



Regulatory effects of antihistamines on the responses to staphylococcal enterotoxin B of human monocyte-derived dendritic cells and CD4⁺ T cells

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KEYWORDS

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Cytokine;
Chemokine;
IFN-gamma;
IL-4;
TNF-alpha;
TARC;
MDC;
IP-10;
Mig;
ELISA;
Proliferation;
ICAM-1;
CD54;
Flow cytometry

Summary

Background: Antihistamines are widely used for the treatment of allergic diseases, such as urticaria and allergic rhinitis. They are also effective for the treatment of diseases in which T cells are mainly involved in the pathogenesis, such as atopic dermatitis (AD) and contact dermatitis. Dendritic cells (DCs) drive polarization of naive T cells into Th1 or Th2 subsets, and are also likely to be involved in AD pathogenesis.

Objectives: The aim of this study was to determine the effects of antihistamines on DCs and CD4⁺ T cells.

Methods: Human monocyte-derived DCs (MoDCs) and autologous CD4⁺ T cells were obtained from healthy subjects, and cultured together or independently in the presence of antihistamines. As a stimulant, we used staphylococcal enterotoxin B or the combination of anti-CD3 monoclonal antibody (mAb) and anti-CD28 mAb. The concentrations of cytokines and chemokines in culture supernatants were measured by ELISA. The expression of surface molecules on MoDCs was measured by flow cytometry. Cell proliferation in the cocultures of MoDCs and CD4⁺ T cells (DC-T cocultures) was measured by a [³H] thymidine incorporation assay.

Results: Antihistamines inhibited the production of IFN- γ , and enhanced the production of IL-4 in DC-T cocultures. Antihistamines inhibited the production of TNF- α , TARC, MDC, IP-10, and Mig from MoDCs. Epinastine, one of antihistamines, suppressed the expression of ICAM-1 (CD54) on MoDCs. Epinastine also inhibited the proliferation of CD4⁺ T cells cocultured with MoDCs.

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Conclusions: Our findings show that antihistamines regulate immune responses by affecting the interaction between DCs and CD4⁺ T cells, and further DCs and CD4⁺ T cells independently, which may partially contribute to the control of allergic diseases such as AD and contact dermatitis.

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1. Introduction

Antihistamines are widely used for the treatment of allergic diseases, such as urticaria and allergic rhinitis. Their clinical effect is believed to be mediated by their antagonistic action against histamine, mainly produced by mast cells. On the other hand, antihistamines are also effective for the treatment of diseases in which T cells are mainly involved in the pathogenesis, such as atopic dermatitis (AD) and contact dermatitis [1–3]. Recent studies have suggested that antihistamines may possess additional mechanisms besides their ability to block histamine receptors [4,5], and that antihistamines exert inhibitory action on cytokine production from T cells in vitro and in vivo [6–10].

Previous reports have suggested that Th1-related chemokines such as IFN- γ -inducible protein-10 (IP-10) and monokine induced by IFN- γ (Mig), and Th2-related chemokines such as thymus and activation regulated chemokine (TARC) and macrophage-derived chemokine (MDC), which may facilitate the recruitment of Th1/Th2 cells [11,12], are important molecules in the pathogenesis of AD [13–15]. Therefore, the regulation of these chemokines may be useful in the management and treatment of allergic diseases, including AD.

Dendritic cells (DCs) could also be targets for the treatment of allergic diseases, since they drive polarization of naive T cells into Th1 or Th2 subsets [16], and are likely to be involved in the pathogenesis of the above diseases [17]. Nevertheless, the effects of antihistamines on the functions of human DCs, such as the production of Th1/Th2-related cytokines and chemokines, and the expression of surface markers, are yet to be determined.

In this study, we evaluated the effects of antihistamines on the function of DCs and autologous CD4⁺ T cells. In certain experiments, CD4⁺ T cells were cocultured with DCs in vitro, because we considered that T cell stimulation in the presence of DCs, as compared to the stimulation without DCs, may better reflect the physiological conditions in which DCs mainly work as antigen presenting cells. Skin colonization with *Staphylococcus aureus* is an almost ubiquitous feature of AD, with rates as high as 75–90% of lichenified plaques compared with only 5% in skin from healthy donors [18]. *S. aureus* strains

producing superantigens were isolated at a high prevalence (37–57%) from AD subjects [19,20]. Staphylococcal enterotoxin B (SEB), one of the superantigens produced by *S. aureus*, has been shown to induce inflammatory reactions following application to intact skin of healthy and atopic subjects [21]. Therefore, we used SEB as a stimulant of immune cells in this study. We observed that antihistamines had considerable effects on DC and T cell function.

