Journal of Bacteriology and Virology 2010. Vol. 40, No. 4 p.151 – 157
DOI 10.4167/jbv.2010.40.4.151

Original Article

Cleavage of p65 Subunit of NF-κB by *Orientia tsutsugamushi*

Mi-Jeong Kim, Sun-Myoung Lee, Se-Hee Kil and Jae-Seung Kang*

Department of Microbiology, Inha University College of Medicine, Incheon, Korea

Orientia tsutsugamushi, a causative agent of scrub typhus, is an obligate intracellular parasite and usually propagates in the cytoplasm of host endothelial cells and macrophages. Macrophages are the first defense line against bacterial infection and NF-κB is activated upon contact with bacteria, resulting in the transcription of inflammatory cytokine to control bacterial infection. In this study, we investigated whether *O. tsutsugamushi* modulates NF-κB activation in the macrophages. We examined the changes of NF-κB proteins upon infection with *O. tsutsugamushi* and found that NF-κB is activated at a slow rate as judged with EMSA and immunoblot analysis. Interestingly, we found that p65 was cleaved generating a 45 kDa fragment. In addition, fragment of p65 is generated only by the virulent serotype strain of *O. tsutsugamushi*, suggesting this cleavage may be associated with the mouse virulence. It is still unknown whether this is a direct result of *O. tsutsugamushi* proteins or enzymes of host cell. Further exploration of the mechanism that modulates NF-κB activity by *O. tsutsugamushi* could contribute to a better understanding of the molecular pathogenesis of *O. tsutsugamushi* infection.

Key Words: *O. tsutsugamushi*, NF-κB, p65, Fragment, Macrophage

INTRODUCTION

Orientia tsutsugamushi is an obligate intracellular parasite that requires a host cell for its replication. It has a cell wall resembling that of gram-negative bacterium without lipopolysaccharide and peptidoglycan (1). *O. tsutsugamushi* invades human through mite biting and replicates in endothelial cells and macrophages (2, 3). It causes tsutsugamushi disease that is endemic in large area of Asia (4) and the number of Korean patient increases sharply in recent years (5).

O. tsutsugamushi infects endothelial cells of various organs and causes systemic inflammatory response causing fever, rash, and eschar (1, 6). The molecule inducing inflammation is known be the heat-stable molecule, but its precise chemical nature remains to be identified. This inflammatory molecule of *O. tsutsugamushi* may activate toll-like receptors and subsequently cause activation of NF-κB pathway as other bacterium (7).

NF-κB is a key transcription factor regulating many aspects of cellular responses including inflammation. This transcription factor plays an important role for the development of various immune cells of innate and adaptive immunity and the production of effector proteins (8). When immune cells recognize pathogenic microorganisms invading body, they activate NF-κB pathway and many signaling pathway associated with protective immunity is initiated. NF-κB consists of five NF-κB family proteins: NF-κB1 (p50), NF-κB2 (p52), Rel A (p65), Rel B, and c-Rel. These proteins pair to form homo- and heterodimers. In the cytoplasm, they are sequestered by the inhibitor kappaB (IκB) proteins containing a tail of ankyrin repeats that are

Received: October 7, 2010/ Revised: November 12, 2010
Accepted: November 16, 2010

*Corresponding author: Dr. Jae-Seung Kang. Department of Microbiology, Inha University College of Medicine, Jungsuk B/D, 3rd Street, Shinheung-Dong, Choong-Gu, Incheon 400-712, Korea.
Phone: +82-32-890-0952, Fax: +82-32-881-8559
e-mail: jaeskang@inha.ac.kr

**This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2008-E00031).

important in binding to NF-κB family proteins. Once in the nucleus, NF-κB is involved in the increased transcription of various defense proteins and IκB proteins that provides a feedback mechanism to limit NF-κB activation (9).

