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Communication

Molecules and Cells

Chemokines Gene Expression of RAW 264.7 Cells by *Actinobacillus actinomycetemcomitans* Lipopolysaccharide Using Microarray and RT-PCR Analysis

Jin Chung[1], Mun Jeoung Choi[1], So Yeon Jeong[1], Jong Suk Oh[2], and Hyung Keun Kim[3,*]

Actinobacillus actinomycetemcomitans* (*A. actinomycetemcomitans*) is an important pathogen casuing aggressive periodontitis. The present study was designed to investigate the chemokines expression regulated by *A. actinomycetemcomitans* lipopolysaccharide (LPS). Chemokines genes expression profiling was performed in Raw 264.7 cells by analyses of microarray and reverse transcription-polymerase chain reaction (RT-PCR). Microarray results showed that the induction of monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1α (MIP-1α), MIP-1β, MIP-1γ, regulated upon activation, normal T-cell expressed and secreted (RANTES), macrophage inflammatory protein-2 (MIP-2), and interferon-γ inducible protein 10 (IP 10) by *A. actinomycetemcomitans* LPS was increased to 12.5, 1.53, 9.09, 17.3, 2.82, 16.1, and 18.1 folds at 18 h, respectively. To check these chemokines expression by *A. actinomycetemcomitans* LPS, we examined gene expressions by RT-PCR, and found that the expression of MIP-1β, MIP-1γ, RANTES, MIP-2, and IP 10 was increased 107.1, 93.6, 106.8, 86.5, and 162.0 folds at 18 h, respectively. These results indicate that *A. actinomycetemcomitans* LPS stimulates the several chemokines expressions (MIP-1α, MIP-1β, MIP-1γ, RANTES, MIP-2, and IP 10) in Raw 264.7 cells.

INTRODUCTION

Periodontal disease which is mainly caused by oral bacteria is one of the most significant causes of tooth loss in adults. Oral microflora colonizes and evokes inflammatory responses in oral cavity (Theilade, 1989). *Actinobacillus actinomycetemcomitans (A. actinomycetemcomitans)* is a gram-negative capnophilic bacillus and associated with localized aggressive periodontitis (Slot, 1999; Zambon, 1985). It has been known that A. *actinomycetemcomitans* induces the expression of several cytokines and chemokines in periodontal inflammatory response (Jiang and Graves, 1999; Oido-Mori et al., 2001).

Chemokines, a family of chemotactic cytokine, are small heparin-binding proteins that direct the movement of circulating leukocytes to site of inflammation and injury (Kim et al., 2004; Lee et al., 2008). Chemokine sequences usually have four conserved cysteine residues, and based on the position of the first two cyteine residues, these proteins can be divided into four subfamilies: the CXC (α), CC (β), C (γ), and CX_3C (δ) families (Baggiolini et al., 1997). Chemokines affect cell by activating surface receptors that are seven-transmembrane-domain G-protein-coupled receptors: leukocyte responses to particular chemokines are determined by their complement of chemokine receptors (Charo and Ransohoff, 2006). The binding of the chemokine to the receptor activates signaling cascades that culminate in the rearrangement, change of shape, and cell movement. Some chemokines including Interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), and regulated-upon-activation normal T-cell expressed and secreted (RANTES) have been reported in associated with human periodontal disease (Gamonal et al., 2001; Tonetti et al., 1994,). However, total analysis for the gene expressions of chemokine and chemokine receptors induced by *A. actinomycetemcomitans* lipopolysaccharide (*A. actinomycetemcomitans* LPS) was not reported.

DNA microarray technology is a new and powerful tool that allows the simultaneous analysis of a large number of nucleic acid hybridizations in a rapid and efficient fashion. Therefore, in this study, using microarray, we investigated the chemokines gene expression induced by *A. actinomycetemcomitans* LPS in RAW 264.7 cells and confirmed the microarray results by reverse transcription-polymerase chain reaction (RT-PCR).

