ACADEMIC PRESS

Biochemical and Biophysical Research Communications 295 (2002) 157–162

BBRC

www.academicpress.com

TIRAP mediates endotoxin-induced NF-κB activation and apoptosis in endothelial cells

Douglas D. Bannerman,[a,*] Ryan D. Erwert,[b] Robert K. Winn,[a] and John M. Harlan[c]

[a] *Department of Surgery, University of Washington School of Medicine, Seattle, WA 98104, USA*
[b] *Department of Pathology, University of Washington School of Medicine, Seattle, WA 98104, USA*
[c] *Department of Medicine, University of Washington School of Medicine, Seattle, WA 98104, USA*

Received 5 June 2002

Abstract

Bacterial lipopolysaccharide (LPS) initiates multiple signaling events in vascular endothelial cells that can result in activation and/or cell death. LPS-induced activation of endothelial cells elicits a wide array of vascular endothelial responses, many of which are dependent on NF-κB activation. Several of the signaling molecules that mediate LPS-induced NF-κB activation, including Tlr-4, MyD88, and IRAK-1, have been similarly reported to mediate LPS pro-apoptotic signaling. Recently, a new signaling molecule, TIRAP, has been identified that mediates LPS-induced NF-κB signaling in monocytes and macrophages. Using a TIRAP dominant negative construct, we have identified a role for TIRAP in mediating LPS-induced NF-κB activation and apoptosis in human endothelial cells. These data identify TIRAP as a dual functioning signaling molecule and suggest the presence of a MyD88-independent LPS signaling pathway in human endothelial cells.

Keywords: Endothelium; Endotoxin; Apoptosis; NF-κB; Toll-like receptor-4

The management of Gram-negative septic patients is often complicated by the development of vascular complications including systemic vascular collapse [1–3], disseminated intravascular coagulation [4,5], and vascular leak syndromes [6–10]. A common denominator of these vascular complications is endothelial cell (EC) activation, injury, and/or dysfunction [1,8,11,12]. Bacterial lipopolysaccharide (LPS), a highly pro-inflammatory molecule, has been implicated in the pathogenesis of Gram-negative sepsis and its attendant vascular complications [6,13–15]. LPS activation of the vascular endothelium elicits several EC responses including: (1) the production of the pro-inflammatory cytokines, IL-6 [16], IL-8 [17,18], and IL-1_β [19], (2) the upregulation of the adhesion molecules E-selectin, ICAM-1, and VCAM-1 [20,21], and (3) expression of tissue factor [22,23]. A common denominator that mediates the upregulation of these gene products is NF-κB activation [24]. In addition to activation, LPS has been reported to induce EC injury both in vitro [25–30], and in vivo [31–33].

Toll-like receptor (Tlr)-4 has been identified as the putative transmembrane receptor for LPS that activates NF-κB signaling [34–36]. Following activation, the adapter protein MyD88 is recruited to the cytoplasmic domain of Tlr-4 through homotypic binding of respective Toll receptor-interleukin-1 receptor (TIR) domains [37,38]. MyD88 contains another highly conserved protein binding domain, a death domain (DD), that facilitates its association with the DD-containing signaling molecule IL-1 receptor-associated kinase-1 (IRAK-1) [39]. Following an autophosphorylation event, IRAK-1 dissociates from MyD88 and interacts with TNF receptor-associated factor-6 (TRAF-6) [40,41], resulting in the activation of a downstream kinase cascade involving NF-κB-inducing kinase (NIK) and IκB kinase (IKK). The IKK-mediated phosphorylation of IκB, an inhibitor of NF-κB, leads to IκB degradation by the proteasome and enables NF-κB to translocate to the nucleus where it can promote new gene expression [39]. Several of the proximal signaling

* Corresponding author. Fax: +1-206-341-5322.
E-mail address: dbannerm@u.washington.edu (D.D. Bannerman).

0006-291X/02/$ - see front matter
PII: S0006-291X(02)00638-1

molecules involved in LPS-induced NF-κB activation, including Tlr-4 [42], MyD88 [43], and IRAK-1 [43], have a similar role in promoting LPS pro-apoptotic signaling. Despite the involvement of common proximal signaling molecules, LPS-induced NF-κB and apoptosis occur independent of one another and the signaling pathways leading to activation of these events diverge upstream of IκB degradation [43].

