Chemokine and Tumor Necrosis Factor Alpha Responses in Murine Peritoneal Macrophages Infected with *Orientia tsutsugamushi*

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This paper was published in The Microbiological Society of Korea, 39(3):186~194(2001)

Abstract

Scrub typhus, caused by *Orientia tsutsugamushi* infection, is clinically and histopathologically characterized by local as well as systemic inflammatory reactions, indicating that orientiae induce mechanisms that amplify the inflammatory response. To reveal the underlying mechanisms of chemoattraction and activation of responding leukocytes, the expression of chemokine and tumor necrosis factor alpha (TNF-a) genes in murine peritoneal macrophages by infection with the obligate intracellular bacterium *O. tsutsugamushi* was investigated. The mRNAs that were upregulated included macrophage inflammatory proteins 1a/b (MIP-1a/b), MIP-2, monocyte chemoattractant protein 1 (MCP-1), RANTES (regulated upon activation, normal T-cell expressed and secreted), gamma- interferon-inducible protein 10 (IP-10), and TNF-a. Peak expression of these chemokines and cytokine was observed between 1 and 3 h after infection. These responses returned to or approached baseline preinfection levels by 6 h after challenge. Semiquantitative reverse transcription (RT)-PCR analysis revealed dramatic increases during infection in the steady-state levels of mRNA coding for the inhibitory subunit of NF-kB (IkBa), transcription of which is enhanced by the binding of NF-kB within the IkBa promoter region. Thus, *O. tsutsugamushi* appears to be a strong inducer of chemokines and TNF-a cytokine which may, by the attraction and activation of phagocytic leukocytes, significantly contribute to inflammation and tissue damage observed in scrub typhus.

Key words: *Orientia tsutsugamushi*, scrub typhus, peritoneal macrophage, chemokine gene expression, TNF-a

Introduction

Orientia tsutsugamushi, an obligate intracellular bacterium, is the causative agent of scrub typhus (tsutsugamushi disease), which is one of the most prevalent febrile illnesses in South Korea (9, 32). The disease is characterized by fever, rash, eschar, pneumonitis, meningitis, and disseminated intravascular coagulation, which leads to severe multiorgan failure in untreated cases (1, 10, 68). This bacterium infects a variety of host cells *in vitro* and *in vivo*, including macrophages, polymorphonuclear leukocytes (PMN), lymphocytes, and endothelial cells, where it replicates in the cytoplasm without being surrounded by a phagolysosomal membrane (32, 40, 45, 53). *O. tsutsugamushi* causes local inflammations accompanying eschars at the site of infection, which then spread systemically (6). Inflammation is initiated by *O. tsutsugamushi*-infected macrophages and endothelial cells in the dermis.

Analysis of early immunologic responses to *O. tsutsugamushi* infection in mice showed that macrophage-mediated cellular immunity is essential for resolution of this infection (8, 41). Resistance to the lethal effects of acute oriential infection is under unigenic dominant control by the *Ric* locus (20). Macrophages infiltrate both susceptible (Ric^S) and resistant (Ric^r) mouse strains in response to *O. tsutsugamushi* infection (26, 41). The resistant strain of mice was reported to have less PMN response to *O. tsutsugamushi* than a susceptible strain did (27). As a result, susceptible mice died within 2 weeks of infection. By contrast, Ric^r strains showed a minimal level of infection over 2 weeks and survived the infection (29, 41). Mononuclear cells such as lymphocytes and macrophages as well as PMN were observed in eschars and rashes caused by scrub typhus (1). Early host inflammatory responses seem to play a key role in determining the fate of the host infected with *O. tsutsugamushi* (41). For these reasons, the regulatory components that determine the quality and magnitude of the cellular influx to the site of the oriential infection need to be analyzed. Proinflammatory cytokines and chemokines (chemotatic cytokines) play an important role in these processes (3, 24). The expression of chemokines and their kinetics, however, have not been elucidated in the disease caused by *O. tsutsugamushi*.