MATERIALS AND METHODS

Bacterial growth

A. actinomycetemcomitans strain ATCC 33384 was cultured in Tryptic soy broth, which contained 5 mg/ml hemin and 0.5 mg/ml of vitamin K at 37°C in an incubator.

[1]Department of Microbiology, School of Dentistry and Research Institute for Oral Biotechnology, Pusan National University, Busan 602-739, Korea, [2]Department of Microbiology, Chonnam National University, Gwangju 501-757, Korea, [3]Cardiovascular Research Institute, Chonnam National University, Gwangju 501-757, Korea
*Correspondence: chemokines@naver.com

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LPS purification

A. actinomycetemcomitans was grown and harvested at the end of the logarithmic phase of growth. LPS extraction was achieved by the hot phenol-water method (Westphal and Jann, 1965). Briefly, the bacterial cell pellet was suspended in pyrogen-free water, and then an equal volume of 90% (v/v) phenol at 60°C was added dropwise for 20 min and stirred constantly. The aqueous phase was separated by centrifugation at 7,000 rpm for 15 min at 4°C and collected. This process was repeated, and the aqueous phase was pooled and dialyzed against deionized water for 3 d at 4°C. The dialyzed LPS preparation was then centrifuged at 40,000 rpm for 1.5 h at 4°C using ultracentrifuge (Beckman, USA). The precipitate was suspended with 30 ml of pyrogen-free water, dialyzed against distilled water for 3 d, lyophilized, and stored at 4°C. LPS samples were separated by SDS-polyacrylamide gel electrophoresis and stained for protein with Coomassie blue to confirm the purity of the LPS moieties.

Cell culture

The mouse macrophage cell line RAW 264.7 was purchased from ATCC (USA). Cells were maintained in DMEM with 10% fetal bovine serum (Life Technologies Inc., Scotland), 100 U of penicillin/ml, and 100 μg of streptomycin/ml and were incubated at 37°C in a humidified atmosphere of 5% CO_2.

RNA preparation

RAW 264.7 were treated for 2 h and 18 h with 0.1 μg/ml of *A. actinomycetemcomitans* LPS. Total RNA from these cells was isolated using TRIzol (Invitrogen, USA) according to the manufacturer's instructions. The concentration of the RNA obtained was determined by measuring the absorbance at 260 and 280 nm using microplate reader (Infinite M200, Tecan Austria GmbH, Austria).

Microarray

The gene expression array analysis was performed using the mouse chemokine and receptor gene GEArray Q series kit (SuperArray Inc., USA) according to the manufacturer's instructions. This array membrane is composed of 67 chemokine and chemokine receptor genes, a plasmid pUC18 negative control, and four housekeeping genes including glyceraldehydes-3-phosphate dehydrogenase (GAPDH), cyclophilin A, ribosomal protein L13a, and β-actin, each of which is printed with tetra spots format. The biotin-16-dUTP-labeled cDNA probes were synthesized from 5 μg of total RNA. After pre-hybridization with GEAhyb Hybridization Solution (SuperArray Inc.) containing 100 μg of DNA/ml of denatured salmon sperm DNA (Invitrogen, USA) for 2 h at 60°C, the array membrane was hybridized with denatured cDNA probes overnight at 60°C. Following washing the membrane twice with 2× sodium citrate dihydrate (SSC), 1% (w/v) SDS and twice with 0.1x SSC, 0.5% (w/v) SDS for 15 min at 60°C each, the membrane was blocked with GEAblocking Solution Q (SuperArray Inc.) for 40 min and incubated with alkaline phosphatase-conjugated streptavidin for 10 min at room temperature. Chemiluminescent detection was performed using CDP-Star chemiluminescent substrate and array image was recorded with X-ray film. The image was scanned with a scanner TouchToss SIS-3800 (Samsung, Korea). The resulting scanned image was converted to raw data file using Scanalyse software. GEArray Analyzer software (SuperArray Inc.) was used for data analysis. The relative expression levels of different genes were estimated by comparing its signal intensity with that of internal control of β-actin.

