Effects of Macrolide Antibiotics on Th1 Cell and Th2 Cell Development Mediated by Langerhans Cells

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ABSTRACT - Background: It is well known that Langerhans cells (LCs) work as the primary orchestrators in the polarization of the immune milieu towards a T helper type 1 (Th1) or a Th2 immune response. In this study, we investigated the effects of macrolide antibiotics on Th1 cell and Th2 cell development mediated by LCs. **Methods:** LC-like dendritic cells (LDCs) were generated from mouse bone marrow cells and used as substitutes for LCs. Mice were primed with ovalbumin (OVA) peptide-pulsed LDCs, which had been treated with each macrolide antibiotic, via the hind footpad. After 5 days, the cytokine response in the popliteal lymph nodes was investigated by enzyme-linked immunosorbent assay. The expression of cell surface molecules on LDCs was investigated using reverse transcriptase polymerase chain reaction. **Results:** Injection of OVA peptide-pulsed LDCs, which had been treated with josamycin or spiramycin, inhibited Th2 cell development as represented by down-regulation of interleukin (IL)-4 production as well as Th1 cell development as represented by down-regulation of interferon (IFN)-γ production. This inhibition of Th1 cell and Th2 cell development was associated with suppression of CD86 and T-cell immunoglobulin and mucin domain-containing protein (TIM)-4 expression, respectively, in LDCs. Furthermore, *Staphylococcus aureus* strains isolated from skin lesions of patients with atopic dermatitis (AD) were more susceptible to josamycin than to spiramycin. **Conclusions:** These results suggest that topical application of josamycin to AD lesions colonized with *S. aureus* would be beneficial for control of AD by acting on both superficial *S. aureus* and epidermal LCs, and inhibiting the development of Th2 cells.

INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disease with immunopathologic features that vary depending on the duration of the lesions. The majority of AD patients show superficial skin colonization by *Staphylococcus aureus* and increased expression of T helper type 2 (Th2) cytokines such as interleukin (IL)-4, IL-5 and IL-13 in their peripheral blood mononuclear cells (1). *S. aureus* can be isolated from 96-100% of skin lesions of AD patients, whereas only 0-10% of healthy individuals show skin colonization by this organism (2, 3). We have also found that the incidence of *S. aureus* detection in the lesioned skin of AD patients is higher than that in non-lesioned skin, and that the *S. aureus* bacterial cell count in lesioned skin is significantly higher than that in non-lesioned skin (3). Furthermore, our recent studies have demonstrated that chronic skin colonization with *S. aureus* may augment Th2 cell development in AD patients (4-6). Therefore, treatment with antibiotics helps to ameliorate AD, not only in patients with impetiginized AD but also in patients without clinical signs of superinfection.

Langerhans cells (LCs) are a subpopulation of bone marrow-derived dendritic cells (DCs). They are antigen-presenting cells (APCs), capable of internalizing and processing antigen (7). Because they reside in epithelium of the skin and mucosal membrane, they become the primary response cell for antigens entering the skin, oral mucosa and

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airways (8-11). After antigen uptake, LCs migrate to regional lymph nodes where peptides, in the context of major histocompatibility complex (MHC) class Ⅱ molecules, are presented to naïve Th cells bearing appropriate Th cell receptors. This initial signal delivered to naïve Th cells, together with a second signal, delivered in part by interaction between the CD80 and CD86 molecules on LCs and CD28 on Th cells, results in activation of the Th cells (12, 13). Furthermore, LCs work as the primary orchestrators in the polarization of immune responses towards a Th1 or a Th2 immune response. The nature of the polarization is influenced by a number of factors and, in particular, development of Th2 cells, producing type 2 cytokines such as IL-4, IL-5 and IL-13, plays pivotal roles in inducing allergic inflammation (14). Therefore, allergic inflammation might be controllable through regulation of LCs. In our previous study, we succeeded in generating LC-like dendritic cells (LDCs) from murine bone marrow cells as substitute cells for LCs, and through them developed Th1 cells or Th2 cells (15). Although it is well known that macrolide antibiotics have immunomodulatory effects in addition to their bactericidal activity (16, 17), it is unclear whether they inhibit Th2 cell development mediated by LCs. In this study, therefore, we investigated the effects of macrolide antibiotics on LDCs.

MATERIALS AND METHODS

Mice

Specific-pathogen-free BALB/c (wild type) mice and DO 11.10 TCR Tg mice (ovalbumin $(OVA)_{323-339}$-specific I-A^d-restricted T cell receptor (TCR)-transgenic mice) were obtained from Japan SLC (Hamamatsu, Japan) and The Jackson Laboratory (Bar Harbor, Maine, USA), respectively, and used at the age of 6 to 8 weeks. They were housed in plastic cages with sterilized paper bedding in a clean, air-conditioned room at 24 °C and allowed free access to a standard laboratory diet and water. All procedures performed on the mice were in accordance with the Guidelines of the Animal Care and Use Committee of Meiji Pharmaceutical University, Tokyo.