Although a role for MyD88 in mediating LPS signaling has been clearly established [37,44,45], there is evidence to suggest that a redundant or alternative MyD88-independent pathway(s) exists as well [44,46,47]. LPS is capable of activating NF-κB in MyD88$^{-/-}$ murine macrophages [44]. Although this response is delayed compared with wild-type macrophages, the induction of NF-κB activity in the MyD88$^{-/-}$ macrophages suggests that cellular activation by LPS can occur independent of MyD88. Recently, a MyD88-like protein has been identified that promotes LPS-induced NF-κB activation [46,47]. This protein, identified as TIR domain-containing adapter protein (TIRAP) [47] or MyD88 adapter-like protein (MAL) [46] by the two groups that co-discovered this molecule, binds directly to Tlr-4. TIRAP, in turn, interacts with IRAK-2 [46], the latter of which associates with TRAF-6 [48]. The existence of this MyD88-independent pathway, linking Tlr-4 to downstream signaling mediators of NF-κB, may explain the lack of inhibition of LPS-induced NF-κB activation in the MyD88$^{-/-}$ macrophages [44]. Whether TIRAP mediates LPS-induced NF-κB activation in EC, a key host target of LPS, has yet to be addressed. Further, whether TIRAP mediates LPS-induced pro-apoptotic signaling in any cell type remains unknown. We therefore decided to investigate whether LPS-induced EC NF-κB activation and apoptosis are mediated by TIRAP.

Materials and methods

Materials. LPS from *Escherichia coli* serotype 0111:B4 was purchased from Sigma Chemical (St. Louis, MO). Recombinant human TNF-α was purchased from R&D Systems, (Minneapolis, MN).

Cell culture. The human dermal microvascular EC line (developed and provided by F.J. Candal and Dr. E. Ades, Centers for Disease Control, and Dr. T. Lawley, Emory University, Atlanta, GA) [49] was cultured in RPMI medium (Biowhittaker, Walkersville, MD), enriched with 10% fetal bovine serum (FBS) (Hyclone Laboratories, Logan, UT), endothelial cell growth factor prepared from bovine hypothalamus, L-glutamine (2 mM), sodium pyruvate (1 mM), and nonessential amino acids, in the presence of penicillin (100 U/ml) and streptomycin (100 μg/ml) (all purchased from Biowhittaker).

Cloning and stable expression of cDNA constructs. cDNA encoding mutant TIRAP containing a proline to histidine substitution at amino acid 125 (a gift of Dr. Ruslan Medzhitov, Yale University School of Medicine, New Haven, CT) [47] was cloned into the bicistronic retroviral expression plasmid, pBMN-IRES-enhanced green fluorescent protein (EGFP) (provided by Dr. Gary Nolan, Stanford University, Stanford, CA) [50]. High-titer retrovirus was prepared from the Phoenix amphotropic packaging cell line (ATCC, Manassas, VA) transfected with 24 μg expression plasmid by calcium phosphate precipitation. Recombinant retroviral supernatants were collected 48 h after transfection and filtered through a Millex-HV 0.45 μM filter (Millipore, Bedford, MA). For infection, 4×10^5 EC were seeded per well of a 6-well plate for 24 h to achieve ~80% confluence. The growth medium was replaced with 2.5 ml retroviral supernatant supplemented with 32 μg/ml polybrene and 10 mM HEPES and the plate was centrifuged for 2 h (1430*g*; 32 °C). The cells were then incubated for 10 h (5% CO_2, 37 °C) after which the retroviral supernatant was replaced with normal growth medium. Cells were analyzed and sorted on the basis of EGFP expression using a FACVantage SE cell sorter (Becton–Dickinson, Franklin Lakes, NJ).

Immunoblotting. Cell monolayers were washed once with phosphate-buffered saline (PBS), lysed with ice-cold modified radioimmunoprecipitation assay lysis buffer [50 mM Tris–HCl (pH 7.4), 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, protease inhibitor cocktail tablet (Roche Molecular Biochemicals, Indianapolis, IN), 1 mM vanadate, and 50 mM NaF], scraped, transferred to microcentrifuge tubes, and centrifuged (16,000*g*, 10 min, 4 °C). Total protein was determined using the BCA protein assay (Pierce Chemical, Rockford, IL). The supernatants were combined with 5× sample buffer (Genomic Solutions, Chelmsford, MA), boiled for 3 min, and 20 μg protein/lane was resolved by SDS–PAGE on a 4–20% Tris–Glycine gradient gel (Novex, San Diego, CA). Protein was subsequently transferred for 1 h at 100 V to polyvinylidene fluoride membrane (Millipore, Bedford, MA). Blots were blocked with 5% dried milk and then incubated with either anti-FLAG (0.5 μg/ml; Upstate Biotechnology, Lake Placid, NY), anti-IκBα (0.4 μg/ml; Santa Cruz Biotechnology, Santa Cruz, CA), or anti-β-tubulin (0.5 μg/ml; Roche Molecular Biochemicals, Indianapolis, IN) antibodies for 1 h at room temperature. The blots were incubated with horseradish-peroxidase-conjugated anti-mouse IgG (0.13 μg/ml; Transduction Laboratories, Lexington, KY), developed with enhanced chemiluminescence (Amersham Life Sciences, Arlington Heights, IL), and exposed to Kodak X-Omat Blue film (NEN Life Sciences, Boston, MA).

