NF-κB is a key modulator in the signaling pathway of *Borrelia burgdorferi* BmpA-induced inflammatory chemokines in murine microglia BV2 cells

ZHENYU ZHAO¹, LVYAN TAO¹,², AIHUA LIU¹,⁶, MINGBIAO MA¹,⁷, HAIYI LI⁸, HUA ZHAO¹, JIARU YANG¹, SHIMING WANG¹, YIRONG JIN¹, XIAN SHAO¹ and FUKAI BAO¹,⁷

¹School of Basic Medical Sciences; ²Department of Biochemistry and Molecular Biology; ³Yunnan Province Key Laboratory for Tropical Infectious Diseases in Universities; ⁴The Institute for Tropical Medicine; ⁵Yunnan Province Integrative Innovation Center for Public Health, Diseases Prevention and Control; ⁶Yunnan Demonstration Base of International Science and Technology Cooperation for Tropical Diseases; ⁷Department of Microbiology and Immunology; ⁸Faculty of Public Health, Kunming Medical University, Kunming, Yunnan 650500, P.R. China

Received May 27, 2017; Accepted November 15, 2017

DOI: 10.3892/mmr.2018.8526

**Abstract.** Lyme disease, caused by the bacterial spirochete *Borrelia burgdorferi*, is a tick-borne zoonosis. Lyme neuroborreliosis is a principal manifestation of Lyme disease and its pathogenesis remains incompletely understood. Recent studies have demonstrated that *Borrelia burgdorferi* lipoproteins caused similar inflammatory effects as exhibited in Lyme neuroborreliosis. Basic membrane protein A (BmpA) is one of the dominant lipoproteins in the *Borrelia burgdorferi* membrane. In addition, nuclear factor κ-B (NF-κB) modulates the regulation of gene transcription associated with immunity and inflammation; however, in unstimulated cells, NF-κB is combined with the inhibitor of NF-κB (IκB-β). Therefore, it was hypothesized that NF-κB may be associated with BmpA-induced inflammation and the occurrence of Lyme neuroborreliosis. Therefore, the aim of the present study was to investigate the role that NF-κB serves in the signaling pathway of BmpA-induced inflammatory chemokines. The present study measured the expression levels of NF-κB, IκB-β and inflammatory chemokines following recombinant BmpA (rBmpA) stimulation of murine microglia BV2 cells. Following stimulation with rBmpA, concentrations of pro-inflammatory cytokines including C-X-C motif chemokine 2, C-C motif chemokine (CCL) 5 and CCL22 were determined by ELISA analysis. Reverse transcription-quantitative polymerase chain reaction and western blotting were used to detect the expression levels of NF-κB p65 and IκB-β. The data demonstrated that concentrations of these chemokines in cell supernatants increased significantly following rBmpA stimulation. NF-κB was overexpressed, but IκB-β expression was significantly decreased. In conclusion, these results suggested that NF-κB serves an important stimulatory role in the signaling pathway of BmpA-induced inflammatory chemokines in BV2 cells.

**Introduction**

Lyme disease (LD) is a multisystem inflammatory, tick-borne disease resulting from infection with *Borrelia burgdorferi* (1,2). LD is present in >80 countries, including China. A total of ~3,000,000 cases of LD are reported each year worldwide, representing an incidence of ~0.111% (2,3). LD seriously affects human health, in addition to economic development. As a result, this widespread disease has been given attention regarding its prevention and treatment by the World Health Organization (3). Lyme neuroborreliosis (LNB) is a principal manifestation of LD and is caused by the inflammatory effects of the spirochete *B. burgdorferi* on the nervous system. LNB causes extensive neurological damage and encephalitic memory impairment, even leading to dementia and personality disorders. Given the high disability rate caused by the disease, researchers and clinicians are giving more concentrated attention to LNB (4,5); however, its pathogenesis remains incompletely understood. It is generally accepted that LNB is caused by an autoimmune response triggered by molecular mimicry (6,7).

