

Overexpression of IL-10 mRNA in gut mucosa of patients with allergic asthma

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Because an airway-like inflammation has been reported in the gut of asthmatic patients, we sought to examine the expression of immunoregulatory cytokines like IL-4, IL-10, and IL-13 by gut mucosa. To establish this, we initiated this study to examine mRNA expressions of IL-4, IL-10, and IL-13 in duodenal mucosa from patients with asthma. Duodenal biopsy specimens were obtained from 20 asthmatic patients (10 allergic, 10 nonallergic) and 8 healthy controls. Cytokine mRNA was quantified with reverse transcriptase-competitive PCR, and results were expressed in proportion to the number of β -actin mRNA in the same sample. IL-10 and IL-4 mRNA were detectable in all patients, whereas no IL-13 mRNA was detected. IL-10 mRNA concentrations were significantly higher in allergic subjects with asthma than in control subjects and nonallergic subjects with asthma. No significant difference was observed for IL-4. IL-10 mRNA expression was not related to asthma severity, FEV₁, blood eosinophilia, or IgE levels. Our results support the hypothesis that IL-10 overexpression may counterbalance the effects of proinflammatory cytokines and mitigate the inflammatory reaction found in gut mucosa of subjects with asthma. (*J Allergy Clin Immunol* 2001;107:739-41.)

Key words: Asthma, IL-10, IL-4, gut

Asthma is characterized by a chronic mucosal inflammation of the airways underlying the 2 main features of the disease, bronchial constriction and bronchial hyperresponsiveness. Interestingly, immune inflammatory abnormalities in asthma are not limited to the lung but can be found in the gastrointestinal tract as well (ie, infiltration of the gut mucosa by T cells, eosinophils, and mast cells expressing high levels of IL-3 and IL-5) in the absence of overt abdominal symptoms.¹ Therefore we hypothesized that immunoregulatory cytokines like IL-10 and IL-4, which have as their major action to down-regulate inflammatory events, may be overexpressed at the intestinal level in asthma.

Abbreviation used

Reverse transcriptase PCR: RT-PCR

METHODS

IL-10, IL-4, and IL-13 mRNA concentrations were measured in duodenal biopsy specimens from 20 subjects with asthma (10 with atopic asthma and 10 with nonatopic asthma) and 8 healthy control subjects. All subjects gave their informed consent for the study, and the protocol was approved by the hospital's ethical committee (CP 94/34). Atopy was defined as the presence of more than 1 positive skin prick test response to common inhalant allergens (house dust mites, animal dander, grass and tree pollens, and molds). Skin prick tests to food allergens yielded negative results in all cases. The serum total IgE and eosinophil counts were measured. Spirometric measurements were performed. Asthma severity was determined according to the Global Initiative for Asthma classification.² Severe subjects with asthma who were treated with oral steroids were not included in the study because it is known that IL-10 production is inhibited by corticosteroids.

No endoscopic lesion was found. Duodenal biopsy specimens were taken during gastroduodenal endoscopy; 2 to 4 biopsy specimens of similar size and a mean weight of 4 ± 1 mg were immediately placed in RNazol (Bioprobe, Montreuil, France) and homogenized by mechanical dispersion. Total RNA was extracted as previously described.³ After treatment at 37°C for 30 minutes with 20 to 50 units of DNase I RNase-free (Boehringer, Mannheim, Germany), the quantity and purity of RNA were estimated by determining absorbance at 260/280 nm. Total RNA (5–10 μ g) was reverse transcribed into cDNA with Murine leukemia virus reverse transcriptase (Gibco-BRL, Cergy Pontoise, France), 5 μ mol/L oligodT16 and 2.5 mmol/L of each of the 4 dNTPs (Pharmacia, Orsay, France) in a final reaction volume of 20 μ L in the presence of 1 U/ μ L of human placenta ribonuclease inhibitor (Promega, Lyon, France). Samples were incubated at 42°C for 60 minutes followed by heating for 5 minutes at 95°C to inactivate the enzyme and stored at –20°C until use. Quantification is based on competitions between unknown amounts of mucosal cDNA and serial dilution of a known amount of competitor, an internal multispecific control.^{4,5} The reaction mixture was amplified by PCR with sense and antisense primers specific for the following: β -actin, 5'-GGG TCA GAA GGA TTC CTA TG-3' and 5'-GGT CTC AAA CAT GAT CTG GG-3'; IL-4, 5'-TGC CTC CAA GAA CAC AAC TG-3' and 5'-AAC GTA CTC TGG TTG GCT TC-3'; IL-10, 5'-AAA TTT GGT TCT AGG CCG GG-3' and 5'-GAG TAC AGG GGC ATG ATA TC-3'; and IL-13, 5'-GAG TGT GTT TGT CAC CGT TG-3' and 5'-TAC TCG TTG GCT GAG AGC TG-3'. All samples were systematically subjected to 40 PCR cycles, consisting of denaturation for 1 minute at 94°C, primer annealing for 1 minute at 53°C (IL-8) or 60°C (β -actin), and primer extension for 1.5 minutes at 72°C with a GeneAmp PCR system 9700 (Perkin Elmer, Coutaboeuf, France). After PCR amplification, quantification of β -actin and cytokine mRNA was performed by electrophoresis in 2% to 3% agarose gel as previously described.^{3,5} Because accurate quantifica-

