTA, 150 mM NaCl). The extracts were incubated with control beads or beads-vizantin at 4°C for 90 min. The beads were washed with lysis buffer and then subjected to SDS-PAGE and Western blotting using anti-TLR-4 and anti-MD-2 Abs.

Binding of Alexa Fluor 488–labeled LPS

THP-1 cells were seeded on a poly-t-lysine glass-bottom dish (MatTek). The cells were then incubated with Alexa Fluor 488–labeled LPS (Invitrogen, Carlsbad, CA) in PBS at room temperature for 60 min, and then washed three times with PBS. The fluorescence level of Alexa Fluor 488–labeled LPS in each cell was analyzed by fluorescence microscopy (BIOREVO BZ-9000; Keyence, Japan) and the associated analysis software package (BZ-H2A).

In silico simulation of vizantin docking to the cavity of MD-2

Molecular modeling studies were performed using Molecular Operating Environment software (MOE, Chemical Computing Group, Montreal, Canada). The x-ray crystallographic structure of the cavity of MD-2 (PDB ID: Z265) was used in the models. The MD-2 structure was prepared for the docking studies as follows. The ligand (ES5664) was removed from the cavity of MD-2, and hydrogen atoms were then added to the structure with a standard geometry. Next, the structure was minimized using an MMFF94s force-field. Finally, MOE a Site Finder was used to identify active sites within the structure of MD-2, and dummy atoms were created from the obtained a spheres. The model was then evaluated using the ASE Dock program (Ryoko Systems, Tokyo, Japan).

Statistical analysis

Most of the presented data are expressed as the mean ± SEM. Mean values among experimental groups were compared using the Student t test, and p < 0.01 was considered statistically significant.

Results

Vizantin induces the release of cytokines and chemokines via TLR-4

We reported that vizantin (Fig. 1A) activates H₂O₂ production and phagocytosis of mouse peritoneal macrophages (14). To investigate the mechanism of vizantin-induced macrophage activation, we analyzed the release of cytokines (IL-12, IL-6, TNF-α, and IL-1β) and chemokines (MIP-1β, MCP-1, and IL-8) from human acute monocytic leukemia (THP-1) cells treated with 100 μM vizantin. Vizantin significantly induced the release of IL-6, MIP-1β, MCP-1, and IL-8, but did not induce the release of IL-12, IL-1β, and TNF-α (Fig. 1B). The effect of highly concentrated vizantin at >200 μM was not confirmed, because the
The compound did not dissolve under the experimental conditions used. TLR-1, TLR-2, TLR-4, TLR-6, and TLR-10 are known to be receptors for glycolipids, glycoproteins, or lipoproteins (20). Therefore, to investigate whether TLRs were involved in the vizantin-induced release of chemokines, we analyzed the effects of siRNA targeted to the various TLRs on the release of MIP-1β from cells treated with vizantin. As shown in Fig. 1C, the release of MIP-1β induced by vizantin significantly decreased in the TLR-4 or MD-2 knockdown cells, but not in the other knockdown cells.

**The release of MIP-1β induced by vizantin is decreased in TLR-4-/- mice**

To assess the role of TLR-4 in mediating signals from vizantin, peritoneal macrophages were isolated from wild-type and gene-disrupted (TLR-4-/-, TLR-2-/-, or MyD88-/-) mice. The cells were then stimulated with vizantin, and MIP-1β levels were measured. In TLR-4-/- mice and MyD88-/- mice, the release of MIP-1β induced by vizantin was significantly lower than in wild-type mice (Fig. 2A). In contrast, the release of MIP-1β from the macrophages isolated from TLR-2-/- mice was similar to wild-type levels (Fig. 2A). Furthermore, compared with the wild-type mice, the release of MIP-1β induced by vizantin in vivo was significantly decreased in the TLR-4-/- and MyD88-/- mice, but not in the TLR-2-/- mice (Fig. 2B).