	A	B	C	D	E	F	G	H
1	5830453M24 Rik	Bir1	Cexcr1	Il8rb (Cmkar2)	Cmkar3	Cmkar4	Cmkbr1	Cmkbr10
2	Cmkbr11l	Cmkbr12	Cmkbr2	Cmkbr3	Cmkbr4	Cmkbr5	Cmkbr6	Cmkbr7
3	Cmkbr8	Cmkbr9	Cmkor1	Cx3cr1	Cxcl16	Cxcr6	D6-pending	Dfy
4	Emap2	Epo	Gro1	Ifna11	Ifnab	Ltb4r2	Pf4	Ppbp
5	Pumag	Scya1	Scya11	Scya12	Scya17	Scya19	Scya2	Scya20
6	Scya21a	Scya22	Scya24	Scya25	Scya27	Scya28	Scya3	Scya4
7	Scya5	Scya6	Scya7	Scya8	Scya9	Scyb10	Scyb11	Scyb13
8	Scyb14	Scyb15	Scyb2	Scyb5	Scyb9	Scyc1	Scyd1	Sdf1
9	Sdf2	Taphp	Tcp10	Blank	Blank	Blank	Blank	Blank
10	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
11	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
12	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
13	PUC18	PUC18	PUC18	Blank	Blank	Blank	Gapd	Gaph
14	Ppia	Ppia	Ppia	Ppia	RPl13a	RPl13a	Actb	Actb

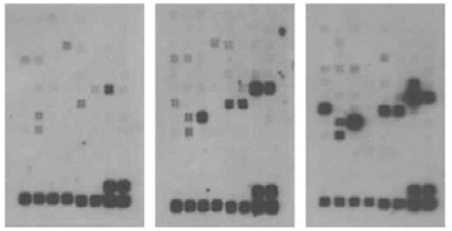

Fig. 1. Microarray spots by *A. actinomycetemcomitans* LPS in RAW264.7 cells.

RT-PCR

Total RNA isolated from each sample was used as a template for the cDNA synthesis. The reverse transcription (RT) of total RNA to cDNA was performed by using AccuPower RT PreMix (Bioneer Co., Korea). cDNA was amplified by PCR with a Perkin-Elmer thermal cycler (denaturation for 30 s at 95°C, annealing for 30 s at 58-70°C, and elongation for 30 s at 72°C) with MIP-1α (416 bp), MIP-1β (351 bp), RANTES (205 bp), MIP-1γ (304 bp), MIP-2 (379 bp), IP-10 (389 bp) and β-actin. The primers used in these analysis were as follows: MIP-1α, 5′-ACT GAA GCC AGC TCT CTC TTC CTC-3′ and 5′-TTC CTT CTT GGG GTC AGC ACA GAC-3′; MIP-1β, 5′-CCT AAC CCC GAG CAA CAC CA-3′ and 5′- GGG CTG AGG AGG CCT GTC C-3′; RANTES, 5′-GCC CAC GTC AAG GAG TAT TTC TAC-3′ and 5′-AGG ACT AGA GCA AGC GAT GAC AGG-3′; MIP-1γ, 5′-GAT CAC ACA TGC AAC AGA GAC AA-3′ and 5′-TTA TTG TTT GTA GGT CCG TGG TTG-3′; MIP-2, 5′-TCT TCC TCG GGC ACT TCC AGA-3′ and 5′-GGA CAG CAG CCC AGG CTC CT-3′; IP-10, 5′-GAT GGC TAG TCC TAA TTG CCC TTG G-3′ and 5′-CTG AGT ATC TTG ATA ACC CCT TGG G-3′; β-actin, 5′-GGT CAG AAC TCC TAT G-3′ and 5′-GTA ACA ATG CCA TGT TCA AT-3′. Products were electrophoresed in 1.5% (w/v) agarose gels containing ethidium bromide and analyzed under UV light. The intensities of the products were analyzed using an image analysis program (Multi Gauge V3.0, Fuji Film, Japan).

