Biochemical and Biophysical Research Communications 391 (2010) 267–271

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Molecular cloning and functional characterization of a mouse gene upregulated by lipopolysaccharide treatment reveals alternative splicing

Kejun Du [a,1], Yaoming Chen [a,1], Zongming Dai [a], Yuan Bi [a], Tongjian Cai [a], Lichao Hou [b], Yubo Chai [c], Qinghe Song [c], Sumin Chen [c], Wenjing Luo [a,*], Jingyuan Chen [a,*]

[a] Department of Occupational and Environmental Health, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, PR China
[b] Department of Anesthesiology, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, PR China
[c] Department of Biochemistry and Molecular Biology, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, PR China

ARTICLE INFO

Article history:
Received 29 October 2009
Available online 12 November 2009

Keywords:
Alternative splicing
Functional characterization
Lipopolysaccharide responsive gene
Molecular cloning

ABSTRACT

Treatment of mouse cells with lipopolysaccharide (LPS) potently initiates an inflammatory response, but the underlying mechanisms are unclear. We therefore sought to characterize cDNA sequences of a new mouse LPS-responsive gene, and to evaluate the effects of MLrg. Full-length cDNAs were obtained from LPS-treated NIH3T3 cells. We report that the MLrg gene produces two alternative splice products (GenBank Accession Nos. DQ316984 and DQ320011), respectively, encoding MLrgW and MLrgS polypeptides. Both proteins contain zinc finger and leucine zipper domains and are thus potential regulators of transcription. Expression of MLrgW and MLrgS were robustly upregulated following LPS treatment, and the proteins were localized predominantly in the nuclear membrane and cytoplasm. In stable transfectants over-expressing MLrgW the proportion of cells in G1 phase was significantly reduced, while in cells over-expressing MLrgS the proportion of cells in G2 was significantly increased; both proteins are thus potential regulators of cell cycle progression. Upregulation of MLrgW and MLrgS may be an important component of the LPS inflammatory pathway and of the host response to infection with GNB.

Introduction

Lipopolysaccharide (LPS) is a glycolipid found in the outer membrane of Gram-negative bacteria (GNB) [1]. LPS is also an effective component of endotoxin with significant impact on human health [2], and in most mammalian species LPS is a powerful activator of innate immune responses [3]. For this reason LPS administration has been widely used in animal models of chronic inflammation including bacterial sepsis [3]. LPS is a robust inducer of the synthesis and release of proinflammatory cytokines, and over-expression of these molecules can progress to septic shock *in vivo* [4]. Nevertheless, the specific receptors and pathways that respond to LPS have not been fully characterized. LPS recognition is thought to be mediated primarily by a small group of immune receptors that activate the inflammatory response [2], notably by pattern recognition receptors (PRRs) that recognize microbe-specific molecular structures or pathogen-associated molecular patterns (PAMPs). PRR binding is thought to represent the initial event in cell recognition of LPS and the induction of the inflammatory response [5]. Toll-like receptors (TLRs), CD14, Scavenger receptor, β_2-integrins, and L-selectin have also been shown to be involved in the process of LPS activation or clearance [6].

In view of the medical importance of LPS, considerable attention has been paid to the development of therapeutics that specifically disrupt LPS signaling [7,8]. Multiple complex pathways appear to be involved in downstream signaling, including MAPKs, PTKs, and NF-κB [9]. Nevertheless, the exact pathways via which LPS induces an inflammatory response have not yet been elucidated [10]. It is furthermore becoming clear that the effects of LPS are modulated by the genetic background of the host, and changes in the expression of specific genes are an important aspect of host adaptation to bacterial infection and/or LPS [11,12]. To gain further insights into the mechanisms whereby LPS is able to induce a potent inflammatory response we have sought to identify mouse genes that are upregulated by LPS. We report here on the molecular characterization of a new LPS-responsive gene, MLrg, and the properties of the encoded protein.