**Vizantin induces the release of IL-8 in HEK293T cells expressing TLR-4 and MD-2**

To observe the involvement of TLR-4 in vizantin signaling, expression plasmids for human TLR-2, TLR-4, and MD-2 were transiently transfected into HEK293T cells, which do not express detectable amounts of endogenous TLR-2, TLR-4, or MD-2. At 48 h after transfection, the expression of the MD-2, TLR-2, and TLR-4 proteins was confirmed by Western blotting. Vizantin treatment of the cells cotransfected with TLR-4 and MD-2 resulted in a remarkable release of IL-8, whereas treatment of the cells...
cotransfected with MD-2, TLR-2, or TLR-4 alone resulted in little IL-8 release (Fig. 3A). The release of TNF-α was not observed under these experimental conditions (Fig. 3B).

Vizantin colocalizes with the TLR-4/MD-2 complex
We next examined whether vizantin colocalizes with TLR-4/MD-2 on the cell surface. A dipyrromethene boron difluoride–conjugated vizantin was synthesized on the basis of a structure–activity relationship study of vizantin (15). The green fluorescent–labeled derivative was named fluorescence-vizantin (Fig. 4A). The level of MIP-1β released from THP-1 cells treated with fluorescence-vizantin was similar to that of cells treated with vizantin (Fig. 4B). HEK293T cells transfected with MD-2, TLR-2, TLR-4, TLR-2/MD-2, or TLR-4/MD-2 were exposed to the fluorescence-vizantin, and the localization of each protein and fluorescence-vizantin was observed via immunofluorescence staining and confocal microscopy. As shown in Fig. 4C, fluorescence-vizantin colocalized with TLR-4/MD-2, but not with TLR-4 or MD-2 alone or with TLR-2 or TLR-2/MD-2. The colocalization of fluorescence-vizantin and TLR-4/MD-2 corresponded to ~70% of total green fluorescence (Fig. 4D).

Vizantin binds to TLR-4/MD-2 in HEK293T cells
To investigate the interaction between vizantin and the TLR-4/MD-2 complex, a pull-down assay was performed using vizantin-coated magnetic beads (beads-vizantin, Fig. 5A). The vizantin-conjugated...
beads were incubated with extracts from HEK293T cells transfected with plasmids for TLR-4 and MD-2. The beads-vizantin-bound proteins were separated by SDS-PAGE and identified by Western blotting with anti-TLR-4 and anti-MD-2 Abs. As shown in Fig. 5B, TLR-4 and MD-2 were detected in the cell extracts treated with beads-vizantin, but not in those treated with the control beads.

Vizantin inhibits the release of inflammatory cytokines and death induced by LPS

Various compounds that inhibit the inflammatory response induced by LPS have been reported (37–43). We therefore examined the effect of vizantin on the release of inflammatory cytokines (TNF-α and IL-1β) from THP-1 cells and human WBCs treated with ultrapure E. coli LPS, which is extracted by successive enzymatic hydrolysis steps and purified by the phenol-TEA-DOC extraction protocol. Vizantin inhibited the release of TNF-α and IL-1β from LPS-treated THP-1 cells and human WBCs (Fig. 6A, 6B) and the binding of Alexa Fluor 488–labeled LPS to THP-1 cells in a dose-dependent manner (Fig. 6C). The IC₅₀ of vizantin was determined to be 53–108 nM in THP-1 cells. It thus appears that vizantin is one of the most potent TLR-4/MD-2 antagonists identified to date (19, 37–43), suggesting that vizantin is a highly active LPS antagonist.

![Chemical structure of control beads and beads-vizantin.](Image)

**FIGURE 5.** Pull down assay with vizantin-conjugated magnetic beads. (A) Chemical structure of control beads and beads-vizantin. The gray circles represent magnetic beads. (B) Western blot showing TLR-4/MD-2 pulled down by the beads-vizantin. HEK293T cells transfected with plasmids for TLR-4 and MD-2 were lysed, and the extracts were incubated with control beads or beads-vizantin. The beads were subjected to SDS-PAGE and western blotting using anti-TLR-4 and anti-MD-2 Abs.

