

THE JOURNAL OF
ALLERGY
AND
CLINICAL IMMUNOLOGY

VOLUME 97

NUMBER 6

Clinical aspects of allergic disease

Increased intestinal permeability in bronchial asthma

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T lymphocytes are a major component of bronchial inflammatory processes in asthma. Because lymphocytes have the ability to migrate from one mucosal site to another, we initiated this prospective study to demonstrate mucosal abnormalities of the digestive barrier in asthma. To establish this we studied intestinal permeability in a group of 37 patients with asthma (21 allergic and 16 nonallergic) by measuring chromium 51-labeled ethylenediaminetetraacetic acid (CrEDTA) urinary recovery. The results were compared with those obtained in a group of 13 nonasthmatic patients with chronic obstructive pulmonary disease and 26 healthy control subjects. Urinary recovery of CrEDTA was significantly higher in patients with asthma ($2.5\% \pm 1.95\%$) than in patients with chronic obstructive pulmonary disease ($1.16\% \pm 0.48\%$) and healthy control subjects ($1.36\% \pm 0.14\%$). There was no significant difference in intestinal permeability between patients with allergic asthma ($2.94\% \pm 2.4\%$) and those with nonallergic asthma ($1.92\% \pm 0.9\%$). Intestinal permeability was not correlated with the severity of asthma as measured by FEV₁. Similarly, intestinal permeability did not significantly vary according to Aas score or steroid treatment. Serum IgE values and eosinophil blood count were not correlated with intestinal permeability. Intestinal permeability was evaluated sequentially in seven patients with asthma (4 allergic and 3 nonallergic) with a mean interval of 7.6 months (range, 2 to 13 months) and did not significantly change. Our results support the hypothesis that a general defect of the whole mucosal system is present as a cause or a consequence of bronchial asthma. (J Allergy Clin Immunol 1996;97:1173-8.)

Key words: Intestinal permeability, Asthma, CrEDTA

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Supported by Centre Hospitalier Régionale Universitaire de Lille (93-02).

Received for publication Dec. 2, 1995; revised June 26, 1995; accepted for publication July 19, 1995.

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Bronchial asthma is an inflammatory disease in which eosinophils and T lymphocytes have a predominant place. Current studies largely emphasize the role of T lymphocytes as the "key" to this inflammatory reaction, playing a role of regulation by the secretion of various cytokines.¹

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0091-6749/96 \$5.00 + 0 1/1/67942

Abbreviations used

COPD:	Chronic obstructive pulmonary disease
CrEDTA:	Chromium 51-labeled ethylenediaminetetraacetic acid
PEG:	Polyethylene glycol

The concept of the common mucosal immune system suggests that activated T lymphocytes are able to migrate from one mucosal site to another.² This hypothesis has been well assessed in animals by studies on rabbits, showing a repopulation of gut and lung with IgA-containing cells after transfer of lymphocytes either from bronchus-associated lymphoid tissue or Peyer's patches.³ Another study in mice demonstrated that after the transfer of lymphocytes from mesenteric lymph nodes, IgA-containing cells were observed in other mucosal sites.⁴

Although few data are available for human beings, one can hypothesize that a lymphocytic infiltrate of the common mucosal immune system may occur in various mucosal sites (e.g., lung, gut, salivary glands) in systemic T lymphocyte-mediated disorders. For example, a subclinical accumulation of activated T lymphocytes has been demonstrated in the lungs of patients with Crohn's disease without respiratory symptoms.⁵⁻⁸ Conversely, a subclinical nongranulomatous T-lymphocyte inflammatory reaction associated with an increased intestinal permeability has been demonstrated in the gut of patients with pulmonary sarcoidosis without intestinal symptoms.⁹ Taken together, these data support the hypothesis that the whole common mucosal immune system may be involved as a cause or a consequence of various T lymphocyte-mediated diseases.

