

Moesin Binds to Bacterial Lipopolysaccharide

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Abstract. Previous work has demonstrated a role for moesin (member of the ERM family proteins) in lipopolysaccharide mediated events; however, no physical binding to lipopolysaccharide has been reported. Therefore, the aim of this experiment was to study the binding of moesin to lipopolysaccharide. Moesin and radixin recombinant proteins, C- and N-terminus truncated proteins were used. Native gel shift assays were performed using the recombinant proteins and purified *E. coli* lipopolysaccharide strain O55:B5. The results of the native Gel Shift assay showed that there was direct binding of moesin to lipopolysaccharide. This binding was only evident with the moesin C-terminus recombinant protein. This finding suggests further the important role of moesin in the initial recognition of lipopolysaccharide.

Keywords: Moesin, Lipopolysaccharide, Inflammation, Signal transduction.

Introduction

The innate immune response is the first line of defense against microbial pathogens^[1]. The principal tasks of the innate immune system are to recognize pathogens, mount an immediate defense response, and activate adaptive immune responses. Lipopolysaccharide (LPS) is the principal component of the outer membrane of Gram-negative bacteria. Monocytes/macrophages orchestrate the innate immune response to LPS

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by expressing a variety of inflammatory cytokines that includes tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β)^[2-4]. Production of these inflammatory cytokines contributes to the efficient control of growth and dissemination of invading pathogens. However, excessive and uncontrolled production of these and other inflammatory cytokines may lead to serious systemic complications; such as microcirculatory dysfunction, disseminated intravascular coagulation, multiple organ failure and septic shock^[5-7].

LPS recognition is initiated by LPS binding protein (LBP), a serum glycoprotein, that first binds to the lipid A moiety of LPS^[8-10]. The LPS-LBP complex is then recognized by CD14^[7,9,11]. Mice with a targeted deletion of the gene encoding CD14 are hypo-responsive to LPS and resistant to the lethal effects of LPS^[11]. However, mice lacking CD14 are still able to respond to high concentrations of LPS^[12]. CD14 is a glycosylphosphatidylinositol-anchor (GPI-anchored) molecule which lacks a cytoplasmic signaling domain, making it incapable of downstream signaling^[13]. Members of a family of proteins, the mammalian homologues of the *Drosophila* Toll protein, identified as Toll-like receptor (TLR) proteins, have been found to mediate the response to LPS^[14]. The TLR proteins possess leucine-rich extracellular repeats that recognize the LBP-CD14 complex^[15]. Considerable progress has been made in identifying the LPS recognition molecules, particularly CD14^[9], TLR4^[16,17] and MD-2^[18].

Previously moesin (Membrane Organizing Extension Spike Protein) was identified as a participant in LPS binding and as a signal transduction^[19]. Moesin was found to be necessary for the detection of LPS, and homozygous moesin knockout mice; exhibited a 3-fold reduction in neutrophil infiltration into LPS injected sites when compared to wild type controls^[20]. Anti-moesin antibody inhibited the release of TNF- α by LPS stimulated monocytes^[19]. Moesin was also found to be expressed on the surface of differentiated human promonocytic cell line, THP-1 cells and primary peripheral blood monocytes. LPS stimulation increased the surface expression of moesin as well as its total protein levels when analyzed by FACS and western blotting, respectively. Furthermore, moesin was found to co-immunoprecipitate with TLR4 after LPS stimulation^[21]. In moesin mRNA knockdown experiments using antisense mRNA, THP-1 cells no longer responded to LPS^[21]; suggesting a role for moesin in LPS signaling. In a recent investigation,

moesin mRNA expression was increased in the tissues of liver, kidney and spleen in mice treated with LPS^[22].

However, no report shows if moesin directly binds to LPS. Therefore, in this study, direct binding of LPS to moesin was studied.

