ORIGINAL ARTICLE

Inhibition of Cytokine-Induced Expression of T-Cell Cytokines by Antihistamines

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Abstract

Objective: To investigate immunomodulatory properties of 4 antihistamines available in Japan.

Method: Isolated peripheral blood T cells from healthy volunteers were preincubated with cetirizine, loratadine, olopatadine, or fexofenadine for 30 minutes and then stimulated with interleukin (IL)-12 or IL-4 to skew immune response towards type 1 or type 2 helper T cells. RNA was extracted 6 hours later and semiquantitative reverse transcription polymerase chain reaction (RT-PCR) was performed using primers for IL-5 and interferon (IFN) γ. Supernatants were collected 24 hours after stimulation, and cytokine production was quantified by enzyme-linked immunosorbent assay (ELISA).

Results: RT-PCR revealed that IL-12–induced expression of IFN-γ was partially suppressed by loratadine and fexofenadine and that all 4 agents tested inhibited IL-4–induced expression of IL-5. ELISA demonstrated that IL-12–induced IFN-γ production was significantly suppressed by cetirizine and fexofenadine and IL-4–induced IL-5 production was downregulated by three agents with the exception of cetirizine. This study demonstrates that antihistamines have varying immunomodulatory properties, suggesting treatment choice for atopic dermatitis can be directed by disease signs and symptoms.

Key words: Atopic dermatitis antihistamines. IL-4. IL-12.

Resumen

Objetivo: Investigar las propiedades inmunomoduladoras de cuatro antihistamínicos disponibles en Japón.

Método: Se preincubaron con cetirizina, loratadina, olopatadina o fexofenadina linfocitos T aislados de sangre periférica durante 30 minutos y, a continuación, se estimularon con interleucina (IL)-12 o IL-4 para desviar la respuesta inmunitaria hacia los linfocitos T cooperadores del tipo 1 o del tipo 2. Se extrajo el ARN seis horas más tarde y se realizó una reacción en cadena de la polimerasa con transcripción inversa semicuantitativa (RT-PCR) utilizando cebadores para la IL-5 y el interferón (IFN)-γ. Los sobrenadantes se recogieron 24 horas después de la estimulación y se cuantificó la producción de citocinas mediante enzimoinmunoanálisis de adsorción (ELISA).

Resultados: La RT-PCR reveló que la expresión de IFN-γ inducida por la IL-12 fue parcialmente suprimida por la loratadina y la fexofenadina y que los cuatro antihistamínicos probados inhibieron la expresión inducida por la IL-4 de la IL-5. La prueba ELISA demostró que la producción de IFN-γ inducida por la IL-12 fue suprimida de forma significativa por la cetirizina y la fexofenadina y la producción de IL-5 inducida por IL-4 sufrió una reducción por la acción de tres de los antihistamínicos, con la excepción de la cetirizina. El estudio probó que los antihistamínicos tienen distintas propiedades inmunomodulatorias, sugiriendo que el tratamiento preferencial para la dermatitis atópica debe decidirse a partir de los signos y síntomas de la enfermedad.

Palabras clave: Dermatitis atópica. Antihistamínicos. IL-4. IL-12.

Introduction

Atopic dermatitis is one of the most frequent skin disorders in infants and children. It manifests as chronic, recurrent, relapsing inflammatory skin rashes and is characterized by episodes of extreme pruritus. The pathogenesis is still unclear, although it is believed that a biphasic inflammatory response is involved [1–3]. Type 2 helper T cell (T_H2) immune responses play a pathogenic role in acute exacerbations, while type 1 (T_H1) responses are predominantly involved in

the chronic eczematous phase [4, 5]. T_H1 cells preferentially produce interferon (IFN)-γ and therefore are involved in contact-hypersensitivity–like chronic eczematous lesions. In contrast, T_H2 cells secrete interleukin (IL)-4 and IL-5, which are crucial in sustaining immediate-type allergic reactions, thereby contributing to acute exacerbations [5]. The development of T_H1 and T_H2 responses is largely dependent on the stimulation of precursor (T_H0) cells with IL-12 and IL-4, respectively. Hence all stages of atopic dermatitis may be attributed to the effects of the local cytokine milieu.