2. Materials and methods

2.1. Media, reagents, and antibodies

The medium used was RPMI1640 (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 2 mM L-glutamine (Nacalai Tesque Inc., Kyoto, Japan), 25 mM HEPES (Wako Pure Chemical Industries, Osaka, Japan), 100 U/ml penicillin, 100 μ g/ml streptomycin (Meiji Seika, Tokyo, Japan), and 10% heat-inactivated foetal bovine serum (Thermo Electron, Melbourne, Australia), defined as complete medium. Recombinant human GM-CSF and recombinant human IL-4 were purchased from PeproTech (Rocky Hill, NJ, USA). Highly purified staphylococcal enterotoxin B was obtained from Toxin Technology Inc. (Florida, USA). Anti-CD80 monoclonal antibody (mAb) (2D10), anti-CD86 mAb (IT2.2), FITC-conjugated anti-CD54 mAb (MEM-111), and FITC-conjugated anti-HLA-DR mAb (L243) were purchased from BioLegend (San Diego, CA, USA). Anti-CD1a mAb (HI149), anti-CD3 mAb (UCHT1), anti-CD28 mAb (CD28.2), anti-CD83 mAb (HB15e), and FITC-conjugated rat anti-mouse IgG1 antibody (A85-1) were purchased from BD PharMingen (San Diego, CA, USA). Control mouse IgG1 and FITC-conjugated control mouse IgG2a were purchased from DakoCytomation (Glostrup, Denmark). FITC-conjugated anti-CD14 mAb (TÜK4) was obtained from Miltenyi Biotec (Bergisch Gladbach, Germany). Anti-CD4 mAb (SFC112T4D11) was obtained from Beckman-Coulter Immunotech (Villepinte, France). The agents used in this study and their sources were as follows, antihistamines: Epinastine, kindly provided by Nihon Boehringer Ingelheim Co. (Kawanishi, Japan); Ketotifen by Novartis Pharma AG (Basel, Switzerland); Loratadine by Shionogi Co. (Osaka, Japan);

and macrolide immunosuppressant: Tacrolimus by Astellas Pharma Inc. (Tokyo, Japan).

2.2. Subjects

Human peripheral blood samples were obtained from healthy controls without a personal or family history of allergic diseases, including bronchial asthma, allergic rhinoconjunctivitis, or AD (aged 24–45 years). The present study was approved by the Ethics Committee of Nara Medical University, and each subject gave written informed consent before the examination.

2.3. Preparation of MoDCs and CD4⁺ T cells

Peripheral blood mononuclear cells were isolated from blood donor buffy coats by density gradient centrifugation with Lymphoprep tube (Axis-Shield, Dundee, Scotland). CD14-positive monocytes were then separated from the mononuclear cells by magnetic cell sorting by using positive selection according to the manufacturer's protocol (Miltenyi Biotec). Briefly, cells were incubated with magnetic microbeads conjugated with mouse anti-human CD14 mAb in MACS buffer for 15 min. Then, cells were run through a MACS column (Miltenyi Biotec) in a magnetic field. The column was then removed from the magnet, and the positive cells were flushed out. Analysis by flow cytometry showed that more than 95% of the purified cells expressed CD14.

Monocytes (10⁶ cells/ml) were seeded into 24-well tissue culture plates and cultured at 37 °C in 5% CO₂ in fresh complete medium supplemented with 100 ng/ml GM-CSF and 100 ng/ml IL-4. At days 3 and 5, half of the medium was replaced by an equivalent volume of fresh medium, containing the same cytokines. On day 7, monocyte-derived dendritic cells (MoDCs) showed the phenotypic characteristics of immature DCs (CD1a^{high}, CD83^{low}, and CD14⁺) as determined by flow cytometry. Contamination of MoDCs with T cells was less than 1%, as assessed by flow cytometry using anti-CD3 mAb.

Autologous CD4⁺ T cells were isolated from the CD14⁺ fraction by negative selection using a CD4⁺ T cell Isolation kit II (Miltenyi Biotec). Analysis by flow cytometry showed that more than 95% of the purified cells expressed both CD3 and CD4.