On the basis of the concept that asthma is a T lymphocyte-mediated inflammatory disease of the bronchus, we hypothesized that the whole mucosal immune system might be involved in bronchial asthma. Along this line, we recently demonstrated an airway-like inflammation of the minor salivary glands in patients with asthma.¹⁰ We initiated this study to further demonstrate the involvement of the mucosal system in asthma by the evaluation of the gut mucosal barrier.

Evaluation of intestinal permeability to chromium 51-labeled ethylenediaminetetraacetic acid (CrEDTA) is a useful tool and provides a good reflection of the integrity of the gut mucosa

barrier when used in evaluation of inflammatory bowel diseases.¹¹ Therefore to demonstrate the involvement of the intestinal mucosal system in bronchial asthma, we initiated a prospective study of intestinal permeability to CrEDTA in patients with allergic and nonallergic asthma. The results were compared with those obtained in patients with chronic obstructive pulmonary disease (COPD) and healthy control subjects.

METHODS

Thirty-seven patients with asthma were studied (15 women and 22 men). The mean age was 44 ± 16 years, and the range was 15 to 65 years. Asthma was defined according to the International Conference Criteria.¹² All patients had a history of episodes of dyspnea and wheezing and had a reversible airway obstruction characterized by a 20% increase in FEV₁ after the inhalation of 200 µg of albuterol. All patients were studied in the same manner. Sensitivity to allergens, including a battery of extracts of common aeroallergens (house dust, *Der-matophagoides pteronyssinus* and *D. farinae* mites; cockroach; cat, dog, and horse dander; grass, tree, and weed pollen; *Aspergillus fumigatus*; *Alternaria*, *Cladosporium*, and *Penicillium* spp.) was evaluated by skin prick tests and total serum IgE level (Phadebas paper RAST; Pharmacia, Uppsala, Sweden) was determined at the time of the study. Atopy was defined as follows: at least two positive skin prick test results (i.e., wheal size greater than that produced by histamine). Serum specific IgE was measured by the Phadebas RAST sorbent test in patients with positive skin prick test responses. Twenty-one patients had allergic asthma, and 16 had nonallergic asthma. All patients had normal sinus and chest x-ray films. Asthma clinical severity was assessed according to bronchial obstruction evaluation by FEV₁, measured with a Medgraphic spirometer (Medgraphic, St. Paul, Minn.) according to the scoring system of Aas (from score 1 for very mild forms to score 5 for incapacitating disease requiring medications). The grading is based on events that took place over a 1-year period and takes into consideration both the symptoms (number and duration of asthma episodes, total duration of symptoms, and presence or absence of symptom-free intervals between attacks) and the requirements for medications. It does not take into account the patient's pulmonary function. A score of 5 would be given to patients who are receiving an antiinflammatory drug. Patients' characteristics are summarized in Table I. There was no difference in the duration of symptoms between patients with allergic asthma and those with nonallergic asthma. No patient had any symptom of eczema or rheumatologic or digestive disease at the time of the study. Anti-gliadin antibody test was performed, and results were negative for all the patients. Skin prick tests with food allergens were performed, and results were negative for all patients. In addition, anti-*Saccharomyces cerevisiae* anti-

TABLE I. Clinical characteristics of patients

	Patients with asthma		Patients with COPD	Control subjects
	Allergic	Nonallergic		
No.	21	16	13	26
Age (yr)	35 ± 3	57 ± 2*	59 ± 1.6*	43 ± 2
Smokers	2	2	13†	14
Mean duration of symptoms (yr)	21.4 ± 1.2	18.3 ± 2.4	17.1 ± 1.1	—
Positive prick test results	21	0	0	0
IgE (IU/ml)	675 ± 172.7†	203 ± 68.2	271 ± 91.2	—
FEV ₁ (% pred)	77 ± 5.9‡	73 ± 5‡	42 ± 3.6†	101 ± 6.1
Blood eosinophilia (/mm ³)	387 ± 106.5	320 ± 102.7	292 ± 73.8	—
Theophylline	2	3	9†	—
Anticholinergic agents	0	0	8†	—
Inhaled steroids	8‡	6‡	3	—
Oral steroids	11‡	10‡	2	0

*Significantly different from patients with allergic asthma and control subjects.