As *O. tsutsugamushi* can survive and replicate in the macrophages, which are effector cells killing many pathogenic bacteria, it may have potent evasion mechanism to ensure its survival in a hostile environment inside macrophages. We therefore hypothesized that *O. tsutsugamushi* might modulate the NF-κB pathway by the following reasons. First, macrophages are major sites of replication of *O. tsutsugamushi* and many bacteria replicating inside macrophages have mechanism for modulating the antibacterial power of macrophages (10). Second, in our previous study (11), we found that *O. tsutsugamushi* inhibits TNF-α production by inducing interleukin 10 secretion in murine macrophage. In that study, however, we also observed that the activation of NF-κB pathway occurred during the intracellular invasion of *O. tsutsugamushi*. Therefore, there might be some mechanism of modulation, which is not the simple inhibition of nuclear translocation of NF-κB proteins. Third, there is a unique feature of NF-κB activation during the invasion of *O. tsutsugamushi* into macrophages. Cho *et al*. reported that p50/p50 homodimeric form of NF-κB might be formed during NF-κB activation by *O. tsutsugamushi* (12). Many studies reported that p50 homodimer has a role in regulating NF-κB activity (13). Therefore, it is highly possible that unknown mechanism modulating NF-κB operates during the infection of this bacterium.

In this work, we observed the changes of NF-κB proteins during the early stage of *O. tsutsugamushi* infection in a murine macrophage cell line, J774A.1. We found that a fragment of p65 subunit of NF-κB is generated by the virulent strain of *O. tsutsugamushi*, suggesting this cleavage might be associated with virulence of this bacterium.

MATERIALS AND METHODS

Cultivation of cells and *O. tsutsugamushi* infection

The Boryong, Kato, and Gilliam serotypes of *O. tsutsugamushi* were cultivated in ECV304 cells, as described previously (11, 14). The infected cells were cultured in M199 (Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco BRL) until the heavily infected cells detached from the culture substrate. The infected cells were harvested and disrupted with glass beads (diameter, 1.0 mm). After centrifugation at 300 × *g* for 5 min, the supernatants, free of large cell debris, were used as bacterial preparations for cellular infection. For the mock-infection, the same number of uninfected ECV304 cells were treated with the same method as infected cells to make cell lysates and used for the negative controls. Murine macrophage cell line, J774A.1 (American Type Culture Collection, Manassas, VA, USA) was maintained in DMEM medium (Life technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum. To infect J774A.1 cells, the bacterial preparations described as above were added to the culture and incubated for 3 hr, and then the media were replaced with fresh media.

Cytoplasmic and nuclear extracts preparation

J774A.1 cells were harvested, washed once with phosphate-buffered saline and suspended in buffer A (10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT and 0.1 mM EDTA). The cells were incubated with buffer B (10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT, 0.1 mM EDTA, 1 mM PMSF and 0.6% Nonidet P-40) for 5 min at 4℃ and then centrifuged for 20 min at 14,000 rpm. The supernatant corresponding to the cytopasmic extract was frozen at -80℃ and the pellet was incubated with buffer C (20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM DTT, 1 mM EDTA, 1 mM PMSF, and 0.6% Nonidet P-40) for 30 min on ice and centrifuged for 20 min at 14,000 rpm. Protein concentration was estimated according to BCA (bicinchoninic acid) solution and copper (Ⅱ) sulfate (Sigma Chemical Co., St. Louis, MO, USA) with bovine serum albumin as a standard.

Electrophoretic mobility shift assay (EMSA)

A double-stranded oligonucleotide containing the NF-κB consensus motif (Santa cruz Biotechnology, Santa cruz,

CA, USA) was end-labeled [γ-^{32}P] ATP using T4 kinase. Unincorporated ATP was removed with G-25 sephadex columns and centrifugation. Before addition of oligonucleotide probe, 10 μg of nuclear extract was added with DNA binding mix [2 μg poly (dI-dC), 20 mM HEPES, pH 7.4, 5% glycerol, 10 mM KCl, 1 mM DTT, and 1 mM $MgCl_2$] and then radiolabeled oligonucleotide was added to the reaction mixture and incubated for 30 min. Samples were separated on 5% polyacrylamide gel electrophoresis (PAGE) in 0.5 × TBE. Gels were dried and subjected to autoradiography.