Luciferase assay. A recombinant adenovirus (KZ142) system (gift of Dr. James Kelly, ZymoGenetics, Seattle, WA) was used to transfect cells with a luciferase reporter construct. The adenoviral construct was created as follows: an oligonucleotide encoding a consensus NF-κB binding site, the tandem NF-κB binding sites of the HIV-1 long terminal repeat [51], two copies of the collagenase AP-1 element, and a single copy of the c-jun TRE [52] were ligated into a luciferase reporter cassette and then placed in the pACCMV.pLpA adenoviral shuttle vector for construction of recombinant adenovirus as described [53]. For transfection of the luciferase reporter construct, EC were seeded into 96-well black view plates (Corning, Corning, NY) at a density of 50,000 cells/well for 24 h and subsequently incubated for 16 h at a multiplicity of infection (m.o.i.) of 2000 in RPMI supplemented with 1% FBS. Following infection, EC were exposed to experimental treatment in Ham's F12 medium supplemented with 2.5% FBS, 20 mM HEPES, and 0.5% BSA for 4 h at 37 °C. Luciferase activity was determined using a commercially available assay kit and a TopCount NXT luminescence counter (both from Packard Instrument, Meriden, CT).

Caspase assay. EC were seeded into 96-well plates at a density of 60,000 cells/well, cultured for 24 h, and treated. Caspase activity was measured with a fluorimetric caspase assay utilizing the caspase-3 substrate, DEVD, conjugated to Rhodamine-110 according to manufacturer's instructions (Roche Molecular Biochemicals). The plates were analyzed on a Cytofluor Series 4000 fluorescence plate reader (Perseptive Biosystems, Framingham, MA) at 485 nm excitation and 530 nm emission and caspase activity was expressed relative to simultaneous medium control. Since human EC sensitization to LPS- and TNF-α-induced apoptosis is dependent upon the inhibition of new protein synthesis by a mechanism that we have previously characterized [26], treatment with these agents was performed in the presence of 40 μg/ml of CHX.

Histone release assay. To assay for the release of histones into the cytoplasm, EC were seeded into 96-well plates at a density of 60,000 cells/well, cultured for 24 h, treated, and histone release was measured with the Cell Death Detection ELISA assay according to manufacturer's instructions (Roche Molecular Biochemicals). The plates were analyzed at 405 nm and 450 nm (reference wavelength) on a microplate reader (Bio-Tech Instruments, Winooski, VT). Background readings defined as EC exposed to medium alone were subtracted from each experimental treatment.

Statistical methods. A *t* test was used to compare the mean responses between a single experimental group and its control. Statistical analyses were performed using GraphPad Prism version 3.00 for Macintosh (GraphPad Software, San Diego, CA). A p value of <0.05 was considered significant.

Results and discussion

Expression of dominant-negative (D/N) TIRAP inhibits LPS-induced NF-κB activation in EC

TIRAP has recently been identified as a signaling molecule that mediates LPS-induced NF-κB activation in monocytes and macrophages [46,47]. By virtue of its direct recruitment to Tlr-4, TIRAP is one of the most proximal intracellular signaling molecules involved in LPS-induced cellular activation. TIRAP interaction with TLR-4 is mediated through reciprocal binding of TIR domains contained within each protein. A proline to histidine mutation at amino acid 125 (P125H) in the TIR domain of TIRAP ablates its ability to bind to the TIR domain of Tlr-4 [46,47]. Correspondingly, expression of TIRAP constructs containing this mutation functions to inhibit signaling mediated by endogenous TIRAP in a dominant-negative manner. To determine whether TIRAP mediates LPS-induced EC NF-κB activation, EC were stably transfected with cDNA encoding either TIRAP with a P125H mutation (TIRAP-D/N) or EGFP-vector alone. Western blot analysis using an anti-FLAG antibody to recognize the FLAG-tagged TIRAP-D/N confirmed expression (Fig. 1A). Expression of the TIRAP-D/N construct inhibited LPS-induced NF-κB activation in EC by ~40% (Fig. 1B). A key step in NF-κB activation is the degradation of its inhibitor, IκB, which enables NF-κB to translocate to the nucleus where it stimulates new gene expression. Since TIRAP is reportedly a proximal signaling mediator that functions upstream of IκB degradation, the ability of TIRAP-D/N to inhibit LPS-induced IκBα degradation in EC was assayed (Fig. 1C). EC expressing either EGFP or TIRAP-D/N demonstrated equivalent levels of IκBα expression under basal conditions. Following LPS (100 ng/ml) treatment for 60 min, IκBα was readily degraded in EC expressing EGFP consistent with intact NF-κB signaling. In contrast, degradation of IκBα in EC expressing D/N TIRAP was greatly impaired.

