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ENHANCED INTERLEUKIN-10 SIGNALING WITH 14-MEMBER MACROLIDES IN LIPOPOLYSACCHARIDE-STIMULATED MACROPHAGES

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ABSTRACT

Immunomodulatory effects of 14-member macrolides, namely erythromycin (EM) and clarithromycin (CAM), have been reported in chronic respiratory infectious diseases. It has been suggested that 14-member macrolides have immunomodulatory effects on various lung cells such as alveolar macrophages. Interleukin (IL)-10 is an immunomodulatory cytokine that performs an irreplaceable role in negatively regulating inflammation, primarily via a mechanism that selectively blocks the expression of pro-inflammatory genes. It activates signal transducer and activator of transcription (STAT)-3, and subsequently induces the suppressor of cytokine signaling-3 (SOCS-3), resulting in the resolution of inflammatory response in macrophages. However, it has been still unclear whether 14-member macrolides exert immunomodulatory effects via IL-10 signaling pathway. We aimed to evaluate whether 14-member macrolides affect the IL-10 signaling pathway. The RAW264.7 macrophage cell line was pretreated with EM or CAM, and stimulated with lipopolysaccharide (LPS). The levels of IL-10, IL-10 receptor, phosphorylated (p) STAT-3, and SOCS-3 were determined by RT-PCR, ELISA and immunoblotting. We observed increased levels of IL-10, p-STAT-3 and SOCS-3 in the treated cells. In addition, while the levels of tumor necrosis factor-α 6 h after LPS stimulation was equal between vehicle-treated and CAM-treated macrophage cells, those of CAM-treated cells were repressed 36 h following LPS stimulation, compared with those of the control cells. Therefore, the 14-member macrolides may initiate an early resolution of inflammation, in part, via the enhancement of the IL-10/STAT-3/SOCS-3 pathway.

Keywords: interleukin-10; immunomodulatory effects; erythromycin; clarithromycin; STAT3; SOCS-3; macrophage

INTRODUCTION

Macrolide antibiotics are a group of antibiotics whose activity stems from the presence of a *macrolide ring*, a large macrocyclic lactone ring to which one or more deoxy sugars, usually cladinose and desosamine, may be attached (Jain and Danziger, 2004). Fourteen, fifteen, and sixteen-membered lactone ring macrolides are available for clinical use. In particular, 14-member macrolides, such as erythromycin (EM) and clarithromycin (CAM), are widely used in patients with respiratory infections (Wierzbowski et al., 2006).

Diffuse panbronchiolitis (DPB) is a respiratory disease observed in the Asian population (Azuma and Kudoh, 2006; Keicho and Kudoh, 2002). Prior to the dis-

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covery of long term EM therapy, DPB was an untreatable disease, which resulted in a lower respiratory infection from *Haemophilus influenzae* or *Pseudomonas aeruginosa*, and many patients with DPB died due to their progressive respiratory failure. Kudoh et al. reported that the long-term use of EM in these patients improved their chest X-rays and lung functions, and resulted in a vast improvement in their survival (Kudoh et al., 1998). Similar therapeutic effects of CAM have been reported in DPB patients (Kadota et al., 2003).

The therapeutic mechanism of macrolides in DPB is not fully understood. The mechanism appears to be independent of the antibiotic effect of macrolides because their therapeutic benefit in DPB is observed at a dosage below the standard antibiotic dosage (Azuma and Kudoh, 2006; Keicho and Kudoh, 2002). Accumulating evidence suggests that an important mechanism in macrolide therapy for DPB may be immunomodulatory effects (Azuma and Kudoh, 2006). We previously demonstrated that CAM suppresses IL-8 production via suppression of AP-1 activity in bronchial epithelial cells (Abe et al., 2000). Others have shown that 14-member macrolides exert immunomodulatory effects on lung epithelial cells (Shinkai et al., 2006).

Alveolar macrophages are resident immune cells that play an important role in host defense and inflammation in the lung (Shibata et al., 2001). Invading pathogens are recognized via pathogen recognition receptors such as toll-like receptor-2, -4, scavenger receptors, and mannose receptors, and are phagocytosed. These macrophages also produce pro-inflammatory cytokines and chemokines, which accumulate and activate other inflammatory cells such as neutrophils and T lymphocytes (Akira, 2003; Plowden et al., 2004). Macrolide antibiotics reportedly repress the production of pro-inflammatory cytokines in alveolar macrophages (Hodge et al., 2006).