Materials and Methods

Reagents / Materials

Moesin and radixin recombinant proteins, C- and N- terminus truncated proteins were purchased from ProMab Biotechnologies, Inc. (Albany, CA). *E. coli* LPS (strain O55:B5) was purchased from Sigma (St. Louis, MO). Protein assay reagents and silver staining kits were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA). Unless, otherwise specified, all other reagents were purchased from Sigma (St. Louis, MO).

Native PAGE Gel Shift Assay for Moesin and LPS

Gel shift assays were performed as previously described^[23]. Briefly, recombinant proteins (30 µg/ml) for moesin and radixin (N- and C- terminal) were incubated with sonicated LPS (0-500µg/ml) in PBS lacking Ca²⁺ and Mg²⁺ with 1mM EDTA at 37°C for 30 min. Glycerol and bromophenol blue were added, and the mixture was electrophoresed using 4-20% nondenaturing PAGE at 100-150 V for 2 h in a running buffer containing 192 mM glycine; 24 mM Tris; pH 8.3, without detergents. Silver staining was performed according to the manufacturer instructions (Bio-Rad Laboratories, Inc., Hercules, CA).

Results

Native PAGE gel shift assay was used to examine the binding of moesin to LPS. Two recombinant fragments of both proteins were examined, C-moesin and N-moesin. Recombinant radixin protein (C- and N- terminus) was used as control. The experiment was run in triplicates to confirm the finding. When LPS was incubated with C-moesin protein, there was retardation in the gel (Fig. 1A). This shift in the protein band suggests a stable binding of LPS to the recombinant C-moesin. No shift was observed when LPS was incubated with N-moesin (Fig. 1B) or with C- or N-radixin (Fig. 1C and D).

Discussion

In this study, it was demonstrated that moesin physically binds to LPS. The binding of LPS to moesin was localized to the carboxyl, half of the moesin recombinant protein using a native PAGE gel shift assay (Fig. 1 A).