Proinflammatory cytokines and chemokines are the main factors responsible for the recruitment of distinct leukocytes into inflamed tissues during inflammatory disease (3, 24). The interaction of different chemokines with their receptors on leukocytes allows selective activation and chemotaxis of neutrophils, lymphocytes, or monocytes necessary for migration to the sites of evolving inflammation. The site-directed immigration of leukocytes into inflamed tissue is provoked by gradients of chemokines that contribute to the adhesion of leukocytes to vascular endothelium, direct transendothelial migration, and movement through the extracellular matrix (24). Members of the CC chemokine subfamily, which include RANTES, MIP-1a, MIP-1b, and MCP-1, preferentially attract monocytes and lymphocytes. Those of the CXC chemokine subfamily, such as Interleukin 8 (IL-8) and MIP-2, are potent neutrophil attractants (3). It has been shown that during infection, infected macrophages produce a subset of chemokines (16, 52 61). Various sets of chemokines are produced by monocytes and macrophages infected with different pathogenic microorganisms (7, 16, 51, 52, 61). The kinetics of chemokine gene expression also varies according to the microorganism studied. The regulation of chemokine gene expression, as a defense mechanism against pathogenic microorganism, seems to be related to the clinical courses of the infected host (34, 36, 39, 50). The mechanisms by which leukocytes are attracted to lesional sites in *O. tsutsugamushi* infections are still incompletely understood. In particular, little attention has been directed to the chemokine family in the pathogenesis of scrub typhus.

Tumor necrosis factor alpha (TNF-a) is a well-characterized proinflammatory cytokine released primarily from monocytes and macrophages upon invasion of the host by a wide variety of pathogens. It mediates a wide range of cellular immune responses to infection or inflammation (5). TNF-a has been shown to be important in host defenses against a variety of intracellular pathogens (64, 70, 71). The development of intracellular killing activity by activated monocytes and macrophages requires the autocrine effects of TNF-a (42). The cytokines gamma interferon (IFN-g) and TNF-a play roles not only in host defense but also in the pathogenesis of *R.prowazekii* infections (66). The effects of these cytokines on *R.prowazekii* infection in cultured cells include inhibition of rickettsial growth, killing of some of the rickettsiae, cytotoxic effects on the host cells, and inhibition of the initial infection of host cells by the rickettsiae (66). Studies of *Rickettsia conorii* infections in mice have demonstrated that both IFN-g and TNF-a are important host defenses against infections with this *Rickettsia* species (15). It was

found that the spleen cells from mice immunized with *O. tsutsugamushi* produced TNF-a in response to *O. tsutsugamushi* antigens, and TNF activity was found in the sera of immune mice after injection with the oriential antigen (30). Recombinant murine TNF-a inhibited intracellular growth of *O. tsutsugamushi* in the mouse embryo cell line, peritoneal exudate macrophages or bone marrow-derived macrophages (19). However, studies delineating the role of TNF-a in host defense and the pathogenesis of *O.tsutsugamushi* infection *in vivo* are not available.

Activation of the nuclear factor k-B/Rel (NF-kB) family of transcription factors is a critical step in the regulation of inflammatory mediators, such as cytokines, acute-phase proteins, and adhesion molecules (4, 18). NF-kB resides in the host cell cytoplasm as homodimers and heterodimers of specific subunits, including p50 (NF-kB1), p65 (RelA), and c-Rel (12). It exists as a pre-formed but inactive pool bound to an inhibitory subunit (IkB) or containing an inhibitory prosequence (p105), and its activation follows the phosphorylation and degradation of these inhibitory components after cell stimulation (48). Following activation, NF-kB is translocated to the nucleus, whereupon it influences transcription by binding to specific sites within promoter. Since many of the chemokine genes are also regulated by NF-kB (67), it is possible that *O. tsutsugamushi* induces the chemokine genes through activation of NF-kB. In this study, we analyzed the expression of a subset of chemokine and TNF-a genes in mouse thioglycollate exudate peritoneal (TEP) macrophages during *O. tsutsugamushi* infection.