Statistical analysis

The results were presented as means ± SDs of three independent measurements performed in triplicate. The statistical significance of differences between untreated control and

Table 1. Chemokines gene table by *A. actinomycetemcomitans* LPS using microarray methods

Receptors	Symbol	Gene name	Description	Con	2 h	18 h
Chemokine receptors	Blr1	CXCR5	Mus musculus Burkitt lymphoma receptor 1	0.13	0.11	0.06
	Ccxcr1	mXCR1	Mus musculus chemokine (C-motif) XC receptor 1	0.17	0.16	0.13
	Cmkar3	CXCR3	Mus musculus chemokine receptor CXCR3 mRNA	0.09	0.13	0.08
	Cmkar4	CXCR4	Mouse mRNA for murine CXCR-4	0.07	0.12	0.07
	Cmkbr1	CCR-1	Mus musculus chemokine (C-C) receptor 10	0.05	0.11	0.07
	Cmkbr1l1	Cmkbr1l1	Mus musculus chemokine (C-C) receptor 1,-like 1	0.08	0.08	0.03
	Cmkbr1l2	Cmkbr1l2	Mus musculus lipopolysaccharide inducible C-C chemokine receptor related (L-CCR)	0.08	0.11	0.19
	Cmkbr2	CCR2	Mus musculus C-C chemokine receptor (mCCR2) mRNA	0.07	0.08	0.06
	Cmkbr3	CCR3	Chemokine (C-C) receptor 1, -like 2, Mus musculus chemokine G-protein-coupled receptor CCR-3 gene	0.06	0.08	0.05
	Cmkbr4	CCR4	Chemokine (C-C) receptor 4	0.05	0.1	0.04
	Cmkbr5	CCR5	Mus musculus mRNA for C-C chemokine receptor 5	0.04	0.09	0.06
	Cmkbr6	CCR6	Mus musculus mRNA for G protein-coupled receptor KY411	0.04	0.12	0.07
	Cmkbr7	CCR7	Mus musculus chemokine (C-C) receptor 7	0.07	0.17	0.09
	Cmkbr8	CCR8	Mus musculus chemokine receptor CCR8 gene	0.07	0.09	0
	Cmkbr9	CCR9	Mus musculus chemokine (C-C) receptor 9	0.07	0.09	0.06
	Cmkbr10	Cmkbr10	Mus musculus mRNA for chemokine receptor CCR9 or chemokine (C-C) receotor 10	0.05	0.13	0.07
	Cmkor1	CMKOR1	Mus musculus chemokine receptor orphan receptor 1	0.06	0.08	0.03
	Cx3cr1	CXCR-1	Mus musculus chemokine receptor CX3CR1 mRNA	0.45	0.41	0.04
	Cxcr6	Cxcr6	Chemokine (C-X-C) receptor 6	0.04	0.14	0.08
Subfamily B (CXC)	Cxcl16	Cxcl16	Cxc chemokine ligand 16	0.1	0.29	0.2
	Gro1	Gro1	GRO1 oncogene	0.05	0.12	0.33
	Scyb2	MIP2	Mouse mRNA for macrophage inflammatory protein-2	0.06	1.05	1.09
	Scyb 5	LIX	Mus musculus CXC chemokine MIP-2gamma precursor, mRNA	0.06	0.06	0.25