Abbreviations: FBS, fetal bovine serum; FCM, flow cytometry; GNB, Gram-negative bacterium; LBP, LPS binding protein; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MLrg, mouse lipopolysaccharide-responding gene; MLrgW, MLrg wild-type; MLrgS, MLrg splicing; NF-κB, nuclear factor-κB; PAMP, pathogen-associated molecular pattern; PRRs, pattern recognition receptors; SABC–FITC, streptavidin–biotin peroxidase complex–fluorescein isothiocyanate

* Corresponding authors. Fax: +86 29 84774863.
E-mail addresses: luowenj@fmmu.edu.cn (W. Luo), jy_chen@fmmu.edu.cn (J. Chen).

[1] These authors have contributed equally to this work.

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.11.047

Materials and methods

Materials. LPS (*Escherichia coli*), Hepes, and DEPC were purchased from Sigma Company (Texas, USA). Plasmids pUC19 and pcDNA3.1(+), Triazol RNA reagent, and DNA purification reagents were purchased from Invitrogen Company (California, USA). The reverse transcriptase kit was purchased from Promega Company (Madison, USA). The plasmid extraction kit (endotoxin free) was purchased from Sangon Company (Shanghai, China). Restriction endonucleases, Taq DNA polymerase, and T4 ligase were purchased from Santa Cruz Company (Santa Cruz, USA). Enhanced chemiluminescence detection system was purchased from Pierce Chemical Company (Rockford, USA). NIH3T3 and L929 cell line, BL21(DE3) cell strain, and pTAT plasmid were provided by the Biochemistry and Molecular Biology Laboratory, Fourth Military Medical University (Xi'an, China).

Cell culture. Mouse NIH3T3 cells were cultured in DMEM. Cells were cultured at 37 °C in an incubator under humidified 5% CO_2. Experimental cells were exposed to LPS (100 μg/L) for 24 h.

RT-PCR amplification of MLrg cDNA. Comparative BLAST searching for homologies between human LRG cDNA (Accession No. NM_018360 [13]) and mouse ESTs in GenBank was used to identify primers for PCR amplification of the mouse cDNA sequences. Total RNA was extracted from LPS-treated NIH3T3 cells using the TRIzol reagent. cDNA synthesis employed reverse transcriptase and oligo-(dT). Complete MLrg sequences were amplified using the upstream primer P1 (GCTGCCGTCACCTCATGG) and the downstream primer P2: (TTCACATCAAGGAACCATCG). The MLrg coding region was amplified using the upstream primer PF (GCGGGTACCGCGACTCGGC TTGAGGAGGTAACGCGA) and downstream PR (CCGCTCGAGGCC TTAAGCAGGAGAACC) containing restriction sites (underlined) for KpnI and XhoI, respectively, to facilitate cloning. PCR amplification employed 30 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 40 s, and extension at 72 °C for 40 s; a further extension step was performed at 72 °C for 30 min. PCR products were separated by electrophoresis on 1% agarose gels and stained with ethidium bromide (EB), excised from the gels, and purified and recovered using a DNA purification kit. PCR products were digested with HincII and inserted between the KpnI and XhoI sites of pTAT vectors prior to transformation into *E. coli* BL21 cells. Transformants were confirmed by DNA sequencing. The nucleotide sequence and the deduced amino acid sequence of MLrg were analyzed using software from the UCSC Genome Bioinformatics Site (http://genome.ucsc.edu); this identified two different forms, MLrgW and MLrgS.

Expression of MLrgW/MLrgS and preparation of immune serum. Plasmids pTAT-MlrgW and pTAT-MLrgS were transformed into *E. coli* BL21 cells. Gene expression was induced by IPTG and the 6His-TAT fusion proteins were purified by nickel–nitrilotriacetic acid (Ni–NTA) column chromatography and SDS–PAGE. Rabbits were immunized according to standard procedures with MLrgW and MLrgS polypeptides, boosted three times, and antisera to MLrgW and MLrgS were collected.