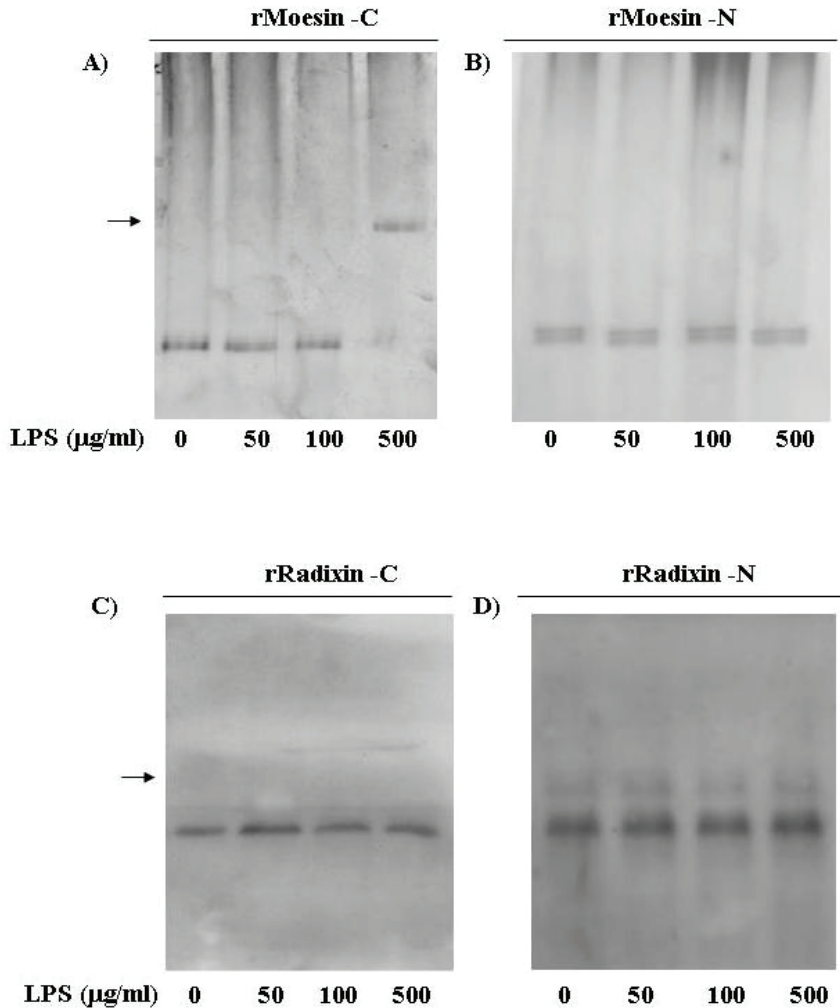


Fig. 1. Native PAGE Gel Shift analysis of moesin – LPS Complex: Carboxyl and amine halves of recombinant moesin and radixin (30 µg) were incubated in the absence and presence of increasing LPS concentrations at 37°C for 30 minutes. A shift in the electrophoresis mobility was only detected when C-moesin was incubated with LPS. No shift was observed for N-moesin or for either C- or N-radixin.

A current understanding of the innate recognition of bacterial LPS is based on the discovery that LPS binds to LPS-Binding Protein (LBP) in serum^[9], which in turn, rapidly catalyzes the transfer of LPS to membrane-bound CD14 (mCD14) or soluble CD14 (sCD14). Although CD14 has been identified as an LPS receptor^[9], it is a GPI anchor protein and therefore, lacks transmembrane and intracellular domains^[13]. Several binding studies have shown that CD14-blocking monoclonal antibodies only partially inhibit LPS-binding, suggesting the existence of alternative receptors^[24-26]. Thus, additional transmembrane receptors must act in concert with the LPS-CD14 complex to initiate the signaling process leading to LPS-induced cellular activation. Using mostly genetic approaches, the human counterpart of *Drosophila* toll receptors reveals that TLR4 is the main toll-like molecule involved in LPS signaling in mice and humans^[27-29]. This was also confirmed by identifying, via positional cloning, the LPS gene as responsible for the failure of LPS to induce pro-inflammatory responses in C3H/HeJ and C57BL/10ScCr mouse strains^[17,30].

Shimazu *et al.* (1999) showed that TLR4 requires an additional molecule, MD-2, which forms a complex with the extracellular domain of TLR4, for effective LPS recognition^[18]. Therefore, the association of MD-2 with TLR4 is critical for LPS responses^[31-32]. CD14 enhances the formation of LPS-TLR4-MD-2 complexes. Akashi *et al.*^[32] stated that their results using mechanically solubilized cell lysates clearly showed a physical association between LPS and TLR-4-MD2, but these were co-immunoprecipitation experiments and direct binding was not actually demonstrated. The only evidence suggesting that there is a direct interaction between LPS and membrane proteins was by forming a membrane receptor complex in transfected cells; demonstrating an interaction between TLR, MD-2, and CD14 (alone or various combinations) with crosslinking to LPS^[33].

In these studies, LPS cross-linked to TLR4 and MD-2 only when co-expressed with CD14. This report showed for the first time the direct binding of LPS to moesin, using a gel shift assay and localizes the binding site to the N-terminus of the moesin molecule.

Previously, several moesin blocking antibodies were employed revealing that TNF- α production was abrogated by anti-moesin antibody at all LPS concentrations tested^[19]. In addition, the inhibition was

specific for the LPS-moesin interaction, since events mediated by unrelated receptors (fMLP and IL-1) were not inhibited by anti-moesin antibody. The response to the gram-positive bacterium *S. aureus* was also not altered by moesin antibody, confirming that the effects were unique to LPS elicited responses^[19]. It was also postulated that CD14 and moesin may physically interact for optimal binding of LPS and transduction of the signal^[19]. Further characterization of cell surface expression of moesin after exposure to LPS in macrophage-like differentiated THP-1 cells revealed that stimulation with LPS resulted in a significant increase of moesin cell surface expression as well as an increase in moesin protein level^[21].