Microglial cells, the resident macrophage cells within the central nervous system (CNS), are important in initiating an immune response to microbial products (8). As a previous study demonstrated, CNS damage and inflammation may activate microglia (9). Reactive microglia produce various cytokines and chemokines causing an acute inflammatory reaction, which leads to neuronal damage and apoptosis. Microglia...
cells are hypersensitive to CNS damage. These cells multiply rapidly, begin to express major histocompatibility complex proteins, migrate and subsequently differentiate into phagocytes that secrete cytokines and other toxic substances (10,11).

Previous studies have suggested that B. burgdorferi basic membrane protein A (BmpA), one of the primary B. burgdorferi pathogenic substances, exhibits a potent pro-inflammatory effect (11-13). In the authors' previous study, an Escherichia coli expression system was established and purified recombinant BmpA (rBmpA) was successfully obtained. When stimulated with rBmpA, murine microglia BV2 cells produced pro-inflammatory chemokines, including C-X-C motif chemokine 2 (CXCL2), C-C motif chemokine (CCL) 5 and CCL22, causing inflammation and damage in mice (13). However, the signal transduction mechanism involved is unclear (13).

The nuclear factor-κB (NF-κB) is a protein complex which was found to specifically bind to the κB sequence (GGGACTTCC) of the immunoglobulin K enhancer and regulated the expression of target genes (14-16). NF-κB has received widespread interest from researchers for its crucial role in the immune system. It has been demonstrated in unstimulated cells that inhibitor of NF-κB (IκB-β) combines with NF-κB and maintains NF-κB in an inactive state. The stimuli that cause NF-κB activation target IκB-β for degradation via a phosphorylation-dependent ubiquitination process (16,17). With the degradation of IκB-β, NF-κB is activated, entering the cell nucleus where it encounters a promoter with DNA binding sites for NF-κB (17,18). The activation of target gene transcription induces the expression of specific mRNAs, and the production of cytokines and chemokines, thereby regulating the activation, multiplication, infiltration, chemotaxis and secretion of immune cells (18-20). As rBmpA stimulates the production of CXCL and CCL chemokines in microglia, it was hypothesized that NF-κB may be closely associated with rBmpA-induced chemokine production in BV2 cells (13).

Based on the conclusions of the authors' previous study demonstrating that rBmpA stimulates the production of inflammatory chemokines in BV2 cells, the present study continued to investigate the signal transduction mechanism of LNB (13). Additionally, the present study investigated the role of NF-κB in the signaling pathway of rBmpA-induced inflammatory chemokines and provides a scientific basis for the prevention and treatment of LBN.

Materials and methods

rBmpA preparation. Recombinant BmpA proteins were produced in E. coli using the bacterial expression vector pGEX-6P1 (GE Healthcare, Chicago, IL, USA). Expression, purification and enzymatic cleavage of the glutathione S-transferase fusion proteins were performed as previously described (14,15).

Cell culture and groups. BV2 cells (Kunming Medical University Biological Engineer Center, Kunming, China) were cultured in Dulbecco’s modified Eagle’s medium (DMEM)/high glucose with 5% cattle serum (CS; HyClone, Logan, UT, USA), 1% penicillin/streptomycin (Sangon Biotech Co., Ltd., Shanghai, China) at 37˚C with 5% CO₂. BV2 cells were seeded into a 6-well plate at a concentration of 3x10⁵ cells/ml. Supernatants were discarded until the cells fully adhered to the plate. Cells were divided into three groups (negative control group, positive control group and experimental group), and were stimulated with 2 ml/well PBS with 5% CS-DMEM medium, 1 µg/ml lipopolysaccharide (LPS; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), and 20 µg/ml rBmpA, respectively. Radioimmunoprecipitation assay buffer (high; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) was used to lyse the cells and cell lysates were collected at 12, 24 and 48 h following stimulation.