Antibiotics

Erythromycin, roxithromycin, clarithromycin, azithromycin, spiramycin and gentamicin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Josamycin and midecamycin were provided by Astellas Pharma Inc. (Tokyo, Japan) and Meiji Seika Pharma Co., Ltd. (Tokyo Japan), respectively.

Generation of LDCs

The preparation and culture of mouse bone marrow cells to generate LDCs were performed according to the method established in our previous study (15). Briefly, bone marrow cells from BALB/c mice were cultured in RPMI 10 (RPMI 1640 medium with L-glutamine (Sigma-Aldrich) containing 10% fetal bovine serum (Sigma-Aldrich), 25 mM Hepes (Sigma-Aldrich), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco RBL, Grand Island, NY, USA) supplemented with recombinant murine GM-CSF (20 ng/mL; PeproTech, Rocky Hill, NJ, USA), recombinant murine IL-4 (100 ng/mL; PeproTech) and recombinant human TGF-β1 (10 ng/mL; PeproTech) at 37 °C in a humidified atmosphere with 5% CO_2. Half of the total volume of the culture medium was changed every 48 h, and 7 days after the start of culture, the grown cells were treated with mouse anti-mouse I-A^d monoclonal antibody (clone 34-5-3s, mouse IgG2a) (1: 200; Cedarlane Laboratories, Ontario, Canada) in RPMI 10 for 1 h on ice. The cells that reacted with the anti-I-A^d antibody were then purified using a CELLection™ Pan Mouse IgG Kit (Invitrogen, Oslo, Norway), and used as LDCs. These I-A^d-positive LDCs were purified to around 95%, as determined by flow cytometry.

Th1 Cell and Th2 Cell Regulation by Antibiotic-Treated LDCs

Th1/Th2 regulation by LDCs was investigated according to the method used in our previous study (18). Briefly, LDCs were adjusted to 2×10^5 cells/mL in RPMI 10 and then incubated with 6 μM OVA peptide (323-ISQAVHAAHAEINEAGR-339; obtained from Operon Biotechnologies, Tokyo, Japan) in the absence or presence of 5-50 μM each macrolide antibiotic at 37°C in a humidified atmosphere with 5% CO_2. The cells were collected after incubation for 18 h, washed in RPMI 10, and injected at a dose of 5×10^4 cells into both hind footpads of DO 11.10 TCR Tg mice. After 5 days, draining popliteal lymph nodes were harvested and adjusted to 1×10^6 cells/mL in RPMI 10. The

cultures (0.2 mL/well) were incubated in 96-well culture plates (Nunc, Roskilde, Denmark) in the presence of Dynabeads® Mouse T-Activator CD3/CD28 (Life Technologies, Oslo, Norway) at 37°C in a humidified atmosphere with 5% CO_2. The culture supernatants were collected after incubation for 48 h, and the interferon (IFN)-γ and IL-4 concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits for quantification of murine IFN-γ and IL-4, respectively (R & D Systems, Minneapolis, MN, USA).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

In order to determine the levels of mRNA expression for various cell surface molecules, mRNA was extracted from LDCs (1×10^5 cells) using a Dynabeads® mRNA DIRECT™ Micro Kit (Life Technologies, Oslo, Norway). The cDNA was then synthesized from the mRNA using a first-strand cDNA synthesis kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK). PCR was performed using the following primers: β-actin (540 bp) 5' primer, 5'-GTGGGCCGCTCTAGGCACCAA-3' and 3' primer, 5'-CTCTTTGATGTCACGCACGATTTC-3'; CD40 (295 bp) 5' primer, 5'-CCTGTAAGGAAGGACAACAC-3' and 3' primer, 5'-ATCACGACAGGAATGACCAG-3'; CD80 (312 bp) 5' primer, 5'-GAAGACCGAATCTACTGGCA-3' and 3' primer, 5'-GGAAGCAAAGCAGGTAATCC-3'; CD86 (302 bp) 5' primer, 5'-AGCCTGAGTGAGCTGGTAGT-3' and 3' primer, 5'-CCTGTTACATTCTGAGCCAG-3'; Delta 1 (318 bp) 5' primer, 5'-TGCACTGACCCAATCTGTCT-3' and 3' primer, 5'-CTCACAGTTGGCACCTGTAT-3'; Delta 3 (331 bp) 5' primer, 5'-CTACTGTGAAGAGCCTGATG-3' and 3' primer, 5'-ACAGACATAGGCAGAGTCAG-3'; Delta 4 (307 bp) 5' primer, 5'-TCACCAGACTGAGCTACTCT-3' and 3' primer, 5'-ATGCTGCAGGTGCCATGGAT-3'; Jagged 1 (314 bp) 5' primer, 5'-ATCCGAGTGACCTGTGATGA-3' and 3' primer, 5'-TTGGTCTCACAGAGGCACTG-3'; Jagged 2 (300 bp) 5' primer, 5'-GCTGTGATGAGAACTACTAC-3' and 3' primer, 5'-TCTCACAGTCACAGTGCCAG-3'; T-cell immunoglobulin and mucin domain-containing protein (TIM)-4 (304 bp) 5' primer, 5'-GTCCAGTTTGGTGAAGTGTC-3' and 3' primer, 5'-ACGTGGTCACTGCTGTACTG-3'. Each PCR was performed using a GeneAmp PCR System 9700 (Perkin-Elmer, Norwalk, CT, USA) in 25 μL of reaction mixture comprising 1.5 μL cDNA, 200 μM deoxynucleotide triphosphate mixture, 400 nM each PCR primer and 25 U/mL Ex Taq DNA polymerase (Takara, Shiga, Japan). The reaction conditions were as follows: one 4-min cycle at 94 °C, 35 cycles comprising 45 s at 94 °C, 45 s at 61 °C and 2 min at 72 °C, followed by one 7-min cycle at 72 °C, and the PCR products were separated on a 2% agarose gel containing ethidium bromide.