The human gene encoding moesin was cloned^[34], and its primary sequence revealed that this protein could be either integrally incorporated into the plasma membrane and/or in the cytoplasm, making it available for free movement upon activation and phosphorylation^[35-37]. Keresztes *et al.*^[38] suggested that upon adhesion of porcine neutrophils to a plastic surface, moesin is translocated to the plasma membrane and the extracellular surface. Ariel *et al.*^[39] reported that moesin could be expressed on the cell surface not only by stimulation with PHA or PMA but also by Calyculin A, a specific inhibitor of phosphatases 1 and 2A, that is capable of inducing moesin phosphorylation^[36]. At its N-terminus, moesin and other proteins from this family have so-called FERM (band four-point-one, ezrin, radixin, moesin homology domain) contain sequences of three subdomains, F1-F3^[40-41]. The cluster of acidic amino acids, especially in the PH homology domain, has been shown to be important in interactions of ERM proteins with lipid layers of the membrane^[42]. Crystallographic studies recently showed that these proteins interact and link with various lipid fractions in the membrane^[41-44].

This report is the first study to show the direct association of moesin with LPS, and have confirmed and localized the direct binding of moesin (C-terminus) to LPS using a native PAGE gel shift assay. Therefore, it appears that there is a specific LPS binding sequence in the C-terminal part of the protein. Furthermore, this observation suggests possible physical association between moesin and CD14, TLR4, and MD-2 making this protein part of the LPS receptor cluster.

Bacterial recognition systems in mammalian cells are complex. Triantafilou and Triantafilou^[45] hypothesize that different receptors are recruited to the site of ligation to form an activation cluster, which is followed by multiple signaling cascades. Thus, the existence of receptor clusters is consistent with the variety of signaling cascades that are triggered by LPS. Diversity in the receptor clusters that are responsible for bacterial recognition clarifies why anti-sepsis therapies have been unsuccessful, since therapeutic interventions target only a single component of the cluster. Therefore, therapeutic approaches may need to target multiple receptors in order to be successful.

In conclusion, the author would like to propose that the innate recognition of LPS involves the dynamic association of multiple receptors forming a cluster of molecules (Fig. 2). The association of moesin with CD14, TLR, and MD-2 remain to be elucidated. Furthermore, more studies are required to investigate the moesin LPS binding domain.

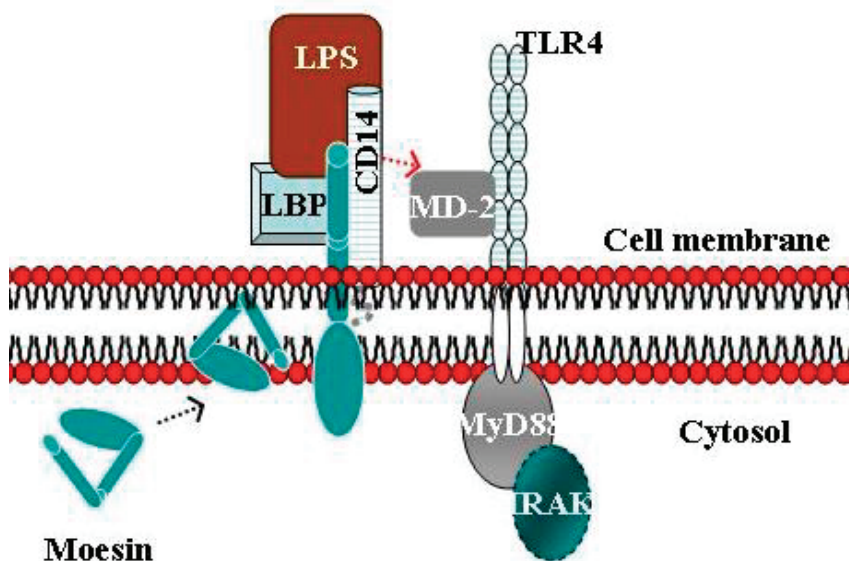


Fig. 2. Proposed Model for LPS recognition. LPS Binds to moesin and CD14. Following LPS stimulation, this cluster binds to the TLR4 and MD-2.

