Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.biomed-online.com

Review article

Activation of Toll-like receptors induces macrophage migration via the iNOS/Src/FAK pathway

Ming-Chei Maa [a,b,*], *Tzeng-Horng Leu* [c,d,e,**]

[a] *Institute of Molecular Systems Biomedicine, China Medical University, Taichung, Taiwan*
[b] *Institute of Medical Science, China Medical University, Taichung, Taiwan*
[c] *Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan*
[d] *Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan, Taiwan*
[e] *Center for Gene Regulation and Signal Transduction Research, College of Medicine, National Cheng Kung University, Tainan, Taiwan*

ARTICLE INFO

Article history:
Received 15 June 2011
Received in revised form
3 July 2011
Accepted 21 September 2011
Available online 8 November 2011

Keywords:
FAK
iNOS
macrophage migration
Src
Toll-like receptors

ABSTRACT

Macrophage motility is crucial in innate immunity. Toll-like receptors (TLRs), members of the interleukin-1 receptor superfamily, are a family of transmembrane pattern recognition receptors that are expressed primarily in antigen-expressing cells such as macrophages. These interleukin-1 receptors play a key role in immune response by recognizing highly conserved microbial molecules known as pathogen-associated molecular patterns. Studies have shown that when the macrophage-like cell line RAW264.7, peritoneal macrophages, and bone marrow-derived macrophages are exposed to lipopolysaccharide (LPS, TLR4 ligand), peptidoglycan (PGN, TLR2 ligand), polyinosinic–polycytidylic acid (polyI:C, TLR3 ligand), or CpG–oligodeoxynucleotides (CpG, TLR9 ligand), there is a marked increase in cell motility and in levels of activated Src (but not Fgr, Hck, and Lyn). Attenuation of Src suppressed LPS-, PGN-, polyI:C-, and CpG-elicited movement as well as the level of FAK Pi-Tyr861, which can be reversed by reintroduction of siRNA-resistant Src. Furthermore, siRNA knockdown of FAK decreased the degree of TLR-mediated motility of macrophages. Strikingly, LPS-, PGN-, polyI:C-, and CpG-enhanced Src expression, FAK Pi-Tyr861, and cell motility were greatly reduced in macrophages devoid of inducible nitric oxide synthase (iNOS, a NF-κB target), which can be induced by the aforementioned TLR ligands. Because NO can upregulate the expression and activity of Src, and because Src can mediate NF-κB activation, we hypothesize that there is a loop of signal amplification that influences the iNOS/Src/FAK axis for macrophage locomotion in response to engagement of TLRs.

1. Introduction

The proto-oncogene product, c-Src, is a nonreceptor tyrosine kinase that is encoded by the cellular homolog (*v-src*) of the transforming gene of Rous sarcoma virus. Mounting evidence indicates that c-Src functions as a co-transducer of transmembrane signals emanating from a spectrum of polypeptide growth factor receptors, including the epidermal growth factor

* *Corresponding author*. Institute of Molecular Systems Biomedicine, China Medical University, Taichung, Taiwan.
** *Corresponding author*. Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan 70101, Taiwan. E-mail addresses: mcmaa@mail.cmu.edu.tw (M.-C. Maa), tzengleu@mail.ncku.edu.tw (T.-H. Leu).

2211-8020/$ – see front matter
doi:10.1016/j.biomed.2011.10.002

receptor [1]. Although the level of Src is low in most cells, neurons and platelets are known to retain high levels and activity of Src [2,3]. However, it has been shown that targeted disruption of the *src* gene in transgenic mice does not result in any obvious phenotypic or functional abnormalities except for osteopetrosis [4]. Osteopetrosis is a skeletal abnormality caused by a defect in osteoclasts [5], which are derived from hematopoietic precursors of the monocyte/macrophage lineage [6]. Osteoclasts function as resorbers of mineralized bone during bone homeostasis [7]. Deletion of Src leads to impaired bone resorption, which can be partly attributable to reduced osteoclast motility [7]. Although Src is indispensable in osteoclast function, its low basal expression conceals its physiological significance in macrophages. Recently, we demonstrated that nitric oxide synthase (iNOS)-induced activation of the Src and FAK pathway is responsible for macrophage migration in cells exposed to lipopolysaccharide (LPS). Strikingly, similar results were obtained in macrophages that had been exposed to peptidoglycan (PGN), polyinosinic–polycytidylic acid (polyI:C), and CpG–oligodeoxynucleotides (CpG). In this review, we focus on the role that Src, FAK, and the upstream signaling protein iNOS play in macrophage motility in response to various Toll-like receptor (TLR) ligands.