Antihistamines have long been employed to manage skin rashes in atopic dermatitis, leading to improvement of symptoms when used concomitantly with steroidal agents [6]. Indeed, the results of a large clinical study in Japan demonstrated that 60 mg of fexofenadine in hydrogen chloride (HCl) solution twice daily as add-on therapy to corticosteroid therapy rapidly reduces pruritus associated with atopic dermatitis [7].

It is well established that oral antihistamines exert their antihistaminergic effect via inhibition of histamine H_1 receptors. However, several antihistamines have also been reported to have additional anti-inflammatory properties. Recently, we demonstrated that terfenadine suppresses IL-4–induced expression of a panel of T-cell cytokines at both mRNA and protein levels. This effect was thought to be mediated via suppressing the activation of a nuclear factor, activator protein-1 [8]. These are the first known data to demonstrate that antihistamines might modulate gene expression and protein production of immune cytokines. However, it is unknown whether other antihistamines have similar immunomodulatory effects. Selection of antihistamines specifically for use in atopic dermatitis would be a helpful clinical tool. Hence this study was undertaken to assess the effects of 4 antihistamines currently available in Japan (cetirizine, loratadine, olopatadine, and fexofenadine) on IL-4– and IL-12–induced expression of IL-5 and IFN-γ in isolated human T-cells.

Subjects and Methods

Subjects

Blood samples (approximately 30 mL) were obtained from 35 healthy volunteers who provided informed consent prior to study entry. The study was approved by the institutional review board of Kinki University School of Medicine and was performed in accordance with the principles of good clinical practice as set out in the Declaration of Helsinki and its amendments.

Isolation of T cells

The preparation of T cells has been described elsewhere [8]. Briefly, peripheral blood mononuclear cells were isolated using density gradient centrifugation (Ficoll-Paque, Amersham Pharmacia Biotech AB, Uppsala, Sweden). Mononuclear cells were recovered from the interface and incubated with magnetic beads coupled with monoclonal antibodies directed against CD19 and CD14 (Dynabeads, Dynal AS, Oslo, Norway). Unbound T cells were resuspended in RPMI1640 medium supplemented with 10% human AB serum, 1% L-glutamine, and 1% antibiotic/antimycotic solution. Serum added to the medium was dialyzed through a membrane (cutoff <5000 Da) to remove internal histamine. Percentage enrichment was quantified by fluorescence-activated cell-sorter analysis using a panel of antibodies (anti-CD3, anti-CD14, and anti-CD19). Approximately 95% of the cells in the preparations were T cells.

Stimulation of T Cells

Concentrations were based on peak serum concentrations (C_{max}) after administration of clinical doses of each agent as described by the manufacturers' product information. A total of 3 concentrations of each drug were used, with the middle concentration consistently corresponding to C_{max}. T cells were preincubated with cetirizine (40 ng/mL, 400 ng/mL, 4000 ng/mL), loratadine (0.7 ng/mL, 7 ng/mL, 70 ng/mL), olopatadine (10 ng/mL, 100 ng/mL, 1000 ng/mL), fexofenadine (7 ng/mL, 70 ng/mL, 700 ng/mL), or vehicle control for 30 minutes. Cells were then stimulated with human IL-4 (1 ng/mL, R&D Systems, Minneapolis, Minnesota, USA), IL-12 (10 ng/mL, R&D Systems) or left unstimulated. In preliminary studies, the induction of IL-5 and IFN-γ by graded concentrations of IL-4 and IL-12 was examined. A series of concentrations of IL-4 (0.1 ng/mL, 1 ng/mL, and 10 ng/mL) and IL-12 (1 ng/mL, 10 ng/mL, and 20 ng/mL) was also examined in preliminary experiments. The results revealed that stimulation with IL-4 1 ng/mL and IL-12 10 ng/mL induced logarithmic increases of mRNA specific for IL-5 and IFN-γ, respectively (data not shown). Three hours later, cytokine-stimulated cells were harvested for RNA preparation.