Immunoblot analysis

J774A.1 cells infected with *O. tsutsugamushi* for the indicated time were harvested and centrifuged at 300 × *g* for 5 min. Proteins of cytoplasmic and nuclear fractions were separated and then mixed with 5 × sample buffer (250 mM Tris, 50% glycerol, 10% SDS, 0.5% bromphenol blue) with or without β-mercaptoethanol and boiled for 5 min. The resulting samples were separated on 12% SDS-PAGE with molecular weight markers (Amersham International, Amersham, UK). The resolved proteins were transferred electrophoretically to polyvinylidene fluoride (PVDF) membrane (Millipore Co., Bedford, MA, USA.). Membrane was blocked with 5% nonfat dry milk and then incubated with the following primary antibodies: rabbit anti-p65 C-terminal (SC-375, Santacruz Biotechnology), rabbit anti-p65 N-terminal (SC-109, Santacruz Biotechnology), and anti-p50 (SC-1190, Santacruz Biotechnology). After membrane was washed three times in TBS with 0.1% Tween-20 (TBST), it was incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Jackson Immunoresearch, West Grove, PA, USA) for 1 hr at room temperature and developed with an enhanced chemiluminescence (ECL) substrate (Amersham International).

RESULTS

NF-κB activation in J774A.1 cells infected with *O. tsutsugamushi*

To examine whether NF-κB is activated in J774A.1 cells infected with *O. tsutsugamushi*, infected or mock-treated J774A.1 cells were incubated for the indicated times and then nuclear proteins were analyzed for the presence of NF-κB complex. As shown in Figure 1, three discrete bands were resolved in nuclear extracts from J774A.1 cells infected with *O. tsutsugamushi* or mock-treated. The upper band is likely composed of p65 and p50; the middle band is a homodimer of p50. At 4 hr after infection, we observed the increase of the intensity of middle band in infected cells as compared with that of mock-treated cells. This result suggests that the infection of macrophage with *O. tsutsugamushi* resulted in the induction of NF-κB activation.

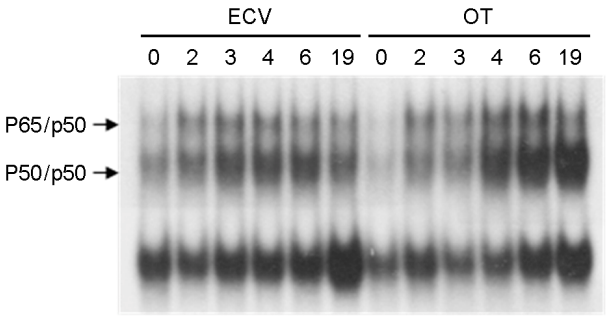

Figure 1. The activation of NF-κB in J774A.1 cells infected with *O. tsutsugamushi*. NF-κB activation was examined by EMSA for nuclear extracts prepared from J774A.1 cells for the indicated times. J774A.1 cells were infected with *O. tsutsugamushi* (OT) or treated ECV cell lysate for the mock-infection (ECV).

Cleavage of p65 in cells infected with *O. tsutsugamushi*

To verify the above finding, we examined the nuclear translocation of p65 of NF-κB by immunblot analysis. The distribution of p65 in the cytosol and the nuclei of the cells infected or treated with LPS were examined after fractionation by SDS-PAGE and immunblot analysis using a C-terminus-specific anti-p65 antibody. Mock-treated cells showed little difference of the band intensity between the cytosol and nuclear fraction, although slight increase of the nuclear bands suggesting the cell lysate used for control of mock-infection itself has a weak stimulatory effect. In sharp contrast, the cells infected with *O. tsutsugamushi* showed the increase of band intensity of nuclear fraction after 3 hr infection, which lasted until 19 hr infection (Fig. 2 upper panel).

Interestingly, the *O. tsutsugamushi*-induced accumulation of NF-κB complex in the nucleus is accompanied by a new protein band with a size of approximately 45 kDa, suggesting the fragmentation of p65 during *O. tsutsugamushi*-infection. This fragment appeared at 3 hr after infection and last until 6 hr after infection (Fig. 2 middle panel). This fragmentation of p65 is not a general inflammatory process, because the cells stimulated with LPS also showed the increase of nuclear p65 and no generation of shorter fragment (Fig. 2 lower panel).