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References:

- [1] **Janeway CA Jr., Medzhitov R.** Innate immune recognition. *Annu Rev Immunol* 2002; **20**(1): 197-216.
- [2] **Beutler B, Krochin N, Milsark IW, Luedke C, Cerami A.** Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. *Science* 1986; **232**(4753): 977-980.
- [3] **Nathan CF.** Secretory products of macrophages. *J Clin Invest.* 1987; **79**(2): 319-326.
- [4] **Raetz CR.** Biochemistry of endotoxins. *Annu Rev Biochem.* 1990; **59**: 129-170.
- [5] **Tracey KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, Lowry SF, Cerami A.** Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature* 1987; **330**(6149): 662-664.
- [6] **Morrison DC, Ryan JL.** Endotoxins and disease mechanisms. *Annu Rev Med* 1987; **38**: 417-432.
- [7] **Ulevitch RJ, Tobias PS.** Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu Rev Immunol* 1995; **13**: 437-457.
- [8] **Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC.** CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 1990; **249**(4975): 1431-1433.
- [9] **Schumann RR, Leong SR, Flaggs GW, Gray PW, Wright SD, Mathison JC, Tobias PS, Ulevitch RJ.** Structure and function of lipopolysaccharide binding protein. *Science* 1990; **249**(4975): 1429-1431.
- [10] **Gegner JA, Ulevitch RJ, Tobias PS.** Lipopolysaccharide (LPS) signal transduction and clearance. Dual roles for LPS binding protein and membrane CD14. *J Biol Chem* 1995; **270**(10): 5320-5325.
- [11] **Haziot A, Ferrero E, Köntgen F, Hijiya N, Yamamoto S, Silver J, Stewart CL, Goyert SM.** Resistance to endotoxin shock and reduced dissemination of gram-negative bacteria in CD14-deficient mice.. *Immunity* 1996; **4**(4): 407-414.
- [12] **Wurfel MM, Monks BG, Ingalls RR, Dedrick RL, Delude R, Zhou D, Lamping N, Schumann RR, Thieringer R, Fenton MJ, Wright SD, Golenbock D.** Targeted deletion of the lipopolysaccharide (LPS)-binding protein gene leads to profound suppression of LPS responses ex vivo, whereas in vivo responses remain intact. *J Exp Med* 1997; **186**(12): 2051-2056.
- [13] **Haziot A, Chen S, Ferrero E, Low MG, Silber R, Goyert SM.** The monocyte differentiation antigen, CD14, is anchored to the cell membrane by a phosphatidylinositol linkage. *J Immuno.* 1988; **141**(2): 547-552.