2.4. Cell culture

CD4⁺ T cells (10⁵ cells/well) cocultured with autologous MoDCs at a ratio of 100:1 were pretreated with various concentrations of epinastine (1, 10, and 100 μM), ketotifen (10 and 100 μM), loratadine

(1 and 10 μM), tacrolimus (1 μM), or left untreated for 2 h. Then, cells were stimulated with SEB (1 ng/ml). MoDCs alone were also pretreated with various concentrations of antihistamines or left untreated for 2 h, then stimulated with SEB (100 ng/ml). The optimal duration of the culture and the optimal concentrations of SEB were predetermined according to the results of preliminary kinetic and titration experiments (data not shown). For the measurement of cytokines and chemokines produced by CD4⁺ T cells independent of DCs, mAb-coated plates were prepared as described previously [22]. In brief, 96-well flat-bottomed plates were coated with 100 μL of phosphate-buffer saline (PBS) containing 10 μg/mL of anti-CD3 mAbs plus 10 μg/mL of anti-CD28 mAbs, and incubated 1 h at 37 °C. Prior to use, these plates were washed once with PBS. CD4⁺ T cells (10⁵ cells/well) were seeded along with various concentrations of epinastine (0, 1, 10, and 100 μM) or tacrolimus (1 μM), and incubated for 48 h. Each drug concentration was chosen on the basis of previous studies and reported physiological concentrations [1,3,9,13,23–26]. Cell viability as measured by means of trypan blue staining before and after the addition of drugs, and all cultures were found to contain more than 95% viable cells.

2.5. Cytokine and chemokine assays

Culture supernatants were recovered 48 h after stimulation and applied to ELISAs specific for various cytokines and chemokines, including IFN-γ, IL-4, TNF-α (Biosource International Inc., Camarillo, USA), IP-10, Mig, TARC, and MDC (R&D Systems, Minneapolis, MN, USA).

2.6. Flow cytometric analysis

After 48 h of stimulation, MoDCs were washed three times and incubated for 30 min at 4 °C with mAbs against various cell surface molecules at saturating concentrations, or isotype-matched negative control mAbs at the same concentration. Cells with FITC-unlabeled mAbs were further incubated with FITC-conjugated anti-mouse IgG antibody for 30 min at 4 °C. After washing three times, the labeled cells (10⁴) were analyzed with a FACScan (Becton Dickinson, San Jose, CA) using CellQuest software (Becton Dickinson).

2.7. T cell proliferation assays

After 3 days of stimulation, CD4⁺ T cells cocultured with MoDCs were pulsed with 1 μCi/well of [³H] thymidine (Amersham Pharmacia Biotech, Little Chalfont, UK) 8 h prior to harvesting, and the incor-

porated radioactivity was quantified using a liquid scintillation counter (Aloka, Tokyo).

2.8. Statistical analysis

Mean values were compared by using the Student's *t*-test. All tests were considered significant for $P < 0.05$.

3. Results

3.1. Effect of antihistamines on cytokine production in the cocultures of CD4⁺ T cells and MoDCs (DC-T cocultures)

We evaluated the production of IFN- γ as Th1-type cytokine and IL-4 as Th2-type cytokine from CD4⁺ T cells cocultured with MoDCs stimulated with SEB for 48 h in the presence of antihistamines at various concentrations. As shown in Fig. 1, SEB-stimulated cells produced significant amounts of IFN- γ and IL-4. All three antihistamines used in this study, epinastine, ketotifen, and loratadine, inhibited IFN- γ pro-