Among the cytokines induced in the inflamed lung, interleukin (IL)-10 is a potent anti-inflammatory cytokine predominantly produced by the alveolar macrophages (Moore et al., 2001). IL-10 suppresses inflammation via the repression of nuclear factor-κB (Shames et al., 1998), and via the activation of the suppressor of cytokine signaling 3 (SOCS-3) (Yoshimura et al., 2003, 2005). Following the binding of IL-10 to its receptor, the signal transducer and activator of transcription (STAT)-3 is phosphorylated. Subsequently, SOCS-3 is induced and it inhibits pro-inflammatory cytokine signals (Yoshimura et al., 2003, 2005). Through this pathway, IL-10 also represses the production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α in macrophages (Yoshimura et al., 2003, 2005). Although CAM reportedly augments the expression of IL-10 in human monocytes (Morikawa et al., 1996), it is unknown whether the immunomodulatory effects of 14-member macrolides on macrophages are associated with this IL-10 pathway.

With this in mind we sought to clarify the effect of 14-member macrolides on the expression of IL-10 in a macrophage cell line. We showed that the production of IL-10 by macrophages pretreated with EM and CAM was enhanced upon stimulation with lipopolysaccharide (LPS), followed by STAT-3 phosphorylation and SOCS-3 up-regulation. The induction of IL-10 may thus be involved in the immunomodulatory effects of 14-member macrolide antibiotics on macrophages.

MATERIALS AND METHODS

Cell culture. The mouse macrophage cell line RAW264.7 was grown in DMEM with 10 % FBS, 100 U/mL penicillin, and 100 U/mL streptomycin in an atmosphere of 5 % CO2 and 95 % air at 37 °C.

Pretreatment with macrolides and stimulation with LPS. RAW264.7 cells were seeded onto a 6 cm dish at 1×10^5 cells/dish and were treated with vehicle (ethanol), EM (Sigma, St. Louis, MO, USA: 1 or 10 μg/mL) or CAM (Sigma: 1 or 10 μg/mL) for 3 days. Subsequently, the cells were stimulated with LPS (Sigma: 20 ng/mL) for 4 h (total RNA isolation), 8 h

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(cell lysate preparation), or 24 h (supernatant collection).

RT-PCR. Total RNA was extracted from cells using TRIzol regent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. Reverse transcription (RT) was performed using SuperScript II reverse transcriptase (Invitrogen) and gene expression was assessed by RT-PCR. Sequences of the specific primers used are as follows: for IL-10 (forward; CTT CAG CCA GGT GAA GAC, reverse; TGG AGT CCA GCA GAC TCA AT), for IL-10 receptor (R) (forward; AGG CAG AGG CAG CAG GCC CAG CAG AAT GCT, reverse; TGG AGC CTG GCT AGC TGG TCA CAG TAG GTC T), for SOCS-3 (forward; ACC AGC GCC ACT TCT TCA CG, reverse; GTG GAG CAT CAT ACT GAT CC), and for GAPDH (forward; ACT CCA CTC ACG GCA AAT TCA ACG G, reverse; AGG GGC GGA GAT GAT GAC CC). PCR products were electrophoresed in 1 % agarose gels containing ethidium bromide and visualized digitally with a UV illuminator (AB1500 Printgraph and AE 6905H Image Saver HR; ATTO Bioscience, Tokyo, Japan). Band intensities were semi-quantified using computer software (Lane Analyzer 3.0; ATTO Bioscience) (Machiya et al., 2007).

ELISA. Supernatant IL-10 concentrations were measured using a sandwich ELISA kit (R&D systems, Minneapolis, MN, USA), according to the manufacturer's protocol. After LPS stimulation, the supernatants were collected and stored at -80 °C until measurement. After removal of all supernatants, adhered cells were lysed with cell lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1 % Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, and 1 μg/mL leupeptin), and protein concentrations determined with the RC DC protein assay kit (Bio-Rad, Hercules, CA, USA). IL-10 concentrations were adjusted for total protein in the cell lysate.

