

Epinastine Hydrochloride Antagonism against Interleukin-4-Mediated T Cell Cytokine Imbalance in vitro

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Key Words

H₁ receptor antagonist · Epinastine hydrochloride · Th2-type cytokine · Human peripheral blood CD4⁺ T cells

Abstract

Background: Interleukin (IL)-4 is well accepted to be a cytokine that plays many roles in the regulation of immune responses. Although the primary pharmacological target of antihistamines has been regarded as the histamine H₁ receptor, there is little information about the influence of antihistamines on IL-4-mediated immune responses. The present study was undertaken to examine whether H₁ receptor antagonists could modulate IL-4-mediated immune responses in vitro. **Methods:** CD4⁺ T cells from normal human peripheral blood (1×10^6 cells/ml) were incubated with various concentrations of epinastine hydrochloride (EP) or chlorpheniramine (CH) for 30 min and then stimulated with 10.0 ng/ml IL-4. After 24 h, culture supernatants were collected and assayed for IL-5, IL-6, IL-13 and interferon- γ by ELISA. The influence of EP on transcription factor activation and mRNA expression for cytokines was also examined. **Results:** Addition of EP into cell cultures at more than 20.0 ng/ml significantly suppressed the production of IL-5, IL-6 and IL-13, which were increased by IL-4 stimulation. EP at more than 20.0 ng/ml also suppresses nuclear factor- κ B

activation, signal transducers and activators of transcription 6 phosphorylation and mRNA expression, which were upregulated by IL-4 stimulation. However, the ability of CD4⁺ T cells to produce interferon- γ was decreased by IL-4 stimulation, which was dramatically restored by treatment with EP at more than 15.0 ng/ml. On the other hand, CH, a first-generation H₁ receptor antagonist, could not inhibit cytokine production from CD4⁺ T cells in response to IL-4 stimulation, even when 90.0 ng/ml of the agent was added to cell cultures. **Conclusion:** The present results strongly suggest that EP, a second-generation H₁ receptor antagonist, interferes with IL-4-activated signaling in CD4⁺ T cells and results in favorable modification of the allergic disease state or conditions.

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Introduction

Antiallergic agents, especially antihistamines, have been used as a medication for allergic diseases for many years. Although the primary pharmacological target of antihistamines is generally recognized as the histamine H₁ receptor, these drugs act as inhibitors of the synthesis and release of chemical mediators from mast cells, eosinophils and other cells following immunological and nonimmunological stimulation. On the other hand, there is much evidence that antihistamines, such as azelastine,

ketotifen, fexofenadine and oxatomide, exert inhibitory action on cytokine production from T cells in vitro and in vivo [1, 2], suggesting that this suppressive effect of the agents on cytokine production may contribute, at least in part, to improve clinical conditions of allergic diseases.

Clinical and experimental observations have revealed the accumulation of eosinophils, neutrophils and mast cells at the site of allergic diseases [3]. It is also recognized that elevated immunoglobulin (Ig)E production is closely related to the severity of allergic diseases [4]. All these processes are orchestrated by T cells, especially Th2-type helper T cells, through the production of several cytokines with widely varying attributes [4]. Among Th2-type cytokines, the first important cytokines are interleukin (IL)-3 and IL-5, which are essential for proliferation and differentiation of mast cells and eosinophils from their committed precursors [5–7]. IL-4 is another important Th2 cytokine and is shown to enhance IgE synthesis from resting B cells [8] and a hemopoietic stem cell colony-forming response to IL-3 [9]. It is also observed that IL-4 could upregulate the expression of both major histocompatibility complex class II molecules and Fc receptor in B cells and monocytes [10]. These reports may suggest that IL-4 is a key player of immune modulation in allergic responses and its contributions to the pathogenesis of allergic diseases have been elucidated in IL-4 gene knockout mouse, which lacks the induction of the Th2-type immune responses [11]. Therefore, in the present study, we assessed the influence of epinastine hydrochloride (EP), a second-generation H₁ receptor antagonist, on IL-4-mediated immune responses by measuring the release of cytokines from CD4⁺ T cells in vitro.

Materials and Methods

Reagents

EP was kindly donated from Nihon Boehringer Ingelheim Co. Ltd. (Kawanishi, Japan) as a preservative free pure powder. Chlorpheniramine (CH) was purchased from Sigma Chemicals (St. Louis, Mo., USA). These two agents were dissolved in RPMI-1640 medium (Sigma Chemicals) supplemented with 10% heat-inactivated fetal calf serum (RPMI-FCS) at appropriate concentrations, sterilized by passing through 0.2- μ m filters and used for experiments, respectively. Recombinant human IL-4 was purchased from R & D System Inc. (Minneapolis, Minn., USA) and diluted with RPMI-FCS at a concentration of 50.0 ng/ml.

Preparation of CD4⁺ T Cells

Human heparinized peripheral blood was obtained from 5 male nonallergic volunteers (40.0 $\pm$ 13.0 years) under written informed consent, which was approved by the Ethics Committee of Showa University. The blood was diluted twice with phosphate-buffered

saline (PBS), layered onto lymphocyte separation medium (Organon Technica, Durham, N.J., USA) and centrifuged at 25 $\pm$ 2°C for 30 min at 1,000 g. Peripheral blood lymphocytes onto plasma and medium interface were then collected, washed several times with PBS and suspended in PBS. CD4⁺ T cells were separated from peripheral blood lymphocytes using a magnetic cell separator (Miltény Biotec, Bergisch Gladbach, Germany) as described previously [12]. The cells were suspended in RPMI-FCS at a concentration of 1 $\times$ 10⁶ cells/ml. The cell purity was more than 95% as judged by a flow cytometer (FACScan; Becton Dickinson, San Jose, Calif., USA).