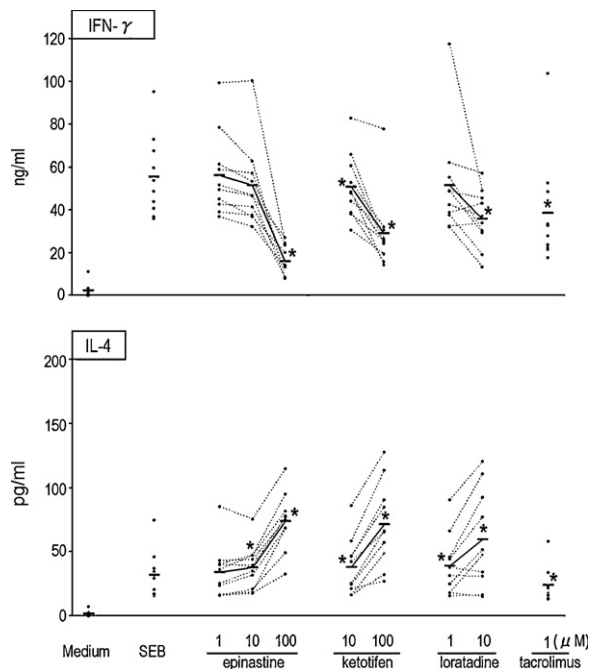


Fig. 1 Effect of antihistamines on cytokine production in DC-T cocultures. CD4⁺ T cell from healthy donors were cocultured with autologous DCs in the presence or absence of epinastine, ketotifen, loratadine, or tacrolimus for 2 h, and stimulated with 1 ng/ml SEB. After 48 h, the culture supernatants were harvested and assayed for IFN- γ and IL-4 by ELISA. The results of ten different subjects are shown. The short horizontal lines indicate the mean values. * $P < 0.05$ versus SEB-stimulated cells in the absence of drugs.

duction, while they enhanced IL-4 production. Tacrolimus, on the other hand, inhibited both IFN- γ and IL-4 production.

3.2. Effect of epinastine on cytokine and chemokine production from CD4⁺ T cells

To assess the effect of antihistamine on CD4⁺ T cells independent of DCs, we evaluated the production of IFN- γ , IL-4 stimulated with anti-CD3 plus anti-CD28 in the presence or absence of epinastine. As shown in Fig. 2, the production of IFN- γ and IL-4 from CD4⁺ T cells was induced to a significant extent by stimulation. Epinastine inhibited the production of these cytokines by antibody-stimulated CD4⁺ T cells in a dose-dependent manner. Tacrolimus also inhibited the production of these cytokines.

3.3. Effect of antihistamines on cytokine and chemokine production from MoDCs

Next, we evaluated the production of cytokines and chemokines from MoDCs in the presence or absence of epinastine or loratadine. We chose TNF- α , TARC,

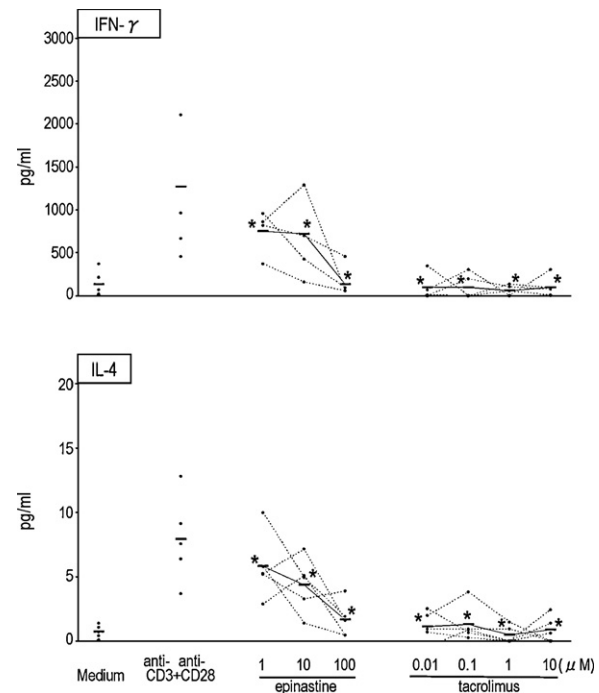


Fig. 2 Effect of antihistamine on cytokine production from CD4⁺ T cells. CD4⁺ T cell from healthy donors were cultured in mAb-coated plates in the presence or absence of epinastine or tacrolimus. After 48 h, the culture supernatants were harvested and assayed for IFN- γ and IL-4 by ELISA. The results of five different subjects are shown. The short horizontal lines indicate the mean values. * $P < 0.05$ versus SEB-stimulated cells in the absence of drugs.