Antimicrobial Susceptibility Testing

S. aureus strains were isolated from the lesional skin of AD patients, and the antibiotic sensitivity of each *S. aureus* strain was measured as the minimum inhibitory concentration (MIC). The MIC of each antibiotic was determined by the broth microdilution method using Mueller Hinton broth, i.e. the standard procedure recommended by the Japanese Society of Chemotherapy.

Statistical Analysis

The data were expressed as means (± SD), and differences between means were analyzed using Student's *t* test with a two-tailed test of significance. Differences at $P < 0.05$ were considered to be statistically significant.

RESULTS

Effects of Macrolide Antibiotics on Th1 Cell and Th2 Cell Development through LDCs

LDCs were pulsed with OVA for 18 h in the absence or presence of erythromycin, roxithromycin, and clarithromycin, respectively, which are 14-membered ring macrolide antibiotics. The LDCs were washed and injected into the hind footpads of DO 11.10 TCR Tg mice, and draining popliteal lymph node cells were harvested 5 days later. Subsequently, T-lymphocytes in lymph nodes cells thus obtained were stimulated for 48 h through their surface CD3/CD28 molecules, and IFN-γ and IL-4 concentrations in the culture supernatants were then determined by ELISA. As shown in Figure 1,

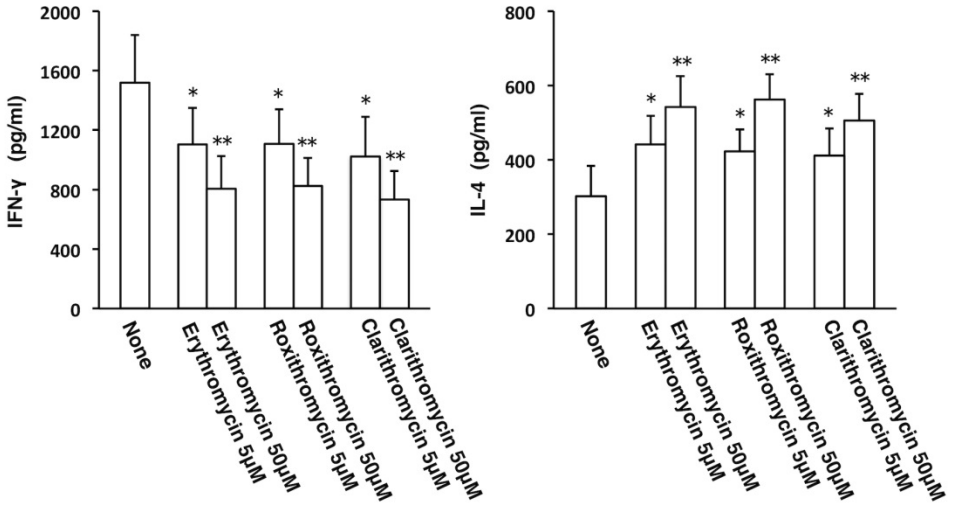

Figure 1. Effects of 14-membered ring macrolide antibiotics on development of Th1 and Th2 cells mediated by LDCs. LDCs were pulsed with OVA peptide for 18 h in the absence or presence of 5-50 μM erythromycin, roxithromycin or clarithromycin. The OVA peptide-pulsed LDCs were then injected into the hind footpads of mice, and lymph node cells were harvested 5 days later. Lymph node cells were stimulated through their surface CD3/CD28 molecules, and the IFN-γ and IL-4 concentrations in the culture supernatants were determined by ELISA. Each culture was prepared in triplicate, and the mean value was obtained as a representative result for one experiment. The same experiment was repeated 6 times, and the results are expressed as means ± SD (n = 6).
**P*<0.05, ***P*<0.01 versus non-treatment.