Materials and Methods

Mice

Specific pathogen free, female BALB/c mice purchased from SLC Inc. (Japan) were kept in the animal facility located in the Cheju National University Medical School. Utmost precautions were taken so the mice remained free from infection from environmental pathogens to ensure that the degree of spontaneous activation of tissue macrophages would be minimal. All mice used for peritoneal macrophages were 8to 12weeks old.

Cell culture

Thioglycollate exudate peritoneal (TEP) macrophages were obtained from BALB/c mice that had been injected intraperitoneally 3days previously with 1.5ml of 4% Brewer's thioglycollate broth (Difco Laboratories, Detroit, MI) by peritoneal lavage with 10 ml of Hanks' balanced salt solution (HBSS, Gibco BRL, Grand Island, NY) containing 10 U/ml heparin (21). The cells were pelleted, washed with cold HBSS, and resuspended in Dulbecco's modified Eagle's medium (Gibco BRL) containing 10% (vol/vol) heat-inactivated fetal bovine serum (FBS) (Gibco BRL), 100g of streptomycin per ml, 100U of penicillin per ml, 50g of gentamicin per ml and 2mM L-glutamine, and plated in six-well plates (Corning Inc., Corning, NY). Nonadherent cells were removed after 2h of incubation in a humidified 5% CO_2 atmosphere at 37℃ by washing the cells three times with cold HBSS. Adherent macrophages were then cultured in a humidified 5% CO_2 atmosphere at 37℃ for 18 h before initiation of each experiment. The prototype strain, *O.tsutsugamushi* Karp (American Type Culture Collection) was propagated in monolayers of L-929 cells as described previously (33, 57). When more than 90% of the cells were infected, as determined by an indirect immunofluorescent-antibody technique (9), the cells were collected, homogenized with a glass Dounce homogenizer (Wheaton Inc., Millville, NJ), and centrifuged at 500*g* for 5min. The supernatant was recovered and stored in liquid nitrogen until use. The infectivity titer of the inoculum was determined as described previously with modification (63). Briefly, five-fold serially diluted oriential samples were inoculated onto L-929 cell layers on 24-well tissue culture plates. After 3days of

incubation, the cells were collected, fixed, and stained as described previously (9). The ratio of infected cells to the counted number of cells was determined microscopically, and infected-cell counting units (ICU) of the oriential sample were calculated as follows (63): ICU=(total number of cells used in infection) (percentage of infected cells)(dilution rate of the orientiae suspension)/100. A total of 1.410^{6} to 1.410^{7} ICU of *O.tsutsugamushi* was used to infect TEP macrophages cultured in six-well plates for the preparation of total RNA. Infection was confirmed by an immunofluorescent-antibody assay 2h after infection (2 to 5 bacteria were found per cell). Lipopolysaccharide (LPS) derived from *Escherichia coli* (Sigma Chemical Co., St. Louis, MO), which induces the production of chemokines in murine and human monocytes/ macrophages (67), was used as a positive control for each experiment.

Semiquantitative RT-PCR

Total RNA was prepared with SV total RNA isolation system (Promega, Madison, WI) as specified by the manufacturer and was quantified spectrophotometrically. Total RNA extracted from each sample (1to 2g per sample) was subjected to first-strand cDNA synthesis at 42C for 15 min in a 20-l reaction mixture containing 10mM Tris-HCl (pH 9.0), 50mM KCl, 5mM $MgCl_2$, 0.1% Triton X-100, 1mM deoxynucleoside triphosphate mixture, 1U of RNasin per l, 0.5g of oligo$(dT)_{15}$ primer, and 10U of avian myeloblastosis virus reverse transcriptase (RT) (all from Promega). The cDNA was heated at 99C for 5min and diluted with water. The cDNA amounts equivalent to 100ng of total RNA were subjected to PCR amplification in a 25-l reaction mixture containing 10mM Tris-HCl (pH 9.0), 50mM KCl, 2mM $MgCl_2$, 0.1% Triton X-100, 0.2mM deoxynucleoside triphosphate mixture, 1M of each primer, and 0.25U of *Taq* DNA polymerase (all from Promega) in a GeneAmp PCR System 9600 (Perkin-Elmer, Norwalk, CT). The reaction mixture was prepared as a master mixture to minimize reaction variation. One PCR cycle consisted of denaturation at 95C for 30s, annealing at 55C for 30s, and extension at 72C for 1min. The PCR products (5-l