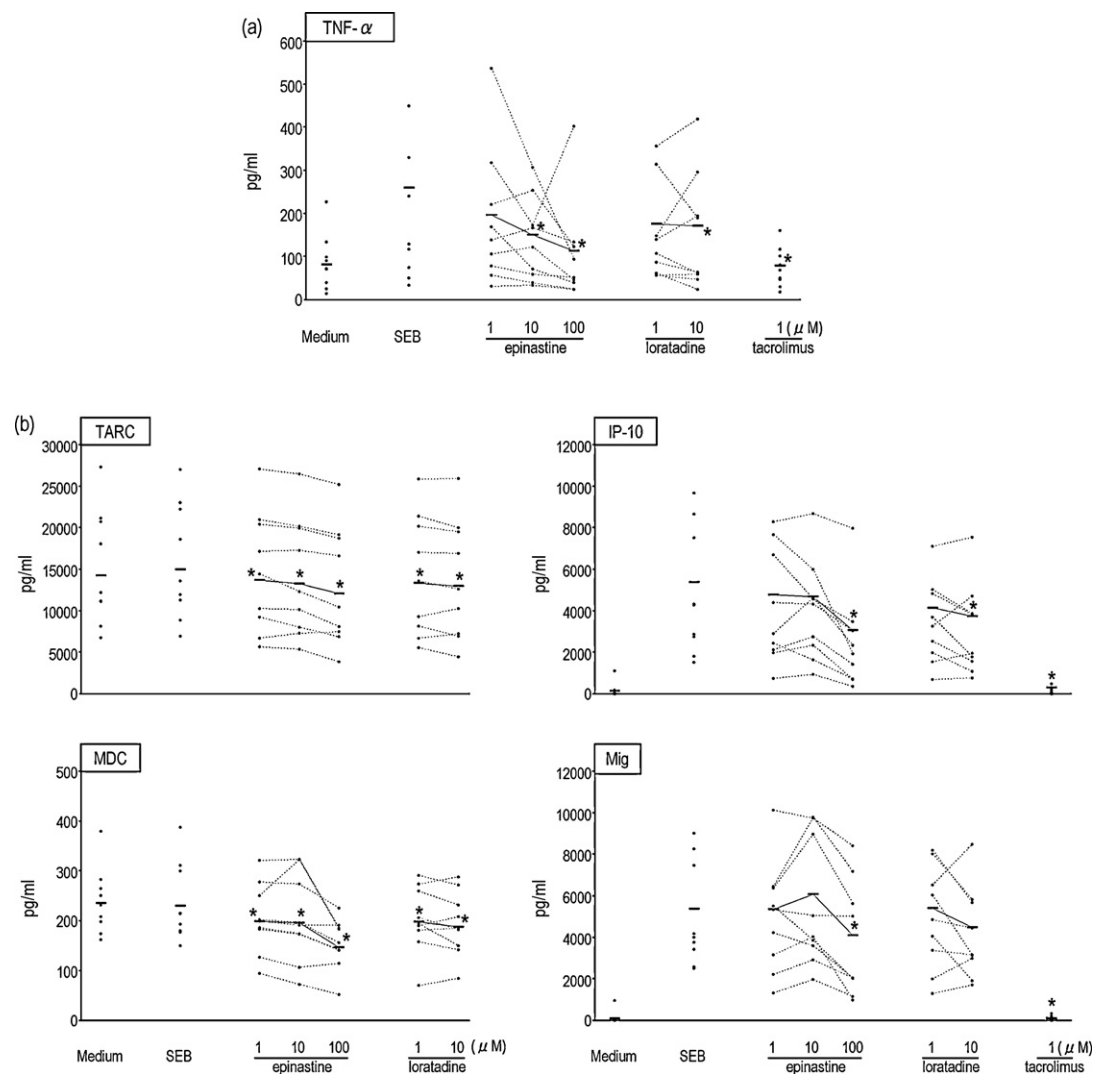


Fig. 3 Effect of antihistamines on the production of cytokine (A) and chemokines (B) from DCs. DCs were cultured in the presence or absence of epinastine, loratadine, or tacrolimus for 2 h, and stimulated with 100 ng/ml SEB. After 48 h, the culture supernatants were harvested and assayed for TNF- α , TARC, MDC, IP-10, and Mig by ELISA. The results of ten different subjects are shown. The short horizontal lines indicate the mean values. * $P < 0.05$ versus SEB-stimulated cells in the absence of drugs.