Localization of MlrgW and MlrgS by immunohistochemistry. L929 cells were cultured in 1640 medium. Cells were fixed with paraformaldehyde and blocked with normal goat serum. Antisera directed against bacterially-produced MLrgW and MLrgS were used to localize MLrgW and MLrgS antigens by immunohistochemistry using a peroxidase-labeled secondary antibody and staining with diaminobenzidine.

LPS-induced changes in MlrgW and MLrgS expression evaluated by Western blotting and immunohistochemistry. Mouse NIH3T3 fibroblasts and L929 fibrosarcoma cells were exposed to LPS (500 μg/L) for 24 h. Cells were lysed and protein extracts were prepared by standard techniques. Fifty micrograms aliquots was separated by SDS–PAGE and transferred to nitrocellulose membranes. Rabbit polyclonal antibodies were used to detect MLrgW and MLrgS polypeptides; blots were developed using horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG and an enhanced chemiluminescence detection system. For immunohistochemistry, NIH3T3 cells were exposed to LPS (500 μg/L) for 24 h, and fixed and blocked prior to the addition of rabbit anti-MLrgW or MLrgS polyclonal antisera, respectively. Bound antibody was detected using biotinylated goat anti-rabbit IgG, stained with streptavidin–biotin peroxidase complex (SABC), developed with FITC, and imaged using laser scanning confocal microscopy.

Cell lines stably expressing MLrgW or MLrgS. pcDNA3.1(+) vector and pTAT-MLrgW/MLrgS plasmids were digested with KpnI and XhoI, ligase treated, and transformed into *E. coli* BL21 cells. Positive pcDNA3.1(+)-MLrgW/MLrgS clones were confirmed by DNA sequencing. These were transfected into NIH3T3 cells using lipofectamine for 48 h and then cultured in DMEM containing G418 (400 μg/mL) for 28 days to generate stable transfectants. Expression of MLrg/MLrgS polypeptides was detected by Western blotting as described above.

Cell cycle distribution assessed by flow cytometry. NIH3T3 cells stably transfected with pcDNA3.1(+) expressing MLrgW/MLrgS were collected, washed, and fixed. Cells were then incubated with propidium iodide and the distribution of different phases of the cell cycle were recorded using a Becton–Dickinson FACSort analyzer.

Results

Amplification of the novel MLrg gene and expression of MLrg protein in E. coli

Comparison of the human LRG sequence (Accession No. NM_018360; [13]) with the database of mouse cDNA sequences was used to identify PCR primers suitable for amplification of the corresponding mouse lipopolysaccharide-responding gene (MLrg) cDNA sequences. These primers were then used for PCR of total oligo-(dT)-primed cDNA prepared from LPS-treated mouse NIH3T3 cells. The full-length cDNA for MLrg was 1905 bp (Fig. S1A; all supplementary figures supplied online) and encompassed the 5′ and 3′ untranslated regions and the open reading frames. Two alternative isoforms named MLrgW and MLrgS (Fig. S1B) were also obtained that appeared to be splice variants (Fig. S1C).

Bioinformatics analysis of MLrgW and MLrgS

By using UCSC Genome Bioinformatics Database (http://genome.ucsc.edu), the MLrg gene is located at qF4 on chromosome X (Fig. S2A). The full-length MLrgW (1554 bp) and MLrgS (1407 bp) cDNAs contained open reading frames encoding proteins of 517 and 468 amino acids, respectively (Fig. S2B). These sequences have been filed at GenBank under Accession Nos. DQ316984 (MLrgW) and DQ320011 (MLrgS). MLrgW comprises 10 exons and 9 introns (Fig. S2C) while MLrgS comprises 11 exons and 10 introns (Fig. S2D), revealing that the two transcripts are generated by alternative splicing. Exon 2 of MLrgW is internally spliced to the independent exon 2 and exon 3 of MLrgS (Fig. S2E). The spliced exon 2 of MLrgW is named independent intron 2 of MLrgS. And this splicing was typical internal splicing (Fig. S2E). Independent exon 2/intron 2/exon 3 of MLrgS is contained within the total exon 2 of MLrgW (Fig. S2E). A zinc finger domain is present in both MLrgW and MLrgS while leucine zipper regions (L-x(6)-L-x(6)-L-x(6)-L) are conserved in both polypeptides (Fig. 1A).

