AAGTTTCAGTTGAACCGTCC), and control β-actin (sense: TGACGGGGTCACCCACACTGTGCCCATCTA; antisense: CTAGA-AGCATTGCGGTGGACGATGG-AGGG) were based on published sequences of IL-12[3,9,18] and IL-13.[14] PCR products were visualized by electrophoresis on 3% agarose followed by ethidium bromide staining. Sizes of PCR products were: IL-12 p35, 358 bp; IL-12 p40: 714 bp (set 1), 311 bp (set 2), 420 bp (set 3); IL-13, 449 bp; and control β-actin, 660 bp.

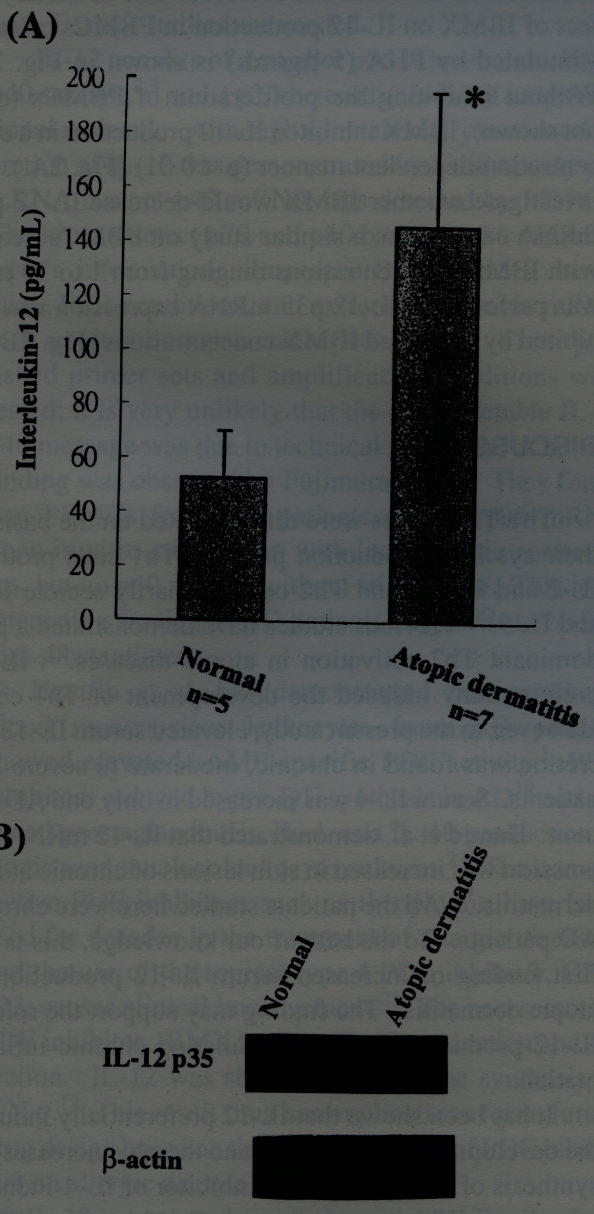

Fig. 1. Interleukin-12 (IL-12) secretion and IL-12 p35 mRNA expression in atopic dermatitis and normal controls. (A) Levels of IL-12 in serum of normal controls (n=5) and patients with atopic dermatitis (n=7). Data were expressed as the mean ± SEM. (normal vs. AD: *$p < 0.05$) (B) Detection of IL-12 p35, and β-actin mRNA expression using RT-PCR. Lane 1, normal control; Lane 2, atopic dermatitis.

ELISA

Quantitative determination of human IL-4, IL-6, IFN-γ, IL-12, and IL-13 was performed using ELISA kits (BioSource International Cytoscreen Human IL-4, IL-6, IFN-γ, IL-12 and IL-13 ELISA kits; the IL-12 kit recognizes both natural and recombinant human IL-12 p40). The sensitivities were 2 pg/mL for IL-4, 2 pg/mL for IL-6, 4 pg/mL for IFN-γ, 0.8 pg/mL for IL-12, and 12 pg/mL for IL-13. In order to measure levels exceeding 500 pg/mL (IL-12), and 2500 pg/mL (IL-13), samples were diluted with standard diluent buffer according to the manufacturer's instructions. PDE inhibitor did not interfere with the IL-12 and IL-13 standard curves.

Statistical Analysis

Data were expressed as the mean ± SEM and analyzed by a two-tailed Student's t-test. A p value less than 0.05 was considered to be significant. Percentage of inhibition for each condition was calculated on the basis of inhibition relative to the stimulated, inhibitor-free mean value subtracted from background counts with media alone in each case. Graphs were generated on a Macintosh LC II by using Cricket Graph (Cricket Software, Malvern, PA).

RESULTS

To evaluate the cytokine patterns in patients with AD, we assayed IL-4, IL-6, IL-12, IL-13, and IFN-γ concentrations in serum by ELISA. No significant differences were seen in serum levels of IL-4, IL-6, IL-13, and IFN-γ between patients with AD and normal controls. The level of IL-12 in serum of patients with AD (n = 7) was increased significantly as compared with normal controls (n = 5) ($p < 0.05$) (Fig. 1A). For the IL-4, IL-6, IL-12, IL-13, and IFN-γ mRNA expression assays, the RT-PCR method was used to detect mRNA in PBMCs from AD patients and normal controls. IL-4 mRNA expression was detected in one patient's serum by ELISA. IL-6, IL-13, and IFN-γ mRNA expressions were non-detectable in PBMCs of all patients and normal controls. IL-12 is a heterodimer consisting of p35