MDC, IP-10, and Mig, because DCs are known to be a major source of these proteins [13,26–31]. As shown in Fig. 3, the production of TNF- α (Fig. 3A), TARC, IP-10, and Mig (Fig. 3B) from MoDCs was induced to a significant extent by SEB. With regard to TNF- α , TARC, and IP-10, both antihistamines inhibited the production all of these proteins in a dose-dependent manner. Mig production was also significantly inhibited by epinastine at 100 μ M, and tended to be lower in the presence of loratadine at 10 μ M. MoDCs constitutively produced large amounts of MDC (Fig. 3B), but stimulation with SEB did not enhance this production. Epinastine and loratadine inhibited the constitutive production of MDC from MoDCs. Tacrolimus significantly inhibited SEB-induced production of TNF- α , IP-10, and

Mig, whereas the production of TARC and MDC was not inhibited by tacrolimus (Fig. S2B). We also examined the production of IL-1 β , IL-10, and IL-12 from MoDCs, but we were unable to detect these cytokines by ELISA, even after adding SEB.

3.4. Effect of epinastine on expression of surface markers on MoDCs

We examined the effect of epinastine on expression of surface molecules on MoDCs. We chose ICAM-1 (CD54), CD80, CD86, and HLA-DR as markers involved in the interaction between DCs and CD4⁺ T cells. As shown in Fig. 4, untreated MoDCs expressed ICAM-1 moderately. Its expression was upregulated by stimulation with SEB, and epinastine

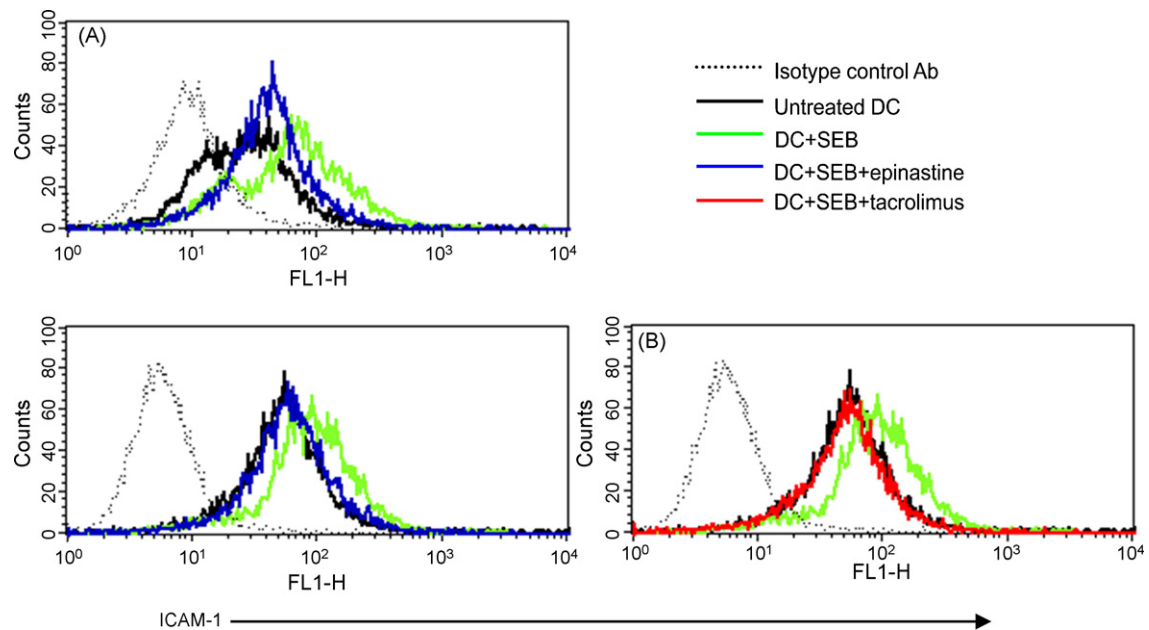


Fig. 4 Effect of antihistamine on expression of surface markers on DCs. (A) DCs were cultured in the presence of epinastine (A) or tacrolimus (B) for 2 h, and stimulated with 100 ng/ml SEB. After 48 h, the expression of ICAM-1 was examined by flow cytometry. In A, two representative results of four-independent experiments from different donors are shown. In B, one representative result is shown.

at 100 μ M downregulated SEB-induced ICAM-1 augmentation, as well as tacrolimus. CD80, CD86, and HLA-DR were also expressed in untreated MoDCs, but their expression levels were not affected by stimulation with SEB, and even with epinastine or tacrolimus (data not shown).

3.5. Effect of epinastine on CD4⁺ T cells proliferation cocultured with MoDCs

Finally, we examined the effect of epinastine on the proliferation of CD4⁺ T cells stimulated with SEB in the presence of MoDCs. Cell proliferation was assessed by examining [³H] thymidine uptake. As shown in Fig. 5, epinastine at 100 μ M as well as 1 μ M tacrolimus significantly suppressed SEB-induced cell proliferation.