ELISA analysis. Culture supernatants were collected at 6, 12 and 24 h following stimulation with rBmpA and were analyzed by ELISA. Mouse CXCL2 (MIP-2), mouse CCL22 (MDC) and mouse CCL5 (RANTES) ELISA kits (RayBiotech, Inc., Norcross, GA, USA; cat. nos. P10889, O88430 and P30882) was used following manufacturer's protocol. A Bio-Rad microplate reader (Model 680; Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to measure the absorbance at 450 nm.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Harvested cells were lysed with RNAiso Plus (Takara Biotechnology Co., Ltd., Dalian, China) and RNA was extracted, following the manufacturer’s protocol. Total RNA was reverse transcribed to cDNA. The RT-qPCR was performed using the real-time PCR System Cfx-Connect (Bio-Rad Laboratories, Inc.). The reaction conditions of the RT-qPCR were as follows: Denaturation at 95˚C for 30 min, annealing at 58˚C for 1 h and extension at 65˚C for 5 sec. The total volume used in the PCR was 25 µl, including 2 µl cDNA, 12.5 µl SYBR (Takara Biotechnology, Co., Ltd.), 8.5 µl dH₂O, 1 µl forward primer and 1 µl reverse primer (Tsingke Biotech Co., Ltd., Kunming, China). The sequences of the specific primers used to amplify NF-κB p65 and GAPDH were as follows: NF-κB p65 forward, 5'−GCT ACA CAG AGG CCA CTGGA −3' and reverse, 5'−TCCCGGG AG TT CAC TCT ATG TTG−3'; IκB-β forward, 5'−GGGAA CAGTC AG TCT GTACCA−3' and reverse, 5'−GCACAT CCG TCT GTT TTT−3'; GAPDH forward, 5'−TCC CAG AGC TGA ACC GGAAG−3' and reverse, 5'−TCAGTG GGC CCTCAG ATGC−3'. The gene expression level was calculated using the method of 2−ΔΔCt (21-23).

Western blot analysis. Total protein quantitation analysis was used as the internal control for quantitative fluorescent western blot analysis (24-27). Cells were lysed in radioimmunoprecipitation lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) with 1 mM phenylmethylsulfonyl fluoride (PVDF) membranes (0.2 µm; Bio-Rad Laboratories, Inc.) using Trans-Blot Turbo Transfer System (p65, 15 V in 4-min protocol; IκB-β, 15 V in 4-min protocol; Bio-Rad Laboratories, Inc.) with Trans-Blot Turbo Midi Transfer
Packs (Bio-Rad Laboratories, Inc.). The PVDF membranes were blocked with 5% skim milk at 37˚C for 2 h. Blots were incubated with specific primary antibodies of NF-κB p65 (cat. no. ab16502; 1:2,000; Abcam, Cambridge, UK) or IκB-β (cat. no. ab7574; 1:1,000; Abcam) overnight at 4˚C, and subsequently incubated with goat anti-rabbit secondary antibodies (cat. no. BS13278; p65 1:10,000; Nanjing Bioworld Biotech Co., Ltd., Nanjing, China; cat. no. ab150077; IκB-β; 1:3,000; Abcam) at room temperature for 2 h. The blots were washed three times between the primary and secondary antibody incubations. The immunoreactive bands were imaged using an infrared imaging system (Bio-Rad Laboratories, Inc.).

Statistical analysis. The results are expressed as the mean ± standard error of the mean and were analyzed using the GraphPad Prism 6 software package (GraphPad Software, Inc., La Jolla, CA, USA) (26). Two-way analysis of variance was used to calculate the P-values, multiple comparison between the groups was performed using the Student-Newman-Keuls method. P<0.05 was considered to indicate a statistically significant difference.