2. Macrophages

Macrophages are important participants in innate immunity. They are ubiquitous and present in most tissues, and function as sensors of endogenous or exogenous danger signals by eliciting numerous immunological, inflammatory, and metabolic processes. In the developing embryo, the progenitors of macrophages differentiate in the yolk sac into monocytic tissue macrophages under the influence of granulocyte macrophage colony stimulating factor and macrophage colony-stimulating factor. In adults, pluripotent stem cells in bone marrow can develop into promonocytes (macrophage progenitors). Unlike the nonproliferating and short-lived monocyte-derived macrophages in inflammatory sites, tissue-derived macrophages maintain their numbers through homeostatic proliferation and appear to survive for at least 6 weeks [8]. With the ability to eliminate opsonized pathogens through diverse surface receptors and antigen presentation, macrophage recruitment to sites of infection as well as mobilization of activated macrophages from the infection area to the regional lymph node are crucial physiological processes in host defense. Disturbed regulation of this process results in pathological disorders such as atherosclerosis, sepsis, and autoimmune disorders.

3. Toll-like receptors

As crucial players in innate immunity, macrophages recognize invaded microorganisms via the interaction between the conserved structure called pathogen-associated molecular patterns (PAMPs) in microorganisms and their pattern-recognition receptors, such as TLRs [9]. TLRs contain N-terminal extracellular leucine-rich repeats that recognize specific pathogen components, a membrane-spanning domain that determines the cellular localization, and a C-terminal intracellular region similar to that of the interleukin-1 (IL-1) receptor known as the Toll/IL-1 receptor (TIR) domain, which is pivotal for downstream signaling [10]. A total of 12 TLRs have been identified in mice and a total of 10 have been identified in humans [9]. Certain TLRs (i.e., TLR1, 2, 4, 5, 6, and 11) are detected on the cell surface, whereas others (i.e., TLR3, 7, 8, and 9) are found almost exclusively in intracellular compartments such as endosomes [9,11]. TLR2 can recognize PGN, a major bacterial cell wall component. TLR4 acts as a signaling receptor for LPS, an outer membrane component of Gram-negative bacteria [12,13]. TLR3 can sense viral double-stranded RNA [14] and TLR9 can detect the unmethylated CpG that are frequently found in bacteria, but not in vertebrate DNA [15]. Subsequent to recognition of a PAMP, TLR will recruit a combination of TIR-containing adaptors, including MyD88, TRIF, TRAM, and TIRAP/Mal. It is well-established that TLR9 utilizes MyD88, TLR3 needs TRIF, TLR2 requires MyD88 and TIRAP, and that TLR4 uses all four of the aforementioned adaptors [10]. Through individually preferential adaptors, engagement of TLRs triggers downstream signaling pathways that activate MAP kinase or NF-κB, which in turn produces proinflammatory cytokines required for host defensive strategies [9,11,16].

4. Inducible nitric oxide synthase

Nitric oxide (NO) is a unique diffusible messenger molecule that is produced via the oxidation of L-arginine by enzymes known as nitric oxide synthases (NOSs) [17]. Three distinct isoforms of the enzyme have been identified and characterized. Whereas Ca^{2+}/calmodulin can regulate the activity of constitutively expressed neuronal (n) and endothelial (e) NOS, the activity of inducible (i) NOS is independent of Ca^{2+}/calmodulin and is only induced by bacterial products as well as inflammatory cytokines. A low level of constitutively produced NO is a crucial mediator for a variety of physiological functions including regulation of neurotransmission, vasodilation, smooth muscle relaxation, and inhibition of platelet aggregation. In contrast, activated TLR pathways trigger a high level of NO production by transcriptional and post-transcriptional mechanisms that enhance the expression of iNOS in macrophages and other effector cells [18]. It is also well-established that some pathological processes such as inflammation and tumor development can be induced by sustained, chronically produced NO. Soluble guanylyl cyclase (sGC) is a cytosolic, heme-containing heterodimer comprising α and β subunits whose activity can be modulated by NO. Binding of NO to the sGC heme prosthetic group activates sGC, which in turn leads to the conversion of guanosine 5′-triphosphate to the secondary intracellular messenger cGMP. Accumulation of cGMP results in transmission of NO signals to downstream effectors such as cGMP-regulated phosphodiesterase, cGMP-dependent protein kinase, and cGMP-gated cation channels, leading to physiological and pathological responses [19].