TNF-α is a well-described activator of EC NF-κB [54]. Distinct receptor membrane complexes and proximal intracellular signaling molecules mediate LPS- and TNF-α-induced NF-κB activation. Further downstream, however, the signaling pathways leading to NF-κB activation converge at the level of NIK and IKK [55]. Consistent with a role for TIRAP in mediating signaling elicited by LPS/Tlr4, but not by TNF-α and its receptor, expression of the TIRAP-D/N protein had no effect on the ability of TNF-α to activate NF-κB (Fig. 1B) or to promote degradation of IκBα (Fig. 1C).

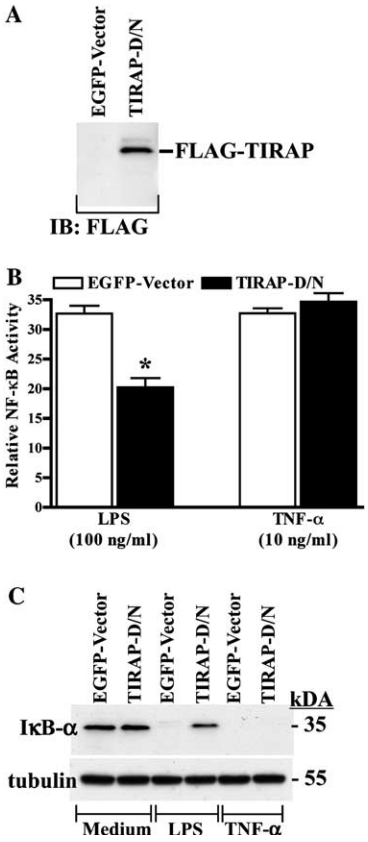

Fig. 1. Expression of dominant-negative (D/N) TIRAP inhibits LPS-induced NF-κB activation in EC. EC were stably transfected with either EGFP-vector alone or cDNA encoding TIRAP with a proline to histidine mutation at amino acid 125 (TIRAP-D/N) (A–C). An anti-FLAG antibody was used to detect FLAG-tagged TIRAP D/N (A). To determine whether the TIRAP-D/N construct mediates LPS-induced NF-κB activation in EC, these cells were treated for 4 h with either medium, LPS (100 ng/ml), or TNF-α (10 ng/ml), lysed, and assayed for NF-κB-dependent luciferase activity (B). In other experiments, EC expressing EGFP-vector or TIRAP D/N were treated with medium (60 min), LPS (100 ng/ml; 60 min), or TNF-α (10 ng/ml; 30 min), and assayed for IκBα degradation (C). Vertical bars represent means (± SE) NF-κB activity (B) relative to simultaneous media controls. * = significantly decreased compared to EGFP-vector transfected EC exposed to identical treatment.

TIRAP D/N inhibits LPS-induced EC apoptosis

We have previously established that LPS induces apoptosis in human EC and that MyD88 and IRAK-1