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Supported by the Programme Hospitalier de Recherche Clinique (PHRC, 1997, No 1916) du Centre Hospitalier et Universitaire de Lille.

Received for publication October 19, 2000; revised January 8, 2001; accepted for publication January 9, 2001.

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0091-6749/2001 \$35.00 + 0 1/54/114111

doi:10.1067/mai.2001.114111

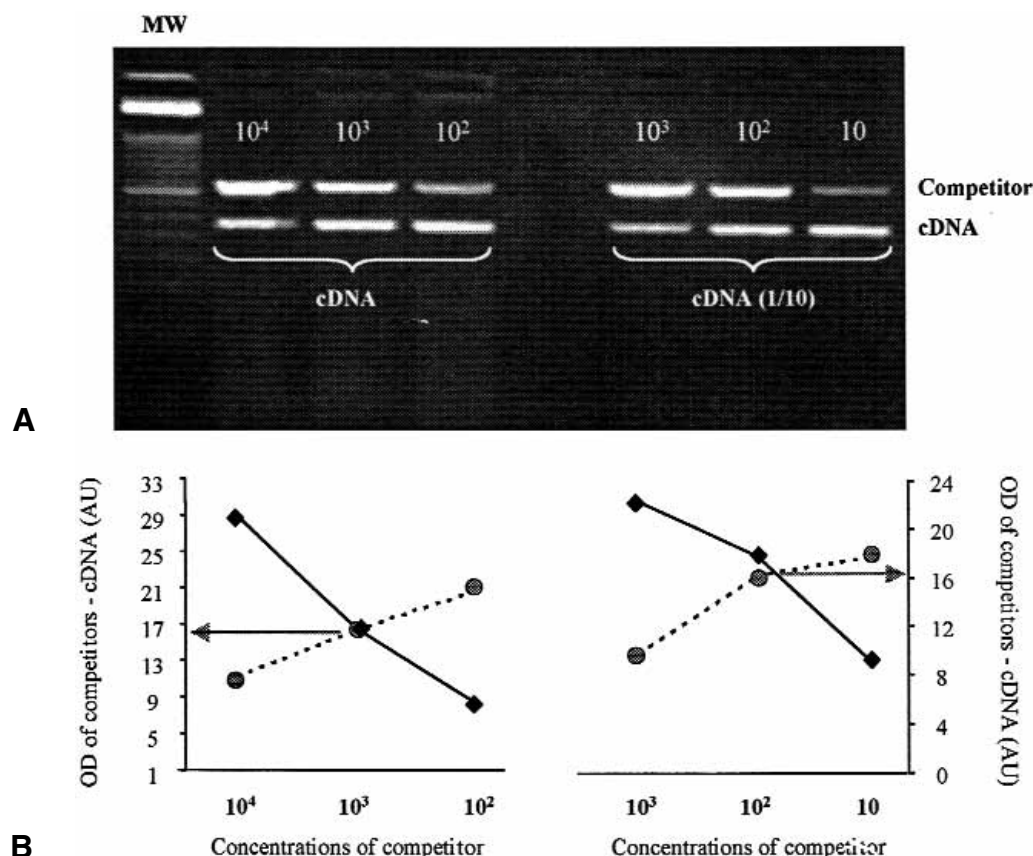


FIG 1. Ethidium bromide-stained agarose gel and quantification of PCR products by competitive RT-PCR. **A**, Successful competition of 2 dilutions of the duodenal IL-10 cDNA (cDNA and cDNA 1/10) with various concentrations of competitor (from 10^4 to 10 pg/mL). **B**, Graph obtained after scanning the agarose gel of different concentrations of competitor (diamond) and cDNA (circle). The quantifications of cDNA and diluted cDNA (1/10) gave concordant results (arrow), respectively, of 16 optical density (OD) at the concentration of 10^3 mg/L of competitor and 16.4 optical density at the concentration of 10^2 mg/L of competitor. MW, Molecular weight. AU, Arbitrary units.