Table 1. Primer sequences used in this study

Primer	Sequences[a]	PCR product size (bp)	No.of PCR cycles
MIP-1 α	5′-GGT CTC CAC CAC TGC CCT TGC-3′ 5′-GGT GGC AGG AAT GTT CGG CTC-3′	357	20
MIP-1 β	5′-AAC CCC GAG CAA CAC CAT GAA G-3′ 5′-TGA ACG TGA GGA GCA AGG ACG C-3′	390	25
MIP-2	5′-AGT TTG CCT TGA CCC TGA AGC C-3′ 5′-CCA TGA AAG CCA TCC GAC TGC A-3′	536	25
MCP-1	5′-TCT CTT CCT CCA CCA CCA TGC AG-3′ 5′-GGA AAA ATG GAT CCA CAC CTT GC-3′	582	25
RANTES	5′-CCT CAC CAT CAT CCT CAC TGC A-3′ 5′-TCT TCT CTG GGT TGG CAC ACA C-3′	215	30
IP-10	5′-CCT ATC CTG CCC ACG TGT TGA G-3′ 5′-GGC GTC GCA CCT CCA CAT AGC T-3′	436	30
TNF-	5′-GCG ACG TGG AAC TGG CAG AAG-3′ 5′-TCC ATG CCG TTG GCC AGG AGG-3′	340	30
I κ B α	5′-GGT GAA GGG AGA CCT GGC-3′ 5′-GTG GCC ATT GTA GTT GGT-3′	266	30
β -actin	5′-TGG AAT CCT GTG GGA TCC ATG AAA C-3′ 5′-TAA AAC GCA GCT CAG TAA CAG TCC G-3′	349	25

[a]For each primer pair, the sense primer is given above the antisense primer.

samples) were electrophoresed in a 2% agarose gel containing 0.5g of ethidium bromide per ml. An 100-bp DNA ladder (Promega) was used at 1g/lane as molecular size markers to provide bands from 100 to 1,500 bp. The amplified DNA fragments in the gels were identified according to their size predicted by cDNA sequences reported previously (2, 14, 22, 31, 46, 47, 58, 59, 65). The densities of the bands were analyzed using a Gel Doc 2000 Gel Documentation System and Quantity One software (Bio-Rad, Hercules, CA). The densitometric intensity was normalized by comparing the ratio of chemokine bands with that of b-actin. PCR was performed for the number of cycles described in Table 1 for each set of primers to ensure that the assay was in the linear range according to the amount of template (data not shown).

Results

TEP macrophages were exposed to *O. tsutsugamushi* (*O. tsutsugamushi*:macrophage ratios of 10:1 and 1:1) for 2 h and the profile of chemokine mRNA expression was analyzed by semiquantitative RT-PCR (Fig. 1). Although there were some variations in the ratio of RNA transcripts for the control and the test groups between the sets of RT-PCR experiments, the changes within a set of experiments were reproducible throughout this study. Macrophages treated with *E.coli* LPS were included as a positive control for each experiment. Macrophages treated with medium alone were used as a negative control. A low ratio (1:1) was sufficient to induce the expression of MCP-1, RANTES and TNF-a , while the dose of *O. tsutsugamushi* required to stimulate a significant production of MIP-1b, MIP-2, and IP-10 was higher (10:1). The mRNA levels of MIP-1b, RANTES and TNF-a showed an approximately eight- to eleven-fold increase in their optical densities. The mRNA levels of MIP-2, MCP-1, and IP-10 in macrophages incubated with *O.tsutsugamushi* increased by approximately two- to three-fold as measured by their optical densities. Compared to control groups, macrophages incubated for 3h with *O.tsutsugamushi* resulted in higher levels of mRNAs of all the chemokines and TNF-a tested. The orientiae-induced MCP-1 expression was only slightly higher than in the untreated control. However, this was due to a relatively strong constitutive MCP-1 expression in the untreated control. Basal levels of this chemokine is also expressed constitutively in monocytes and macrophages (16, 61). In the cells stimulated with 1g of LPS per ml for 2h, comparable but slightly smaller amounts of chemokine mRNAs were detected compared with those in *O.tsutsugamushi*-infected cells, suggesting that *O. tsutsugamushi* alone was capable of inducing an almost maximal expression of chemokine and TNF-a genes.