	Scyb 9	MIG	Mouse monokine induced by gamma interferon	0.06	0.07	0.18
	Scyb 10	IP-10	Interferon-gamma induced protein CRG-2 precursor	0.06	0.85	0.97
	Scyb 11	SCYB11	Mus musculus CxC chemokine SCYB11.pep gene	0.07	0.23	0.24
	Scyb 13	BLC/BCA-1	Mus musculus B lymphocyte chemoattractant BLC	0.05	0.15	0.2
	Scyb 14	BRAK	Mus musculus CXC chemokine MIP-2gamma precursor, mRNA	0.07	0.09	0.11
	Scyb 15	Sbyb15	Small inducible cytokine subfamily B, member 15	0.46	0.55	0.86
Other subfamily members	Scyc1	lymphotactin	Mus musculus cytokine lymphotactin mRNA	0.06	0.06	0.18
	Scyd1	Fractalkine	Small inducible cytokine subfamily D, 1	0.08	0.08	0.09
Chemokine receptors	Blr1	CXCR5	Mus musculus Burkitt lymphoma receptor 1	0.13	0.11	0.06
	Ccxcr1	mXCR1	Mus musculus chemokine (C-motif) XC receptor 1	0.17	0.16	0.13
	Cmkar3	CXCR3	Mus musculus chemokine receptor CXCR3 mRNA	0.09	0.13	0.08
	Cmkar4	CXCR4	Mouse mRNA for murine CXCR-4	0.07	0.12	0.07
	Cmkbr1	CCR-1	Mus musculus chemokine (C-C) receptor 10	0.05	0.11	0.07
	Cmkbr1l1	Cmkbr1l1	Mus musculus chemokine (C-C) receptor 1,-like 1	0.08	0.08	0.03
	Cmkbr1l2	Cmkbr1l2	Mus musculus lipopolysaccharide inducible C-C chemokine receptor related (L-CCR)	0.08	0.11	0.19
	Cmkbr2	CCR2	Mus musculus C-C chemokine receptor (mCCR2) mRNA	0.07	0.08	0.06
	Cmkbr3	CCR3	Chemokine (C-C) receptor 1, -like 2, Mus musculus chemokine G-protein-coupled receptor CCR-3 gene	0.06	0.08	0.05
	Cmkbr4	CCR4	Chemokine (C-C) receptor 4	0.05	0.1	0.04
	Cmkbr5	CCR5	Mus musculus mRNA for C-C chemokine receptor 5	0.04	0.09	0.06
	Cmkbr6	CCR6	Mus musculus mRNA for G protein-coupled receptor KY411	0.04	0.12	0.07
	Cmkbr7	CCR7	Mus musculus chemokine (C-C) receptor 7	0.07	0.17	0.09
	Cmkbr8	CCR8	Mus musculus chemokine receptor CCR8 gene	0.07	0.09	0
	Cmkbr9	CCR9	Mus musculus chemokine (C-C) receptor 9	0.07	0.09	0.06
	Cmkbr10	Cmkbr10	Mus musculus mRNA for chemokine receptor CCR9 or chemokine (C-C) receotor 10	0.05	0.13	0.07
	Cmkor1	CMKOR1	Mus musculus chemokine receptor orphan receptor 1	0.06	0.08	0.03
	Cx3cr1	CXCR-1	Mus musculus chemokine receptor CX3CR1 mRNA	0.45	0.41	0.04
	Cxcr6	Cxcr6	Chemokine (C-X-C) receptor 6	0.04	0.14	0.08