Cell Culture

CD4⁺ T cells (1 $\times$ 10⁶ cells/ml) were stimulated with 10.0 ng/ml IL-4 [2] in the absence or presence of EP in 24-well culture plates for 24 h in a final volume of 2.0 ml. The culture supernatants were collected after pelleting cells by centrifugation at 1,000 g for 5 min at 4°C and stored at –40°C until used. To prepare cells for examining transcription factor activities and mRNA expression, CD4⁺ T cells were cultured in a similar manner for 1 and 4 h, respectively. In cases of examining the influence of EP on cell proliferation, 100 μ l cell suspension (1 $\times$ 10⁵ cells) was introduced into each well of 96-well flat-bottomed microculture plates in triplicate that contained various concentrations of EP and concanavaline A (Con A; 10.0 μ g/ml) in a final volume of 200 μ l. After 20 h, the cells were pulsed with 1.0 μ Ci of ³H-thymidine (³H-TdR) for the final 4 h of the culture [12]. ³H-TdR incorporation into DNA was measured by a liquid scintillation photometer. In all cases, treatment of cells with EP was started 30 min before stimulation.

Assay for Cytokines

IL-5, IL-6, IL-13 and interferon (IFN)- γ levels in culture supernatants were assayed by commercially available human cytokine ELISA test kits (R & D). The ELISA was done in duplicate according to the manufacturer's instructions. The minimum detectable concentration of these ELISA test kits were 3.0 pg/ml for IL-5, 0.7 pg/ml for IL-6, 32.0 pg/ml for IL-13 and 8.0 pg/ml for IFN- γ , respectively.

Assay for Transcription Factor Activities

Nuclear factor- κ B (NF- κ B) activity was analyzed by a commercially available ELISA test kit (Active Motif, Co. Ltd., Carlsbad, Calif., USA) that contains sufficient reagents and monoclonal antibodies against p50 and p65 according to the manufacturer's recommended procedures. In brief, nuclear extract (5.0 μ g protein) from cells was introduced into each well of 96-well microtiter plates precoated with oligonucleotide containing the NF- κ B consensus site (5'-GGGACTTCC-3') in a volume of 20.0 μ l and incubated for 1 h at 25°C. After washing three times, 100 μ l of monoclonal antibody against p50 or p65 was added to the appropriate wells and incubated for a further 1 h at 25°C. Then, anti-IgG horseradish peroxidase conjugate in a volume of 100 μ l was added and incubated 1 h at 25°C. Absorbance at 450 nm was measured after the addition of tetramethylbenzidine solution. Signal transducers and activators of transcription (STAT)-6 activity was also analyzed by a commercially available ELISA test kits (Active Motif) that contained sufficient reagents and antibodies against total and phosphorylated STAT-6. Briefly, cells (1 $\times$ 10⁶) fixed with 8% formaldehyde were treated with antibody blocking buffer for 1 h at 25°C. After washing two times, 40 μ l of primary antibody against total

and phosphorylated STAT-6 was added to wells in triplicate and incubated for 12 h at 4°C. Then, anti-IgG horseradish peroxidase conjugate in a volume of 100 µl was added and incubated for a further 1 h at 25°C. Absorbance at 450 nm was measured after the addition of tetramethylbenzidine solution.

Real-Time Polymerase Chain Reaction

mRNA was extracted from cells cultured for 4 h using µMACS mRNA isolation kits (Miltény Biotec) according to the manufacturer's instructions. The first-strand cDNA synthesis from 1.0 µg mRNA was performed using the SuperScript Preamplification System for cDNA synthesis (Gibco BRL, Gaithersburg, Md., USA). Polymerase chain reaction (PCR) was then carried out using a GeneAmp 5700 Sequence Detection System (Applied Biosystems, Foster City, Calif., USA). The PCR mixture consisted of 2.0 µl of sample cDNA solution (10.0 ng/µl), 25.0 µl of SYBR-Green Mastermix (Applied Biosystems), 0.3 µl of both sense and antisense primers, and distilled water to give a final volume of 50.0 µl. The reaction was conducted as follows: 4 min at 95°C, followed by 40 cycles of 15 s at 95°C and 60 s at 60°C. β-Actin was amplified as an internal control. mRNA levels were calculated by using the comparative parameter threshold cycle and normalized to β-actin. The nucleotide sequence of the primers was as follows [13]: for IL-5, 5'-GCTTCTGCATTTGAGTTTGCTAGCT-3' (sense) and 5'-TGGCCGTCATGTATTTCTTTATTAAG-3' (antisense), for IL-6, 5'-ATGAACCTCTCTCCACAAGCGC-3' (sense) and 5'-GAAGAGCCCTCAGGCTGGACTG-3' (antisense), for IL-13, 5'-CCACGGTCATTGCTCTCACTTGCC-3' (sense) and 5'-CCTTGTGCGGGCAGAATCCGCTCA-3' (antisense), and for β-actin, 5'-GTGGGGCGCCCCAGGCACCA-3' (sense) and 5'-GTCCTTAATGTCACGCACGATTTC-3' (antisense).