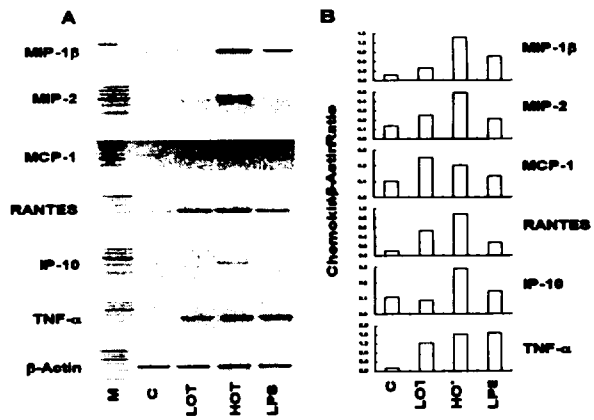

Fig. 1. Determination of chemokine and TNF-a mRNA induction in TEP macrophages treated with *O. tsutsugamushi* strain Karp. (A) The levels of chemokine and TNF-a mRNAs were compared by semiquantitative RT-PCR after incubation of murine peritoneal macrophages for 2 h with medium only (C), low ($(1.4\times10^6$ ICU, LOT) or high dose (1.4×10^7 ICU, HOT) of *O. tsutsugamushi*, or LPS derived from *E. coli* (LPS). M, 100-bp DNA ladder. (B) The densitometric band intensities were determined with Quantity One software, and normalized with mRNA level of -actin.

Before and after exposure of TEP macrophages to

O.tsutsugamushi, the levels of chemokine transcripts were assayed at each time point by semiquantitative RT-PCR (Fig. 2). The mRNAs for MIP-1a, MIP-1b, MCP-1, IP-10, and TNF-a were up-regulated and peaked at 1h, and began to decrease from 1to 3h after infection. While the MIP-1b mRNA persisted after incubation for 6h, the levels of transcripts for MIP-1a, MCP-1, IP-10 and TNF-a were reduced to the levels in uninfected cells by 6h. The transcript for RANTES was also detectable as early as 1 h after infection. Expression of RANTES mRNA was up-regulated and characterized by slower kinetics compared to those of other induced chemokine mRNAs.

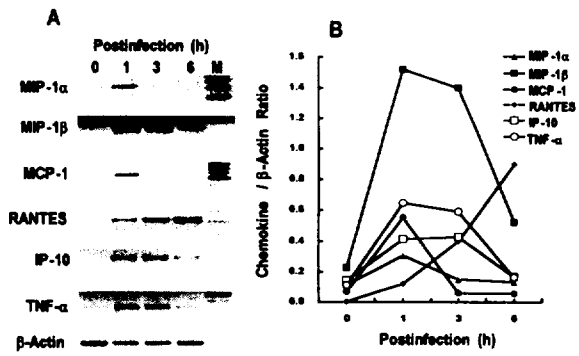

Fig. 2. Kinetics of *O. tsutsugamushi*-stimulated chemokine and TNF-a induction by TEP macrophages. (A) mRNA levels induced by the infection of *O. tsutsugamushi* (1.4×10^7 ICU), analyzed by semiquantitative RT-PCR at each time point. M, 100-bp DNA ladder. (B) Normalized expression level of each chemokine and TNF-a mRNA determined as for the experiment in Fig. 1B.

NF-kB positively regulates the expression of IkBa, since sequences within the IkBa promoter bind several NF-kB species including p50-p65 (35). Therefore, an increase in the steady-state level of IkBa mRNA is a sensitive indicator of NF-kB activation. The levels of IkBa mRNA, as measured by RT-PCR, which was barely detectable in uninfected macrophages, increased approximately three-fold (Fig. 3A and B). Furthermore, IkBa mRNA was clearly increased at 3 h after infection (Fig. 3C and D). No detectable alteration in levels of the housekeeping mRNA species, b-actin, occurred due to infection (Fig. 3).