Immunoblot assay for phosphorylated STAT-3 (p-STAT-3). After cells lysis, 15 μg of protein was blotted onto a PVDF membrane (Amersham, Piscataway, NJ, USA). The membranes were blocked with milk and treated with rabbit phospho-STAT-3 antibody (1:1000 dilution; Cell Signaling Technology, Danvers, MA, USA) and horseradish peroxidase (HRP)-conjugated anti-rabbit IgG immunoglobulin (1:2000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Subsequently, p-STAT-3 was detected using the ECL plus system (Amersham, Piscataway, NJ, USA). The membranes were stripped and total STAT-3 levels were evaluated using mouse STAT-3 antibody (1:1000 dilution; BD Transduction Laboratories, Mississauga, ON, Canada) and HRP-conjugated anti-mouse IgG immunoglobulin (1:3333 dilution; Santa Cruz Biotechnology).

Time course study of TNF-α gene expression. RAW264.7 cells were seeded onto a 6 cm dish at 1×10^5 cells/dish and pre-treated with vehicle (ethanol) or CAM (10 μg/mL) for 3 days, followed by stimulation with LPS (20 ng/mL). Total RNA was extracted 0, 6, 12 and 36 h after LPS stimulation, and RT-PCR was performed using specific primers for GAPDH and TNF-α (forward; AAC TAG TGG TGC CAG CCG AT, reverse; CTT CAC AGA GCA ATG ACT CC), as described above.

Statistical analysis. Multiple comparesons were performed by 1-way ANOVA followed by the Student-Newman-Keuls method. In the figures, results are expressed as the mean ± SD. Significance was inferred for results for which $P < 0.05$. Statistical analyses were done using computer software (SigmaStat 3.1, Hulinks).

RESULTS

Expression of IL-10 and IL-10R following pre-treatment with a 14-member macrolide

Minimal gene expression of IL-10 was observed in the EM-treated RAW cells, while no expression was detected in the control (vehicle) or CAM-treated RAW264.7 cells (Figure 1A, top). IL-10R gene expression remained unchanged following EM or CAM treatment, compared with the control cells (Figure 1A, middle).

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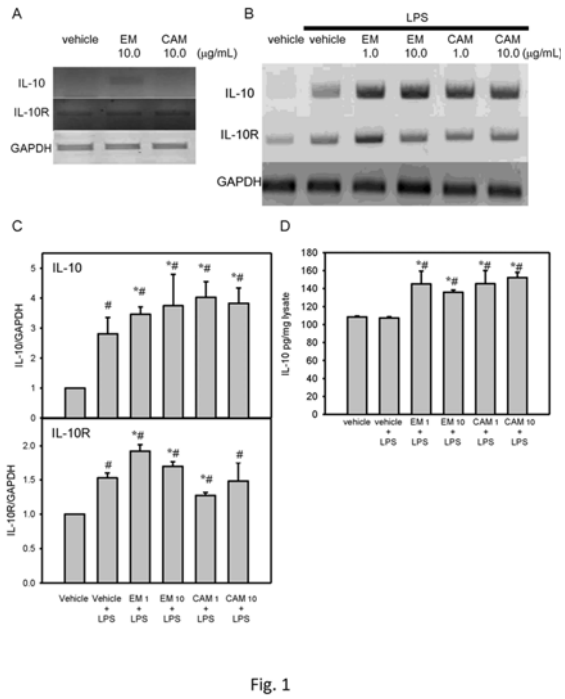

Fig. 1

Figure 1: The expression of IL-10 and IL-10R following treatment with erythromycin (EM) or clarithromycin CAM

(A) Following treatment of RAW264.7 cells with EM (10 μg/mL) or CAM (10 μg/mL) for 3 d, IL-10 and IL-10R expression was evaluated by RT-PCR. Minimal IL-10 expression was observed in the EM-treated cells (top panel), but not the CAM-treated cells. The expression of IL-10R remained unchanged (middle panel).

(B) The macrophage cells were pretreated with EM or CAM for 3 d, and stimulated with LPS for 4 h (total RNA isolation), or 24 h (supernatant collection). The expression of IL10 and IL10R was evaluated by RT-PCR or sandwich ELISA. IL-10 gene expression was enhanced by EM- or CAM-treatment compared with the control (vehicle treated) cells after LPS stimulation. Following LPS stimulation, IL-10R expression was enhanced by EM treatment, but not by CAM treatment. These images are representative of 6 independent experiments.