contribute to LPS pro-apoptotic signaling [43]. Although both MyD88 and IRAK-1 have a similar role in promoting NF-κB signaling, LPS-induced apoptosis and NF-κB activation are distinct events that occur independent of one another [43]. The bifurcation in the signaling pathways leading to these events occurs upstream of IκBα degradation. Interestingly, we have previously reported that expression of two different MyD88-D/N constructs that block >85% of LPS-induced NF-κB activation could only inhibit ~30% of LPS-induced caspase activation [43]. This may suggest the presence of a redundant pro-apoptotic signaling pathway involving another mediator. Since LPS-induced apoptosis is dependent on Tlr-4 signaling [42], other Tlr-4 binding proteins may similarly transduce LPS pro-apoptotic signaling. To determine whether TIRAP promotes LPS pro-apoptotic signaling, EC expressing EGFP or TIRAP-D/N were treated with LPS (100 ng/ml) for 6 h and assayed for caspase activation (Fig. 2A). EC exposed to LPS demonstrated a marked increase in caspase activity relative to EC exposed to medium alone. This activation of caspases, which are highly specific effector proteases activated during apoptosis [56], is consistent with prior studies demonstrating LPS-induced EC apoptosis by other criterion, including PARP cleavage, nuclear histone release, and DNA laddering [26,57]. EC expressing the TIRAP-D/N, however, demonstrated a >45% decrease in caspase activity, following LPS exposure compared with control EC (Fig. 2A). Another hallmark of apoptosis is the release of histones into the cytoplasm [58]. Expression of the TIRAP-D/N construct inhibited LPS-induced histone release by >35% relative to EC expressing vector alone (Fig. 2B). In contrast to its effect on LPS signaling, the TIRAP-D/N failed to inhibit TNF-α-induced apoptosis, consistent with a specific role for TIRAP in mediating LPS/Tlr-4 signaling (Fig. 2). Together, these data suggest that TIRAP specifically mediates LPS-induced pro-apoptotic signaling. The inability to completely inhibit LPS-induced NF-κB activation and apoptosis using the TIRAP-D/N construct is not surprising considering the fact that endogenous TIRAP is still expressed. Further, the presence of a redundant signaling pathway(s) involving MyD88 and/or other molecules may contribute to these signaling events, independent of TIRAP.

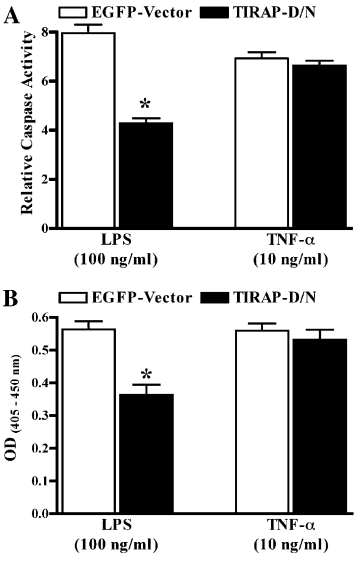

Fig. 2. Expression of TIRAP-D/N inhibits LPS-induced apoptosis. EC stably expressing either EGFP or TIRAP-D/N were treated for 6 h with either medium, LPS (100 ng/ml), or TNF-α (10 ng/ml), and assayed for caspase activity (A). Vertical bars represent means (± SE) caspase activity relative to simultaneous media controls. In other experiments, EC were treated for 8 h as above and assayed for histone release (B). Mean (± SE) histone release is reported in OD units. * = significantly decreased compared to EGFP-vector transfected EC exposed to identical treatment.

In summary, we have identified a role for TIRAP in mediating LPS-induced NF-κB activation in EC. Further, this is the first report to demonstrate that TIRAP promotes pro-apoptotic signaling in any cell type. Future studies will be needed to identify the downstream signaling mediators that link proximal LPS/Tlr-4 signaling molecules to those that initiate caspase activation and the onset of apoptosis.

Acknowledgments

This work was supported by National Institutes of Health Grants GM07037, GM42686, HL18645, and HL03174.

References

[1] L.B. Hinshaw, Sepsis/septic shock: participation of the microcirculation: an abbreviated review, Crit. Care Med. 24 (1996) 1072–1078.
[2] G.D. Martich, A.J. Boujoukos, A.F. Suffredini, Response of man to endotoxin, Immunobiology 187 (1993) 403–416.
[3] R.J. Ulevitch, C.G. Cochrane, P.M. Henson, D.C. Morrison, W.F. Doe, Mediation systems in bacterial lipopolysaccharide-induced hypotension and disseminated intravascular coagulation. I. The role of complement, J. Exp. Med. 142 (1975) 1570–1590.
[4] M. Levi, H. ten Cate, T. van der Poll, S.J. van Deventer, Pathogenesis of disseminated intravascular coagulation in sepsis, JAMA 270 (1993) 975–979.
[5] L.G. Thijs, J.P. de Boer, M.C. de Groot, C.E. Hack, Coagulation disorders in septic shock, Intensive Care Med. 19 (1993) S8–15.
[6] P. Brandtzaeg, P. Kierulf, P. Gaustad, A. Skulberg, J.N. Bruun, S. Halvorsen, E. Sorensen, Plasma endotoxin as a predictor of multiple organ failure and death in systemic meningococcal disease, J. Infect. Dis. 159 (1989) 195–204.
[7] K.L. Brigham, B. Meyrick, Endotoxin and lung injury, Am. Rev. Respir. Dis. 133 (1986) 913–927.
[8] M.A. Martin, H.J. Silverman, Gram-negative sepsis and the adult respiratory distress syndrome, Clin. Infect. Dis. 14 (1992) 1213–1228.