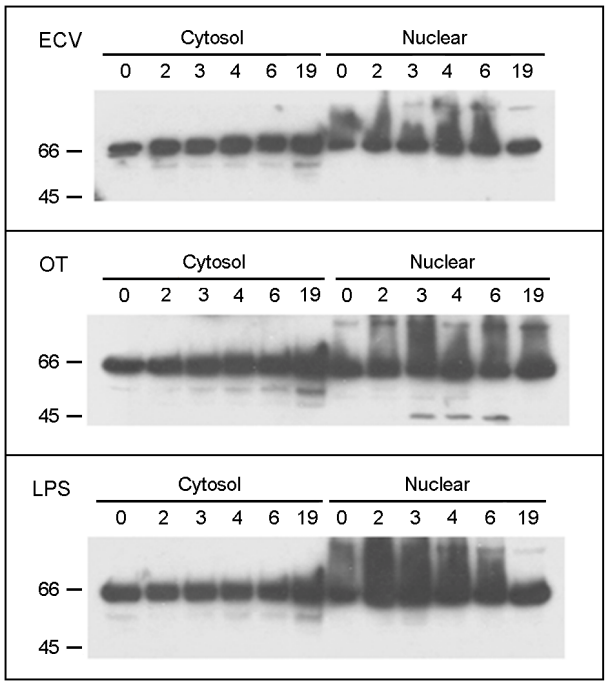

Figure 2. The cleavage of p65 in J774A.1 cells infected with *O. tsutsugamushi*. The p65 was visualized with the immunoblot analysis of protein extracts from the cytosol and nuclei of the J774A.1 cells treated ECV cell lysate (upper panel), infected with *O. tsutsugamushi* (middle panel), or treated with LPS (lower panel) for the indicated times.

The fragment of p65 was recognized by C-terminus-specific anti-p65 antibody but not by N-terminus-specific anti-p65 antibody (Fig. 3). In contrast to the results obtained with anti-p65 antibody, we could not observe any change of p50, which is another component of NF-κB complex (Fig. 3), indicating the 45 kDa band is not a fragment of p50.

Difference of p65 cleavage according to serotype of *O. tsutsugamushi*

O. tsutsugamushi has multiple serotypes according to the 56 kDa protein that is a most abundant surface protein of this bacterium. Strains of different serotype vary in both of antigenicity and animal virulence. Virulent strains, such as Kato, kill the mice even at low doses; less virulent strain, such as Gilliam that propagates slowly in mice, requires large number of bacteria to kill mice (4). To examine whether the different serotype strains of *O. tsutsugamushi* show distinct pattern of p65 cleavage, we infected J774A.1 cells with Kato and Gilliam serotype strains. To our interest, the pattern of p65 cleavage is different between two serotypes (Fig. 4). Only Kato serotype strain showed fragment of p65 after 5 hr infection. This result suggests the possibility that the generation of p65 during NF-κB activation may be related with the virulence of this bacterium in mice.

DISCUSSION

NF-κB is important for the protective immune response against microbial pathogens (15) and pathogens modulate

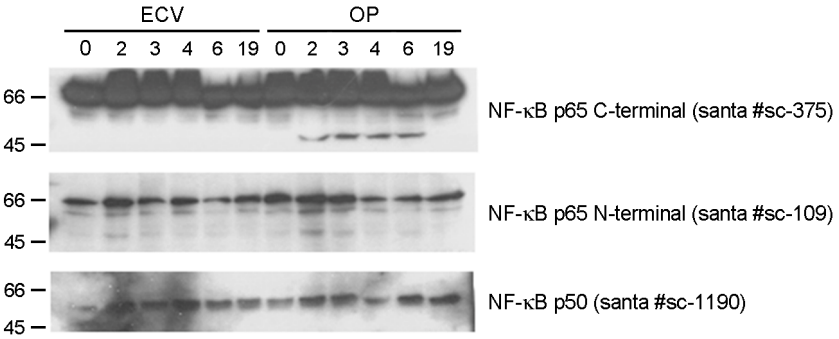

Figure 3. The cleavage of p65 observed only in western blot using p65 C- and N-terminal antibodies. The p65 was visualized with the immunoblot analysis of protein extracts from the nuclei of J774A.1 cells treated ECV cell lysate (ECV) and infected with *O. tsutsugamushi* (OT) using the indicated primary antibodies.