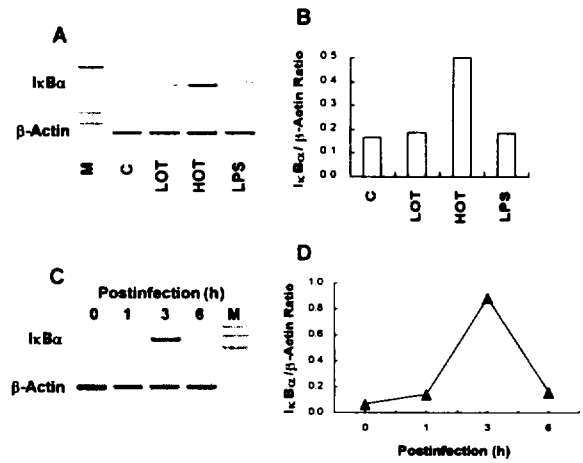

Fig. 3. Measurement of IkBa mRNA by RT-PCR in TEP macrophages following *O. tsutsugamushii* infection. (A) The levels of mRNA were compared by semiquantitative RT-PCR after incubation of murine TEP macrophages for 2 h with medium only (C), low ($(1.4\times10^6$ ICU, LOT) or high dose (1.4×10^7 ICU, HOT) of *O. tsutsugamushi*, or LPS derived from *E. coli* (LPS). (B) Normalized expression level of mRNA determined as for the experiment in Fig. 1B. (C) Time course of *O. tsutsugamushi*-stimulated IkBa induction by TEP macrophages. mRNA levels analyzed by semiquantitative RT-PCR at each time point after infection. (D) Normalized expression level of mRNA. M, 100-bp DNA ladder.

Discussion

It has been well documented that macrophages play a pivotal role in early immune responses to *O.tsutsugamushi* infection (29, 41, 43, 44). Although the inactive tissue macrophages could support the growth of *O.tsutsugamushi* at the site of infection, subsequent cellular influxes, especially of activated macrophages and lymphocytes, have been suggested to be important in protection against *O.tsutsugamushi* infection (26, 27).

Early PMN responses seem to provide a cellular population for oriential replication instead of providing antioriential activity *in vivo* (27). The cellular recruitment is controlled largely by chemokines which are secreted by stimulated cells such as macrophages at the site of primary infection (36, 67).

In this study, I showed that murine peritoneal macrophages induced the expression of MIP-1a, MIP-1b, MIP-2, MCP-1, RANTES, IP-10 and TNF-a in response to *O.tsutsugamushi* infection. With the exception of RANTES, the induction of the chemokine and TNF-a genes peaked transiently between 1and 3h. The inducibility and the kinetics of these chemokines and TNF-a are different from those of murine macrophages infected with other pathogenic microorganisms (51, 52). The differences in the patterns of early chemokine responses to various pathogens are likely to be related to disease manifestations (55). Although I did not perform assays to confirm the secretion of active chemokine and TNF-a protein following gene induction, several recent studies have shown a correlation between mRNA expression and chemokine protein secretion (7, 16, 52, 61).

This study revealed that murine peritoneal macrophages induced the expression of TNF-a in response to *O.tsutsugamushi* infection, which is compatible to the previous result, indicating *O. tsutsugamushi* stimulates TNF-a production in a macrophage cell line (11). In addition to its essential protective effects in the generation of immunity against pathogens, TNF-a has been shown in many systems to induce immunopathology *in vivo*. Significant increases in TNF-a levels in serum were observed during the convalescent phase in patients (25). TNF-a may be involved in the development of a condition such as hemophagocytic syndrome (HPS) during the advanced stage of tsutsugamushi disease in some patients (25). This disorder represents a hyperreaction of the immune system that is mediated by an upregulation of the cytokine network. This cytokine may thus have a specific role in disease progression. However, it should be determined whether the elevated production of this cytokine will contribute to host defense or pathogenesis.