[9] B. Meyrick, Pathology of the adult respiratory distress syndrome, Crit. Care Clin. 2 (1986) 405–428.
[10] P.E. Parsons, G.S. Worthen, E.E. Moore, R.M. Tate, P.M. Henson, The association of circulating endotoxin with the development of the adult respiratory distress syndrome, Am. Rev. Respir. Dis. 140 (1989) 294–301.
[11] C.E. Hack, S. Zeerleder, The endothelium in sepsis: source of and a target for inflammation, Crit. Care Med. 29 (2001) S21–27.
[12] M. Mutunga, B. Fulton, R. Bullock, A. Batchelor, A. Gascoigne, J.I. Gillespie, S.V. Baudouin, Circulating endothelial cells in patients with septic shock, Am. J. Respir. Crit. Care Med. 163 (2001) 195–200.
[13] R.L. Danner, R.J. Elin, J.M. Hosseini, R.A. Wesley, J.M. Reilly, J.E. Parillo, Endotoxemia in human septic shock, Chest 99 (1991) 169–175.
[14] A. Haziot, E. Ferrero, F. Kontgen, N. Hijiya, S. Yamamoto, J. Silver, C.L. Stewart, S.M. Goyert, Resistance to endotoxin shock and reduced dissemination of gram-negative bacteria in CD14-deficient mice, Immunity 4 (1996) 407–414.
[15] M. Pollack, C.A. Ohl, Endotoxin-based molecular strategies for the prevention and treatment of gram-negative sepsis and septic shock, Curr. Top. Microbiol. Immunol. 216 (1996) 275–297.
[16] F.R. Jirik, T.J. Podor, T. Hirano, T. Kishimoto, D.J. Loskutoff, D.A. Carson, M. Lotz, Bacterial lipopolysaccharide and inflammatory mediators augment IL-6 secretion by human endothelial cells, J. Immunol. 142 (1989) 144–147.
[17] J. Anrather, V. Csizmadia, C. Brostjan, M.P. Soares, F.H. Bach, H. Winkler, Inhibition of bovine endothelial cell activation in vitro by regulated expression of a transdominant inhibitor of NF-kappaB, J. Clin. Invest. 99 (1997) 763–772.
[18] B. Zhao, R.A. Bowden, S.A. Stavchansky, P.D. Bowman, Human endothelial cell response to gram-negative lipopolysaccharide assessed with cDNA microarrays, Am. J. Physiol. Cell Physiol. 281 (2001) C1587–1595.
[19] P. Libby, J.M. Ordovas, K.R. Auger, A.H. Robbins, L.K. Birinyi, C.A. Dinarello, Endotoxin and tumor necrosis factor induce interleukin-1 gene expression in adult human vascular endothelial cells, Am. J. Pathol. 124 (1986) 179–185.
[20] C.C. Chen, C.L. Rosenbloom, D.C. Anderson, A.M. Manning, Selective inhibition of E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 expression by inhibitors of I kappaB-alpha phosphorylation, J. Immunol. 155 (1995) 3538–3545.
[21] H.P. Jersmann, C.S. Hii, J.V. Ferrante, A. Ferrante, Bacterial lipopolysaccharide and tumor necrosis factor alpha synergistically increase expression of human endothelial adhesion molecules through activation of NF-kappaB and p38 mitogen-activated protein kinase signaling pathways, Infect. Immun. 69 (2001) 1273–1279.
[22] M. Colucci, G. Balconi, R. Lorenzet, A. Pietra, D. Locati, M.B. Donati, N. Semeraro, Cultured human endothelial cells generate tissue factor in response to endotoxin, J. Clin. Invest. 71 (1983) 1893–1896.
[23] D.T. Golenbock, R.R. Bach, H. Lichenstein, T.S. Juan, A. Tadavarthy, C.F. Moldow, Soluble CD14 promotes LPS activation of CD14-deficient PNH monocytes and endothelial cells, J. Lab. Clin. Med. 125 (1995) 662–671.
[24] R. De Martin, M. Hoeth, R. Hofer-Warbinek, J.A. Schmid, The transcription factor NF-kappaB and the regulation of vascular cell function, Arterioscler. Thromb. Vasc. Biol. 20 (2000) E83–88.
[25] D.D. Bannerman, M. Sathyamoorthy, S.E. Goldblum, Bacterial lipopolysaccharide disrupts endothelial monolayer integrity and survival signaling events through caspase cleavage of adherens junction proteins, J. Biol. Chem. 273 (1998) 35371–35380.
[26] D.D. Bannerman, J.C. Tupper, W.A. Ricketts, C.F. Bennett, R.K. Winn, J.M. Harlan, A constitutive cytoprotective pathway protects endothelial cells from lipopolysaccharide-induced apoptosis, J. Biol. Chem. 276 (2001) 14924–14932.