LDCs treated with erythromycin, roxithromycin and clarithromycin, respectively, inhibited the development of Th1 cells in dose-dependent manner, as represented by suppressed IFN-γ production. However, Th2 cell development was augmented, as verified by enhanced production of IL-4.

In the same way, when mice were injected with LDCs that had been treated with a 15-membered ring macrolide antibiotic, azithromycin, Th1 cell development in lymph node cells was inhibited whereas Th2 cell development was augmented, as was the case when mice were injected with LDCs that had been treated with 14-membered ring macrolide antibiotics (Fig. 2).

In addition, when LDCs were treated with the 16-membered ring macrolide antibiotics, josamycin, spiramycin and midecamycin, respectively, development of Th1 cells in mouse lymph node cells was inhibited, as was the case when mice were injected with 14-membered ring macrolide antibiotic-treated LDCs and 15-membered ring macrolide antibiotic-treated LDCs, respectively (Fig. 3). However, the levels of inhibition of Th1 cell development induced by 16-membered ring macrolide antibiotic-treated LDCs were slightly lower than those induced by 14-membered ring macrolide antibiotic-treated LDCs and 15-membered ring macrolide antibiotic-treated LDCs. On the other hand, although development of Th2 cells in lymph node cells of mice that had received midecamycin-treated LDCs tended to be slightly augmented, that of mice which received josamycin-treated LDCs and spiramycin-treated LDCs, respectively, was significantly inhibited in a dose-dependent manner. Furthermore, the levels of inhibition of Th2 cell development induced by josamycin-treated LDCs were superior to those induced by spiramycin-treated LDCs.

Effects of Macrolide Antibiotics on mRNA Expression of Cell Surface Molecules in LDCs

Subsequently, in order to clarify the mechanisms responsible for regulation of Th1 cell and Th2 cell development via macrolide antibiotic-treated LDCs, LDCs were pulsed with OVA for 18 h in the absence or presence of erythromycin, roxithromycin and clarithromycin, respectively, which are

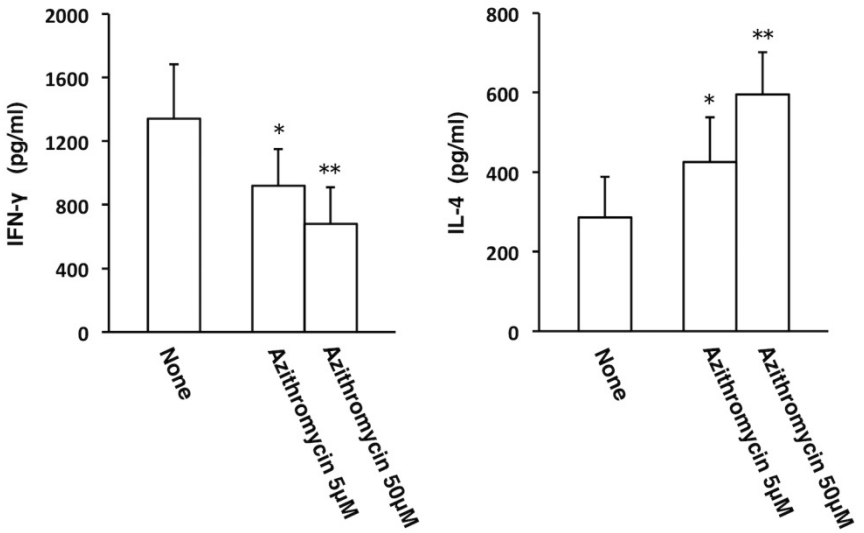

Figure 2. Effects of a 15-membered ring macrolide antibiotic on development of Th1 and Th2 cells mediated by LDCs. LDCs were pulsed with OVA peptide for 18 h in the absence or presence of 5-50 μM azithromycin. The OVA peptide-pulsed LDCs were then injected into the hind footpads of mice, and lymph node cells were harvested 5 days later. Lymph node cells were stimulated through their surface CD3/CD28 molecules, and the IFN-γ and IL-4 concentrations in the culture supernatants were determined by ELISA. Each culture was prepared in triplicate, and the mean value was obtained as a representative result for one experiment. The same experiment was repeated 6 times, and the results are expressed as means ± SD (n = 6).
**P*<0.05, ***P*<0.01 versus non-treatment.