(C) The intensity of each band from (B) was semi-quantified as described in *Materials and Methods*.

(D) Protein concentrations of IL-10 in the supernatant, following LPS stimulation of the macrophages, were enhanced by EM- or CAM-treatment compared with the control cells (n = 5 for each group).

All data are expressed as the mean ± SD. #P < 0.05 vs. *vehicle*, *P < 0.05 vs. *vehicle + LPS*

Expression of IL-10 and IL-10R in 14-member macrolide-treated RAW264.7 cells following LPS stimulation

Following pre-treatment with EM or CAM, cells were stimulated with LPS for 6 h and IL-10 and IL-10R gene expression evaluated by RT-PCR. LPS induced IL-10 and IL-10R gene expression in the macrophage cell line. The expression levels of IL-10 in EM-or CAM-treated cells were greater than the control cells after LPS stimulation (Figure 1B top, Figure 1C top). The expression of IL-10R in the EM-treated RAW264.7 cells was higher than the control cells, while IL-10R expression levels of the CAM-treated RAW264.7 cells were slightly lower (1.0 μg/mL), or similar (10.0 μg/mL) to the control cells after LPS stimulation (Figure 1B middle, Figure 1C bottom). In addition, IL-10 protein levels in the lysates of EM- or CAM-pretreated RAW264.7 cells were significantly increased compared with control cells after LPS stimulation. However, IL-10 protein levels in the lysates of control cells stimulated with LPS were not increased compared with unstimulated cells (Figure1D).

Enhanced p-STAT-3 expression following LPS stimulation in 14-member macrolide-treated RAW264.7 cells

Levels of p-STAT-3 after LPS stimulation were enhanced by pre-treatment with EM or CAM in a dose-dependent manner (Figure 2).

Expression of SOCS-3 after LPS stimulation in 14-member macrolide-treated RAW264.7 cells

Since SOCS-3 is a key molecule involved in the suppression of cytokine signaling by IL-10, we evaluated SOCS-3 gene expression after LPS stimulation in macrolide-pretreated cells. LPS induced SOCS-3 gene expression in RAW264.7 cells. EM and CAM enhanced the expression of SOCS-3 compared with the control cells after LPS stimulation (Figure 3A and 3B).

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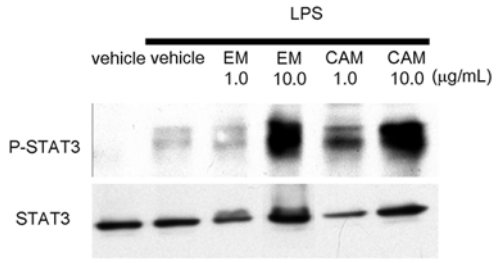

Fig. 2

Figure 2: Levels of phosphorylated (p)-STAT-3 in LPS stimulated RAW264.7 macrophages were enhanced by pre-treatment with macrolides

Following the pre-treatment of RAW264.7 cells with erythromycin (EM) or clarithromycin (CAM) for 3 d, cells were stimulated with LPS for 8 h. Cell lysates were immunoblotted to evaluate p-STAT-3 and STAT-3 levels, as described in *Materials and Methods*. Levels of p-STAT3 after LPS stimulation were enhanced by pre-treatment with either EM or CAM. Representative images of 3 independent experiments are shown.

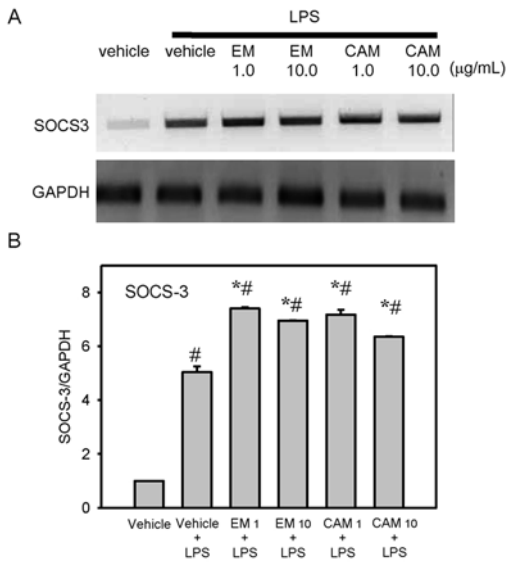

Fig. 3

Figure 3: Suppressor of cytokine signaling-3 (SOCS-3) gene expression following LPS stimulation in 14-member macrolide-treated RAW264.7 cells

Following the pretreatment of RAW264.7 macrophages with erythromycin (EM) or clarithromycin (CAM) for 3 d, cells were stimulated with LPS for 4 h. RT-PCR was performed using specific primers, as described in *Materials and Methods*. (A) SOCS-3 gene expression after LPS stimulation was enhanced with EM- or CAM-treatment, compared with the control cells. Representative images of 6 independent experiments are shown. (B) The intensity of each band was semi-quantified, as described in *Materials and Methods*.