Statistical Analysis

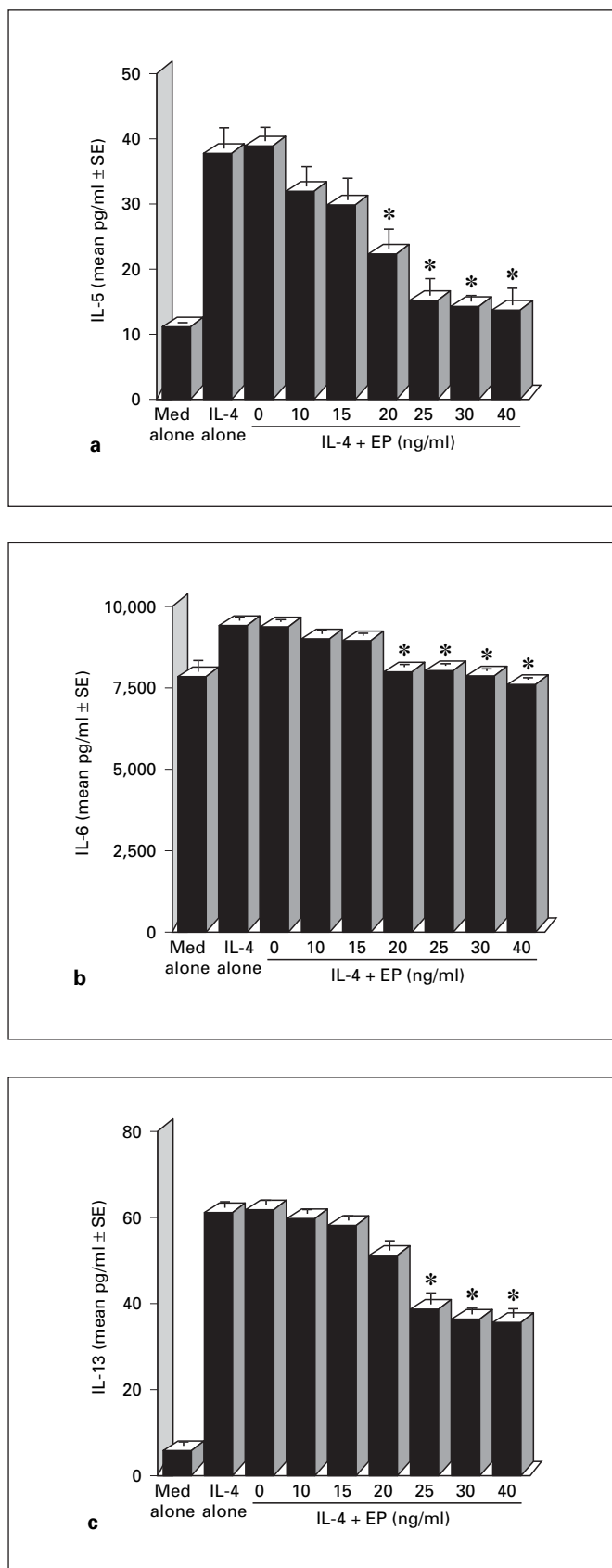
The statistical significance of the data between the control and experimental groups was analyzed by ANOVA followed by Fisher's PLSD test. A p value <0.05 was accepted as statistically significant.

Results

Influence of EP on T Cell Cytokine Production

The first set of experiments was designed to examine the influence of EP on Th2 cytokine production from CD4⁺ T cells in response to IL-4 stimulation. The cells were stimulated with IL-4 in the presence of various concentrations of EP, and Th2 cytokine, IL-5, IL-6 and IL-13 levels in culture supernatants were measured by ELISA. As shown in figure 1a, treatment of cells with EP at

Fig. 1. Influence of EP on Th2-type cytokine production from human peripheral blood CD4⁺ T cells after IL-4 stimulation in vitro. CD4⁺ T cells (1×10^6 cells/ml) were stimulated with 10.0 ng/ml IL-4 in the absence or presence of various concentrations of EP for 24 h. Cytokine levels in culture supernatants were assayed by ELISA. **a** IL-5. **b** IL-6. **c** IL-13. * p < 0.05 versus IL-4 alone.



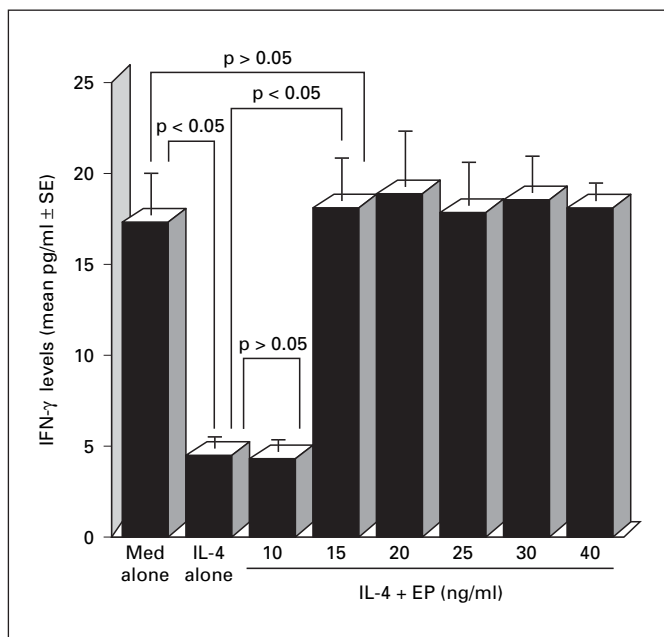


Fig. 2. Influence of EP on IFN- γ production from human peripheral blood CD4 $^{+}$ T cells after IL-4 stimulation. CD4 $^{+}$ T cells (1×10^6 cells/ml) were stimulated with 10.0 ng/ml IL-4 in the absence or presence of various concentrations of EP for 24 h. IFN- γ levels in culture supernatant were assayed by ELISA.