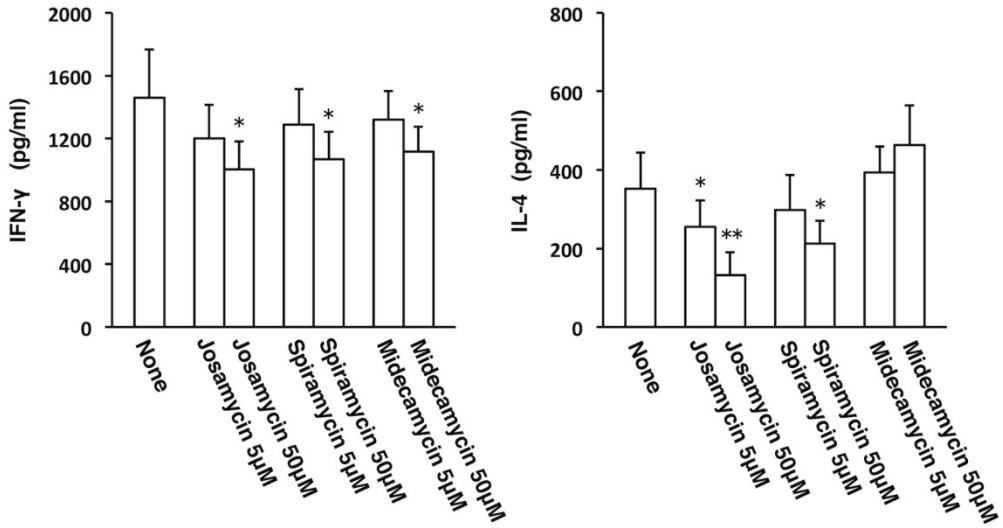

Figure 3. Effects of 16-membered ring macrolide antibiotics on development of Th1 and Th2 cells mediated by LDCs. LDCs were pulsed with OVA peptide for 18 h in the absence or presence of 5-50 μM josamycin, spiramycin or midecamycin. The OVA peptide-pulsed LDCs were then injected into the hind footpads of mice, and lymph node cells were harvested 5 days later. Lymph node cells were stimulated through their surface CD3/CD28 molecules, and the IFN-γ and IL-4 concentrations in the culture supernatants were determined by ELISA. Each culture was prepared in triplicate, and the mean value was obtained as a representative result for one experiment. The same experiment was repeated 6 times, and the results are expressed as means ± SD (n = 6).
**P*<0.05, ***P*<0.01 versus non-treatment.

14-membered ring macrolide antibiotics, and expression of mRNA for the cell surface molecules, CD40, CD80, CD86, Delta 1, Delta 3, Delta 4, Jagged 1, Jagged 2 and TIM-4 was investigated using RT-PCR. As shown in Figure 4, although LDCs treated with erythromycin, roxithromycin and clarithromycin, respectively, inhibited the expression of both CD40 and Delta 1 mRNA, expression of TIM-4 mRNA was conversely augmented.

When LDCs were treated with the 15-membered ring macrolide antibiotic, azithromycin, the expression of both CD40 and Delta 4 mRNA was suppressed, whereas expression of TIM-4 mRNA was conversely enhanced (Fig. 5).

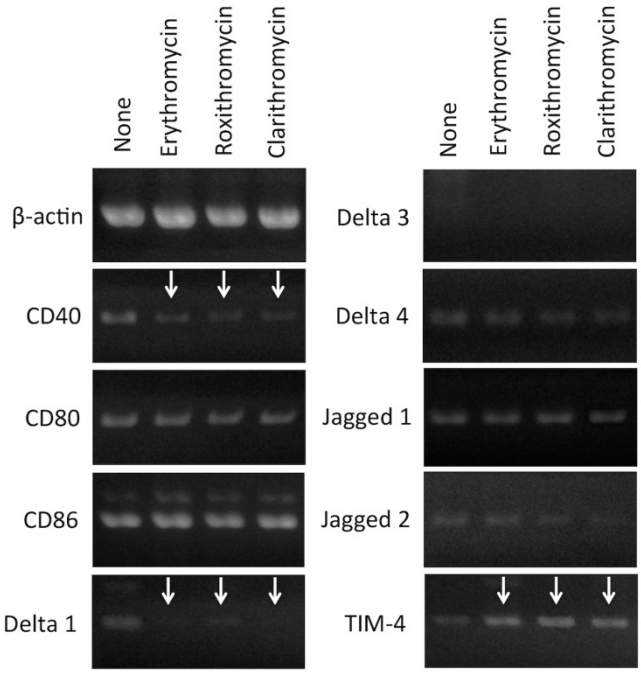

Figure 4. Effects of 14-membered ring macrolide antibiotics on expression of mRNAs for cell surface molecules on LDCs. LDCs were pulsed with OVA peptide for 18 h in the absence or presence of 50 μM erythromycin, roxithromycin or clarithromycin. Cytoplasmic mRNA was extracted from LDCs, reverse-transcribed, and amplified by PCR using primer sets for β-actin, CD40, CD80, CD86, Delta 1, Delta 3, Delta 4, Jagged 1, Jagged 2 and TIM-4. The data shown are the representative results of five independent experiments.