All data are expressed as the mean ± SD. #*P* < 0.05 vs. *vehicle*, **P* < 0.05 vs. *vehicle* + *LPS*

Time course of TNF-α expression after LPS stimulation in 14-member macrolide-treated RAW264.7 cells

We pretreated cells with CAM or vehicle, and compared the induction of TNF-α in these cells after LPS stimulation. Six hours after LPS stimulation, TNF-α expression in CAM-treated cells was not different from the control cells (Figure 4). At 12 h after LPS stimulation, TNF-α levels in CAM-treated cells were slightly decreased compared with control cells, while at 36 h after LPS stimulation, TNF-α mRNA levels were distinctly lower in the CAM-treated RAW264.7 cells than in the control cells (Figure 4).

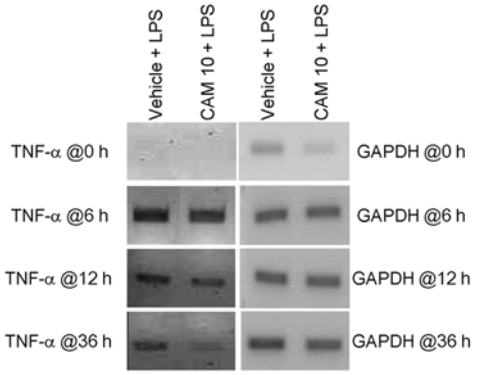

Fig. 4

Figure 4: The expression of TNF-α in clarithromycin (CAM)-treated RAW264.7 cells following LPS stimulation

After pretreatment of the macrophages with CAM (10 μg/mL) or vehicle for 3 d, the cells were stimulated with LPS for 0, 6, 12, and 36 h and the expression of TNF-α evaluated by RT-PCR. The expression of TNF-α 6 h after LPS stimulation in CAM-treated cells was not different to the control cells. At 12 h after LPS stimulation, TNF-α levels in the CAM-treated cells were slightly decreased compared with the controls, while at 36 h after LPS stimulation, TNF-α mRNA levels were clearly lower in the CAM-treated RAW264.7 cells than the control cells. Representative images of 3 independent experiments are shown.

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DISCUSSION

We have demonstrated that 14-member macrolide treatment enhances the induction of IL-10 in macrophages following LPS stimulation. Moreover, we observed enhanced STAT-3 phosphorylation and SOCS-3 expression in EM- and CAM-treated cells following LPS stimulation. Although the level of TNF-α production 6 h after LPS stimulation was similar between the control and CAM-treated macrophage cells, at 36 h after LPS stimulation, the levels of TNF-α in the CAM-treated cells were low compared with those of the control cells. Thus CAM-treatment may result in the early disappearance of TNF-α following LPS stimulation, leading to an early recovery of the cells.

Inflammation is generated by pro-inflammatory cytokines, and is resolved with anti-inflammatory cytokines, such as IL-10 (Moore et al., 2001). Lung-specific overexpression of IL-10 reduced lung inflammation after intra-tracheal administration of LPS in mice (Spight et al., 2005). Pro-inflammatory cytokines activate the Janus family kinases associated with their connate receptors (Murray, 2007). These kinases, associated with their receptors, phosphorylate the receptor cytoplasmic domains, creating docking sites for Src-homology2 domain-containing signaling proteins (Murray, 2007). The substrates for tyrosine phosphorylation are members of the STAT family proteins (Murray, 2007). Interestingly, the anti-inflammatory cytokine IL-10 also activates STAT-3. Activated (phosphorylated) STAT-3 translocates into the nucleus and stimulates the expression of SOCS-3. Finally, SOCS-3 binds to the phosphorylated tyrosine residues of cytokine receptors via their Src-homology2 domains, thereby negating the pro-inflammatory cytokine signals (Yoshimura et al., 2003, 2005). Moreover, the ability of IL-10 to induce *de novo* synthesis of SOCS-3 in macrophages correlates with its ability to inhibit the expression of many genes in these cells, including endotoxin-inducible cytokines such as TNF-α and IL-1β (Ito et al., 1999). Thus, the ability of IL-10 to inhibit gene expression in macrophages is associated with its ability to rapidly induce synthesis of SOCS-3.