Results

rBmpA significantly stimulates the production of the inflammatory cytokines CXCL2, CCL5 and CCL22 in BV2 cells. To determine whether rBmpA induces the production of pro-inflammatory chemokines in BV2 cells, analysis of CXCL2, CCL5 and CCL22 concentrations in the cell supernatants was assayed by ELISA. The results demonstrated that rBmpA significantly increased CXCL2, CCL5 and CCL22 expression at all time points tested compared with the PBS only group (P<0.05; Fig. 1). The expression of the three chemokines demonstrated an increasing trend between 12 and 48 h, except for a slight decline in CCL5 between 24 and 48 h (Fig. 1B).

rBmpA stimulation induces an increase in NF-κB and decrease in IκB-bmRNA in BV2 cells. rBmpA stimulation significantly increased CXCL2, CCL5 and CCL22 expression compared with the PBS control (P<0.05). Therefore, the rBmpA-induced nuclear translocation of NF-κB p65 and degradation of cytosolic IκB-β was investigated. RT-qPCR was used to analyze the expression of NF-κB p65 and IκB-β mRNA. As exhibited in Fig. 2, the expression of NF-κB p65 and IκB-β mRNA in the group treated with rBmpA was significantly increased (P<0.01) compared with the PBS-only control at 12, 24 and 48 h and rBmpA treatment significantly decreased the intracellular levels of IκB-β (P<0.05).

rBmpA stimulation induces an increase in NF-κB p65 and the degradation of IκB-bproteins in BV2 cells. Western blot analysis was used to detect the protein expression levels of NF-κB p65 and IκB-β in BV2 cells. As exhibited in Fig. 3, following stimulation with rBmpA for 12, 24 and 48 h, NF-κB p65 protein expression was significantly increased (P<0.05) and the protein level of IκB-β was significantly decreased (P<0.05) in the rBmpA treatment group compared with the PBS-only control. Increased expression levels of NF-κB p65 protein were observed in the rBmpA and LPS treatment groups. rBmpA significantly decreased the intracellular levels of IκB-β protein compared with the PBS control (P<0.05).
Discussion

NF-κB is a nuclear transcription factor which regulates the immune response, stress response and apoptosis (28). Upregulation of NF-κB has been demonstrated in various tumors, pulmonary disease and hepatic disease. Previous in vitro studies have suggested that NF-κB is a key signal transduction molecule in the downstream pathway of Toll-like receptors (TLRs) (29,30). Once the TLRs are stimulated by various pathogenic factors, the degradation of IκB-β is initiated and NF-κB is free to enter the cell nucleus, where NF-κB combines with the IκB motif controlling the transcription of a number of cytokines, including tumor necrosis factor-α (TNF-α), CXCL13 and interleukin (IL)-6. Studies have demonstrated that NF-κB is a transducer of various common inflammatory signaling pathway reactions (27-30). Therefore, methods of inhibiting NF-κB signaling have potential therapeutic applications in inflammatory diseases. At present, a number of anti-inflammatory drugs and anti-rheumatic drugs, including corticosteroids and aspirin, have been confirmed to be inhibitors of NF-κB activation (31,32).
It has been reported that the *B. burgdorferi* bmpA/B gene operon exhibits the most marked upregulation in mouse and human joints (33). BmpA possesses stimulatory activity through functional domains that trigger the inflammatory response. For example, the activated NF-κB kinase signalling pathway in articular-synovial cells was demonstrated to produce pro-inflammatory cytokines including TNF-α and IL-1β, triggering prostaglandin E2 receptor EP4 subtype (PGE-2) (15,34). Rasley et al (35) confirmed that *B. burgdorferi* was an important stimulus that induced microglia to produce IL-6, TNF-α and PGE-2, which was associated with increased expression of NF-κB, TLR2 and cluster of differentiation 14 in BV2 cells. By activating signaling molecules, *B. burgdorferi* stimulated immune cells to produce multiple inflammatory substances (35). Further investigation by Sun et al (36) suggested that substance P was able to prompt *B. burgdorferi*-induced NF-κB activation by upregulating NF-κB subunit p65, causing a significant increase in the production of inflammatory cytokines (36). NF-κB serves an important role in the inflammatory cytokine signaling pathway of immune cells. However, rBmpA and NF-κB associated pathogenesis in LNB, to the best of the authors' knowledge, has not been reported in the literature. A recent report indicated that rBmpA induced activation of BV2 cells with a concentration-dependent secretion of inflammatory chemokines (13). Therefore, rBmpA may be associated with the inflammatory chemokines produced by BV2 cells and the occurrence of LNB. However, the precise mechanism of the signal transduction pathway remains uncertain.