Antibody production and subcellular localization

To characterize the two polypeptides, coding regions were inserted into the pTAT expression vector to generate a hybrid coding

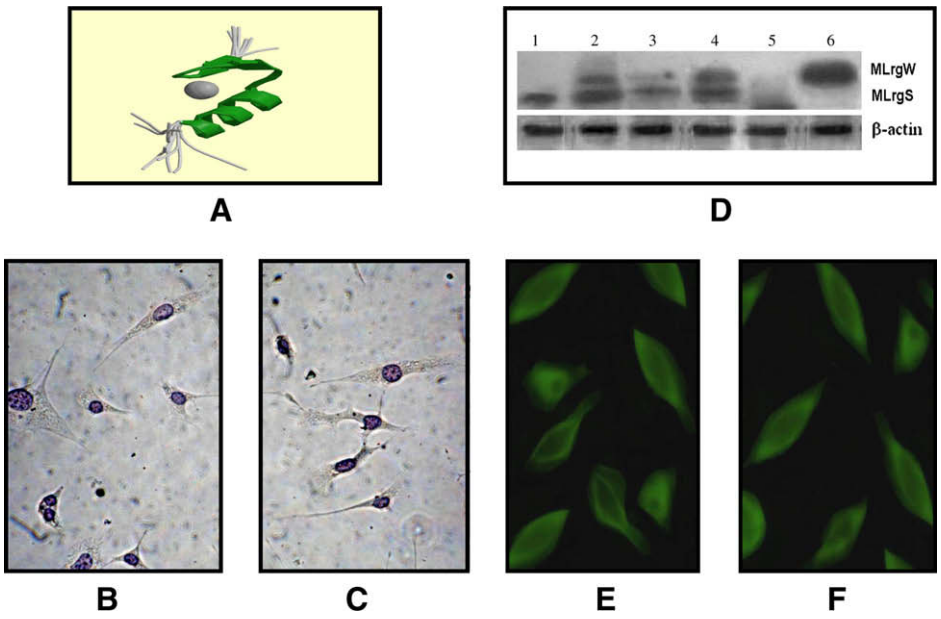

Fig. 1. (A) Analysis of functional domains within MLrgW and MLrgS with conserved leucine zipper motifs in both molecules. (B,C) Localization of MlrgW and MlrgS in L929 cells. MLrgW (B) and MLrgS (C) proteins were localized predominantly in the nuclear membrane and cytoplasm. (D) Western blotting of MLrgW and MLrgS proteins in NIH3T3 and L929 cells exposed to LPS. Lane 1, 6His-TAT-MLrgS; lane 2, NIH3T3 cells exposed to LPS; lane 3, untreated NIH3T3 cells; lane 4, L929 cells exposed to LPS; lane 5, untreated L929 cells; lane 6, 6His-TAT-MLrgW. Immunohistochemistry of MLrgW (E) and MLrgS (F) proteins in NIH3T3 cells exposed to LPS.

sequence in which MLrg sequences were fused in-frame to 6His-TAT. The 6His-TAT-MLrgW fusion protein and 6His-TAT-MLrgS fusion protein were then expressed in *E. coli* where the expressed products were present in inclusion bodies (Fig. S1D). The polypeptides were then purified by nickel–nitrilotriacetic acid (Ni–NTA) affinity chromatography and used to immunize rabbits. Titers of the antisera obtained exceeded 1:1000.