Viability Changes of T Cells by Antihistamines

To examine T-cell viability, the cells were pre-incubated with various concentrations of antihistamines (0.1 ×, 1 ×, and 10 × C_{max}-equivalent doses) and stimulated with IL-4 (1 ng/mL) and IL-12 (10 ng/mL) 30 minutes later. Viability of T cells was checked 24 hours later by trypan-blue exclusion assays.

RNA Preparation and Extraction

RNA was extracted using the acidic phenol procedure. The amount of extracted RNA in each sample was measured photometrically at a wavelength of 260 nm and reverse transcribed. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) amplification was performed as previously described using 5-μg samples of total RNA. All cDNA samples were amplified with primers specific for β-actin using various cycle numbers and dilution

factors to determine equal input for each sample and the logarithmic increase in amplification. Samples were then amplified with primers specific for IL-5 and IFN-γ. All RT-PCR primers were purchased from Clontech (Palo Alto, California, USA).

Measurement of Cytokine Production

Levels of IL-5 and IFN-γ production were measured by enzyme-linked immunosorbent assay (ELISA). Briefly, T cells were preincubated with cetirizine, loratadine, olopatadine, fexofenadine, or an equal volume of respective vehicle control for 30 minutes then stimulated with IL-4 or IL-12 or left unstimulated. Supernatants were collected 24 hours later and IL-5 and IFN-γ production was quantified. Each sample was tested in triplicate. Densitometric analyses were also conducted. The signal intensity of PCR products amplified by cytokine primers was divided by the signal intensity of β-actin of the same cDNA sample for normalization. Relative signal intensity of non-stimulated cells was calibrated as one. Accordingly, the modulated expression of cytokine mRNA was indicated as a ratio to that of unstimulated cells.

Statistical Analysis

All data are expressed as mean ±SD. A Student *t* test was used to compare groups, and *P* values less than .05 were considered statistically significant.

Results

Viability of Antihistamine-Induced Changes of T cells

None of the antihistamines significantly impaired the viability of T cells with the concentrations used.

Cytokine mRNA Expression by T cells

Loratadine 70 ng/mL (10 × the C_{max}-equivalent dose) and fexofenadine HCl at 7, 70, and 700 ng/mL suppressed IL-12–induced mRNA expression of IFN-γ (Figure 1). In contrast, olopatadine and cetirizine had no inhibitory effect on the T_H1 response (Figure 1). Loratadine, olopatadine, and fexofenadine prevented IL-4–induced mRNA expression of IL-5 at all concentrations examined (Figure 2). Cetirizine was found to downregulate IL-5 mRNA expression only at the highest dose (10 × the C_{max}-equivalent dose). The data were further confirmed densitometrically with T-cells stimulated with IL-12 (10 ng/mL) and IL-4 (1 ng/mL) (Figures 1 and 2).

Cytokine Production from T Cells

The effects of the 4 antihistamines on cytokine response are summarized in the table. IL-12 and IL-4 induced secretion of IFN-γ and IL-5, respectively, from T cells (Figure 3). Preincubation with cetirizine 400 ng/mL and fexofenadine HCl 70 ng/mL resulted in significant downregulation of T-cell secretion of IFN-γ. No such downregulation was seen with loratadine and olopatadine. In contrast, fexofenadine HCl 70 ng/mL, loratadine 7 ng/mL, and olopatadine 100 ng/mL significantly suppressed IL-4–induced production of IL-5 ($P < .05$), whereas cetirizine did not.