- [14] **Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, Ghosh S, Janeway CA Jr.** MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol Cell* 1998; **2**(2): 253-258.
- [15] **Poltorak A, Ricciardi-Castagnoli P, Citterio S, Beutler B.** Physical contact between lipopolysaccharide and toll-like receptor 4 revealed by genetic complementation. *Proc Natl Acad Sci U S A* 2000; **97**(5): 2163-2167.
- [16] **Medzhitov R, Preston-Hurlburt P, Janeway CA, Jr.** A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* 1997; **388**(6640): 394-397.
- [17] **Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B.** Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* 1998; **282**(5396): 2085-2088.
- [18] **Shimazu R, Akashi S, Ogata H, Nagai Y, Fukudome K, Miyake K, Kimoto M.** MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J Exp Med* 1999; **189**(11): 1777-17782.
- [19] **Tohme ZN, Amar S, Van Dyke TE.** Moesin functions as a lipopolysaccharide receptor on human monocytes. *Infect Immun* 1999; **67**(7): 3215-3220.
- [20] **Amar S, Oyaisu K, Li L, Van Dyke T.** Moesin: a potential LPS receptor on human monocytes. *J Endotoxin Res* 2001; **7**(4): 281-286.
- [21] **Iontcheva I, Amar S, Zawawi KH, Kantarci A, Van Dyke TE.** Role for moesin in lipopolysaccharide-stimulated signal transduction. *Infect Immun* 2004; **72**(4): 2312-2320.
- [22] **Li J, Liu Y, Fang J, Chen X, Xie W.** Effect of Radix Isatidis on the expression of moesin mRNA induced by LPS in the tissues of mice. *J Huazhong Univ Sci Technolog Med Sci* 2007; **27**(2): 135-137.
- [23] **Hailman E, Lichenstein HS, Wurfel MM, Miller DS, Johnson DA, Kelley M, Busse LA, Zukowski MM, Wright SD.** Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. *J Exp Med* 1994; **179**(1): 269-277.
- [24] **Lynn WA, Liu Y, Golenbock DT.** Neither CD14 nor serum is absolutely necessary for activation of mononuclear phagocytes by bacterial lipopolysaccharide. *Infect Immun* 1993; **61**(10): 4452-4461.
- [25] **Troelstra A, Antal-Szalmas P, de Graaf-Miltenburg LA, Weersink AJ, Verhoef J, Van Kessel KP, Van Strijp JA.** Saturable CD14-dependent binding of fluorescein-labeled lipopolysaccharide to human monocytes. *Infect Immun* 1997; **65**(6): 2272-2277.
- [26] **Triantafilou M, Triantafilou K, Fernandez N.** Rough and smooth forms of fluorescein-labelled bacterial endotoxin exhibit CD14/LBP dependent and independent binding that is influenced by endotoxin concentration. *Eur J Biochem* 2000; **267**(8): 2218-2226.
- [27] **Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, Takeda K, Akira S.** Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the *Lps* gene product. *J Immunol* 1999; **162**(7): 3749-3752.
- [28] **Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, Frees K, Watt JL, Schwartz DA.** TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000; **25**(2): 187-191.
- [29] **Lien E, Means TK, Heine H, Yoshimura A, Kusumoto S, Fukase K, Fenton MJ, Oikawa M, Qureshi N, Monks B, Finberg RW, Ingalls RR, Golenbock DT.** Toll-like receptor 4 imparts ligand-specific recognition of bacterial lipopolysaccharide. *J Clin Invest* 2000; **105**(4): 497-504.