TABLE I. Clinical and functional features of asthmatic and control subjects and IL-10 and IL-4 mRNA concentrations in duodenal biopsy specimens

	Control (n = 8)	Asthma (n = 20)	Nonatopic asthma (n = 10)	Atopic asthma (n = 10)
Age (y)	50 ± 4.6	45 ± 3.3	50 ± 4.7	40 ± 4.7
IgE (kIU/L)	92 ± 34	648 ± 259*	111 ± 21	1,245 ± 483*†
Eosinophils (/mm ³)	228 ± 69	524 ± 78*	556 ± 113*	488 ± 112*
FEV ₁ (% predicted values)	104 ± 4.8	82.5 ± 5*	79.7 ± 7*	85 ± 8*
FEV ₁ /FVC	86.6 ± 1.2	68.7 ± 2.8*	64 ± 3.3‡	73 ± 4*
IL-4 mRNA§				
Mean ± SEM	70 ± 39	843 ± 434	542 ± 505	1,177 ± 740
Median (IQR)	22 (53)	6.3 (163)	6.3 (102)	6.3 (1437)
IL-10 mRNA				
Mean ± SEM	24.7 ± 10	23,494 ± 23,021*	150 ± 67	49,432 ± 48,555†‡
Median (IQR)	11 (19)	1,176 (408)*	46 (189)	176 (2015)†‡

IQR, Interquartile range.

Mann-Whitney *U* test: **P* < .05, different from control subjects; †*P* < .05, different from patients with nonatopic asthma; ‡*P* < .005, different from control subjects.

§Results expressed as 1 molecule IL-4 per 10^5 molecules β -actin.

||Results expressed as 1 molecule IL-10 per 1 molecule β -actin.

tion of RNA in a sample is often difficult and imprecise,^{4,5} the number of cytokine mRNA molecules was expressed as compared with the number of mRNA molecules of an internal control, β -actin, in the same sample.

Statistical analysis was performed with the nonparametric Kruskal-Wallis test for differences among the groups. When this test indicated significant difference, each pairing was examined by means of the Mann-Whitney *U* test.

RESULTS

IL-10 and IL-4 mRNA were detectable in all patients (Table I), whereas no IL-13 mRNA was detected. A representative example of competitive RT-PCR is shown in Fig 1. IL-10 mRNA concentrations were significantly increased in duodenal biopsy specimens from subjects with atopic asthma as compared with control subjects ($P < .005$), and a trend was found toward a nonstatistically significant increase of IL-10 mRNA concentrations in subjects with nonatopic asthma as compared with control subjects. In addition, IL-10 mRNA concentrations were significantly higher in subjects with atopic asthma than in those with nonatopic asthma. No significant difference for IL-4 mRNA concentrations in duodenal biopsy specimens was observed between asthmatics and control subjects. No correlation was found between IL-10 mRNA concentrations in duodenal biopsy specimens and FEV₁, FEV₁/FVC ratio, asthma severity, eosinophil levels, and IgE levels.

DISCUSSION

IL-10 is a cytokine that has pleiotropic anti-inflammatory and immunomodulatory properties. IL-10 inhibits the synthesis of proinflammatory cytokines, T_H2 type cytokines, and chemokines by monocytes/macrophages, neutrophils, and eosinophils. IL-10 also inhibits antigen-specific T-cell proliferation and antigen-induced cellular recruitment into the airways of mouse models.⁶ In asthma, local and systemic production of IL-10 was reported to be deficient in comparison with that in control subjects.⁷ In addition, a low IL-10-producing haplotype was found to be associated with more severe asthma.⁸ Thus it is hypothesized that diminished production of IL-10 could contribute to the chronic airway inflammation found in bronchial asthma.

In contrast, our results show that IL-10 mRNA is overexpressed in duodenal mucosa from patients with asthma when compared with control subjects, especially in allergic asthma. Interestingly, in the gut, IL-10 plays an important role in downregulating pathologic inflammation.⁹ Consis-

tent with these results in the intestinal tract is the finding of increased IL-10 mRNA-positive cells in bronchoalveolar lavage of asthmatic subjects after allergen challenge, also suggesting that IL-10 may participate in the resolution of allergen-induced inflammation.¹⁰ Because there is some evidence that, when an antigen is inhaled, a large proportion is translocated into the intestinal tract, it is conceivable that IL-10 overexpression may counterbalance the effects of proinflammatory cytokines, mitigate the inflammatory reaction found in gut mucosa of asthmatic subjects in response to ingested allergens, and prevent the development of a symptomatic abdominal disease.

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