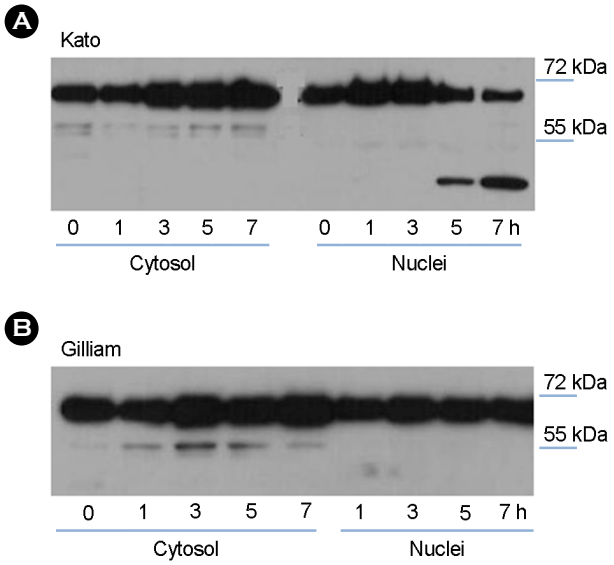

Figure 4. The p65 cleavage by different serotypic strains of *O. tsutsugamushi*. The p65 was visualized with the immunoblot analysis of protein extracts from the nuclei of the J774A.1 cells infected with Kato and Gilliam strains of *O. tsutsugamushi*.

the NF-κB pathway to survive in the host. In this study, we examined the change of NF-κB pathway during *O. tsutsugamushi* infection and found that p65 was cleaved generating a 45 kDa fragment. Furthermore, we found that p65 is degraded only by the virulent serotype strain of *O. tsutsugamushi*.

The signaling pathway of NF-κB activation is well characterized (8, 16). The five NF-κB family proteins pair to form homo- and heterodimers that are sequestered in the cytoplasm by IκB proteins. A variety of signaling pathways lead to the phosphorylation and degradation of IκB proteins, revealing the nuclear localization signal of the NF-κB protein and so allow nuclear translocation of NF-κB dimer. In the nucleus, NF-κB binds to the corresponding region of DNA and results in the increased transcription of many genes encoding proteins for the defense against invading microorganisms. To survive this powerful defensive action of host, many bacterial pathogens modulate some point of NF-κB pathways by various mechanisms. For example, *Salmonella* inhibits the ubiquitination of phosphorylated IκB, which is necessary for the degradation of IκB and subsequent activation of NF-κB (15).

Although studies on the modulation of NF-κB pathway during *O. tsutsugamushi* infection are lacking, there are a few data about the actions of closely related bacteria including *Rickettsia* and *Ehrlichia*. *R. rickettsii*, a causative agent of Rocky mountain spotted fever, results in biphasic activation of NF-κB, which consists of early activation for 3 hr and then second peak of activation until 24 hr (17). This result suggested us that some rickettsial mechanism may operate for the maintenance of NF-κB pathway that is necessary for intracellular survival of this bacterium by the inhibition of apoptosis. Another intracellular bacterium, *E. chaffeensis*, adopts a different strategy (18). This bacterium causes a weak activation of NF-κB by a unique mechanism: down-regulation of several pattern recognition receptors such as TLR2, TLR4, and CD14 that are necessary for recognition and defense for bacteria. Through this mechanism, *E. chaffeensis* could survive and replicate in the hostile environment of monocyte and macrophage.

At present, there are a few reports about the effect of *O. tsutsugamushi* on the signaling pathway of NF-κB pathway. Some previous reports suggested that this modulation of NF-κB may operate by this bacterium. The early study by Jerrells *et al*. (19) found that *O. tsutsugamushi* does not induce the production of TNF-α by mouse peritoneal macrophages unlike *R. conorii* and *R. prowazekii*. These results lead the authors the conclusion that *O. tsutsugamushi* has no cellular components inducing the inflammatory cytokines. However, the following studies by other researchers and us (11, 12) indicate that *O. tsutsugamushi* has components that are recognized by host receptor and activate NF-κB pathway with different kinetics. Furthermore, our studies clearly indicate that *O. tsutsugamushi* has mechanisms suppressing the production of inflammatory cytokines induced by its heat-stable molecules, most possibly by modulating NF-κB pathway (14). The mechanism by which *O. tsutsugamushi* suppresses the induction of inflammatory cytokine is not simple lack of molecule that trigger host NF-κB pathway but seems to be some specific action of *O. tsutsugamushi*. Preinfection with *O. tsutsugamushi* causes the release of unknown factor that stimulates IL-10 release in culture medium and this conditioned medium could inhibit the TNF-α release by LPS stimulation as well as by