The transcription factor NF-kB/Rel family plays a central role in the regulation of a variety of genes involved in host innate immunity, including various chemokines (18). *O. tsutsugamushi* infection resulted in an increased steady-state level of IkBa mRNA (Fig. 3), the promoter of which is specifically activated by several NF-kB species including p50-p65, p50c-Rel, and p65c-Rel (35, 62). Induction of IkBa transcription by NF-kB probably allows for replenishment of cytoplasmic IkBa following its degradation (48, 62) and serves as a sensitive marker of NF-kB activation. This result suggests that peritoneal macrophage infection with *O. tsutsugamushi* results in activation of NF-kB.

For all the chemokine genes tested in this study, regulation by NF-kB either has been demonstrated or is suggested by the presence of the NF-kB consensus motif in the promoter (13, 17, 67, 69). It is highly likely that the activation of transcription factor NF-kB is involved in the induction of chemokine genes in peritoneal macrophages by *O. tsutsugamushi* infection. However, direct evidence was not provided in this study to examine whether NF-kB activation is involved in the chemokine induction of *O. tsutsugamushi*-exposed peritoneal macrophages. Therefore it should be explored whether the induction of the chemokine mRNAs by *O. tsutsugamushi* is affected by using inhibitors of NF-kB activation, pyrrolidinedithiocarbamate (PDTC), an antioxidant (56) and *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK), a proteasome inhibitor (38).

Furthermore, previous studies had already demonstrated that various cytokines such as TNF-a or IL-1 stimulate chemokine production in macrophages (3, 42). Therefore, the role of newly synthesized host proteins in chemokine gene expression should be examined using cycloheximide, an eukaryotic protein

synthesis inhibitor.

Further studies on stimulatory components of *O.tsutsugamushi* and signal transduction pathways in host cells during oriential infection will provide valuable insights into the mechanisms controlling inflammatory responses during *O.tsutsugamushi* infection.

Protective immunity against *O.tsutsugamushi* is largely due to cell-mediated immune responses, particularly those provided by macrophages and T cells (28, 43, 57). The explanation for a susceptible/resistant mouse phenotype to *O.tsutsugamushi* infection was provided by the analysis of the early T-lymphocyte activation 1(*Eta-1*)/osteopontine (*Op*) gene, which maps to the *Ric* locus (20, 49). Eta-1/Op has been thought to enhance resistance to oriential infection by affecting the ability of macrophages to migrate to sites of infection and/or to express bactericidal activity (49). However, the infiltration of T lymphocytes and their secretion of Eta-1/Op in the early stage of infection should be preceded by activation of macrophages and their chemokine secretions, which recruit specific and nonspecific immune cells. In other studies, genetic susceptibility to infectious disease has been shown to be associated with the expression of different cytokine profiles (23). Members of the CC chemokine subfamily, which include RANTES, MIP-1a, MIP-1b, and MCP-1, preferentially attract monocytes and lymphocytes. Those of the CXC chemokine subfamily, such as IL-8 and MIP-2, are potent neutrophil attractants (3). Furthermore, a correlation between chemokines and a subset of T-cell responses has been described (37, 54, 60). While the CC chemokines MIP-1a, MIP-1b, and RANTES were found to be efficient chemoattractants for Th1 cells, Th2 cells were not attracted by these chemokines (60). Stimulation of T cells in the presence of MIP-1a enhanced gamma interferon production by Th1 cells, while stimulation of T cells in the presence of MCP-1 led to an increased IL-4 production (37). Based on these studies, I hypothesize that a delicate balance of chemokines exists between the induction of a resistant and a susceptible immune response to oriential infection. Further study is required to determine whether qualitative and quantitative differences in the production of chemokines can be correlated with the resistant or susceptible mouse phenotype.

Acknowledgment

This work was supported by a grant from the Cheju National University Development Foundation made in the program year of 2000 to Y.-S. Koh. I thank Dr. Ik-Sang Kim in the Department of Microbiology and Immunology, Seoul National University College of Medicine for sending *O. tsutsugamushi* strains and anti-*O. tsutsugamushi* antibodies.

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