4. Discussion

In this study, we first showed that antihistamines inhibited IFN- γ production and enhanced IL-4 production in DC-T cocultures (Fig. 1), while antihistamine suppressed both IFN- γ and IL-4 production from CD4⁺ T cells stimulated with anti-CD3 and anti-CD28 (Fig. 2). Previous studies also demonstrated that antihistamines suppressed IL-4 production [7–9]. It is reported that blocking the interaction between ICAM-1 and its receptor LFA-1 in DC-T cocultures reduced production of IFN- γ and increased produc-

tion of IL-4 from T cells stimulated with SEB [32]. In this study, antihistamine was found to suppress the expression of ICAM-1 on MoDCs (Fig. 4). Therefore, the observed difference in IL-4 production between culture of SEB-stimulated DC-T and that of antibody-stimulated CD4⁺ T cells may be explained by the presence of MoDCs in the former coculture systems. Another possible explanation is the differences in T cell population activated by different stimulants. It is known that in healthy subjects, SEB, but not anti-CD3

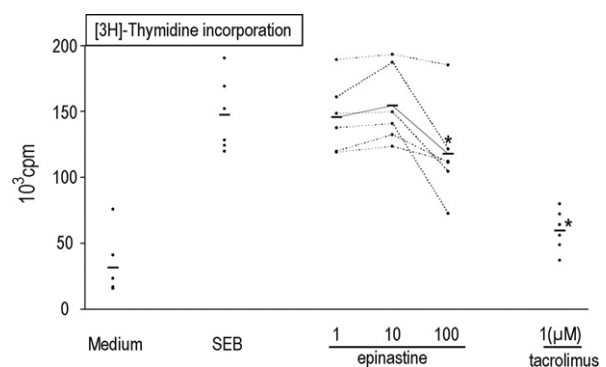


Fig. 5 Effect of antihistamine on CD4⁺ T cell proliferation cocultured with DCs. CD4⁺ T cells from healthy donors were cocultured with autologous DCs in the presence or absence of epinastine or tacrolimus for 2 h, and stimulated with 1 ng/ml SEB. Cells were pulsed with 1 μ Ci/well of [³H] thymidine during the last 8 h of the 3-day culture. The results of six different subjects are shown. The short horizontal lines indicate the mean values. * P < 0.05 versus SEB-stimulated cells in the absence of drugs.

plus anti-CD28, preferentially induces the development of Th1 cells producing IFN- γ [33]. Thus, antihistamines are thought to mainly suppress IFN- γ -producing T cell activation by SEB in DC-T cocultures. Since IFN- γ and IL-4 could counteract each other, the downregulation of IFN- γ production by antihistamines might induce IL-4 production from Th2 cells.

Present results demonstrated the effect of antihistamines on inhibition of the production of both Th1- and Th2-type chemokines, and TNF- α from MoDCs (Fig. 3). We also demonstrated the inhibitory effect of antihistamine on cell proliferation in DC-T cocultures (Fig. 5). It may be partially accounted for by the fact that antihistamine suppressed ICAM-1 expression on MoDCs (Fig. 4), because ICAM-1 was known to induce important costimulatory signal for activation and proliferation of T cells [34].

Taken together, our findings have shown that antihistamines regulate immune responses by affecting not only mast cells but also the interaction between DCs and CD4⁺ T cells, both of which are possibly regulated independently. The concentrations of antihistamines used in this study were much higher than in vivo concentrations from a single dose of antihistamines. However, in many clinical cases patients usually take antihistamines for a long period of time. Therefore, the in vitro data presented herein are likely to correspond to the in vivo effects of antihistamines. Previous studies have indicated that dysregulated production of cytokines and chemokines, which were examined in our study, is observed in AD [13–15,35–39]. Thus, present results suggest that antihistamines partially contribute to the control of allergic diseases such as AD or contact dermatitis, though the precise mechanism of the effect of antihistamines on DCs and CD4⁺ T cells is not elucidated in the present study. Currently, our investigation is in progress to clarify the mechanism, which could support the clinical applicability of antihistamines on a variety of allergic diseases or non-allergic diseases related to Th1/Th2 imbalance.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jdermsci.2008.04.004.

Conflict of interest

None.

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