The mechanism as to how 14-member macrolides enhance the expression of IL-10 following LPS stimulation is still unclear. Also, other mechanisms, independent of IL-10, may be involved in the immunomodulatory effects of the 14-member macrolides. For example, CAM has been previously shown to modify inflammation by suppressing IL-8 production via AP-1 and nuclear factor-κB (Kikuchi et al., 2002). Yamaryo and colleagues demonstrated that 14-member macrolides promote the phosphatidylserine receptor-dependent phagocytosis of apoptotic neutrophils by alveolar macrophages (Yamaryo et al., 2003). Recently, it was reported that the ability of mannose receptor-mediated apoptotic cell clearance in alveolar macrophages was enhanced by azithromycin, a 15-member macrolide (Hodge et al., 2008). However, our study indicates that the immunomodulatory effects of 14-member macrolides on macrophages are associated with the IL-10/SOCS-3 pathway. Demartini and colleagues demonstrated that CAM significantly increased plasma levels of IL-10 on the 3rd and 7th day when compared with basal levels in patients with community acquired pneumonia (Demartini et al., 2004). Therefore, the activation of the IL-10/SOCS-3 pathway may be an important mechanism for the immunosuppressive effects of 14-member macrolides.

Macrolides are suitable antibiotics for treating respiratory tract infections with respect to their superior drug delivery into lung tissue. The levels of CAM after oral administration (500 mg) are reported to be 4.0 μg/mL in serum, 16.8 mg/kg in bronchial biopsies, 20.5 μg/mL in epithelial lining fluid, and 372.7 μg/mL in alveolar cells (Honeybourne et al., 1994). Thus, the concentrations of macrolide antibiotics tested in this study are compatible with their clinical use.

As described above, macrolide antibiotics have diverse inflammatory suppressive effects on lung cells. Accumulating evi-

dence suggests that macrolides are effective, not only for patients with DPB, but also for those with cystic fibrosis (McArdle and Talwalkar, 2007). Thus, clarifying the anti-inflammatory effects of macrolide antibiotics may assist in the development of new therapies against many other refractory inflammatory diseases.

We have demonstrated the involvement of the IL-10/STAT-3/SOCS-3 pathway in the immunomodulatory effects of 14-member macrolide on macrophages. Our results suggest a potent effect of macrolide antibiotics on macrophages in chronic, infectious diseases.

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REFERENCES

Abe S, Nakamura H, Inoue S, Takeda H, Saito H, Kato S, Mukaida N, Matsushima K, Tomoike H. Interleukin-8 gene repression by clarithromycin is mediated by the activator protein-1 binding site in human bronchial epithelial cells. Am J Respir Cell Mol Biol 2000;22:51-60.

Akira S. Toll-like receptor signaling. J Biol Chem 2003;278:38105-8.

Azuma A, Kudoh S. Diffuse panbronchiolitis in East Asia. Respirology 2006;11:249-61.

Demartini G, Esposti D, Marthyn P, Lapidari A, Fraschini F, Scaglione F. Effect of multiple doses of clarithromycin and amoxicillin on IL-6, IFNgamma and IL-10 plasma levels in patients with community acquired pneumonia. J Chemother 2004;16:82-5.

Hodge S, Hodge G, Brozyna S, Jersmann H, Holmes M, Reynolds PN. Azithromycin increases phagocytosis of apoptotic bronchial epithelial cells by alveolar macrophages. Eur Respir J 2006;28:486-95.

Hodge S, Hodge G, Jersmann H, Matthews G, Ahern J, Holmes M, Reynolds PN. Azithromycin improves macrophage phagocytic function and expression of mannose receptor in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2008;178:139-48.

Honeybourne D, Kees F, Andrews JM, Baldwin D, Wise R. The levels of clarithromycin and its 14-hydroxy metabolite in the lung. Eur Respir J 1994;7:1275-80.