(continued)

Receptors	Symbol	Gene name	Description	Con	2h	18h
Chemokine receptors	D6	D6	Mus musculus D6 beta-chemokine receptor	0.06	0.16	0.1
	IL8rb	CXCR2	Chemokine (CXC) receptor 2 (interleukin-8 receptor)	0.09	0.09	0.06
	Ltb4r2	Ltb4r2	Mus musculus leukotriene B4 receptor 2	0.04	0.09	0.08
	Pumag	Pumag	Mus musculus interferon-gamma inducible gene, Puma-g	0	0.13	0.15
	Tapbp	Tapbp	Mus musculus TAP binding protein	0.39	0.55	0.97
Other related genes	Dfy	DFY	Mus musculus Duffy glycoprotein mRNA	0.05	0.17	0.09
	Emap2	Emap2	Mus musculus endothelial-monocyte activating polypeptide II	0.31	0.38	0.24
	Epo	Epo	Mus musculus Erythropoietin	0.24	0.32	0.28
	Ifna11	IFN-a11	Interferon alpha family, gene 11	0.02	0.08	0.06
	Ifnab	IFN-ab	Mus musculus Interferon alpha family, gene B	0.03	0.13	0.05
	Pf4	PF 4	Mus musculus mRNA for platelet factor 4	0.04	0.13	0.12
	Ppbp	Ppbp	Pro-platelet basic protein	0.05	0.17	0.15
	Sdf1	SDF-1	Mus musculus cytokine (SDF-1-alpha)	0.1	0.14	0.1
	Sdf2	SDF-2	Mus musculus stromal cell derived factor 2	0.07	0.08	0.11
	Tcp10	Tcp10	Mus musculus t-complex protein-10 complex	0.05	0.04	0.22

Table 2. Genes expressed in RAW264.7 cells induced by *A. actinomycetemcomitans* LPS

Chemokines	Symbol	Gene name	Description	2 h (fold)	18 h (fold)
Subfamily A (CC)	Scya2	MCP-1	Mus musculus strain BALB small inducible cytokine A2 precursor (Scya2) mRNA	3.7	12.5
	Scya3	MIP-1α	Macrophage inflammatory protein (MIP)	1.52	1.53
	Scya4	MIP-1β	M.musculus MIP-1β gene for macrophage inflammatory protein 1β	9.6	9.09
	Scya5	RANTES	Small inducible cytokine A5 (RANTES)	6.83	17.3
	Scya9	MIP-1γ	Mus musculus C10-like cytokine	2.29	2.82
Subfamily B (CXC)	Scyb2	MIP-2	Mouse mRNA for macrophage inflammation protein-2	14.1	16.1
	Scyb10	IP-10	Interferon-gamma induced protein CRG-2 precursor, mRNA	17.5	18.1

treated groups were determined using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

LPS is a major component of the outer membrane of gram-negative bacteria. LPS is known to be able to induce the response of local cells to secrete high level of several cytokines that lead to periodontal tissue destruction (Birkedal-Hansen, 1993). It has been reported that low concentration of *A. actinomycetemcomitans* LPS stimulates macrophage to produce interleukins and tumor necrosis factor (Saglie et al., 1990). *A. actinomycetemcomitans* induces the immune response associated with periodontal disease. *A. actinomycetemcomitans* extract induced a time-dependent and dose-dependent expression of interleukin-8 in gingival epithelial cell (Sfakianakis et al., 2001). *A. actinomycetemcomitans* stimulated 500 to 5000 times more potent of MCP-1 production than *Porphyromonas gingivalis* in human peripheral blood mononuclear cell (Jiang et al., 1996). Moreover, MCP-1, MIP-1α, MIP-1β, IP-10, and RANTES- producing cells were found to be present in inflamed human gingival tissue (Kabashima et al., 2002). In this study, we used *A. actinomycetemcomitans* LPS as an inducer for the mRNA expressions of chemokine and chemokine receptors in RAW 264.7 cells. *A. actinomycetemcomitans* LPS stimulated the mRNA expressions of chemokines and chemokine receptors on microarray analysis in RAW 264.7 cells (Fig. 1; Table 1).