5. The Src family kinases

Cellular Src is a 60-kDa nonreceptor, membrane-associated tyrosine kinase that serves as the archetype for a group of Src

family tyrosine kinases (SFKs), including Yes, Fyn, Lck, Fgr, Hck, Lyn, and Blk [20]. Several SFKs are expressed in multiple isoforms because of the use of alternative start codons or alternative splicing patterns. Alignment of the amino acid sequence of the SFK members reveals their structural similarity. The conserved regions comprise an N-terminal myristylation signal that is responsible for the association of SFK with the plasma membrane; a unique region where the greatest sequence divergence among SFK members occurs; an SH3 domain and an SH2 domain that are responsible for direct protein–protein interaction; an SH2/kinase linker; an enzymatic domain; and a C-terminal regulatory region. Tyr416 and Tyr527 are two important phosphorylation sites that are located within the kinase domain and the C-terminal regulatory region, respectively. Unlike the self-mediated phosphorylation of Tyr416 (Pi-Tyr416) that enhances the catalytic activity of Src, CSK (a C-terminal Src kinase)-mediated phosphorylation of Tyr527 (Pi-Tyr527) downregulates Src activity. Based on mutational studies and X-ray crystallographic analyses, a model in which Src is held in an inactive conformation by the intramolecular association between SH2 and Pi-Tyr527 as well as SH3 and the SH2/kinase linker is proposed [21]. Disruption of these interactions will cause Src to become active and mediate Pi-Tyr416.

6. Constitutive expression of myeloid-specific Src family kinases

Src, Yes, and Fyn are widely expressed in most tissues, whereas the distribution of other members of the SFK family exhibits a more tissue-specific distribution, mainly in cells of hematopoietic lineage [22]. Lyn, Fgr, and Hck are myeloid-specific SFK members that are predominantly expressed in macrophages. Given that the release of eicosanoid mediators from LPS-treated RAW264.7 macrophages and the development of tumoricidal activity of LPS-stimulated murine peritoneal macrophages (PEMs) were impaired by herbimycin A (a tyrosine kinase inhibitor) [23] and that TLR4 did not possess intrinsic tyrosine kinase activity, Lyn, Fgr, and Hck seemed to be responsible for LPS-mediated tyrosyl phosphorylation and macrophage activation. Surprisingly, macrophages derived from mice with the combined deficiency of Lyn, Fgr, and Hck still retain full LPS responsiveness [24], a finding that implies that these three myeloid SFKs are not obligatory for LPS-evoked macrophage activation and that their exerted effects might be compensated by other tyrosine kinases.