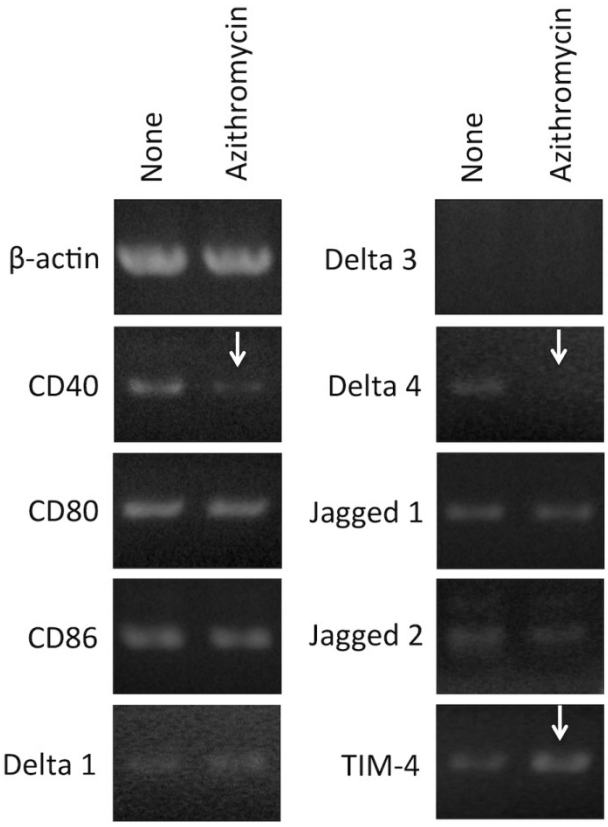

Figure 5. Effects of a 15-membered ring macrolide antibiotic on expression of mRNAs for cell surface molecules on LDCs. LDCs were pulsed with OVA peptide for 18 h in the absence or presence of 50 μM azithromycin. Cytoplasmic mRNA was extracted from LDCs, reverse-transcribed, and amplified by PCR using primer sets for β-actin, CD40, CD80, CD86, Delta 1, Delta 3, Delta 4, Jagged 1, Jagged 2 and TIM-4. The data shown are the representative results of five independent experiments.

On the other hand, when LDCs were treated with the 16-membered ring macrolide antibiotics, josamycin, spiramycin and midecamycin, respectively, inhibition of CD40 mRNA expression was confirmed in only midecamycin-treated LDCs (Fig. 6). Expression of CD86 and TIM-4 mRNA in LDCs was suppressed by treatment with josamycin and spiramycin, but not by treatment with midecamycin. Furthermore, LDCs treated with josamycin also inhibited the expression of Jagged 1 mRNA, in addition to that of CD86 and TIM-4 mRNA.

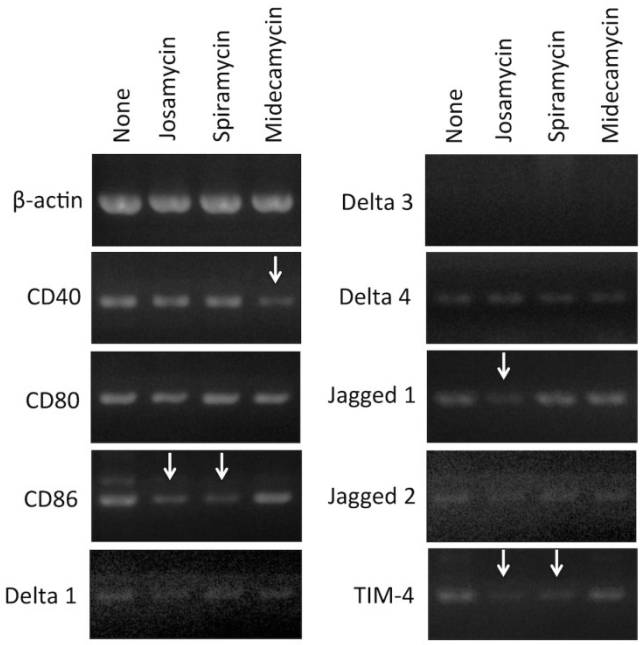

Figure 6. Effects of 16-membered ring macrolide antibiotics on expression of mRNAs for cell surface molecules on LDCs. LDCs were pulsed with OVA peptide for 18 h in the absence or presence of 50 μM josamycin, spiramycin or midecamycin. Cytoplasmic mRNA was extracted from LDCs, reverse-transcribed, and amplified by PCR using primer sets for β-actin, CD40, CD80, CD86, Delta 1, Delta 3, Delta 4, Jagged 1, Jagged 2 and TIM-4. The data shown are the representative results of five independent experiments.