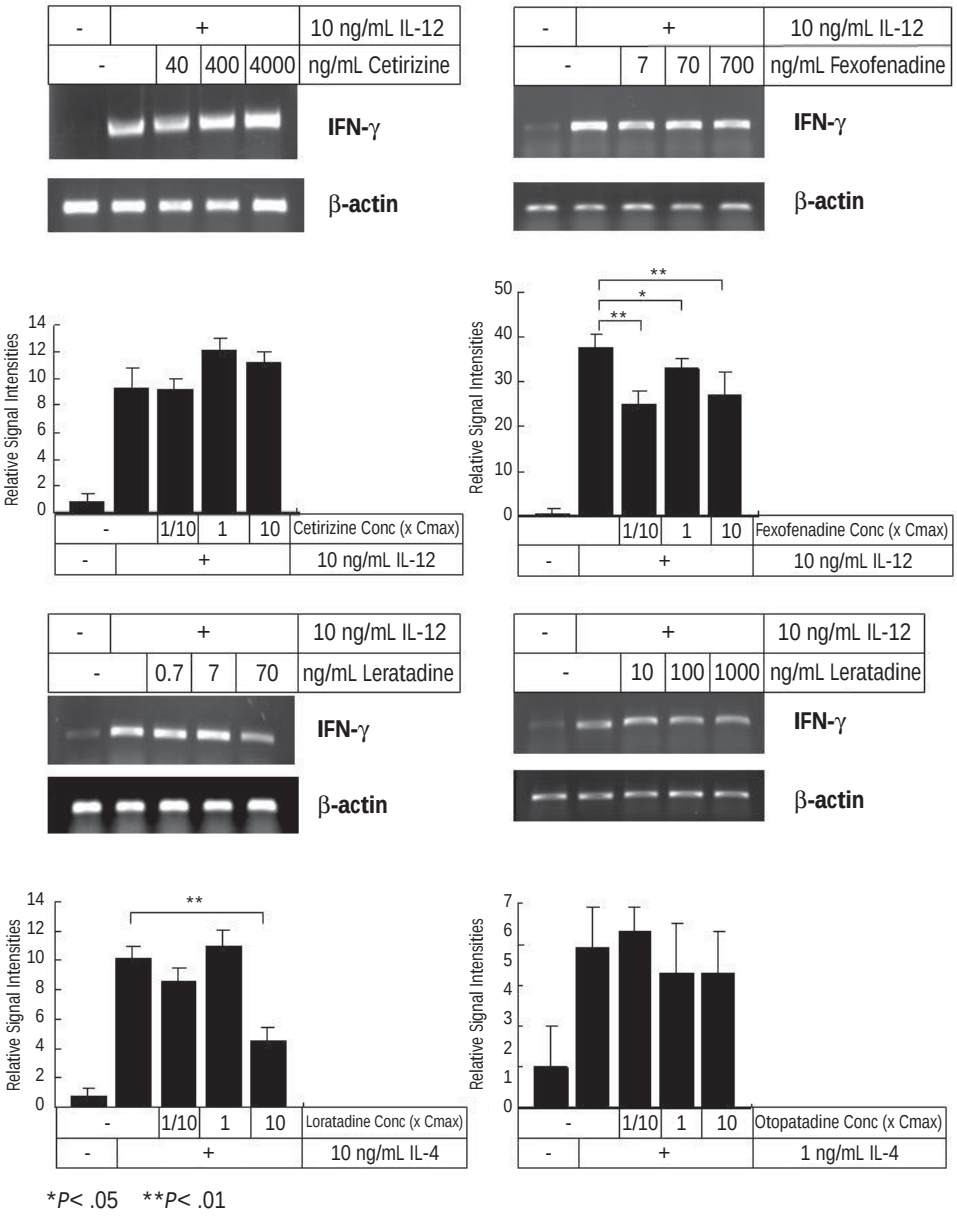
Discussion

We studied the immunomodulatory effects of the currently available antihistamines in Japan by preincubating T cells with these agents followed by stimulation with IL-4 and IL-12 to test the agents' ability to suppress expression of IL-5 and IFN-γ. The findings presented here demonstrate that all of the tested antihistamines downregulated IL-4–stimulated IL-5 mRNA expression, while only loratadine and fexofenadine successfully prevented IL-12–induced IFN-γ mRNA expression. The protein data show that all of the antihistamines except cetirizine downregulated IL-4–induced production of IL-5, while only cetirizine and fexofenadine suppressed IL-12–induced secretion of IFN-γ.