In the present study, whether NF-κB was a key modulator in the inflammatory chemokines (CXCL2, CCL5 and CCL22) signaling pathway stimulated by rBmpA in BV2 cells was investigated. The results of the present study demonstrated that CXCL2, CCL5, CCL22, NF-κB mRNA and NF-κB protein increased and the protein and mRNA levels of IkB-β decreased following stimulation with rBmpA in BV2 cells. It was demonstrated that NF-κB served a key role in the signaling pathways stimulated by rBmpA in BV2 cells, resulting in the production of various inflammatory chemokines. This result increases the understanding of the pathogenesis of LNB. Future studies will investigate the key proteins in inflammatory chemokine signaling pathways stimulated by rBmpA and, most importantly, investigate whether these signaling pathways also modulate the pathogenesis of Lyme arthritis, dermatitis and carditis. The long-term aim is to investigate whether preventive and therapeutic medicines for LNB may be developed that target these proteins (37).

In conclusion, following stimulation by rBmpA, BV2 cells overexpressed NF-κB and exhibited significantly reduced expression of IkB-β in the inflammatory cytokine-signaling pathway. This result suggested that NF-κB is important in the inflammatory cytokines signaling pathways stimulated by rBmpA and may be associated with the occurrence of LNB. The present study further clarified the mechanism underlying the rBmpA-induced inflammatory chemokines signaling pathway in microglial cells, and provides a scientific basis for the prevention and treatment of LNB.

Acknowledgements

The present study was supported by the National Natural Science Foundation of China (grant nos. 81560596 and 31560005) and the Natural Foundation of Yunnan Province (grant nos. 2012FB011, 2014FA011, 2014FB001 and 2017FF467-001).