7. Inducible expression of Src

Mounting evidence indicates that sustained activity of SFKs is indispensable for LPS-elicited responses [25]. Because knockout of Lyn, Fgr, and Hck does not hamper macrophage activation, nonmyeloid-specific SFKs are likely to compensate for the loss of their myeloid-predominant relatives. To circumvent the obstacle of their low basal protein expression as well as the need for their sustained activation, the potential tactic that macrophages might utilize is to have the nonmyeloid SFK members become LPS-inducible. Given that Src is indispensable for resorbing activity of macrophage-related osteoclasts, Src is likely to be the elusive and long-sought after SFK responsible for LPS-mediated macrophage activation. Indeed, LPS augments the expression of Src in both RAW264.7 macrophages and PEMs in a time-dependent manner. The upregulation of Src observed in macrophages recovered from LPS-challenged rats further implicates its physiological significance [25]. In agreement with the results obtained from $lyn^{-/-}fgr^{-/-}hck^{-/-}$ macrophages, Src with its expression induced by LPS, is an attractive, compensating candidate for the defect of Lyn, Fgr, and Hck. Intriguingly, Src induction is also detected in RAW264.7 macrophages, PEMs and bone marrow-derived macrophages (BMDMs) treated with PGN, polyI:C, and CpG. This LPS-, PGN-, polyI:C-, and CpG-induced Src expression can be attributed to the increased level of the *src* transcript [26]. In contrast, the expression of Lyn, Fgr, and Hck is almost unaltered in RAW264.7, PEMs, and BMDMs in response to various TLR ligands [26]. The inducible characteristic of Src implicates its importance in relaying signals triggered by diverse PAMPs.

8. Focal adhesion kinase

Focal adhesion kinase (FAK) was initially identified as an Src target by immunological screening of the cDNA library with a panel of monoclonal antibodies directed against putative v-Src substrates [27]. FAK is an intracellular tyrosine kinase that participates in a wide-ranging spectrum of integrin-elicited biological activities including proliferation, survival, cell adhesion, and migration [28]. Deficiency of FAK results in embryonic lethality, characterized by impaired fibroblast motility [29]. Upon integrin stimulation, FAK becomes activated and autophosphorylated at Tyr397, the binding site for Src. This process facilitates Src-mediated FAK phosphorylation on several tyrosine residues including Tyr861, whose phosphorylation promotes FAK Pi-Tyr397 [30]. FAK was initially thought to be absent or expressed at low levels in monocyte/macrophages [31]. However, studies have shown that FAK is present in macrophages [32]. To study the physiological significance of FAK in macrophages, Owen et al. [33] generated myeloid-specific conditional FAK-knockout mice. Intriguingly, macrophages devoid of FAK displayed motility defects that coincided with reduced levels of adhesion turnover, elevated protrusive activity at the cell periphery, and a marked inability to form lamellipodia. In addition, recruitment of macrophages to sites of inflammation was shown to be impaired in the absence of FAK [33]. These findings indicate that FAK plays a major role in the regulation of macrophage mobility.

9. The role of iNOS/Src/FAK axis in Toll-like receptor-mediated macrophage motility

Based on the findings from seminal studies that NOS inhibitors suppress human monocyte chemotaxis [34] and that decreased polymorphonuclear granulocyte extravasation in peritoneal cavity was detected in zymosan-challenged iNOS-deficient mice [35], NO is now believed to be a key regulator of leukocyte locomotion during inflammation and tissue injury. Indeed, consistent with reduced LPS-, PGN-, polyI:C-, and CpG-

mediated macrophage migration by 1400 W (an iNOS-selective inhibitor), suppressed migration evoked by these TLR agonists was observed in iNOS null PEMs [26,36]. This iNOS deficiency caused defects in LPS-, PGN-, polyI:C-, and CpG-induced motility was not due to the general inability of cells to mobilize, because analogs of iNOS downstream mediators, SNAP (a NO donor) and 8-bromo-cGMP (8-Br-cGMP, a permeable cGMP analogue), were able to augment the migratory ability of iNOS-deficient macrophages in a way comparable to that of wild-type macrophages. These results indicated the participation of the NO/cGMP pathway in macrophage movement in response to activated TLRs [26,36]. Given that LPS-, PGN-, polyI:C-, and CpG-evoked macrophage motility was inhibited by PP2 (an SFK inhibitor), one interesting question turned out to be the relation between iNOS and SFKs. Because (1) LPS-mediated Src induction was labile to 1400 W and ODQ (an sGC inhibitor); (2) Src, but not its myeloid relatives, could be induced by SNAP and 8-Br-cGMP; (3) there was a concomitant decrement of Src and cell migration in iNOS-null PEMs exposed to LPS, PGN, polyI:C, and CpG; and (4) the motility and Src induction in LPS-stimulated PEMs devoid of iNOS could be restored by SNAP and 8-Br-cGMP to a level comparable to that detected in wild-type PEMs inspired us to question whether Src might be the main SFK required for TLR-mediated NO/cGMP-dependent macrophage locomotion. Indeed, Src attenuation led to reduced LPS-, PGN-, polyI:C-, CpG-, and SNAP-evoked motility in RAW264.7 macrophages, and ectopically expressed avian Src rescued this defect. These results support the hypothesis that Src induction mediated by NO/cGMP is involved in macrophage mobilization in response to engagement of TLRs. As a downstream target of Src, the elevated level of Tyr861 phosphorylated FAK in LPS-, PGN-, polyI:C-, and CpG-treated macrophages was suppressed by Src attenuation and reversed by reintroduction of siRNA-resistant Src. The reduction of LPS-elicited FAK Pi-Y861 in both RAW264.7 and rat PEMs pretreated with iNOS inhibitor indicated that Src-mediated FAK Pi-Y861 might be responsible for LPS/iNOS-induced cell migration. Indeed, LPS-mediated FAK Pi-Y861 was inhibited in PEMs devoid of iNOS. Similar results were obtained in iNOS-null PEMs that had been stimulated with PGN, polyI:C, or CpG. Notably, FAK attenuation impaired macrophage motility in response to LPS, PGN, polyI:C, and CpG, revealing the importance of the iNOS/Src/FAK axis in TLR ligand-elicited macrophage migration.