Susceptibility of *S. aureus* Strains to Gentamicin, Spiramycin and Josamycin

The susceptibility of *S. aureus* strains isolated from the lesional skin of AD patients to gentamicin, spiramycin and josamycin, respectively, was also investigated. As shown in Figure 7, 97.0% (32/33) of *S. aureus* strains were markedly susceptible to josamycin, whereas these strains were less susceptible to spiramycin and gentamicin, and in particular, gentamycin-resistant bacterial strains were prominent.

DISCUSSION

Th1/Th2 immune balance is closely related to various immunological diseases, including allergy. Many investigators have revealed that Th2 immunity is responsible for allergic immune responses and the subsequent pathogenesis of allergic inflammatory diseases (19, 20). AD is one of these allergy-related diseases and AD patients show a marked increase in the number of Th2 cells in both peripheral blood and acute skin lesions (1). Therefore, it has been proposed that the Th2 immune response plays a key pathogenetic role in AD, and this is supported by the presence of blood eosinophilia and enhanced serum IgE levels in most AD patients (21). However, no immunoregulatory method for preventing Th2 cell development in AD patients has yet been established.

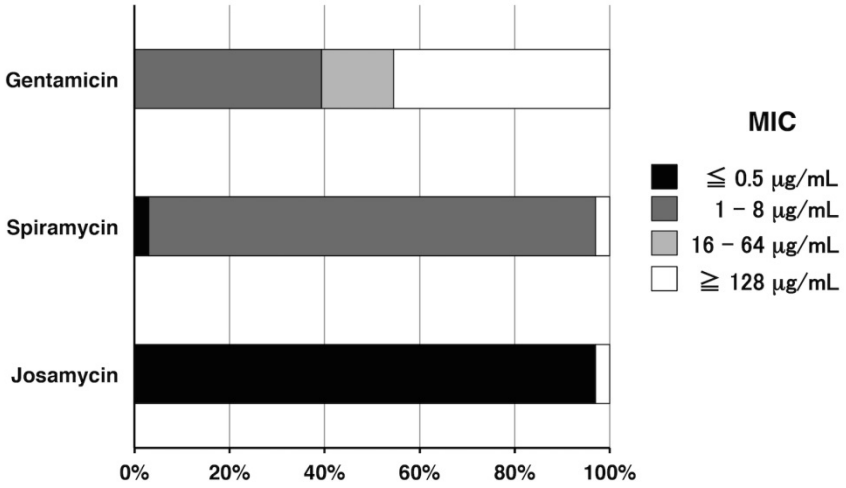

Figure 7. Susceptibility of *S. aureus* strains isolated from atopic dermatitis patients to gentamycin, spiramycin and josamycin. The susceptibility of 33 strains of *S. aureus* to each antibiotic was compared by estimating the minimum inhibitory concentration (MIC). The number of *S. aureus* strains with each MIC value is expressed as a percentage of the 33 strains.

In the present study, we observed that injection of LDCs that had been treated with 14-membered ring macrolide antibiotics and a 15-membered ring macrolide antibiotic suppressed the production of the Th1 cytokine, IFN-γ, and increased the production of the Th2 cytokine, IL-4, respectively, in lymph node cells of mice. To clarify the mechanism responsible for this change in Th1/Th2 immune balance, expression of cell surface molecules on LDCs was confirmed by RT-PCR, which indicated that mRNAs for CD40, CD80, CD86, Delta 1, Delta 4, Jagged 1, Jagged 2 and TIM-4, but not for Delta 3, were expressed spontaneously in LDCs and predicted the presence of the respective cell surface molecules. However, treatment with 14-membered ring macrolide antibiotics and with a 15-membered ring macrolide antibiotic inhibited the expression of CD40 mRNA in LDCs. Furthermore, the expression of mRNA for Delta 1 was suppressed in LDCs treated with 14-membered ring macrolide antibiotics, but not in those treated with a 15-membered ring macrolide antibiotic. The expression of mRNA for Delta 4 was suppressed only in LDCs that had been treated with a 15-membered ring macrolide antibiotic. In mammals, four Notch receptors (Notch 1-4) and five Notch ligands (Delata1, Delta 3, Delta 4, Jagged 1 and Jagged 2) have been identified (22). Amsen *et al*. (23) have presented evidence that different Notch ligands expressed on APCs are responsible for initiating Th1/Th2 differentiation in mice, and have concluded that, in APCs, Th1 adjuvant induces Th1 cell development through the expression of Delta members, whereas Th2 adjuvant induces Th2 cell development through the expression of Jagged members. Therefore, inhibition of Th1 cell development by 14-membered ring macrolide antibiotics and a 15-membered ring macrolide antibiotic would be induced by down-regulation of Delta 1 and Delta 4 expression, respectively, on LDCs. Moreover, treatment with 14- and 15-membered ring macrolide antibiotics suppressed the expression of CD40 mRNA. Since it is known that CD40 promotes IL-12 production from APCs and induces Th1 differentiation (24), the inhibition of Th1 cell development would also be explained by inhibition of CD40 expression on LDCs. However, the up-regulation of Th2 cell development induced by 14- and 15-membered ring macrolide antibiotics could not be explained by the levels of expression of mRNAs for Jagged members in LDCs. TIM-4 is expressed by DCs in lymphoid organs, and its ligand, TIM-1, is expressed by T cells. These molecules have been found to be critical regulators of Th2 cell development (25). In the present study, the expression of TIM-4 mRNA was enhanced by treatment with 14- and 15-membered ring macrolide antibiotics, which would explain why LDCs thus treated were capable of enhancing Th2 cell development.