- [30] **Qureshi ST, Larivière L, Leveque G, Clermont S, Moore KJ, Gros P, Malo D.** Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (TLR4). *J Exp Med* 1999; **189**(4): 615-625.
- [31] **Nagai Y, Akashi S, Nagafuku M, Ogata M, Iwakura Y, Akira S, Kitamura T, Kosugi A, Kimoto M, Miyake K.** Essential role of MD-2 in LPS responsiveness and TLR4 distribution. *Nat Immunol* 2002; **3**(7): 667-672.
- [32] **Akashi S, Saitoh S, Wakabayashi Y, Kikuchi T, Takamura N, Nagai Y, Kusumoto Y, Fukase K, Kusumoto S, Adachi Y, Kosugi A, Miyake K.** Lipopolysaccharide interaction with cell surface Toll-like receptor 4-MD-2: higher affinity than that with MD-2 or CD14. *J Exp Med* 2003; **198**(7): 1035-1042.
- [33] **da Silva Correia J, Soldau K, Christen U, Tobias PS, Ulevitch RJ.** Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex. transfer from CD14 to TLR4 and MD-2. *J Biol Chem* 2001; **276**(24): 21129-21135.
- [34] **Lankes WT, Furthmayr H.** Moesin: a member of the protein 4.1-talin-ezrin family of proteins. *Proc Natl Acad Sci U S A* 1991; **88**(19): 8297-8301.
- [35] **Nakamura F, Amieva MR, Furthmayr H.** Phosphorylation of threonine 558 in the carboxyl-terminal actin-binding domain of moesin by thrombin activation of human platelets. *J Biol Chem* 1995; **270**(52): 31377-85.
- [36] **Nakamura F, Amieva MR, Hirota C, Mizuno Y, Furthmayr H.** Phosphorylation of 558T of moesin detected by site-specific antibodies in RAW264.7 macrophages. *Biochem Biophys Res Commun* 1996; **226**(3): 650-656.
- [37] **Ivetic A, Ridley AJ.** Ezrin/radixin/moesin proteins and Rho GTPase signalling in leucocytes. *Immunology* 2004; **112**(2): 165-176.
- [38] **Keresztes M, Lajtos Z, Fischer J, Dux L.** Moesin becomes linked to the plasma membrane in attached neutrophil granulocytes. *Biochem Biophys Res Commun* 1998; **252**(3): 723-727.
- [39] **Ariel A, Hershkoviz R, Altbaum-Weiss I, Ganor S, Lider O.** Cell surface-expressed moesin-like receptor regulates T cell interactions with tissue components and binds an adhesion-modulating IL-2 peptide generated by elastase. *J Immunol* 2001; **166**(5): 3052-3060.
- [40] **Pearson MA, Reczek D, Bretscher A, Karplus PA.** Structure of the ERM protein moesin reveals the FERM domain fold masked by an extended actin binding tail domain. *Cell* 2000; **101**(3): 259-270.
- [41] **Finnerty CM, Chambers D, Ingraffea J, Faber HR, Karplus PA, Bretscher A.** The EBP50-moesin interaction involves a binding site regulated by direct masking on the FERM domain. *J Cell Sci* 2004; **117**(Pt 8): 1547-1552.
- [42] **Niggli V.** Structural properties of lipid-binding sites in cytoskeletal proteins. *Trends Biochem Sci* 2001; **26**(10): 604-611.
- [43] **Simons PC, Pietromonaco SF, Reczek D, Bretscher A, Elias L.** C-terminal threonine phosphorylation activates ERM proteins to link the cell's cortical lipid bilayer to the cytoskeleton. *Biochem Biophys Res Commun* 1998; **253**(3): 561-565.
- [44] **Edwards SD, Keep NH.** The 2.7 Å crystal structure of the activated FERM domain of moesin: an analysis of structural changes on activation. *Biochemistry* 2001; **40**(24): 7061-7068.
- [45] **Triantafilou M, Triantafilou K.** Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. *Trends Immunol* 2002; **23**(6): 301-304.

ارتباط بروتين المويسين (moesin) بغلاف بكتيريا مادة ليبوبولي ساكارايد (Lipopolysaccharide)

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المستخلص. أثبتت أعمال سابقة دور المويسين في التعرف على غلاف البكتيريا السام لليبوبولي ساكارايد، ومع ذلك لم يتم حتى الآن إثبات ماهية الترابط بين لليبوبولي ساكارايد و المويسين، ولذلك كان الهدف من هذه التجربة هو دراسة إمكانية وجود ارتباط بينهما. تمت دراسة إمكانية وجود ارتباط بين بروتين راديكسين (Radixin) (مسيطر سلبي) وبروتين المويسين (Moesin) بوجود لليبوبولي ساكارايد، وقد تم إجراء التجربة باستخدام هلامية الاكريلاميد بعد احتضان كل من البروتينات مع لليبوبولي ساكارايد . أظهرت نتائج الدراسة وجود ارتباط مباشر بين بروتين المويسين في الاعتراف الأولي لليبوبولي ساكارايد.