The polypeptides were then expressed in stable transfectants of L929 cells and subcellular localization was analyzed by immunohistochemistry. As shown in Fig. 1B and 1C, both proteins were localized predominantly in the nuclear membrane and cytoplasm.

Effects of LPS on MLrgW and MLrgS expression

Mouse NIH3T3 and L929 cell lines were treated with LPS and levels of MLrgW and MLrgS was quantitated by Western blotting using antisera directed against the purified proteins. As shown in Fig. 1D, expression of both polypeptides was barely detectable in untreated control cells but was strongly upregulated after LPS treatment. This result was then confirmed by immunochemistry, revealing that specific staining of MLrgW (Fig. 1E) and MLrpS (Fig. 1F) was robustly increased following LPS treatment.

Cell cycle alterations in stable transfectants expressing MLrgW and MLrgS

Coding sequences for MLrgW and MLrgS were inserted into the pcDNA3.1(+) expression vector and hybrid plasmids were transfected into NIH3T3 cells. Stable cell lines harboring the expression constructs were isolated following G418 selection. Protein expression was analyzed using Western blotting. As shown in Fig. 2A, little if any MLrgW protein was detected in control cells, but was readily detected in the stable transfectants.

To study possible cell effects of MLrgW expression, the distribution of the different phases of the cell cycle were analyzed by propidium iodide staining and flow cytometry. In controls, 64.1% of cells were in the G1 phase, whereas in the transfectants overexpressing only 47.0% were in G1 (Fig. 2B). Significant changes of G2 and S phase have not taken place between control and experimental groups. These findings indicate that expression of MLrgW can alter the cell cycle progression of NIH3T3 cells.

For MLrgS protein, stable transfectants were also analyzed by Western blotting, confirming over-expression of MLrgS (Fig. 2C). Cell cycle distribution was also examined. In controls, 8.9% of cells were found to be in G2 phase, while this was increased to 18.8% in transfectants expressing MLrgS (Fig. 2D). Significant changes of G1 and S phase have not taken place between control and experimental groups. These findings are consistent with the conclusion that MLrgS expression affects cell cycle progression.

Discussion

Animals and plants live in a 'sea' of microorganisms [14]. Humans are constantly exposed to a variety of pathogenic microorganisms including Gram-negative bacteria (GNB) [15], and GNB and their endotoxins present a constant environmental challenge [16]. GNB sepsis is a major cause of death in hospital intensive care units [17]. Although endotoxins can potently mobilize host defenses against invading GNB, they can potentially induce severe pathology [16]. Host defense against invading GNB depends on innate immune recognition of LPS [17]. LPS stimulation of mammalian cells occurs through a series of interactions with several proteins including LBP, CD14, MD-2, and TLR4 [18], and the complex signaling pathway activated by LPS provides several opportunities for pharmacological intervention designed to attenuate endotoxin-induced inflammation [17]. Nevertheless, there are major differences in individual responses to LPS stimulation, and the development of inflammatory disease is dependent on gene–gene and/or gene–environment interactions [19].

We report the cloning and characterization of a mouse gene, MLrg, whose expression is induced by LPS. Two isoforms generated by alternative splicing, MLrgW and MLrgS, were identified. Alternative splicing is an important and prevalent mechanism of gene regulation in higher eukaryotes [20] because it can create multiple mRNA transcripts from a single gene [21], and the resulting increase in protein diversity is exploited in many biological processes