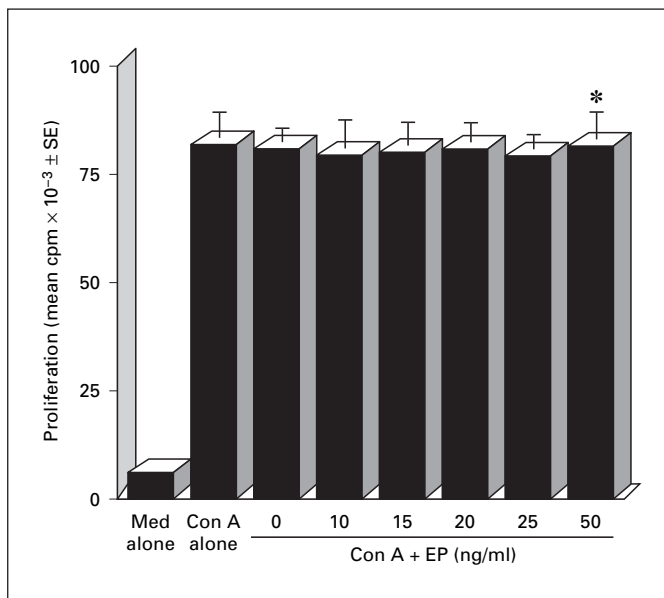


Fig. 3. Influence of EP on Con A-induced T cell proliferation. CD4 $^{+}$ T cells (1×10^5 cells/100 μ l) were stimulated with 10.0 μ g/ml Con A in the absence or presence of EP in 96-well culture plates for 24 h in a final volume of 200 μ l. Cells were pulsed with 1.0 μ Ci of ^{3} H-TdR for 4 h, and proliferation was measured by a liquid scintillation photometer. * $p > 0.05$ versus Con A alone.

<15.0 ng/ml could not suppress IL-5 production from T cells after IL-4 stimulation: IL-5 levels in experimental culture supernatants were nearly identical (not significant) to that in IL-4 stimulation alone. Addition of EP at 20.0 ng/ml could suppress the ability of cells to produce IL-5 in response to IL-4 stimulation. This suppressive activity of EP was further strengthened, when ≥ 25.0 ng/ml of the agent was added to cell cultures. We next examined whether EP could also decrease the ability of cells to produce IL-6 by IL-4 stimulation. The data in figure 1b clearly show the suppressive activity of EP on IL-6 production from cells stimulated with IL-4 as in the case of IL-5 production. The third experiment was performed to examine whether EP could suppress IL-13 production from CD4 $^{+}$ T cells by IL-4 stimulation. Although the data in figure 1c clearly show that EP could suppress the ability of cells to produce IL-13 in response to IL-4 stimulation as in the cases of IL-5 and IL-6, the minimum concentration of EP which causes significant suppression of IL-13 production was 25.0 ng/ml. The fourth experiment was carried out to examine the influence of EP on Th1 cytokine production from CD4 $^{+}$ T cells stimulated with IL-4. As shown in figure 2, IL-4 stimulation of CD4 $^{+}$ T cells caused a significant decrease in IFN- γ levels in culture supernatants. Addition of EP at 10.0 ng/ml into cell cultures could not antagonize the suppressive activity of IL-4 on IFN- γ production. However, treatment of cells at ≥ 15.0 ng/ml of EP could inhibit downregulation of IFN- γ production by IL-4 stimulation (fig. 2).

Influence of EP on CD4 $^{+}$ T Cell Proliferation

This study was carried out to examine whether the suppressive activity of EP on IL-4-induced cytokine production from CD4 $^{+}$ T cells was owing to cytotoxic activity of the agent. CD4 $^{+}$ T cells were stimulated with Con A in the presence of various concentrations of EP, and the cell cytotoxicity was assessed by examining the incorporation of ^{3} H-TdR into T cells. The data in figure 3 clearly show the negative suppressive effects of EP on ^{3} H-TdR uptake: the proliferative responses of cells treated with 50.0 ng/ml EP was nearly identical (not significant) to that observed in cells stimulated with Con A without EP.