References

1. Borchers AT, Keen CL, Huntley AC and Gershwin ME: Lyme disease: A rigorous review of diagnostic criteria and treatment. J Autoimmun 57: 82-115, 2015.
2. Pal U, Wang P, Bao F, Yang X, Samanta S, Schoen R, Wormser GP, Schwartz I and Fikrig E: Borrelia burgdorferi basic membrane proteins A and B participate in the genesis of Lyme arthritis. J Exp Med 205: 133-141, 2008.
3. Ramamoorthy N, Narasimhan S, Pal U, Bao F, Yang X, Fish D, Anguita J, Norgard MV, Kanter FS, Anderson JF, et al: The Lyme disease agent exploits a tick protein to infect the mammalian host. Nature 436: 573-577, 2005.
4. Lantos PM, Shapiro ED, Auwaerter PG, Baker PJ, Halperin JI, McSweenan E and Wormser GP: Unorthodox alternative therapies marketed to treat Lyme disease. Clin Inf Dis 60: 1776-1782, 2015.
5. Marques AR: Lyme neuroborreliosis. Continuum (Minneap Minn) 21 (6 Neuroinfectious Disease): 1729-1744, 2015.
6. Brezem D and DeWeerdt L: Oral doxycycline for Lyme neuroborreliosis with symptoms of encephalitis, myelitis, vasculitis or intracranial hypertension. Eur J Neurol 21: 1162-1167, 2014.
7. Cerar T, Ogrinc K, Lortič‑furlan S, Lotric‑furlan S, Kobal J, Leviničaker‑Stezinak S, Strle F and Razči‑Sabljic E: Diagnostic value of cytokines and chemokines in Lyme neuroborreliosis. Clin Vaccine Immunol 20: 1578-1584, 2013.
8. Kim C, Cho ED, Kim HK, You S, Lee HJ, Hwang D and Lee SJ: β1-integrin-dependent migration of microglia in response to neuron-released α-synuclein. Exp Mol Med 46: e91, 2014.
9. Togna AR, Latina V, Trefilletti G, Guiso M, Moschini S and Togna GI: 1-Phenil-6,7-dihydroxy-isochroman inhibits inflammatory activation of microglia. Brain Res Bull 95: 33-39, 2013.
10. Beresette CA, Houdek HM, Floden AM and Rosenberger TA: Acetate supplementation reduces microglia activation and brain interleukin-1β levels in a rat model of Lyme neuroborreliosis. J Neuroinflammation 9: 249, 2012.
11. Kuhlow CJ, Garcia-Monco JC, Coleman JL and Benach JL: Murine microglia are effective phagocytes for Borrelia burgdorferi. J Neuroimmunol 168: 183-187, 2005.
12. Battisti JM, Bono JL, Rosa PA, Schrumpf ME, Schwang TG and Policastro PF: Outer surface protein A protects Lyme disease spirochetes from acquired host immunity in the tick vector. Infect Immun 77: 5228-5237, 2008.
13. Zhao H, Liu A, Cui Y, Liang Z, Li B and Bao F: Borrelia burgdorferi basic membrane protein A could induce chemokine production in murine microglia cell line BV2. Microbiol Pathog 111: 174-181, 2017.
14. Simpson WJ, Schrumpf ME and Schwang TG: Reactivity of human Lyme borreliosis sera with a 39-kilodalton antigen specific to Borrelia burgdorferi. J Clin Microbiol 28: 1329-1337, 1990.
15. Yang X, Izadi H, Coleman AS, Wang P, Ma Y, Fikrig E, Anguita J and Pal U: Borrelia burgdorferi lipoprotein BmpA activates pro-inflammatory responses in human synovial cells through a protein moiety. Microbes Infect 10: 1300-1308, 2008.
16. Baldwin AS Jr: The NF-kappa B and I kappa B proteins: New discoveries and insights. Annu Rev Immunol 14: 649-683, 1996.
17. Whiteside ST, Epinat JC, Rice NR and Israel A: I kappa B epsilon, a novel member of the I kappa B family, controls RelA and cRel NF-kappa B activity. EMBO J 16: 1413-1426, 1997.
18. Bell S, Degitz K, Quirling M, Jilg N, Page S and Brand K: Involvement of NF-kappab signaling in skin physiology and disease. Cell Signal 15: 1-7, 2003.
19. Vinayagamoorthy R, Koner BC, Kavitha S, Nandakumar DN, Padma Priya P and Goswami 56-60.: Nature 436: 573-577, 2005.
20. Colleran A, Collins PE and Carmody RJ: Assessing Sites of NF-kB DNA binding using chromatin immunoprecipitation. Methods Mol Biol 1280: 47-59, 2015.
21. Leal VO, Saldanha JF, Stockler-Pinto MB, Cardozo LF, Santos FR, Albuquerque AS, Leite M Jr and Mafra D: NRF2 and NF-κB mRNA expression in chronic kidney disease: A focus on nondialysis patients. Int Urol Nephrol 47: 1985-1991, 2015.

22. Naranjo V, Ayllón N, Pérez de la Lastra JM, Galindo RC, Kocan KM, Blouin EF, Mitra R, Alberdi P, Villar M and de la Fuente J: Reciprocal regulation of NF-κB (Relish) and subolesin in the tick vector, Ixodes scapularis. PLoS One 8: e65915, 2013.