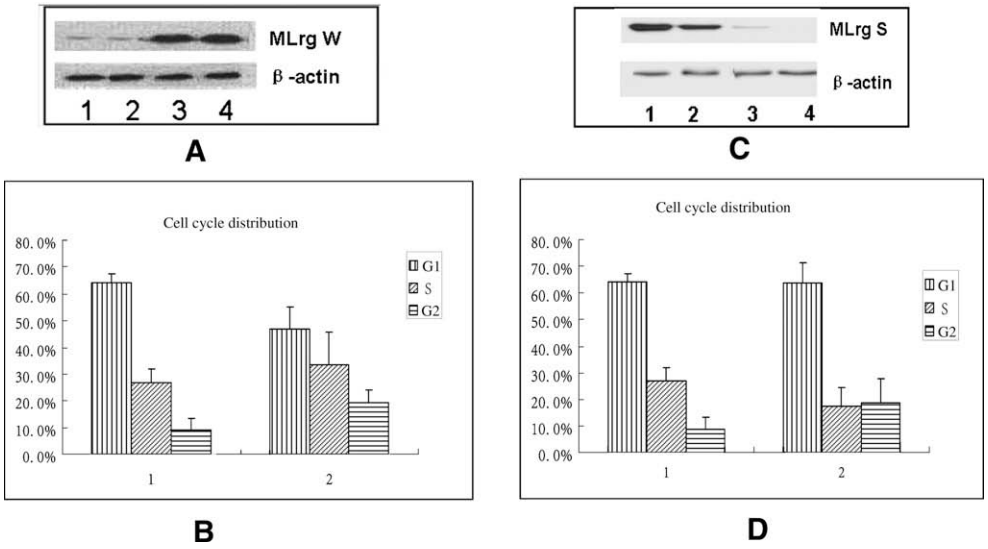

Fig. 2. (A) Western blotting of MLrgW protein in NIH3T3 cells stably transfected with pcDNA3.1(+)-MLrgW or control pcDNA3.1(+) vector. Lanes l–2, NIH3T3 cells transfected with pcDNA3.1(+)vector; lanes 3–4, NIH3T3 cells transfected with pcDNA3.1(+)-MLrgW. (B) Effects of over-expression of MLrgW on cell cycle distribution in NIH3T3 cells. Logarithmically growing NIH3T3 cells were stained with propidium iodide and subjected to cell sorting. 1, NIH3T3 cells transfected with control pcDNA3.1(+) vector; 2, NIH3T3 cells transfected with MLrgW-pcDNA3.1(+). The experiment was repeated three times; representative images are shown in the figure. The difference in the percentages of cells in G1 between controls and cells expressing MLrgW was statistically significant; $^{*}p < 0.05$. (C) Western blotting of MLrgS protein in NIH3T3 cells stably transfected with pcDNA3.1(+)-MLrgS or with control pcDNA3.1(+) vector. Lanes l–2, NIH3T3 cells transfected with pcDNA3.1(+)-MLrgS; lanes 3–4, NIH3T3 cells transfected with pcDNA3.1(+)vector. (D) Effects of over-expression of MLrgS on cell cycle distribution in NIH3T3 cells. Logarithmically growing NIH3T3 cells were stained with propidium iodide and subjected to cell sorting. 1, NIH3T3 cells transfected with pcDNA3.1(+) vector; 2, NIH3T3 cells transfected with MLrgS-pcDNA3.1(+). The experiment was repeated three times and representative images are shown in the figure. The difference in the percentages of cells in G2 between controls and cells expressing MLrgW was statistically significant; $^{*}p < 0.05$.

including cell fate determination and apoptosis [22]. The impact of alternative splicing is well recognized in animal systems as a key regulator of gene expression and proteome complexity [23]; nearly three quarters of human multi-exon genes are subject to alternative splicing [24].

Leucine zipper regions are relatively well conserved between MLrgW and MLrgS proteins. Dimeric basic leucine zipper (bZIP) factors constitute one of the most important classes of enhancer-type transcription factors [25]. In vertebrates, bZIP factors are involved in many cellular processes, including cell survival, learning and memory, cancer progression, lipid metabolism, and a variety of developmental processes [26,27].

