Modulatory Effects of Epinastine Hydrochloride on IL-4 mRNA Expression by Peripheral Blood Mononuclear Cells

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Key Words
Antiallergic drug
Histamine receptor antagonist
IL-4

Introduction
T lymphocytes, with their capacity to produce Th2 cytokines from CD4+ cells, are thought to play an important role in orchestrating the inflammatory process in various allergic diseases. Among these cytokines, IL-4 and IL-5 have been strongly implicated in the pathophysiology of such disorders, since the former promotes IgE synthesis and the latter regulates eosinophil and basophil differentiation and maturation [1].

Epinastine hydrochloride (epinastine) is a newly developed histamine receptor antagonist possessing antiallergic activity, as is evident from its inhibitory action on histamine and leukotriene C4 release from human mast cells and basophils, and has been employed in the treatment of allergic rhinitis, atopic dermatitis and bronchial asthma in Japan with good clinical results [2]. Based on the finding that this compound can suppress Ca2+ uptake into the cells [3], the present investigation was carried out to evaluate whether epinastine can modulate T helper cell functions by affecting IL-4 and interferon (IFN)-γ mRNA expression on peripheral blood mononuclear cells (PBMC) of allergic patients.

Materials and Methods
Subjects
Fifteen nonsmoking asthmatic subjects were selected from the outpatient clinic of the St. Marianna University Hospital who were judged to be sensitive to house dust mite (H DM) on the basis of clinical history and positive IgE CAP RAST (score ≥ 2). Informed consent was obtained from these subjects, and the study was approved by the hospital ethical committee.

Blood Sampling and Cell Culture
PBMC were separated from heparinized venous blood samples by Mono-poly resolving media (ICN Flow, Costa Mesa, Calif, USA) density gradient centrifugation, resuspended in culture medium consisting of RPMI-1640 (Gibco, Grand Island, N.Y, USA), supplemented with 10% heat-inactivated fetal calf serum (Gibco), l-glutamine, streptomycin and penicillin after appropriate washing and adjusted to 1 × 10⁶/tube. The cells were preincubated with or without 1 µM epinastine for 15 min, followed by incubation at 37 °C for 19 h in a humidified atmosphere with CO2 in the presence of phytohemagglutinin (PHA, 1:100) or HDM (1 µg/ml; Torii, Tokyo, Japan).

After the culture period, total RNA was isolated using Isogen (Nippon Gene, Tokyo, Japan). Reverse-Transcriptase-Polymerase Chain Reaction IL-4, IFN-γ and β-actin mRNA of each sample were first reverse-transcribed into complementary DNA using DTT, dNTP, DEPC-dH20 and oligo dT – primer, which was then subjected to conventional PCR amplification with the specific primer designed by oligo-Probe Design Station (Advanced Gene Computing Technology, Irvine, Calif, USA). PCR products were then resolved by agarose gel electrophoresis, stained with ethidium bromide, visualized and photographed under UV light and finally analyzed by the NIH image analyzer. The results were expressed as the ratio of the IL-4 or IFN-γ PCR products divided by the β-actin PCR product.

Results and Discussion

Of the PBMC of 15 asthmatic subjects studied, PBMC from 4 patients did not respond to PHA or to HDM to express detectable IL-4 mRNA, although mRNA for β-actin was clearly identified on the agarose gel. Accordingly, results for IL-4 gene expression from 11 subjects were analyzed and summarized in figure 1 as the ratio to β-actin gene expression. It is evident that IL-4 gene expression was intensified upon PHA stimulation (1.26 ± 0.77 vs. 0.63 ± 0.36; p < 0.05 by Student’s t tests) and that this increased expression tended to be inhibited by the simultaneous addition of 1 µM epinastine (1.26 ± 0.77 vs. 0.74 ± 0.39; 0.05 < p < 0.1). HDM stimulation, however, did not intensify IL-4 gene expression in these subjects. Similar results were also observed with IFN-γ gene expression by PBMC from asthmatic subjects (data not shown). In addition, preliminary experiments performed up to now indicate that PHA could also intensify IL-5 mRNA expression by PBMC and that epinastine also inhibited this increase in these asthmatic patients.

Our above data indicated that epinastine might modulate T helper cell functions by affecting IL-4 (and IL-5) gene expression by CD4+ cells. This is in accordance with the previous report [4] which showed that azelastine and terfe-nadine, other histamine receptor antagonists possessing
antiallergic activities and also employed for clinical use as antiallergic drugs, inhibited IL-4 and IL-5 production from PBMC of healthy donors stimulated with concanavalin A. Although the precise mechanism(s) of this suppression by these agents is not clarified at the moment, it is speculated that the inhibition of Ca2⁺ uptake, as the essential step of cell activation, may be involved [3].

HDM +
PHA +
HDM
PHA
Stimulus Epinastine
Fig. 1. Effect of epinastine on PHA-induced IL-4 mRNA expression by PBMC from asthmatic subjects (n = 11).

In conclusion, this modulation of T helper cell functions of epinastine, besides the capacity to inhibit chemical mediator release from mast cells and basophils, may contribute to its clinical efficacy in the treatment of allergic disorders.

Acknowledgments
The authors wish to thank Ms. Kaori Okano for her secretarial help and Ms. Misa Yoshida for her technical assistance.

References