†Significantly different from the other groups.

‡Significantly different from patients with COPD and control subjects.

body ELISA (against phosphopeptidomannane) was performed, and results were negative for all the patients.

A first control group of 13 nonasthmatic smokers with COPD was studied. They were all men (mean age, 59 ± 6 years), had no allergic disease, and had never had asthma. A second control group included 26 healthy, nonsmoking, nonallergic subjects.

Measurement of intestinal permeability

Intestinal permeability to CrEDTA was evaluated as previously described.⁹ Briefly, a stock solution of CrEDTA (specific activity, 17 to 75 MBq/mg, CIS International, GIF sur Yvette) was diluted with water so that 10 ml had an activity of approximately 100 mCi (3.7 MBq). At 8.00 AM, after an overnight fast, the subjects drank 10 ml of CrEDTA solution, followed by 125 ml of water. They fasted for an additional 2 hours, after which they were permitted normal food and fluid intake, except for alcoholic beverages. Urine was collected during 24 hours. All subjects were encouraged to remain active during the day. Three 5 ml samples of the pooled 24-hour urine were counted in a gamma counter equipped with a 3-inch NaI (TI) crystal (Gammatic I; Kontron Instruments, Inc., Everett, Mass.). A 1:5000 dilution of the CrEDTA test solution was prepared, and a 5 ml volume was counted with the urine samples for each subject. Percentage urinary recovery of the orally administered dose was calculated: (cpm 5 ml urine ÷ cpm 5 ml 1:5000 dilution of dose) × 20 × (urine volume [liters ingested mass]), and radioactivity was expressed as the percentage of the total orally administered dose. The estimated radiation dose was smaller than 0.05 mGy to the whole body and 0.2 mGy to the gonads.

Statistical analysis

Statistical analysis was performed with a Macintosh computer (Apple Computer, Inc., Cupertino, Calif.)

with Statview II software (Abacus Concepts, Inc., Berkeley, Calif.). When applicable, data were expressed as means ± SEM. The Kruskal-Wallis analysis of variance procedure was applied first to the quantitative data. When this was significant, each pairing was examined by means of the Mann-Whitney U test. Correlation between the different parameters and intestinal permeability was determined by means of Spearman's regression test. Comparison of qualitative data between groups was done with Pearson's chi square probability or Fisher's exact test, two-tailed, for small samples. Sequential evaluation of intestinal permeability was examined by means of the Wilcoxon test. Only *p* values less than 0.05 were considered significant.

RESULTS

Clinical characteristics of the patients are summarized in Table I. Healthy control subjects and patients with allergic asthma were younger than those with nonallergic asthma and COPD. Airflow obstruction was more severe in the COPD group (*p* = 0.002). Patients with asthma were more frequently treated with oral and inhaled steroids than patients with COPD. In contrast, patients with COPD were more frequently treated with theophylline and anticholinergics than patients with asthma.

Urinary recovery of CrEDTA was significantly higher in asthmatic patients (2.5% ± 1.95%) than in patients with COPD (1.16% ± 0.48%) and healthy control subjects (1.36% ± 0.14%) (Fig. 1). There was no significant difference between patients with allergic asthma (2.94% ± 2.4%) and those with nonallergic asthma (1.92% ± 0.9%).