These findings are consistent with results obtained in rodent models, which revealed that olopatadine inhibits induction of IL-4 expression by mast cells in vivo and in vitro [9] and decreases the ability of Langerhans cells to present hapten to primed T cells with decreased expression of costimulatory and major histocompatibility complex class II molecules [10]. A recent cetirizine study revealed reduced levels of IL-4, IL-5, and IFN-γ in nasal-associated lymphoid tissue in cetirizine-treated mice compared with mice given saline [11]. These findings were consistent with decreases of allergic symptom score, histamine threshold, and eosinophil count in the nasal septal mucosa in the cetirizine group. Mice models have also shown that fexofenadine decreases T_H2 cytokine production, lymphocyte numbers, and bronchoalveolar lavage and tissue eosinophils [12]. Furthermore, in vitro research has shown that fexofenadine reduces intercellular adhesion molecule (ICAM)-1 expression and partially reduces soluble ICAM-1 release in human epithelial and fibroblast cells [13]. Interestingly, significant decreases of endothelial leukocyte adhesion molecule-1, vascular cell adhesion molecule-1, and tryptase expression were found in chronic idiopathic patients who presented with significant symptom improvement after administration of fexofenadine HCl 180 mg [14].

Our findings should be interpreted with caution. Although a high concentration (70 ng/mL) of loratadine suppressed expression of IFN-γ mRNA by IL-12, loratadine at a concentration of 7 ng/mL, which is in the therapeutic range, failed to downregulate IFN-γ production in the protein analysis. Although we did not test the effect of the 10-fold higher concentration (70 ng/mL) in the protein analysis, we can surmise that doses of loratadine within the therapeutic range do not affect IL-12–induced production of IFN-γ.

The data for cetirizine were also unexpected. Cetirizine prevented IL-12–stimulated production of IFN-γ but failed to



*P< .05 **P< .01

Figure 1. Effect of loratadine, olopatadine, fexofenadine, and cetirizine on interleukin (IL)-12–induced mRNA expression of interferon (IFN)-γ in isolated T cells. Representative data from at least 1 of 3 replicate experiments are presented. Densitometric analysis of T cells stimulated with IL-12 (10 ng/mL) are also represented; they confirm the polymerase chain reaction data.

downregulate IL-12–stimulated expression of IFN-γ mRNA. Conversely, cetirizine suppressed IL-4–induced expression of IL-5 mRNA, but had no effect at the protein level. These results suggest that cetirizine may possess modulatory properties at the post-transcriptional level, but further studies are necessary to investigate this issue.

The different modes of suppression of cytokine production among the 4 antihistamines are quite suggestive. Since in this experimental setting no histamine was included (since serum was dialyzed before use) and contamination by histamine-producing cell population such as mast cells and basophils was negligible, it seems that the antihistamines used in this study did not block endogenous histamine in the culture. We previously reported that IL-4–induced production and expression of T_H2 cytokines were suppressed by the antihistamine terfenadine [8]. That paper suggested that terfenadine may possibly bind to receptors other than the H_1 receptor on T cells, leading to transduction of antagonizing signals to IL-4–induced intracellular signals. In this context, it is worth mentioning that an antihistamine is capable of means other than H_1 receptor binding to some proteins, specifically to S100 proteins [15], or heat shock protein 90 [16]. Hence, it is interesting to exploit cell surface target proteins of antihistamines, which may transduce negative signals to IL-4 signals.

Atopic dermatitis can be divided into 2 immunologic phenotypes according to the disease condition [4,5]. The T_H2 immune phenotype is predominantly involved in acute exacerbation of the disease, while T_H1 immunity plays a pathogenic role in the chronic stage. As is well known, T_H1

