by macrophages, B cells, and polymorphonuclear cells.[9,10] IL-12 has an important role in immunological defense against infections and tumors.[8] Recent studies also suggest the possible role of IL-12 in the pathogenesis of atopic dermatitis (AD).[11-13] IL-13 is mainly produced by activated Th2 cells, the T cell subset which provides help for humoral immune responses.[14] IL-13 suppresses production of proinflammatory cytokines and facilitates a Th2 response resulting in Ab synthesis.[15,16] It has been demonstrated that IL-13 expression is increased in acute skin lesions of AD.[11] It is suggested that cytokines behave as upstream regulatory factors in the development of Th1/Th2 differentiation.

In this study, we designed experiments to examine whether differential expression of cytokines could be determined in serum samples of patients with AD, and to investigate whether IL-12 and IL-13 showed differential responses to a non-selective PDE inhibitor. Levels of serum IL-12, IL-13, and other cytokines (IFN-γ, IL-4, and IL-6), their mRNA expressions in PBMC of patients with AD, and their modulations by a PDE inhibitor were analyzed.

## MATERIALS AND METHODS

### Patients

Non-atopic control subjects consisted of 5 adults, aged 21-25 yr. Normal blood donors had no history of atopic diseases and were taking no medication at the time of the study. Totally of 7 adults with atopic dermatitis, aged 18-45 yr (mean 26 yr) were included in the present study. Patients with AD were chosen according to well-defined criteria described previously.[17] All patients were adults with chronic, moderate to severe dermatitis. Neither normal nor AD donors had ingested caffeine or other methylxanthine-containing beverages for at least 12 h prior to blood drawing.

### PBMC Isolation and Cell Culture

For each experiment, 20 mL of peripheral venous blood was drawn from a single volunteer and collected in tubes coated with the anti-coagulant, sodium heparin. PBMCs were separated by Ficoll-Paque (Pharamia Biotech, Inc.) density-gradient centrifugation as previously described.[18] Briefly, recovered PBMCs were washed 3 times with RPMI-1640 medium (Life Technology). For cytokine synthesis and cellular proliferation assay, PBMCs were suspended in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (Life Technology), 100 U/mL penicillin, and 100 μg/mL streptomycin (Life Technology), at 37 °C with 5% $CO_2$.

### Proliferation Assay

For the proliferation assay, cells were suspended at a concentration of $1 \times 10^5$ cells/mL and placed in 96-well round-bottom culture plates in the presence or absence of PHA (5 μg/mL) or IBMX with concentrations ranging from 1 to 10 μM. After 72 h, 1 μCi/mL [methyl-$^3$H]thymidine (Amersham Life Science) was added, and cells were incubated for an additional 18 h. Cells were harvested onto filter paper, and radioactivities were counted by liquid scintillation counter (Beckman Instrument). All samples were assayed in triplicate.

### Cytokine Synthesis Assays

For measurement of IL-4, IL-5, IFN-γ, IL-12, and IL-13 production, cells were cultured at a density of $1 \times 10^6$ cells/mL in a sterile tube in the presence or absence of PHA (5 μg/mL), and IBMX (ranging from 1 to 10 μM). After a 48-h incubation, cells were harvested and subjected to RNA isolation and cytokine gene expression analysis by reverse transcription-polymerase chain reaction (RT-PCR); the supernatants were assayed for levels of the cytokine proteins by enzyme linked-immuno-sorbent assay (ELISA; see below).

### RT-PCR

mRNA was isolated from PBMC with an mRNA Isolation Kit (Boehringer Mannheim) according to the manufacturer's instructions. The amount of mRNA was quantitated by spectrophotometry after elution. Single-strand cDNA was synthesized using equal amounts of mRNA by using a cDNA Synthesis Kit (Boehringer Mannheim). Then, PCR was performed with equal amounts of cDNA to amplify messages specific for IL-12 p35, p40, and IL-13. Sense and antisense primers for IL-12 p35 (sense: AACTAATGGG-AGTTGCCTG; antisense: GGGACCTGCGCTTTTT-AGGAAG), IL-12 p40 (Set 1: sense: TCACAAAGGA-GGCGAGGTTC; antisense: ATCAGAACCTAACTG-GAGG. Set 2: sense: CCTGCTGGTGGCTGACGA-CAAT; antisense: CTTCAGCTGC-AAGTTGTTGGGT. Set 3: sense: CAGCAGTTGGTCATCTCTTG; antisense: CCAGCAGGTGAAACGTCCA), IL-13 (sense: TATGCATCCGCTCCTCAA-TCCTC; antisense: CG-