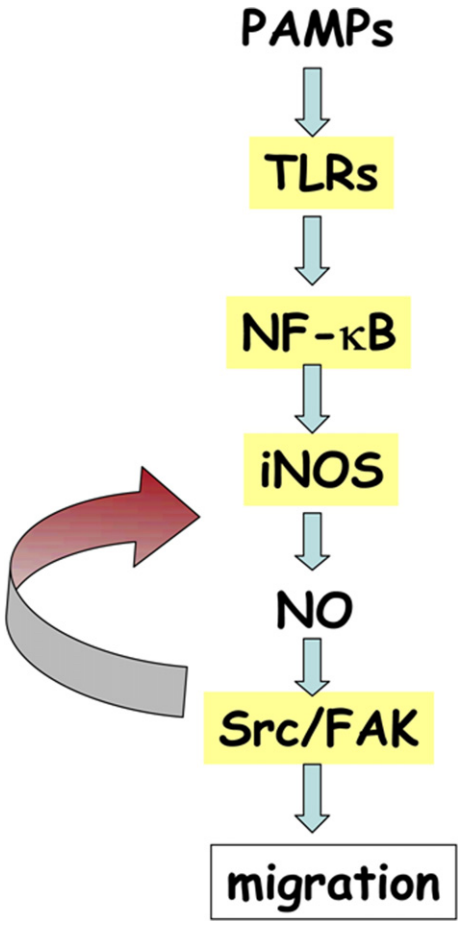

Fig. 1 – Engagement of TLRs leads to macrophage migration. As various PAMPs (i.e., LPS, PGN, polyI:C, and CpG) activate their respective TLRs, NF-κB will be activated and increase the expression of iNOS. NO, the second messenger generated by iNOS, will elevate the expression and activity of Src, which causes FAK activation and macrophage motility. Because Src increases the activity of NF-κB, thereby a signal amplification loop can be constructed. This model reveals a general mechanism utilized in macrophages when their TLRs are occupied.