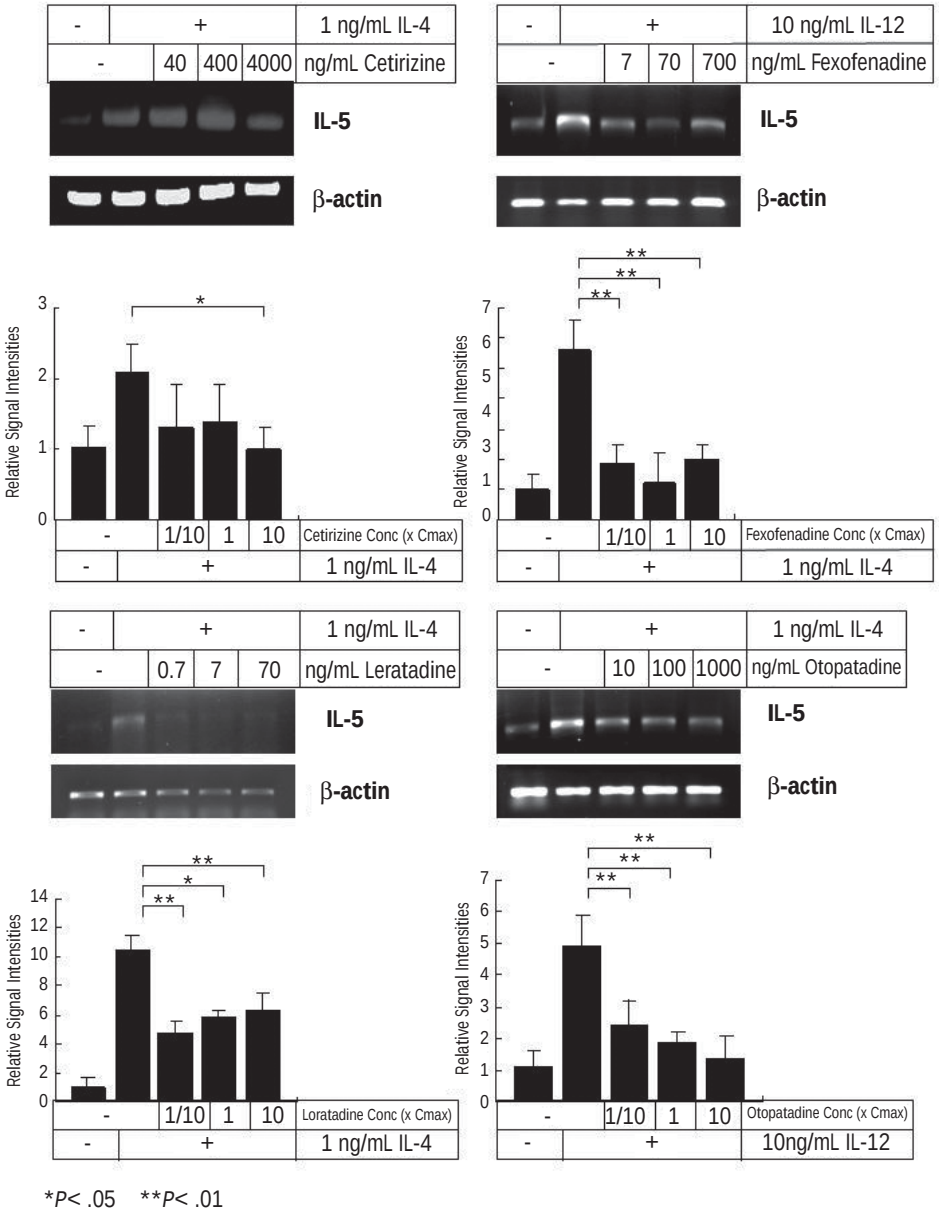


Figure 2. Effect of loratadine, olopatadine, fexofenadine, and cetirizine on interleukin (IL)-4–induced mRNA expression of IL-5 in isolated T cells. Representative data from at least 1 of 3 replicate experiments are presented. Densitometric analysis of T cells stimulated with IL-4 (1 ng/mL) are also represented; they confirm the polymerase chain reaction data.

Effects of Fexofenadine, Cetirizine, Olopatadine, and Loratadine on Cytokine Secretion*

T cells	Cytokine	Fexofenadine	Cetirizine	Olopatadine	Loratadine
T_H2	IL-4/IL-5	+	–	+	+
T_H1	IL-12/IFN-γ	+	+	–	–

*T_H1 indicates type 1 helper T cell; T_H2, type 2 helper T cell; IL, interleukin; IFN, interferon.