We next investigated whether vizantin might protect mice from LPS-induced endotoxin shock. A group of mice were injected i.p. with 20 mg/kg LPS. In this setting, which better reflects the complexity of endotoxin shock, mice developed ruffled fur, diarrhea, ataxia, hunched posture, and lethargy, and death occurred between 24 and 48 h after injection. Mice that were i.p. administered vizantin generally showed only mild signs of endotoxia and were protected from death in a dose-dependent manner. The survival rates at 72 h after the administration of LPS in mice treated with 5 mg/kg and 20 mg/kg vizantin were ~40% and 70%, respectively (Fig. 6D).

Vizantin docks within the cavity of MD-2

E5564 is a known LPS antagonist and specifically binds to MD-2 (17, 44). To confirm the mode of vizantin binding to the cavity of MD-2, we conducted a tertiary structure analysis using MOE software simulation based on the structure of the E5564/MD-2 complex. Our results showed that vizantin fit almost perfectly into the cavity of MD-2 in a manner that was similar to E5564 binding (Fig. 7A, 7B). In this model, the trehalose backbone of vizantin is fully exposed to solvent, and the docking energy of vizantin is −36.3 kcal/mol.

**Discussion**

We previously reported that vizantin can activate macrophages and inhibit lung metastasis of melanoma cells (14, 15). In the current study, we revealed that vizantin-induced macrophage activation occurs via specific binding of vizantin to the TLR-4/MD-2 complex.

It has been reported that TDM, which is the lead compound of vizantin, interacts with the TLR-2/CD14 complex (45). In the current study, several lines of evidence indicated that vizantin induces signal transduction through the TLR-4/MD-2 complex: siRNA for TLR-4 inhibited the release of MIP-1β from THP-1 cells treated with vizantin; the response of TLR-4−/− mice to vizantin was lower than that of wild-type mice; and vizantin-induced MIP-1β release was inhibited in MD-2 knockdown cells, but not in CD14 knockdown cells (Fig. 1D). We also observed that vizantin-induced signal transduction via TLR-4/MD-2 was dependent on MyD88 (Fig. 2A, 2B). LPS signaling via TLR-4/MD-2 is divided into a CD14-dependent pathway, which uses the adaptors TRAM and TRIF to activate IRF-3, and a CD14-independent pathway, which uses the adaptors Mal and MyD88 to activate NF-κB (46, 47). Our results suggest that the release of MIP-1β by vizantin involves a CD14-independent and MyD88-dependent response.

To determine whether vizantin interacts directly with TLR-4/MD-2, we constructed fluorescence-vizantin and beads-vizantin using click chemistry (14, 15), and found that they colocalized and interacted, respectively, with TLR-4/MD-2. These results suggest that vizantin binds to the TLR-4/MD-2 complex. This finding was bolstered by the results of docking simulation analysis; vizantin was shown to fit into the cavity of MD-2, which harbors the binding pocket for glycolipids such as LPS and E5564. Together, our results suggest that vizantin binds to MD-2, and that this is followed by TLR-4 activation and the release of MIP-1β and IL-8 through signal transduction that depends on MyD88, but not CD14.

TLR-4 and MD-2 are essential for the recognition of LPS, which is an outer membrane glycolipid of Gram-negative bacteria and a well-known inducer of the innate immune response (23, 24, 32, 48, 49). We found that vizantin competitively inhibited the binding of ultrapure E. coli LPS to cells and the release of inflammatory cytokines (Fig. 6). In addition, vizantin inhibited the...
lethality induced by LPS in a dose-dependent manner. These observations strongly support the finding that vizantin binds to the TLR-4/MD-2 complex. Artner et al. (50) reported that replacement of the flexible three-bond (1→6)-linked backbone of lipid A by the conformationally confined (1→1) glycosidically connected diglucosamine scaffold (trehalose type) resulted in abrogation of species-specific agonistic activity of lipid IVa. Vizantin is the trehalose-based glycolipid. It is therefore suggested that vizantin represents the potential inhibition of MD-2/TLR4 for human and mouse. One of the reasons for the rapid deactivation of the failed antisepsis drug candidate E5564 was hydrolysis of the anomeric phosphate functionality, leading to an inactive metabolite (51, 52).