[27] E.A. Frey, B.B. Finlay, Lipopolysaccharide induces apoptosis in a bovine endothelial cell line via a soluble CD14 dependent pathway, Microb. Pathog. 24 (1998) 101–109.
[28] D.G. Hoyt, R.J. Mannix, J.M. Rusnak, B.R. Pitt, J.S. Lazo, Collagen is a survival factor against LPS-induced apoptosis in cultured sheep pulmonary artery endothelial cells, Am. J. Physiol. 269 (1995) L171–177.
[29] K. Maeda, P.A. Abello, M.R. Abraham, R.C. Wetzel, J.L. Robotham, T.G. Buchman, Endotoxin induces organ-specific endothelial cell injury, Shock 3 (1995) 46–50.
[30] K. Zen, A. Karsan, A. Stempien-Otero, E. Yee, J. Tupper, X. Li, T. Eunson, M.A. Kay, C.B. Wilson, R.K. Winn, J.M. Harlan, NF-kappaB activation is required for human endothelial survival during exposure to tumor necrosis factor-alpha but not to interleukin-1beta or lipopolysaccharide, J. Biol. Chem. 274 (1999) 28808–28815.
[31] M. Fujita, K. Kuwano, R. Kunitake, N. Hagimoto, H. Miyazaki, Y. Kaneko, M. Kawasaki, T. Maeyama, N. Hara, Endothelial cell apoptosis in lipopolysaccharide-induced lung injury in mice, Int. Arch. Allergy Immunol. 117 (1998) 202–208.
[32] A. Haimovitz-Friedman, C. Cordon-Cardo, S. Bayoumy, M. Garzotto, M. McLoughlin, R. Gallily, C.K. Edwards 3rd, E.H. Schuchman, Z. Fuks, R. Kolesnick, Lipopolysaccharide induces disseminated endothelial apoptosis requiring ceramide generation, J. Exp. Med. 186 (1997) 1831–1841.
[33] M. Kawasaki, K. Kuwano, N. Hagimoto, T. Matsuba, R. Kunitake, T. Tanaka, T. Maeyama, N. Hara, Protection from lethal apoptosis in lipopolysaccharide-induced acute lung injury in mice by a caspase inhibitor, Am. J. Pathol. 157 (2000) 597–603.
[34] J.C. Chow, D.W. Young, D.T. Golenbock, W.J. Christ, F. Gusovsky, Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction, J. Biol. Chem. 274 (1999) 10689–10692.
[35] A. Poltorak, X. He, I. Smirnova, M.Y. Liu, C.V. Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, M. Freudenberg, P. Ricciardi-Castagnoli, B. Layton, B. Beutler, Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene, Science 282 (1998) 2085–2088.
[36] S.T. Qureshi, L. Lariviere, G. Leveque, S. Clermont, K.J. Moore, P. Gros, D. Malo, Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4), J. Exp. Med. 189 (1999) 615–625.
[37] R. Medzhitov, P. Preston-Hurlburt, E. Kopp, A. Stadlen, C. Chen, S. Ghosh, C.A. Janeway Jr., MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways, Mol. Cell. 2 (1998) 253–258.
[38] M. Muzio, G. Natoli, S. Saccani, M. Levrero, A. Mantovani, The human toll signaling pathway: divergence of nuclear factor kappaB and JNK/SAPK activation upstream of tumor necrosis factor receptor-associated factor 6 (TRAF6), J. Exp. Med. 187 (1998) 2097–2101.
[39] J.M. Daun, M.J. Fenton, Interleukin-1/Toll receptor family members: receptor structure and signal transduction pathways, J. Interferon Cytokine Res. 20 (2000) 843–855.
[40] L. Li, S. Cousart, J. Hu, C.E. McCall, Characterization of interleukin-1 receptor-associated kinase in normal and endotoxin-tolerant cells, J. Biol. Chem. 275 (2000) 23340–23345.
[41] J.L. Swantek, M.F. Tsen, M.H. Cobb, J.A. Thomas, IL-1 receptor-associated kinase modulates host responsiveness to endotoxin, J. Immunol. 164 (2000) 4301–4306.
[42] H. Karahashi, F. Amano, Lipopolysaccharide (LPS)-induced cell death of C3H mouse peritoneal macrophages in the presence of cycloheximide: different susceptibilities of C3H/HeN and C3H/HeJ mice macrophages, J. Endotoxin Res. 6 (2000) 33–39.
[43] D.D. Bannerman, J.C. Tupper, R.D. Erwert, R.K. Winn, J.M. Harlan, Divergence of bacterial lipopolysaccharide pro-apoptotic signaling downstream of IRAK-1, J. Biol. Chem. 277 (2002) 8048–8053.