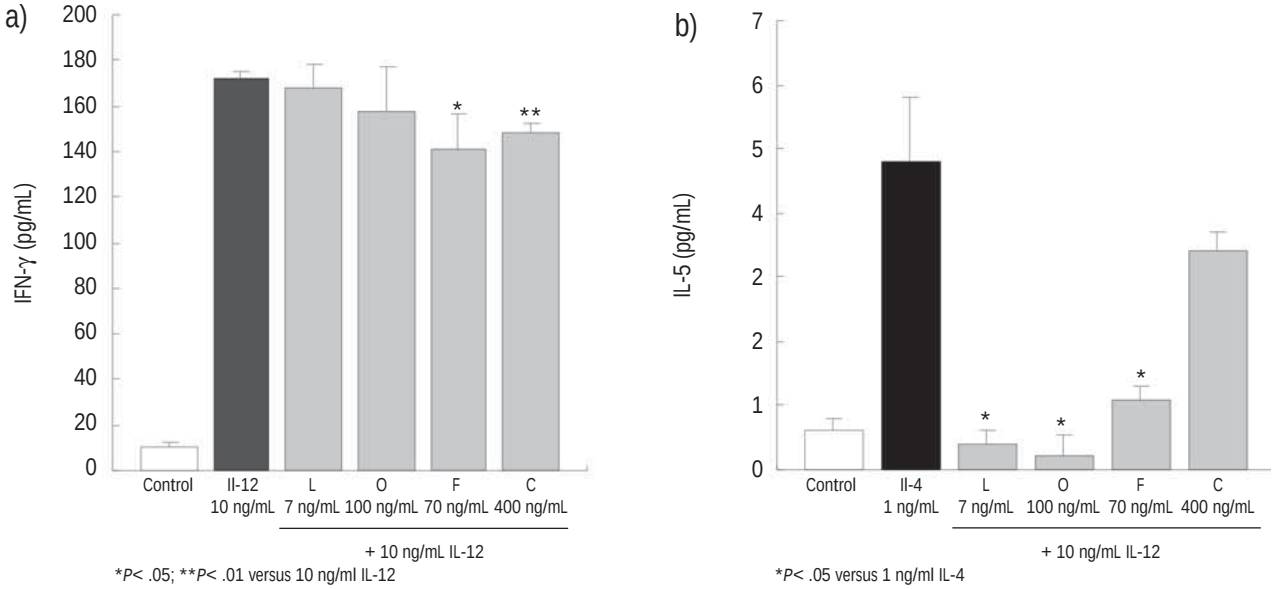


Figure 3. Effect of loratadine (L), olopatadine (O), fexofenadine (F), and cetirizine (C) on (a) interleukin (IL)-12–induced interferon (IFN)-γ production and (b) IL-4–induced IL-5 production in isolated T cells.

and T_H2 immunity are mediated by the immune cytokines IL-12 and IL-4, respectively [17,18]. Therefore we studied the effects of antihistamines on the stimulation of T-cell cytokine production by these immune cytokines. Fexofenadine had effects on T_H2 and T_H1 responses, whereas cetirizine affected the chronic but not the acute stage. Conversely, olopatadine and loratadine affected the acute but not the chronic stage. Overall, the findings from this study suggest that these antihistamines, which are commonly used in Japan, may have immunomodulatory properties, as demonstrated by the downregulation of mRNA and inhibition of IFN-γ and IL-5 in isolated T cells. Therefore in addition to demonstrating the modulatory impact of antihistamines on cytokine responses, our findings suggest that antihistamines have distinct immunologic effects that may allow the choice of treatment to be directed by atopic dermatitis signs and symptoms.

Additional studies are warranted to investigate the molecular basis of these immunomodulatory observations and to provide a thorough understanding of the impact of antihistamines in atopic dermatitis. The key question to address is whether antihistamines are able to modulate cytokine production by T cells activated in the presence of immune cytokines (IL-4, IL-12) and antigens causing atopic dermatitis, such as house dust and mite allergens. In addition, it has not yet been determined whether antihistamines affect antigen presentation processes through effects on antigen-presenting dendritic cells.

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