Influence of CH on IL-4-Induced Cytokine Production from CD4 $^{+}$ T Cells

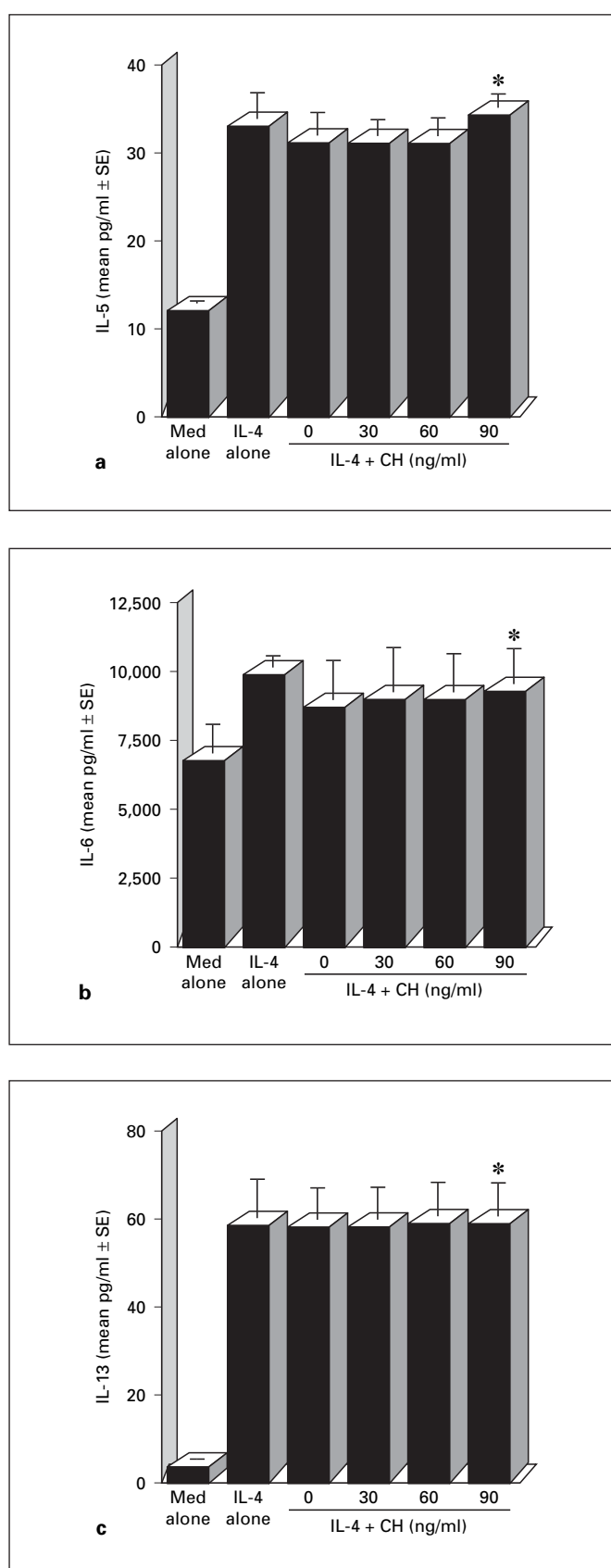
This study was designed to examine whether CH, a first-generation antihistamine, could also suppress IL-4-induced cytokine production. CD4 $^{+}$ T cells were stimulated with IL-4 in the presence of various concentrations of CH for 24 h. Cytokine, IL-5, IL-6 and IL-13 levels

in culture supernatants were examined by ELISA. As shown in figures 4a and b, CH could not suppress IL-5 and IL-6 production from CD4⁺ T cells in response to IL-4 stimulation: IL-5 and IL-6 levels in culture supernatants prepared from cells treated with 90.0 ng/ml CH were similar (not significant) to those from cells treated with IL-4 alone. The data in figure 4c also show that CH could not suppress the ability of cells to produce IL-13 in response to IL-4 stimulation, even when 90.0 ng/ml of the agent was added to cell cultures.

Influence of EP on Transcription Factor Activation and Cytokine mRNA Expression

The final set of experiments was carried out to examine the possible mechanisms by which EP could suppress the production of IL-5, IL-6 and IL-13 from T cells stimulated with IL-4. CD4⁺ T cells were cultured with IL-4 in the presence of various concentrations of EP. Transcription factor, NF- κ B and STAT-6 activation in 1-hour-cultured cells were examined by ELISA. As shown in figure 5, EP at <15.0 ng/ml scarcely affected NF- κ B, p50 and p65 activation. However, addition of EP at ≥ 20.0 ng/ml caused significant suppression of the activation of NF- κ B, which was increased by IL-4 stimulation (fig. 5). The next experiments were undertaken to examine the influence of EP on STAT-6 phosphorylation. Although treatment of cells with IL-4 and EP did not cause changes in total STAT-6 contents (fig. 6a), EP could significantly suppress the phosphorylation of STAT-6 induced by IL-4 stimulation (fig. 6b). The minimum concentration of EP, which caused significant suppression of STAT-6 phosphorylation was 20.0 ng/ml (fig. 6b). The third experiments in this section were performed to examine the influence of EP on mRNA expression for IL-5 and IL-6 in 4-hour-cultured cells by real-time reverse transcription PCR. Addition of EP at 10.0 ng/ml scarcely affected mRNA expression for IL-5, IL-6 and IL-13, but ≥ 15.0 ng/ml of EP significantly suppressed mRNA expression, which was increased by IL-4 stimulation (fig. 7).

Fig. 4. Influence of CH on Th2-type cytokine production from human peripheral blood CD4⁺ T cells after IL-4 stimulation in vitro. CD4⁺ T cells (1×10^6 cells/ml) were stimulated with 10.0 ng/ml IL-4 in the absence or presence of various concentrations of CH for 24 h. Cytokine levels in culture supernatants were assayed by ELISA. **a** IL-5. **b** IL-6. **c** IL-13. * $p > 0.05$ versus IL-4 alone.