Ito S, Ansari P, Sakatsume M, Dickensheets H, Vazquez N, Donnelly RP, Larner AC, Finbloom DS. Interleukin-10 inhibits expression of both interferon alpha- and interferon gamma-induced genes by suppressing tyrosine phosphorylation of STAT1. Blood 1999;93:1456-63.

Jain R, Danziger LH. The macrolide antibiotics: a pharmacokinetic and pharmacodynamic overview. Curr Pharm Des 2004;10:3045-53.

Kadota J, Mukae H, Ishii H, Nagata T, Kaida H, Tomono K, Kohno S. Long-term efficacy and safety of clarithromycin treatment in patients with diffuse panbronchiolitis. Respir Med 2003;97:844-50.

Keicho N, Kudoh S. Diffuse panbronchiolitis: role of macrolides in therapy. Am J Respir Med 2002;1:119-31.

Kikuchi T, Hagiwara K, Honda Y, Gomi K, Kobayashi T, Takahashi H, Tokue Y, Watanabe A, Nukiwa T. Clarithromycin suppresses lipopolysaccharide-induced interleukin-8 production by human monocytes through AP-1 and NF-kappa B transcription factors. J Antimicrob Chemother 2002;49:745-55.

Kudoh S, Azuma A, Yamamoto M, Izumi T, Ando M. Improvement of survival in patients with diffuse panbronchiolitis treated with low-dose erythromycin. Am J Respir Crit Care Med 1998;157:1829-32.

Machiya J, Shibata Y, Yamauchi K, Hirama N, Wada T, Inoue S, Abe S, Takabatake N, Sata M, Kubota I. Enhanced expression of MafB inhibits macrophage apoptosis induced by cigarette smoke exposure. Am J Respir Cell Mol Biol 2007;36:418-26.

McArdle JR, Talwalkar JS. Macrolides in cystic fibrosis. Clin Chest Med 2007;28:347-60.

Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001;19:683-765.

Morikawa K, Watabe H, Araake M, Morikawa S. Modulatory effect of antibiotics on cytokine production by human monocytes in vitro. Antimicrob Agents Chemother 1996;40:1366-70.

Murray PJ. The JAK-STAT signaling pathway: input and output integration. J Immunol 2007;178:2623-9.

Plowden J, Renshaw-Hoelscher M, Engleman C, Katz J, Sambhara S. Innate immunity in aging: impact on macrophage function. Aging Cell 2004;3:161-7.

Shames BD, Selzman CH, Meldrum DR, Pulido EJ, Barton HA, Meng X, Harken AH, McIntyre RC Jr. Interleukin-10 stabilizes inhibitory kappaB-alpha in human monocytes. Shock 1998;10:389-94.

Shibata Y, Berclaz PY, Chroneos ZC, Yoshida M, Whitsett JA, Trapnell BC. GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU.1. Immunity 2001;15:557-67.

Shinkai M, Foster GH, Rubin BK. Macrolide antibiotics modulate ERK phosphorylation and IL-8 and GM-CSF production by human bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 2006;290:L75-L85.

Spight D, Zhao B, Haas M, Wert S, Denenberg A, Shanley TP. Immunoregulatory effects of regulated, lung-targeted expression of IL-10 in vivo. Am J Physiol Lung Cell Mol Physiol 2005;288:L251-L65.

Wierzbowski AK, Hoban DJ, Hisanaga T, DeCorby M, Zhanel GG. The use of macrolides in treatment of upper respiratory tract infections. Curr Allergy Asthma Rep 2006;6:171-81.

Yamaryo T, Oishi K, Yoshimine H, Tsuchihashi Y, Matsushima K, Nagatake T. Fourteen-member macrolides promote the phosphatidylserine receptor-dependent phagocytosis of apoptotic neutrophils by alveolar macrophages. Antimicrob Agents Chemother 2003;47:48-53.

Yoshimura A, Mori H, Ohishi M, Aki D, Hanada T. Negative regulation of cytokine signaling influences inflammation. Curr Opin Immunol 2003;15:704-8.

Yoshimura A, Nishinakamura H, Matsumura Y, Hanada T. Negative regulation of cytokine signaling and immune responses by SOCS proteins. Arthritis Res Ther 2005;7:100-10.