**FIGURE 6.** Effect of vizantin on TNF-α production and death in mice treated with LPS. THP-1 cells (A) and human WBCs (B) were incubated with LPS and the indicated concentration of vizantin for 3 h, respectively. (C) THP-1 cells were incubated with Alexa Fluor 488-labeled LPS and the indicated concentration of vizantin for 1 h. The release of inflammatory cytokines (TNF-α and IL-1β) and the binding of fluorescent LPS were assayed as described in Materials and Methods. Values represent mean ± SEM; n = 5; *p < 0.01, **p < 0.005 compared with cells treated with LPS plus vehicle. (D) C57BL/6 mice were injected i.p. with 20 mg/kg of ultrapure E. coli O111:B4 LPS alone (10 mice, △) or in combination with 5.0 mg/kg (○) or 20 mg/kg (●) vizantin (10 mice). The p values calculated by the Kaplan–Meier method are indicated.

**FIGURE 7.** Docking simulation analysis of vizantin and MD-2. (A) The docking model of vizantin and MD-2 was constructed using MOE software. The most stable structure of vizantin on the molecular electrostatic potential surface is shown. The red and blue surfaces indicate the negative and positive electrostatic potential surfaces, respectively. The green, red, orange, and purple in the compounds indicate carbon, oxygen, phosphate, and nitrogen, respectively. (B) The three-dimensional structure of the complex of E5564 and MD-2 is shown.
Because vizantin has no phosphate group, it appears that vizantin is stable in vivo.

The cell wall skeleton of *Mycobacterium bovis* bacillus Calmette-Guérin was reported to enhance the cytotoxic activity of T cells and macrophages against cancer cells (53), probably through TLR-4 (54). Bacillus Calmette-Guérin cell wall skeleton induced TNF-α secretion from dendritic cells through TLR-2 and TLR-4 signaling and thus induced the maturation of dendritic cells (53, 55). Moreover, OK-432, a penicillin-killed and lyophilized preparation of *Streptococcus pyogenes*, exhibited potent immunotherapeutic effects against cancer (56). The findings of this study expand the range of potential uses of vizantin, and suggest that it might be a safe clinical therapeutic agent for the treatment of endotoxemia, including sepsis-like and septic shock. The findings of this study expand the range of potential uses of vizantin, and suggest that it might be a safe clinical therapeutic agent for the treatment of endotoxemia, including sepsis-like and septic shock.

Vizantin competes with LPS suggests that it might be a safe clinical therapeutic agent for the treatment of endotoxemia, including sepsis-like and septic shock. The findings of this study expand the range of potential uses of vizantin, and suggest that it might be a safe clinical therapeutic agent for the treatment of endotoxemia, including sepsis-like and septic shock.

MD-2/TLR4.

Findings suggest that vizantin is an inhibitor of human and mouse inflammatory cytokines (TNF-α and IL-1β) induced by LPS. These findings suggest that vizantin is an inhibitor of human and mouse MD-2/TLR4.

The data presented in the current study identify TLR-4/MD-2 as炎性 cytokines (TNF-α and IL-1β) induced by LPS. These findings suggest that TLR-4 activators such as vizantin induce antitumor immunity through TLR-4 signaling.

As is shown in Figs. 1 and 6, high concentrations of vizantin (μM order) induced the release of MIP-1β and IL-8, whereas low concentrations of vizantin (nM order) inhibited the release of inflammatory cytokines (TNF-α and IL-1β) induced by LPS. These findings suggest that vizantin is an inhibitor of human and mouse MD-2/TLR4.

The data presented in the current study identify TLR-4/MD-2 as a pivotal receptor for vizantin. Furthermore, the observation that vizantin competes with LPS suggests that it might be a safe clinical therapeutic agent for the treatment of endotoxemia, including sepsis-like and septic shock. The findings of this study expand the range of potential uses of vizantin, and suggest that it might complement the current treatment options for inflammatory diseases and cancer.

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Disclosures

The authors have no financial conflicts of interest.

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