O. tsutsugamushi infection (11).

In this study, we examined the changes of signaling pathway of NF-κB activation to explore the mechanism of cytokine inhibition during *O. tsutsugamushi* infection. Our data confirmed the previous reports and showed a novel finding that occurs in the signaling events during the activation of NF-κB by *O. tsutsugamushi*. We first observed the activation of NF-κB occurs during infection by EMSA in agreement with the previous results by us and others (11, 12). Cho *et al*. also reported the activation of NF-κB by EMSA and suggested the possible formation of p50/p50 homodimer (12). However, in our study we could not demonstrated the increased formation of homodimer and the amount of p50 protein in nucleus did not change significantly as judged by immunoblot analysis. To our interest, the immunoblot analysis showed the formation of new protein band with a size of approximately 45 kDa, suggesting the cleavage of p65 during *O. tsutsugamushi*-infection.

The cleavage of p65 has been reported to have a role for suppressing NF-κB activation (20, 22) and observed to occur during the infection by various pathogens. Poliovirus replication in the HeLa cells results in the cleavage of NF-κB complex, which coincides with the onset of intensive poliovirus protein synthesis and the appearance of the activity of viral protease. Authors suggest that this proteolytic cleavage of p65 is a mechanism for the suppression of the innate immune responses (22). *Chlamydia trachomatis*, an obligate intracellular bacterium and human pathogen, also causes p65 cleavage into an N terminus-derived p40 fragment and a p22 of the C terminus by bacterial protease. Thus, it has been suggested that *Chlamydia* converts a regulatory molecule of host inflammatory response to a dominant negative initiator potentially to minimize inflammation (23). In our study, we could not identify the specific protease that digests the p65. It may be originated from *O. tsutsugamushi* or host protein that is activated during the infectious process. When we searched the genome database of NCBI for the protease, *O. tsutsugamushi* has three potential proteases including metalloprotease and periplasmic serine protease (24). Further studies are required for the identification of the specific protease that cleaves p65 during *O. tsutsugamushi* infection.

One of the interesting findings of this study is that the pattern of p65cleavage is different between two serotypes; only Kato serotype strain showed fragment of p65 after 5 hr infection in contrast to Gilliam strain. *O. tsutsugamushi* comprises multiple strains, which differ in both antigenicity and virulence in mammalian hosts. It is well known that Kato strain is more virulent than Gilliam strain (1, 4). Therefore, our finding that only virulent Kato strain resulted in the cleavage of p65 suggests strongly that the cleavage might be related with mouse virulence. We suggest that *O. tsutsugamushi* modulates NF-κB activation by the cleavage of p65 to counteract the hostile action of macrophages and this modulation results in virulence in mouse.

In conclusion, we examined whether NF-κB is modulated during infection with *O. tsutsugamushi* and found that NF-κB is activated at slow rate and p65 was cleaved to generate a 45 kDa fragment. In addition, the fragment of p65 is generated only by the virulent serotype strain of *O. tsutsugamushi*, suggesting this cleavage might be associated with the mouse virulence of this bacterium.

REFERENCES

1) Seong SY, Choi MS, Kim IS. *Orientia tsutsugamushi* infection: overview and immune responses. Microbes Infect 2001;3:11-21.
2) Cho KA, Jun YH, Suh JW, Kang JS, Choi HJ, Woo SY. *Orientia tsutsugamushi* induced endothelial cell activation via the NOD1-IL-32 pathway. Microb Pathog 2010;49:95-104.
3) Moron CG, Popov VL, Feng HM, Wear D, Walker DH. Identification of the target cells of *Orientia tsutsugamushi* in human cases of scrub typhus. Mod Pathol 2001;14: 752-9.
4) Kelly DJ, Fuerst PA, Ching WM, Richards AL. Scrub typhus: the geographic distribution of phenotypic and genotypic variants of *Orientia tsutsugamushi*. Clin Infect Dis 2009;48:S203-30.
5) Kweon SS, Choi JS, Lim HS, Kim JR, Kim KY, Ryu SY, *et al*. Rapid increase of scrub typhus, South Korea,