10. Conclusions and future perspectives

In contrast to the large repertoire of rearranged receptors utilized by B and T cells in adaptive immunity, the innate immune response mechanisms recognize microorganisms via a limited number of germline-encoded PAMP recognition receptors including TLRs. Irrespective of their localization and utilization of different TIR-containing adaptors, engagement of TLRs lead to the activation of NF-κB and increased expression of iNOS, and proinflammatory cytokines. Given that the aforementioned TLRs are located on either plasma membranes (i.e., TLR2 and TLR4) or endosome (i.e., TLR3 and TLR9), and their mediated signaling pathways can be divided into MyD88-dependent (i.e., TLR2, TLR4, and TLR9) and -independent (i.e., TLR3) pathways, the iNOS-mediated upregulation of Src and activation of FAK in response to engagement of various TLRs might be a general mechanism of macrophage motility. Given that Src elevated the activity of NF-κB [37,38], thereby a signal amplification loop can be established. With what we have learned from studies conducted in murine RAW264.7 macrophage cell line as well as PEMs recovered from normal versus iNOS$^{-/-}$ mice, a simple model illustrating the responsible mechanism for TLR ligands-ignited, iNOS-dependent macrophage motility was proposed in Fig. 1. Considered mobilization of activated macrophages from infection sites to the regional lymph node where they present antigens to naïve CD4^{+} T cells is an essential and crucial step in activating the adaptive immunity [11]; therefore, we speculate that the iNOS –> Src –> FAK signaling pathway might be an important general mechanism utilized by TLR-engaged macrophages required in host defenses. Considering the fact that the accumulation of macrophages contributes to progression of diseases such as cancer and chronic inflammatory diseases, our data highlight the potential

of iNOS, Src, and FAK as therapeutic targets to control infection, inflammation, and the maintenance of tissue homeostasis.

Acknowledgments

This work was supported in part by grants from the National Science Council (NSC 98-2311-B-039-002-MY3 to M.-C.M.), National Health Research Institute (NHRI-EX98-9828B1 to T.-H.L.), and China Medical University (CMU 99-S-07 to M.-C.M.).