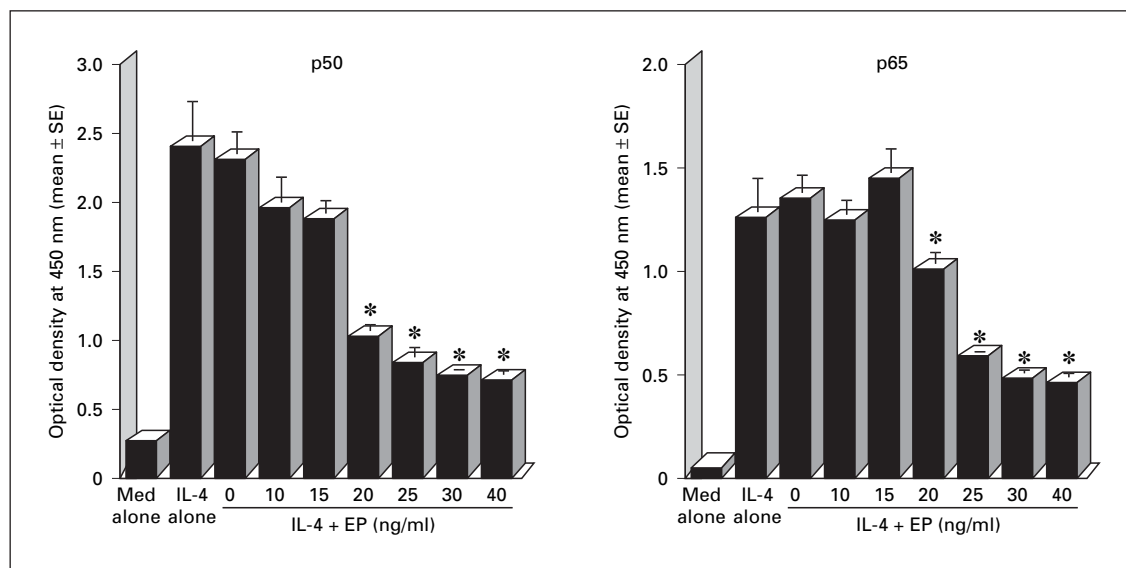


Fig. 5. Influence of EP on NF- κ B activation in human peripheral blood CD4⁺ T cells after IL-4 stimulation in vitro. CD4⁺ T cells (1×10^6 cells/ml) were stimulated with 10.0 ng/ml IL-4 in the absence or presence of various concentrations of EP for 1 h. NF- κ B, p50 and p65 activation was measured by ELISA. * $p < 0.05$ versus IL-4 alone.

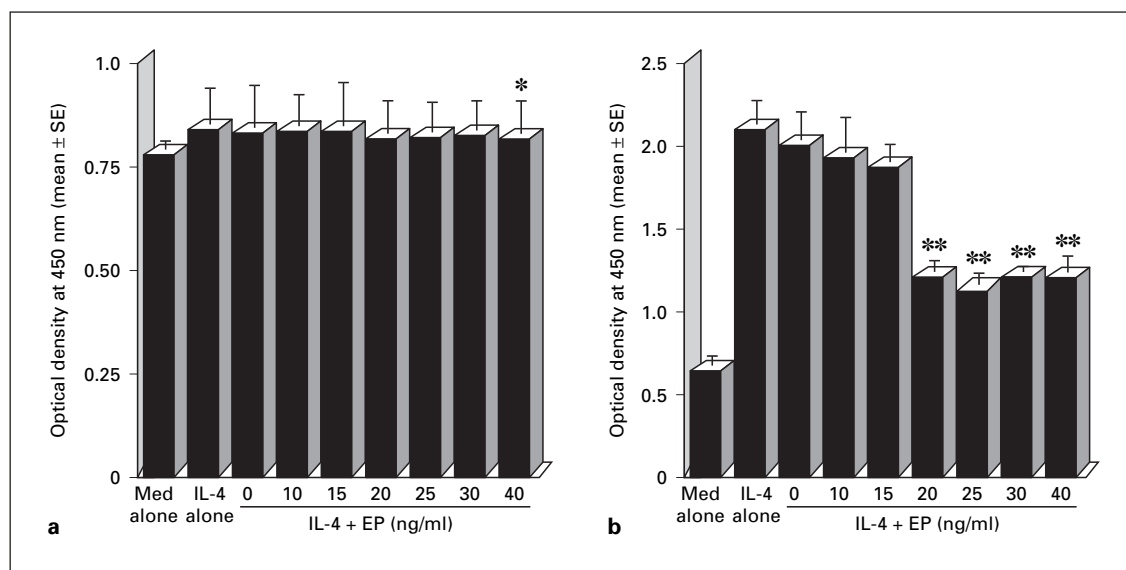
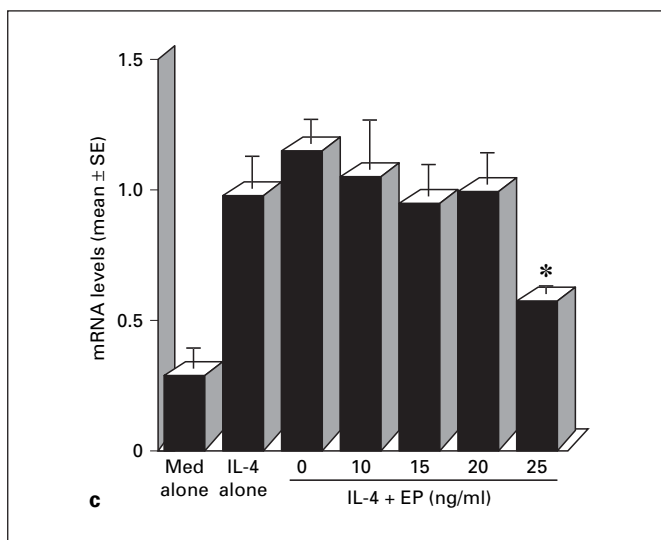
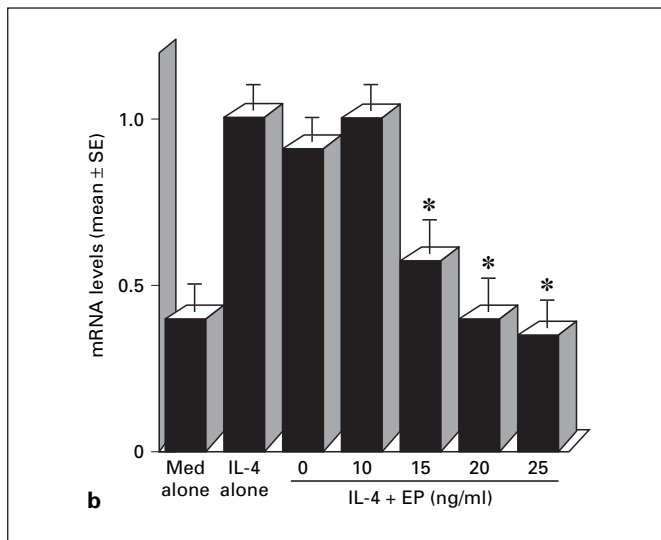
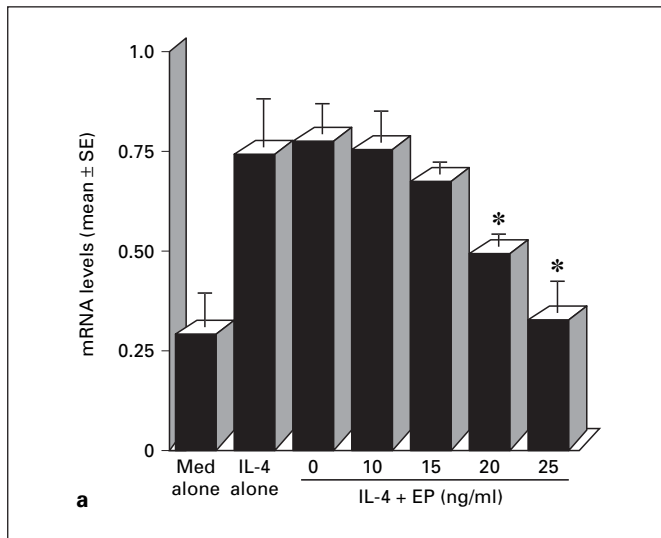