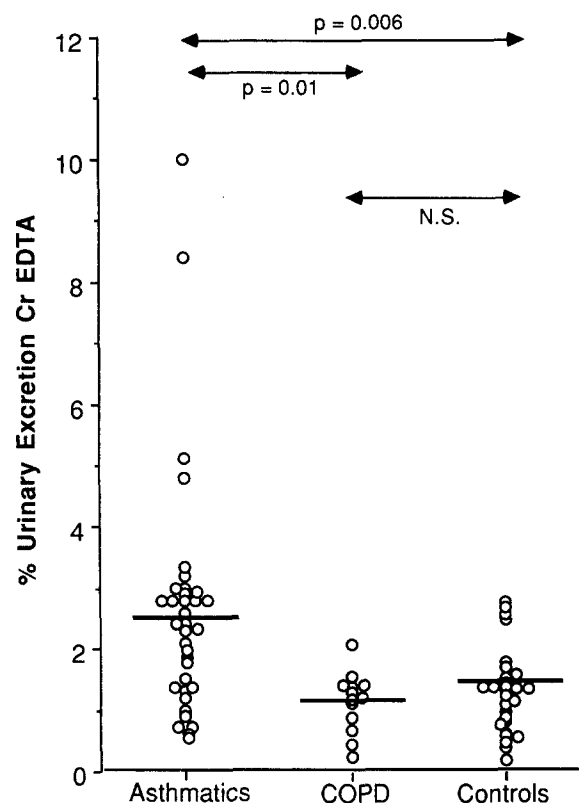


FIG. 1. Intestinal permeability in patients with asthma, patients with COPD, and control subjects. Results are expressed as the percentage of urinary excretion of CrEDTA. N.S., Not significant.

Both patients with allergic asthma and those with nonallergic asthma were significantly different from the control groups.

Intestinal permeability was not correlated with the severity of asthma as judged by FEV₁ measurement. Similarly, intestinal permeability did not significantly vary according to Aas score or steroid treatment (Table II). Serum IgE values and eosinophil blood count were not correlated with intestinal permeability.

Sequential evaluation was performed for seven patients with asthma (4 allergic and 3 nonallergic) with a mean interval of 7.6 months (range, 2 to 13 months). Intestinal permeability did not significantly change between the two measurements ($3.4\% \pm 0.9\%$ and $3.6\% \pm 1.3\%$, $p = 0.18$), although individual FEV₁ values and treatment did (2 had different doses of steroids and 2 had only one measurement when treated with steroids) (Table III). In addition, there was a strong correlation between the two successive measurements of intestinal permeability ($r = 0.98$, $p = 0.0001$).

TABLE II. Intestinal permeability in patients with asthma according to oral steroid treatment or Aas score

Condition	No. of patients	Intestinal permeability (%)
Oral steroids	21	2.25 ± 0.38
No oral steroids	16	2.8 ± 0.54
Aas1	8	3.3 ± 0.99
Aas2	5	1.76 ± 0.22
Aas3	4	1.12 ± 0.39
Aas4	8	2.18 ± 0.35
Aas5	12	2.93 ± 0.63

DISCUSSION

Our results demonstrate that intestinal permeability to CrEDTA is significantly increased in patients with asthma compared with patients with COPD and healthy control subjects. Intestinal permeability did not differ between patients with allergic asthma and those with nonallergic asthma and did not correlate with serum IgE level, blood eosinophilia, severity of disease, or steroid treatment.

Measurement of urinary excretion of CrEDTA offers a very high sensitivity and a low specificity for evaluation of intestinal permeability.^{13, 14} Several other methods have been proposed for the study of intestinal permeability. Low molecular weight molecules such as monosaccharides, mannitol, and polymers of polyethylene glycol (PEG) 400 diffuse across the aqueous pore (polar regions of the lipid cell membrane). Disaccharides, as well as EDTA, diffuse across the mucosa by an intercellular route probably involving tight junctions. Results of the studies with sugars are expressed as a ratio between a monosaccharide or mannitol and a disaccharide, because physiologic parameters affect them equally but do not affect the ratio.¹⁴ PEG requires a complex analysis and high-performance liquid chromatography for good results. CrEDTA permeability analysis is much easier, and its biochemical and physiologic characteristics, as well as its stability and strictly extracellular location, offer great precision. Recovery of 24-hour urine provides the best reproducibility and separation between patients and control subjects.^{15, 16} The influence of variations in gastric emptying, intestinal transit time, and renal function are reduced. However, although predominance of the small bowel is most probable, CrEDTA does not indicate the exact site of the altered permeability.¹⁷