Chemokine classification is base on whether the two amino terminal cysteine residues are immediately adjacent or separated by one amino acid. Most CXC chemokines are chemoattractants for neutrophils whereas CC chemokines generally attract monocytes, lymphocytes, basophils, and eosinophils. These chemokines are released locally at sites of inflammation, attract immune cells, and play a central role in the inflammatory process and the final outcome of immune response (Baggiolini et al., 1997). In the present study, in CC chemokines, *A. actinomycetemcomitans* LPS induced the MCP-1, MIP-1α, MIP-1β, RANTES, and MIP-1γ expression levels to 3.7, 1.52, 9.6, 6.83, and 2.29 folds at 2h and to 12.5, 1.53, 9.09, 17.3, and 2.82 folds at 18 h after treatment with *A. actinomycetemcomitans* LPS compared with the control (without *A. actinomycetemcomitans* LPS), respectively (Table 2). In CXC chemokines, *A. actinomycetemcomitans* LPS induced the MIP-2, and IP-10 expression levels to 14.1 and 17.5 folds at 2 and to 16.1 and 18.1 folds at 18 h after treatment with *A. actinomycetemcomitans* LPS compared with the control, respectively (Table 2). In addition, the expression of MCP-1 and RANTES was more increased to 3.4 and 2.5 folds at 18 h after *A. actinomycetemcomitans* LPS treatment than 2 h, respectively (Table 2).

In order to confirm the results of microarray, RT-PCR was carried out. In accordance with the microarray results, *A. actinomycetemcomitans* LPS enhanced the mRNA expressions of MIP-1β, RANTES, MIP-1γ, MIP-2, and IP-10 in RT-PCR analysis except for MIP-1α (we couldn't detect this chem-

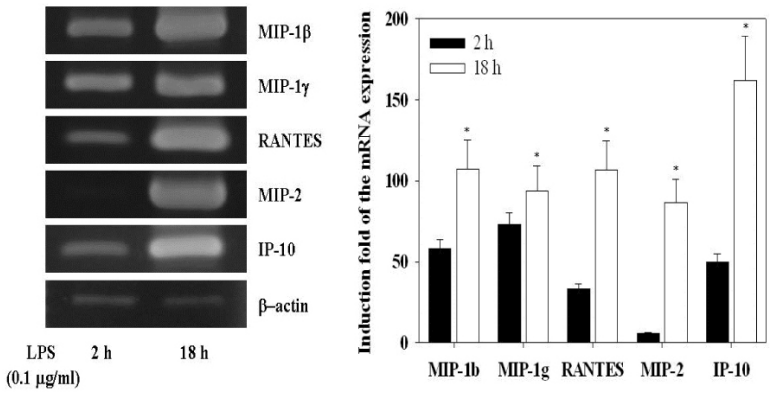

Fig. 2. Expressions of chemokines gene mRNA in RAW 264.7 cells. RAW 264.7 were treated for 2 h and 18 h with 0.1 μg/ml of *A. actinomycetemcomitans* LPS. Total RNA from these cells was isolated using TRIzol (Invitrogen, USA) according to the manufacturer's instructions. The ratios of the RT-PCR products of MIP-1β, MIP-1γ, RANTES, MIP-2, and IP 10 to control (control data not shown) were calculated using an image analysis program (Multi Gauge V3.0, Fuji Film, Japan). Induction folds are represented as the means ± SDs of three separate experiments. Statistical significance; $*p < 0.05$ vs. control.

okine by RT-PCR). MIP-1β, MIP-1γ, RANTES, MIP-2, and IP 10 increased 57.8, 72.9, 33.1, 5.9, and 49.7 folds at 2 h, respectively. In 18 h, MIP-1β, MIP-1γ, RANTES, MIP-2, and IP 10 increased 107.1, 93.6, 106.8, 86.5, and 162.0 folds, respectively (Fig. 2). These results indicate that *A. actinomycetemcomitans* LPS induces the migration of mainly T lymphocytes and monocytes to the inflammatory site by stimulating the production of the chemokines described above from macrophages.

CONCLUSION

This study indicates that *A. actinomycetemcomitans* LPS in RAW 264.7 cells stimulates the mRNA expressions of MIP-1β, RANTES, MIP-1γ, MIP-2, and IP-10. Furthermore, these kinds of chemokines might be involved the pathologic process of localized aggressive periodontitis by directing the movement of T lymphocytes, monocytes, or neutrophils to the inflammatory site.

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