The amino acid sequences of both MLrgW and MLrgS contain a zinc finger domain of the Cys2-His2 (C2H2) class, one of the most common DNA-binding motifs in eukaryotes [28]. This domain allows different proteins to interact with or bind to DNA, RNA, or other proteins, and the motif is present in the proteomes of many different organisms [29]. The simple mode of DNA recognition by C2H2-type zinc finger domains has been exploited for protein design [30], and recent studies suggest that C2H2 zinc finger proteins could function as key transcriptional repressors involved in the defense against and acclimatization to different environmental stress conditions [28].

Immunohistochemistry revealed that MLrgW and MLrgS are predominantly localized in the nuclear membrane and cytoplasm. However, over-expression of MLrgW and MLrgS affected cell cycle progression in stable NIH3T3 transfectants. The percentage of cells in the G1 phase was significantly decreased by over-expression of MLrgW, whereas the percentage of cells in G2 was increased by over-expression of MLrgS. These findings indicated that MLrgW and MLrgS could be involved in cell cycle regulation. Both proteins are strongly upregulated by LPS treatment and, in view of their conserved zinc finger and bZIP domains, are potential transcription regulators. It is therefore possible that MLrgW and MLrgS are involved in transcriptional regulation following activation of the LPS signaling pathway.

In summary, we report two polypeptides, MLrgW and MLrgS, generated by alternative splicing of transcripts from the mouse LPS-responsive MLrg gene. Studies on transfectants over-expressing MLrgW and MLrgS revealed that the proteins are localized in the nuclear membrane and cytoplasm and their expression can modulate cell cycle progression. The molecules contain conserved zinc finger and bZIP domains, and may therefore be regulators of transcription that could potentially contribute to cell regulation following LPS stimulation. Further investigations will be required to determine if pharmacologic intervention in MLrg pathways might be of therapeutic value in shock induced by endotoxin exposure.

Acknowledgments

This work was supported by Grants 30571544 (J.C.) and 30972429, 30571586, 30400413 (K.D.) from the National Natural Science Foundation of China and by Grant 20060391015 (K.D.) from the Chinese Postdoctoral Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.11.047.

References

[1] G. Nagy, T. Pál, Lipopolysaccharide: a tool and target in enterobacterial vaccine development, Biol. Chem. 389 (2008) 513–520.
[2] B. Beutler, E.M. Moresco, The forward genetic dissection of afferent innate immunity, Curr. Top. Microbiol. Immunol. 321 (2008) 3–26.
[3] K.H. Yang, M.G. Lee, Effects of endotoxin derived from *Escherichia coli* lipopolysaccharide on the pharmacokinetics of drugs, Arch. Pharm. Res. 31 (2008) 1073–1086.
[4] M.S. Trent, C.M. Stead, A.X. Tran, J.V. Hankins, Diversity of endotoxin and its impact on pathogenesis, J. Endotoxin Res. 12 (2006) 205–223.
[5] D.J. Philpott, S.E. Girardin, The role of Toll-like receptors and Nod proteins in bacterial infection, Mol. Immunol. 41 (2004) 1099–1108.