Intestinal permeability variations in celiac dis-

TABLE III. Sequential evaluation of intestinal permeability in seven patients with asthma

Patient No.	First evaluation			Second evaluation		
	Intestinal permeability (%)	FEV ₁ (% pred)	Steroids (mg)	Intestinal permeability (%)	FEV ₁ (% pred)	Steroids (mg)
1	3	114	0	2.9	104	0
2	5.1	27	0	4.8	57	60
3	2.3	60	20	2	49	5
4	0.9	93	0	0.7	90	50
5	3	91	0	2.7	82	0
6	1.3	60	40	1.4	41	40
7	8.4	41	60	11	55	20

ease and inflammatory bowel disease, especially in Crohn's disease, when different probes are used, are correlated with the activity of the disease.¹⁸ When biopsies were performed to evaluate inflammatory infiltrate and villous atrophy, increased CrEDTA permeability persisted even as biopsy findings returned to normal, or when gluten was withdrawn in celiac disease.^{11, 14} Gut mucosa alterations of the tight junctions leading to a leaky mucosa have been proposed to explain the persistence of the intestinal permeability abnormalities.^{13, 19} The tight junctions control the paracellular pathway with highly selective entrance criteria. Increased permeability may be a primary event by enhancing absorption of allergens as evoked in Crohn's disease pathogenesis.²⁰ Finally, intestinal permeability was significantly increased in healthy relatives of patients with Crohn's disease successively with lactulose, CrEDTA, and PEG, suggesting that the primary event of the disease may be a mucosal defect.²¹

The presence of a defect of the mucosal permeability has been studied in the past in atopy. The investigators principally wanted to demonstrate the absorption of inhaled antigens through the nasal or respiratory mucosa in atopic and nonatopic patients²²⁻²⁶ but did not evaluate intestinal permeability in bronchial disease. The mechanisms responsible for increased intestinal permeability in asthma remain unclear. The main causes of increased intestinal permeability could be ruled out in our patients: no one had alcoholism or took nonsteroidal antiinflammatory drugs. In addition, smoking, which is known to increase intestinal permeability, cannot induce a bias because smokers were predominant in the COPD group.²⁷ Previous studies explored the relationship between atopy and increased intestinal permeability. Food allergy was thought to be involved in the patho-

genesis of atopic eczema, and digestive investigations were performed in patients with atopic eczema with or without food allergy by means of intestinal permeability evaluation. Studies were successively performed with PEG and sugars.²⁸⁻³¹ The results suggest that a mucosal defect exists in eczema, but whether this abnormality is primary or secondary remains unclear. However, there was no correlation of increased permeability with extent or severity of eczema, and sequential evaluation before and after dietary restriction failed to demonstrate any significant decrease after elimination diet, suggesting that increased intestinal permeability is a consequence of eczema or atopy itself rather than of food allergy.³⁰ In asthma, it is not yet known whether increased intestinal permeability is correlated with gut inflammatory infiltrate. Preliminary results suggest that an airway-like inflammation of intestinal mucosa is present in patients with asthma.³² However, it is unlikely that intestinal permeability may be a "bronchus to gut" consequence of bronchial inflammatory process because no correlation with the severity of the disease, airflow obstruction, or steroid treatment could be demonstrated. It is clear that bronchial inflammatory infiltrate is linked with disease severity and that it decreases with steroid treatment.¹ In marked contrast, intestinal permeability was similarly independent of the clinical status, as demonstrated by the sequential evaluation. This argues for a primary general mucosal defect present simultaneously in several organs and clinically expressed in a single organ after an exogenous stimulation, such as antigenic or environmental factors. Nevertheless, increased permeability was found in many cases of nonallergic asthma too, suggesting that the common response mechanism may be IgE-independent.³³ According to intestinal permeability abnormalities in allergic asthma, it

would be of interest to study intestinal permeability in healthy relatives of patients with allergic asthma to determine whether increased intestinal permeability is one of the primary defects in asthma.

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