Fig. 6. Influence of EP on phosphorylation of STAT-6 in human peripheral blood CD4⁺ T cells after IL-4 stimulation in vitro. CD4⁺ T cells (1×10^6 cells/ml) were stimulated with 10.0 ng/ml IL-4 in the absence or presence of various concentrations of EP for 1 h. Total and phosphorylated STAT-6 were measured by ELISA. **a** Total STAT-6. **b** Phosphorylated STAT-6. * $p > 0.05$ versus IL-4 alone; ** $p < 0.05$ versus IL-4 alone.



Discussion

EP is a well-tolerated antiallergic drug, whose therapeutic spectra widely span various allergic disorders, including allergic rhinitis, bronchial asthma, urticaria and atopic dermatitis, among others [14–16]. Although its effect was mainly described from the perspective of a histamine receptor antagonist that affected mast cells, the precise mechanisms of the action of EP on allergic diseases are not fully understood [17].

In allergic immune reaction, various inflammatory cells contribute to the initial triggering and subsequent maintenance of the reaction through cell to cell interactions or the production of soluble biological factors [3, 10, 18]. CD4+ T cells play a key role in triggering the inflammatory response. Namely, cytokine production by Th2-type helper T cells is indispensable to initiating and maintaining the allergic inflammatory responses [10, 18]. The hypothesis has been generated that eosinophils are the most important final effector cells in allergic inflammation, capable of causing most of the morphological and functional alterations observed in allergic diseases such as asthma and atopic dermatitis [3, 10, 18]. Several cytokines can affect eosinophils, but despite possible redundancy with IL-3 and granulocyte macrophage colony-stimulating factor, IL-5 seems to be the primarily essential cytokine involved in vivo in the production, differentiation, maturation and activation of eosinophils [19]. This has been confirmed by several lines of investigations: IL-5 transgenic mice show the prolongation of eosinophil survival, whereas granulocyte macrophage colony-stimulating factor transgenes show increased numbers of mononuclear cells and neutrophils, but not eosinophils [20]. Similar findings emerge from knockout mice [21]. Furthermore, in IL-5 knockout mice, local administration of CC chemokine such as eotaxin cannot induce tissue eosinophilia, even if donor eosinophils have been administered to restore or increase the circulating pool, a procedure which in wildtype animals clearly enhances the tissue eosinophilia by eotaxin [22]. This report strongly suggests the importance of IL-5 even in the hom-

Fig. 7. Influence of EP on mRNA expression for Th2-type cytokine in human peripheral blood CD4+ T cells after IL-4 stimulation in vitro. CD4+ T cells (1×10^6 cells/ml) were stimulated with 10.0 ng/ml IL-4 in the absence or presence of various concentrations of EP for 4 h. mRNA levels were analyzed by real-time reverse transcription PCR. **a** IL-5. **b** IL-6. **c** IL-13. * $p < 0.05$ versus IL-4 alone.

ing reaction to chemotactic factors of eosinophils. IL-13 is a pleiotropic cytokine produced in large quantities by activated Th2-type T cells [23]. It has a variety of effects that are relevant to allergic diseases and other Th2-dominated inflammatory disorders, including the ability to induce IgE production [24] and vascular cell adhesion molecule 1 expression [25]. It is also reported that IL-13 can activate and inhibit the apoptosis of eosinophils [23] and promote epithelial cell damage and goblet cell hyperplasia with mucus hypersecretion [26]. Together with these reports, the present results show the suppressive effect of EP at therapeutic blood levels [17] on the production of Th2-type cytokine, IL-5 and IL-13 from CD4⁺ T cells after IL-4 stimulation and provide possible mechanism that could explain the favorable effects of EP on allergic diseases.