2001-2006. Emerg Infect Dis 2009;15:1127-9.
6) Hwang TS, Chu YC, Kim YB, Lim BU, Kang JS. Pathologic study of mice infected with *Rickettsia tsutsugamushi* R19 strain. J Korean Med Sci 1993;8: 437-45.
7) Kumar H, Kawai T, Akira S. Toll-like receptors and innate immunity. Biochem Biophys Res Commun 2009; 388:621-5.
8) Naumann M. Nuclear Factor-kappa B activation and innate immune response in microbial pathogen infection. Biochem Pharmacol 2000;60:1109-14.
9) Li Q, Verma IM. NF-kappa B regulation in the immune system. Nat Rev Immunol 2002;2:725-34.
10) al-Ramadi BK, Chen YW, Meissler JJ Jr, Eisenstein TK. Immunosuppression induced by attenuated *Salmonella*. Reversal by IL-4. J Immunol 1991;147: 1954-61.
11) Kim MJ, Kim MK, Kang JS. *Orientia tsutsugamushi* inhibits tumor necrosis factor alpha production by inducing interleukin 10 secretion in murine macrophages. Microb Pathog 2006;40:1-7.
12) Cho NH, Seong SY, Huh MS, Han TH, Koh YS, Choi MS, *et al*. Expression of chemokine genes in murine macrophages infected with *Orientia tsutsugamushi*. Infect Immun 2000;68:594-602.
13) Zhong H, May MJ, Jimi E, Ghosh S. The phosphorylation status of nuclear NF-kappa B determines its association with CBP/p300 or HDAC-1. Mol Cell 2002;9:625-36.
14) Kim MK, Kang JS. *Orientia tsutsugamushi* suppresses the production of inflammatory cytokines induced by its own heat-stable component in murine macrophages. Microb Pathog 2001;31:145-50.
15) Tato CM, Hunter CA. Host-pathogen interactions: subversion and utilization of the NF-kappa B pathway during infection. Infect Immun 2002;70:3311-7.
16) Silverman N, Maniatis T. NF-kappa B signaling pathways in mammalian and insect innate immunity. Genes Dev 2001;15:2321-42.
17) Sporn LA, Sahni SK, Lerner NB, Marder VJ, Silverman DJ, Turpin LC, *et al*. *Rickettsia rickettsii* infection of cultured human endothelial cells induces NF-kappaB activation. Infect Immun 1997;65:2786-91.
18) Lin M, Rikihisa Y. *Ehrlichia chaffeensi*s downregulates surface Toll-like receptors 2/4, CD14 and transcription factors PU.1 and inhibits lipopolysaccharide activation of NF-kappa B, ERK 1/2 and p38 MAPK in host monocytes. Cell Microbiol 2004;6:175-86.
19) Jerrells TR, Geng P. The role of tumor necrosis factor in host defense against scrub typhus rickettsiae. II. Differential induction of tumor necrosis factor-alpha production by *Rickettsia tsutsugamushi* and *Rickettsia conorii*. Microbiol Immunol 1994;38:713-9.
20) Kim HS, Chang I, Kim JY, Choi KH, Lee MS. Caspase-mediated p65 cleavage promotes TRAIL-induced apoptosis. Cancer Res 2005;65:6111-9.
21) Neuzil J, Schröder A, von Hundelshausen P, Zernecke A, Weber T, Gellert N, *et al*. Inhibition of inflammatory endothelial responses by a pathway involving caspase activation and p65 cleavage. Biochemistry 2001;40: 4686-92.
22) Neznanov N, Chumakov KM, Neznanova L, Almasan A, Banerjee AK, Gudkov AV. Proteolytic cleavage of the p65-RelA subunit of NF-kappa B during poliovirus infection. J Biol Chem 2005;280:24153-8.
23) Lad SP, Li J, da Silva Correia J, Pan Q, Gadwal S, Ulevitch RJ, *et al*. Cleavage of p65/RelA of the NF-kappaB pathway by *Chlamydia*. Proc Natl Acad Sci USA 2007;104:2933-8.
24) Cho NH, Kim HR, Lee JH, Kim SY, Kim J, Cha S, *et al*. The *Orientia tsutsugamushi* genome reveals massive proliferation of conjugative type IV secretion system and host-cell interaction genes. Proc Natl Acad Sci USA 2007;104:7981-6.