23. Irvine M, Okitsu C and Hsieh CL: Q-PCR in combination with ChIP assays to detect changes in chromatin acetylation. Methods Mol Biol 791: 213-223, 2011.

24. Eaton SL, Roche SL, Llaverio Hurtado M, Oldknow KJ, Farquharson C, Gillingwater TH and Wishart TM: Western blotting and GAPDH and housekeeping protein immunodetection in Western blotting of human skeletal muscle in aging, making stain-free technology a superior loading control. J Appl Physiol (1985) 118: 386-394, 2015.

25. Rigal D, Filiot E, Schmitz F, Alzari PM, Mapara MY, Moens R, Delaunay C, Chevreul R, Cuvelier M, Kok JG: ding Control for Quantitative Fluorescent Western Blotting. Plos One 8: e72457, 2013.

26. Vigelsø A, Dybboe R, Hansen CN, Dela F, Helge JW and Guadalupe Grau A: GAPDH and β-actin protein decreases with aging, making stain-free technology a superior loading control in Western blotting of human skeletal muscle. J Appl Physiol (1985) 118: 386-394, 2015.

27. Rivero-Gutiérrez B, Anzola A, Martínez-Augustin O and de Medina FS: Stain-free detection as a reliable loading control for quantitative fluorescent western blotting. PLoS One 8: e72457, 2013.

28. Rasley A, Marriott I, Halberstadt CR, Bost KL and Anguita J: Substance P enhances NF-κB transactivation and chemokine response in murine microglia. J Immunol 172: 5707-5713, 2004.

29. Sun J, Ramnath RD, Zhi L, Tamizhselvi R and Bhatia M: Substance P enhances NF-κB transactivation and prostaglandin E2 production by murine microglia. J Immunol 172: 5707-5713, 2004.

30. Sadik CD, Hanfield KP, Bachmann M, Kraiczcy P, Eberhardt W, Brade V, Pfeilschifter J and Mühle H: Systematic analysis highlights the key role of TLR2/NF-κappaB/MAP kinase signaling for IL-8 induction by macrophage-like THP-1 cells under influence of Borrelia burgdorferi lysates. Int J of Biochem Cell Biol 40: 2508-2521, 2008.

31. Ottonello L, Bertolotto M, Montecucco F, Bianchi G and Dallegrì F: Delayed apoptosis of human monocytes exposed to immune complexes is reversed by oxaprozin: Role of the Akt/IkappaB kinase/nuclear factor kappaB pathway. Br J Pharmacol 157: 294-306, 2009.

32. Carvalho L, Jacinto A and Matova N: The toll/NF-κB signaling pathway is required for epidermal wound repair in Drosophila. Proc Natl Acad Sci USA 111: E5373-E5382, 2014.

33. Baum E, Hue F and Barbour AG: Experimental infections of the reservoir species Peromyscus leucopus with diverse strains of Borrelia burgdorferi, a Lyme disease agent. MBio 3: e00434-12, 2012.

34. Bernardino AL, Myers TA, Alvarez X, Hasegawa A and Philipp MT: Toll-like receptors: Insights into their possible role in the pathogenesis of lyme neuroborreliosis. Infect Immun 76: 4385-4395, 2008.

35. Sun J, Ramnath RD, Zhi L, Tamizhselvi R and Bhatia M: Substance P enhances NF-κB transactivation and chemokine response in murine macrophages via ERK1/2 and p38 MAPK signaling pathways. Am J Physiol Cell Physiol 294: C1586-C1596, 2008.

36. Sadik CD, Hanfield KP, Bachmann M, Kraiczcy P, Eberhardt W, Brade V, Pfeilschifter J and Mühle H: Systematic analysis highlights the key role of TLR2/NF-κappaB/MAP kinase signaling for IL-8 induction by macrophage-like THP-1 cells under influence of Borrelia burgdorferi lysates. Int J of Biochem Cell Biol 40: 2508-2521, 2008.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.