CD4⁺ T cells can be divided into two major subsets of helper cells by the different pattern of cytokine secretion: Th1 cells, which secrete IL-2 and IFN- γ , and Th2 cells, which secrete IL-4, IL-5 and IL-13, among others. Although the Th1 responses have been strongly implicated in cell-mediated immune responses related to killing the intracellular pathogens, Th1 cells may be protective against Th2-related immune diseases such as asthma and allergy through the suppression of Th2 cytokine production by IFN- γ [27]. Accumulating evidence also demonstrated that IFN- γ can inhibit activation, differentiation and recruitment of eosinophils by the suppression of eosinophil receptor expression and induction of eosinophil apoptosis [28]. Another critical role of IFN- γ in allergic diseases is its ability to inhibit immunoglobulin class switching to IgE and to enhance IgG production, which is involved in neutralization and capture of allergens [29, 30]. Judging from these reports, restoration of the ability to produce IFN- γ of CD4⁺ T cells by EP may partially account for the therapeutic mechanisms of the agent on allergic diseases.

IL-6 is well known to be a multifunctional cytokine released in inflammatory diseases. This cytokine has been implicated in mast cell development and maturation and in the regulation of IgE synthesis [31]. IL-6 levels in bronchoalveolar lavage fluid and in blood were increased significantly after allergic challenge in asthmatic patients [32]. Therefore, inhibition of IL-6 production from CD4⁺ T cells by EP may also have important therapeutic implications for allergic diseases.

Although the present results clearly show the attenuating effect of EP on IL-4-mediated allergic immune responses, the precise mechanisms by which EP could modulate the immune responses by IL-4 stimulation are not

fully understood. IL-4 exerts its biological effect through a specific interaction with the cell surface IL-4 receptor α -chain that is associated with transcription factor STAT-6 [33], which is essential for Th2-type helper T cell activation including cytokine secretion [34]. These reports may suggest that immunomodulatory action of EP is, in part, owing to its suppressive activity on STAT-6 signal pathway. This speculation may be supported by the present observations that the treatment of CD4⁺ T cells with EP at >20 ng/ml could suppress the phosphorylation of STAT-6 induced by IL-4 stimulation. In addition to the STAT signal pathway, the binding of IL-4 to the γ c-chain (in common with the receptors for IL-2 and IL-7, among others) can induce the dissociation of NF- κ B from the inhibitor protein I κ B, which leads to the translocation of NF- κ B to the nucleus and starts the induction of several types of proteins, including cytokine mRNA expression [35]. Together with the present results, there is a possibility that EP also inhibits the NF- κ B signal pathway and results in suppression of Th2-type cytokine production from CD4⁺ T cells in response to IL-4 stimulation. This speculation may be supported by our previous report showing that the suppressive activity of EP on thymus- and activation-regulated chemokine production from human peripheral blood CD4⁺ T cells through the inhibition of NF- κ B activation was induced by antigenic stimulation [36].

Antihistamines are well recognized to comprise a broad class of pharmacologic agents and to include the first-generation, central-acting H₁ receptor antagonists (e.g., CH and hydroxyzine hydrochloride) and the second-generation, nonsedating H₁ blocker (e.g., EP and fexofenadine). Other histaminic agents, such as cimetidine, primarily target at H₂ receptors, and still other experimental antihistamines act on H₃ receptors. Although the first-generation antihistamines are used for the treatment of sleep disorders, these agents are also frequently used for allergic diseases with successful effects, prompting us to explore whether the first-generation antihistamines also exert immunomodulatory action on CD4⁺ T cells as in the case of the second-generation antihistamines, such as EP. We then examine the influence of CH on IL-4-mediated immune responses. The present data clearly show that CH could not modulate immune responses induced by IL-4 stimulation, even when 90.0 ng/ml, i.e. approximately 5 times the therapeutic blood level, was added to cell cultures [37]. On the other hand, the present results clearly show the suppressive activity of EP on immune responses induced by IL-4 stimulation at 20.0 ng/ml, which is similar to therapeutic blood levels

(26.9 ± 9.1 ng/ml) when 20 mg of the agent was orally administered to the patients [38]. The reasons for this discrepancy are not clear at present. Stimulation of immune cells with IL-4 activates the tyrosine phosphorylation of STAT-6 through Jak1/Jak3- and Ca²⁺-dependent mechanisms, promoting its homodimerization and nuclear translocation [33, 34]. Furthermore, the IL-4 and γ c-chain complex activates protein kinase C- ζ /NF- κ B, involved in dissociation and translocation of NF- κ B in Ca²⁺-independent mechanisms [10, 23, 35]. These reports may suggest that CH could not suppress the Ca²⁺-independent signal pathway and resulted in negative suppression of Th2-type cytokine production from CD4⁺ T cells induced by IL-4 stimulation, since CH at >12 ng/ml could suppress the increase in Ca²⁺ in various animal cells in vitro [39].

In vitro treatment of CD4⁺ T cells with EP at >20.0 ng/ml caused significant, but not complete, suppression of the production of IL-5, IL-6 and IL-13 in response to IL-4 stimulation, whereas the same treatment of cells almost completely inhibited the expression of mRNA for

these cytokines. The reasons for this discrepancy are not clear at present. It is possible that the important events such as specific mRNA expression may occur during 0–4 h in CD4⁺ T cells by IL-4 stimulation in the presence of EP. Then, the lower levels of proteins will be secreted and stored in culture medium without degradation for 24 h after stimulation. This may be responsible for the difference between protein levels measured and mRNA expression. Further experiments are needed to clarify this points.

In conclusion, the present results demonstrate that the modulation of EP on IL-4-mediated immune responses, especially Th2 cytokine production, consists, in part, of a therapeutic mode of action of the agent on allergic diseases, including atopic allergy and pollinosis.

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