REFERENCES

[1] Ishizawar R, Parsons SJ. c-Src and cooperating partners in human cancer. Cancer Cell 2004;6:209–14.
[2] Cotton PC, Brugge JS. Neural tissues express high levels of the cellular src gene product pp60$^{c\text{-}src}$. Mol Cell Biol 1983;3:1157–62.
[3] Golden A, Nemeth SP, Brugge JS. Blood platelets express high levels of the pp60$^{c\text{-}src}$-specific tyrosine kinase activity. Proc Natl Acad Sci USA 1986;83:852–6.
[4] Soriano P, Montgomery C, Geske R, Bradley A. Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. Cell 1991;64:693–702.
[5] Marks Jr SC. Congenital osteopetrotic mutations as probes of the origin, structure, and function of osteoclasts. Clin Orthop Relat Res 1984;189:239–63.
[6] Ash P, Loutit JF, Townsend KM. Osteoclasts derived from haematopoietic stem cells. Nature 1980;283:669–70.
[7] Sanjay A, Houghton A, Neff L, Didomenico E, Bardelay C, Antoine E, et al. Cbl associates with Pyk2 and Src to regulate Src kinase activity, alpha(v)beta(3) integrin-mediated signaling, cell adhesion, and osteoclast motility. J Cell Biol 2001;152:181–95.
[8] Plowden J, Renshaw-Hoelscher M, Engleman C, Katz J, Sambhara S. Innate immunity in aging: impact on macrophage function. Aging Cell 2004;3:161–7.
[9] Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell 2010;140:805–20.
[10] Kawai T, Akira S. Signaling to NF-κB by toll-like receptors. Trends Mol Med 2007;13:460–9.
[11] Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 2006;124:783–801.
[12] Akira S, Sato S. Toll-like receptors and their signaling mechanisms. Scand J Infect Dis 2003;35:555–62.
[13] Kopp E, Medzhitov R. Recognition of microbial infection by Toll-like receptors. Curr Opin Immunol 2003;15:396–401.
[14] Jin MS, Lee JO. Structures of the toll-like receptor family and its ligand complexes. Immunity 2008;29:182–91.
[15] Krieg AM. Development of TLR9 agonists for cancer therapy. J Clin Invest 2007;117:1184–94.
[16] Beutler BA. TLRs and innate immunity. Blood 2009;113: 1399–407.
[17] Murad F. Nitric oxide and cyclic GMP in cell signaling and drug development. N Eng J Med 2006;355:2003–11.
[18] Bogdan C. Nitric oxide and the immune response. Nat Immunol 2001;2:907–16.
[19] Lucas KA, Pitari GM, Kazerounian S, Ruiz-Stewart I, Park J, Schulz S, et al. Guanylyl cyclases and signaling by cyclic GMP. Pharmacol Rev 2000;52:375–414.
[20] Leu TH, Maa MC. Functional implication of the interaction between EGF receptor and c-Src. Front Biosci 2003;8: s28–38.
[21] Xu W, Harrison SC, Eck MJ. Three-dimensional structure of the tyrosine kinase c-Src. Nature 1997;385:595–602.
[22] Lowell CA. Src-family kinases: rheostats of immune cell signaling. Mol Immunol 2003;41:631–43.
[23] Weinstein SL, Gold MR, DeFranco AL. Bacterial lipopolysaccharide stimulates protein tyrosine phosphorylation in macrophages. Proc Natl Acad Sci USA 1991;88:4148–52.
[24] Meng F, Lowell CA. Lipopolysaccharide (LPS)-induced macrophage activation and signal transduction in the absence of Src-family kinases Hck, Fgr and Lyn. J Exp Med 1997;185:1661–70.
[25] Leu TH, Charoenfuprasert S, Yen CK, Fan CW, Maa MC. Lipopolysaccharide-induced c-Src expression plays a role in nitric oxide and TNFα secretion in macrophages. Mol Immunol 2006;43:308–16.
[26] Maa MC, Chang MY, Li J, Li YY, Hsieh MY, Yang CJ, et al. The iNOS/Src/FAK axis is critical in Toll-like receptor-mediated cell motility in macrophages. Biochim Biophys Acta 2011; 1813:136–47.
[27] Schaller MD, Borgman CA, Cobb BS, Vines RR, Reynolds AB, Parsons JT. pp125^{FAK} a structurally distinctive protein-tyrosine kinase associated with focal adhesions. Proc Natl Acad Sci USA 1992;89:5192–6.
[28] Parsons JT. Focal adhesion kinase: the first ten years. J Cell Sci 2003;116:1409–16.
[29] Ilic D, Furuta Y, Kanazawa S, Taketa N, Sobue K, Nakatsuji N, et al. Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature 1995;377:539–44.
[30] Maa MC, Leu TH. Tyr-863 phosphorylation enhances focal adhesion kinase autophosphorylation at Tyr-397. Oncogene 2002;21:6992–7000.
[31] Lin TH, Yurochko A, Kornberg L, Morris J, Walker JJ, Haskill S, et al. The role of protein tyrosine phosphorylation in integrin-mediated gene induction in monocytes. J Cell Biol 1994;126:1585–93.
[32] Rovida E, Lugli B, Barbetti V, Giuntoli S, Olivotto M, Sbarba PD. Focal adhesion kinase is redistributed to focal complexes and mediates cell spreading in macrophages in response to M-CSF. Biol Chem 2005;386:919–29.
[33] Owen KA, Pixley FJ, Thomas KS, Vicente-Manzanares M, Ray BJ, Horwitz AF, et al. Regulation of lamellipodial persistence, adhesion turnover, and motility in macrophages by focal adhesion kinase. J Cell Biol 2007;179:1275–87.
[34] Belenky SN, Robbins RA, Rubinstein I. Nitric oxide synthase inhibitors attenuate human monocyte chemotaxis in vitro. J Leukocyte Biol 1993;53:498–503.
[35] Ajuebor MN, Virag T, Flower RJ, Perretti M, Szabo C. Role of inducible nitric oxide synthase in the regulation of neutrophil migration in zymosan-induced inflammation. Immunology 1998;95:625–30.
[36] Maa MC, Chang MY, Chen YJ, Lin CH, Yu CJ, Yang YL, et al. Requirement of inducible nitric-oxide synthase in lipopolysaccharide-mediated Src induction and macrophage migration. J Biol Chem 2008;283:31408–16.
[37] Abu-Amer Y, Ross FP, McHugh KP, Livolsi A, Peyron JF, Teitelbaum SL. Tumor necrosis factor-α activation of nuclear transcription factor-κB in marrow macrophages is mediated by c-Src tyrosine phosphorylation of IκBα. J Biol Chem 1998; 273:29417–23.
[38] Hung WC, Chen JJ, Chen CC. c-Src-dependent tyrosine phosphorylation of IKKβ is involved in tumor necrosis factor-α-induced intercellular adhesion molecule-1 expression. J Biol Chem 2003;278:9944–52.