[6] H. Bosshart, M. Heinzelmann, Targeting bacterial endotoxin: two sides of a coin, Ann. NY Acad. Sci. 1096 (2007) 1–17.
[7] R. Jerala, Structural biology of the LPS recognition, Int. J. Med. Microbiol. 297 (2007) 353–363.
[8] S.M. Opal, The host response to endotoxin, antilipopolysaccharide strategies, and the management of severe sepsis, Int. J. Med. Microbiol. 297 (2007) 365–377.
[9] S.H. Diks, D.J. Richel, M.P. Peppelenbosch, LPS signal transduction: the picture is becoming more complex, Curr. Top. Med. Chem. 4 (2004) 1115–1126.
[10] B. Wang, S.R. Cai, C. Gao, Lipopolysaccharide results in a marked decrease in hepatocyte nuclear factor 4 alpha in rat liver, Hepatology 34 (2001) 979–989.
[11] F. Zhu, W. Yan, Z.L. Zhao, Improved PCR-based subtractive hybridization strategy for cloning differentially expressed genes, Biotechniques 29 (2000) 310–313.
[12] K.J. Du, Y.B. Chai, L.C. Hou, et al., Over-expression and siRNA of a novel environmental lipopolysaccharide-responding gene on the cell cycle of the human hepatoma-derived cell line HepG2, Toxicology 243 (2008) 303–310.
[13] S. Nogami, S. Satoh, S. Tanaka-Nakadate, et al., Identification and characterization of taxilin isoforms, Biochem. Biophys. Res. Commun. 319 (3) (2004) 936–943.
[14] I.M. Samuel, K.E. Robert, W.B. Martin, LPS, TLR4 and infectious disease diversity, Nat. Rev. Microbiol. 3 (2005) 36–46.
[15] M. Golec, Cathelicidin LL-37: LPS-neutralizing, pleiotropic peptide, Ann. Agric. Environ. Med. 14 (2007) 1–4.
[16] H. Schultz, J.P. Weiss, The bactericidal/permeability-increasing protein (BPI) in infection and inflammatory disease, Clin. Chim. Acta 384 (2007) 12–23.
[17] T.L. Gioannini, J.P. Weiss, Regulation of interactions of Gram-negative bacterial endotoxins with mammalian cells, Immunol. Res. 39 (2007) 249–260.
[18] K. Miyake, Innate immune sensing of pathogens and danger signals by cell surface Toll-like receptors, Semin. Immunol. 19 (2007) 3–10.
[19] T.D. LeVan, J.W. Bloom, T.J. Bailey, A common single nucleotide polymorphism in the CD14 promoter decreases the affinity of Sp protein binding and enhances transcriptional activity, J. Immunol. 167 (2001) 5838–5844.
[20] Y. Xing, Genomic analysis of RNA alternative splicing in cancers, Front. Biosci. 12 (2007) 4034–4041.
[21] M. Pascual, M. Vicente, L. Monferrer, R. Artero, The Muscleblind family of proteins: an emerging class of regulators of developmentally programmed alternative splicing, Differentiation 74 (2006) 65–80.
[22] B.J. Haas, Analysis of alternative splicing in plants with bioinformatics tools, Curr. Top. Microbiol. Immunol. 326 (2008) 17–37.
[23] C.G. Simpson, D. Lewandowska, J. Fuller, et al., Alternative splicing in plants, Biochem. Soc. Trans. 36 (2008) 508–510.
[24] W.B. Barbazuk, Y. Fu, K.M. McGinnis, Genome-wide analyses of alternative splicing in plants: opportunities and challenges, Genome Res. 18 (2008) 1381–1392.
[25] C.D. Deppmann, R.S. Alvania, E.J. Taparowsky, Cross-species annotation of basic leucine zipper factor interactions: insight into the evolution of closed interaction networks, Mol. Biol. Evol. 23 (2006) 1480–1492.
[26] R. St-Arnaud, B. Elchaarani, Identification of additional dimerization partners of FIAT, the factor inhibiting ATF4-mediated transcription, Ann. NY Acad. Sci. 1116 (2007) 208–215.
[27] K. Ameri, A.L. Harris, Activating transcription factor 4, Int. J. Biochem. Cell Biol. 40 (2008) 14–21.
[28] S. Negi, M. Imanishi, M. Matsumoto, et al., New redesigned zinc-finger proteins: design strategy and its application, Chemistry 14 (2008) 3236–3249.
[29] S. Ciftci-Yilmaz, R. Mittler, The zinc finger network of plants, Cell. Mol. Life Sci. 65 (2008) 1150–1160.
[30] K.J. Brayer, D.J. Segal, Keep your fingers off my DNA: protein–protein interactions mediated by C2H2 zinc finger domains, Cell Biochem. Biophys. 50 (2008) 111–131.