[44] T. Kawai, O. Adachi, T. Ogawa, K. Takeda, S. Akira, Unresponsiveness of MyD88-deficient mice to endotoxin, Immunity 11 (1999) 115–122.
[45] F.X. Zhang, C.J. Kirschning, R. Mancinelli, X.P. Xu, Y. Jin, E. Faure, A. Mantovani, M. Rothe, M. Muzio, M. Arditi, Bacterial lipopolysaccharide activates nuclear factor-kappaB through interleukin-1 signaling mediators in cultured human dermal endothelial cells and mononuclear phagocytes, J. Biol. Chem. 274 (1999) 7611–7614.
[46] K.A. Fitzgerald, E.M. Palsson-McDermott, A.G. Bowie, C.A. Jefferies, A.S. Mansell, G. Brady, E. Brint, A. Dunne, P. Gray, M.T. Harte, D. McMurray, D.E. Smith, J.E. Sims, T.A. Bird, L.A. O'Neill, Mal (MyD88-adapter-like) is required for Toll-like receptor-4 signal transduction, Nature 413 (2001) 78–83.
[47] T. Horng, G.M. Barton, R. Medzhitov, TIRAP: an adapter molecule in the Toll signaling pathway, Nat. Immunol. 2 (2001) 835–841.
[48] M. Muzio, J. Ni, P. Feng, V.M. Dixit, IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL- 1 signaling, Science 278 (1997) 1612–1615.
[49] E.W. Ades, F.J. Candal, R.A. Swerlick, V.G. George, S. Summers, D.C. Bosse, T.J. Lawley, HMEC-1: establishment of an immortalized human microvascular endothelial cell line, J. Invest. Dermatol. 99 (1992) 683–690.
[50] S. Kinoshita, L. Su, M. Amano, L.A. Timmerman, H. Kaneshima, G.P. Nolan, The T cell activation factor NF-ATc positively regulates HIV-1 replication and gene expression in T cells, Immunity 6 (1997) 235–244.
[51] K. Wiegmann, S. Schutze, T. Machleidt, D. Witte, M. Kronke, Functional dichotomy of neutral and acidic sphingomyelinases in tumor necrosis factor signaling, Cell 78 (1994) 1005–1015.
[52] R.P. Ryseck, R. Bravo, c-JUN, JUN B, and JUN D differ in their binding affinities to AP-1 and CRE consensus sequences: effect of FOS proteins, Oncogene 6 (1991) 533–542.
[53] H. Blumberg, D. Conklin, W.F. Xu, A. Grossmann, T. Brender, S. Carollo, M. Eagan, D. Foster, B.A. Haldeman, A. Hammond, H. Haugen, L. Jelinek, J.D. Kelly, K. Madden, M.F. Maurer, J. Parrish-Novak, D. Prunkard, S. Sexson, C. Sprecher, K. Waggie, J. West, T.E. Whitmore, L. Yao, M.K. Kuechle, B.A. Dale, Y.A. Chandrasekher, Interleukin 20: discovery, receptor identification, and role in epidermal function, Cell 104 (2001) 9–19.
[54] L.A. Madge, J.S. Pober, TNF signaling in vascular endothelial cells, Exp. Mol. Pathol. 70 (2001) 317–325.
[55] E.B. Kopp, R. Medzhitov, The Toll-receptor family and control of innate immunity, Curr. Opin. Immunol. 11 (1999) 13–18.
[56] N.A. Thornberry, Y. Lazebnik, Caspases: enemies within, Science 281 (1998) 1312–1316.
[57] X. Hu, E. Yee, J.M. Harlan, F. Wong, A. Karsan, Lipopolysaccharide induces the antiapoptotic molecules, A1 and A20, in microvascular endothelial cells, Blood 92 (1998) 2759–2765.
[58] E. Bonfoco, D. Krainc, M. Ankarcrona, P. Nicotera, S.A. Lipton, Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with *N*-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures, Proc. Natl. Acad. Sci. USA 92 (1995) 7162–7166.