On the other hand, in mice injected with LDCs that had been treated with 16-membered ring macrolide antibiotics, the results were slightly complicated. Slightly weakened production of the Th1 cytokine, IFN-γ, in lymph nodes was observed after treatment with all of three antibiotics. However, suppressed production of the Th2 cytokine, IL-4, in lymph nodes was observed only after treatment with josamycin and spiramycin. The results of RT-PCR showed that the expression of CD86 and TIM-4 mRNAs in LDCs was suppressed by josamycin and spiramycin, but not by midecamycin. Our observation that suppression of CD86 expression was correlated with that of Th2 cell development is similar to the findings of Serebrisky *et al.* (26), and was compatible with the fact that CD86, but not CD80, preferentially costimulates the initial production of IL-4 (27). However, since the levels of expression of CD86 mRNA did not necessarily correlate with those of TIM-4 mRNA, as was the case for treatment with 14- and 15-membered ring macrolide antibiotics, the suppression of CD86 expression induced by josamycin and spiramycin might be associated with suppression of Th1 cell, rather than Th2 cell, development as suggested by Whelan *et al.* (28). In addition, although the suppression of CD40 expression by midecamycin explains the suppressed development of Th1 cells, this Th1 suppression and the suppressive effect of josamycin and spiramycin were not necessarily associated with the suppressed expression of Delta members, as was observed after treatment with 14- and 15-membered ring macrolide antibiotics. Therefore, the less marked suppression of Th1 cell development by 16-membered ring macrolide antibiotics may be explained by non-responsiveness to the expression of Delta members. Furthermore, since treatment with josamycin, but not spiramycin, suppressed the expression of Jagged 1 mRNA in addition to that of TIM-4 mRNA, this would explain why josamycin

had a more pronounced suppressive effect on Th2 cell development than did spiramycin. In any events, it seems that the regulation of Th2 cell development by macrolide antibiotics is well correlated with the level of TIM-4 expression, and that this could be employed as a good marker of Th2 cell development.

The data from this study suggest that josamycin may have superior ability to inhibit Th2 cell development in AD patients. Furthermore, the data show that *S. aureus* strains isolated from the lesional skin of AD patients are particularly susceptible to josamycin in comparison with spiramycin, another Th2 inhibitor characterized in the present study, and gentamicin, which is used widely in Japan for treatment of AD in combination with steroids for topical application. Since the skin of most AD patients shows superficial *S. aureus* colonization and barrier disruption due to a decrease of filaggrin (29), bacterial products such as staphylococcal enterotoxins, lipoteichoic acid and peptidoglycan would be expected to penetrate the skin and augment skin inflammation based on the Th2 immune response caused by bacterial product-induced Th2 cell development and chemokine production (1, 4-6, 30).

CONCLUSIONS

Topical application of josamycin to the lesional skin of AD patients would appear to have unique effectiveness in that it has superior bactericidal action against *S. aureus*, an inhibitory effect against the subsequent Th2 immune response, and a capacity to control allergen-specific Th2 cell development mediated by LCs, unlike the immunosuppressant tacrolimus or steroids. Since it is thought that topical application of josamycin to the skin would have few side effects, it might be possible to increase its concentration in ointment to one that would inhibit the Th2 immune response in AD lesions. Our preliminary study showed that topical application of 0.1% josamycin ointment to AD-like skin lesions in NC/Nga mice, which is equivalent to 5 μg josamycin/cm^2 of skin, inhibited Th2 cell development (data not shown). Therefore, the concentration of josamycin used for *in vitro* stimulation in this study (5-50 μM) is a realistic concentration in epidermis of AD patients. Thus, topical application of josamycin might be beneficial as a new therapeutic strategy for AD lesions with superficial *S. aureus* colonization.

ACKNOWLEDGMENTS

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