Immunoreactivity for Interleukin 3 and 5 and Granulocyte/Macrophage Colony-stimulating Factor of Intestinal Mucosa in Bronchial Asthma

By Benoit Wallaert,* Pierre Desreumaux,‡ Marie Christine Copin,§ Isabelle Tillie,* Anne Benard,* Jean Frédéric Colombel,‡ Bernard Gosselin,§ André Bernard Tonnel,* and Anne Janin§

*From the *Service de Pneumologie et Immuno-allergologie, Hôpital A. Calmette and Unité Institut National de la Santé et de la Recherche Médicale U 416, Institut Pasteur; ‡Service des Maladies de l'Appareil Digestif, Hôpital C. Huriez; and §Service d'Anatomie Pathologique, Hôpital A. Calmette, 59037 Lille, France*

Summary

T lymphocytes and eosinophils are important components of the inflammatory cell infiltrate in bronchial mucosa in asthma. Because activated lymphocytes migrate through the thoracic duct and the general circulation to remote glandular and mucosal sites, we initiated this study to evaluate pathological abnormalities and immunoreactivity for interleukin (IL) 3, IL-5, and granulocyte/macrophage colony-stimulating factor (GM-CSF) of intestinal mucosa in bronchial asthma. 15 asthmatic patients, 8 nonasthmatic patients with chronic obstructive pulmonary disease, 6 atopic nonasthmatic healthy controls, and 6 nonatopic healthy controls were studied. Duodenal biopsies were performed by endoscopy. A significantly increased number of intraepithelial lymphocytes and eosinophils and a significant accumulation of mononuclear cells (lymphocytes and mast cells) and eosinophils in the lamina propria were detected in asthmatics and atopic controls. Immunostaining with antibodies directed against IL-3, IL-5, and GM-CSF was positive in asthmatics and atopic controls, whereas no staining was observed in nonatopic controls and chronic obstructive pulmonary disease. Combined ultrastructural study and immunogold labeling demonstrated that IL-3, IL-5, and GM-CSF were localized in eosinophils and mast cells. Although devoid of gastrointestinal symptoms, asthmatics and asymptomatic atopics had duodenal pathological abnormalities mimicking those observed in the bronchial mucosa in asthma, suggesting that the whole mucosal immune system is involved in bronchial asthma.

In bronchial asthma, the chronic inflammation of the airways is an important feature that underlies the bronchoconstriction and bronchial hyperresponsiveness characteristic of the disease. Recent studies have emphasized the potential role of lymphocytes in the pathogenesis of asthma (1): (*a*) T lymphocyte, predominantly $CD4^+$, cell infiltrate has been demonstrated in bronchial biopsies in chronic asthma; and (*b*) the modulation of bronchial inflammation is exerted through the release of lymphokines. IL-3, IL-5, and GM-CSF are of particular importance in the development and activation of eosinophils and mast cells, which are constantly found in the bronchial mucosa of asthmatics (1–4). In addition, eosinophils and mast cells can themselves synthesize IL-3, IL-5, and GM-CSF, which can affect their own functioning and interact with lymphocytes, neutrophils, or macrophages (5–9).

The mucosal immune system comprises a series of specialized lymphoid tissues having the complex task of protecting the most vulnerable surfaces of the body that interact with the external environment (10). Both lung and gut possess mucosa-associated lymphoid tissues, and there is a selective traffic of lymphocytes between them. Animal experiments have demonstrated that precursors originating from bronchus-associated lymphoid tissue may migrate to the intestinal mucosa and other sites with mucosa-associated lymphoid tissue (11). Sensitization of bronchus-associated lymphocytes may thus be responsible for the induction of an immune response in the minor salivary glands (12). Along this line, we recently demonstrated an airway-like inflammation of the minor salivary glands of patients with asthma (13). The proposed explanation is that activated lymphocytes that have the ability to recognize specific antigens in bronchial mucosal lymphoid tissue might migrate through the thoracic duct and the systemic circulation to other glandular and mucosal sites. According to this concept of homing of specific lymphocytes, it was reasonable to hypothesize that a similar airway-like inflammation might occur in other mucosal tissues of asthmatics. To es-

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Table 1. *Characteristics of 15 Patients with Bronchial Asthma, 8 Patients with COPD, 6 Nonatopic Healthy Controls, and 6 Atopic Healthy Controls*

	Asthmatics	COPD	Nonatopic controls	Atopic controls
Number	15	8	6	6
Sex ratio (female/male)	6/9	1/7	3/3	3/3
Age (mean ± SEM)	44 ± 4.1	58 ± 3.2*	46 ± 2	44 ± 3.1
Positive cutaneous prick tests	7	0	0	6
Total IgE (IU/ml)	401 ± 120‡	124 ± 102	92 ± 12	329 ± 150‡
FEV_1 (% predicted)	78 ± 6	71 ± 4	104 ± 2§	101 ± 2§
Blood eosinophils (per mm^3)	216 ± 44*	45 ± 16	62 ± 24	54 ± 22
Oral steroids therapy (number)	7	2	0	0
Inhaled steroids (number)	11	0	0	0

Cutaneous prick test was defined as positive when the wheal size was similar or superior to the positive control (histamine).
*Significantly different from the other groups.
‡Significantly different from COPD and nonatopic controls.
§Significantly different from asthmatics and COPD ($P < 0.05$).

tablish this, we initiated a prospective study of the immunopathological abnormalities of gut mucosa in 15 patients with bronchial asthma, free of symptoms of gastrointestinal disease. Our results clearly demonstrated the presence of an airway-like inflammation of gut mucosa in asthmatics and atopic healthy controls as judged by both an increased number of lymphocytes, plasma cells, eosinophils, and mast cells in the lamina propria, and an immunoreactivity for IL-3, IL-5, and GM-CSF in the gut mucosa, which was not observed in the control groups.

Materials and Methods

15 nonsmoking patients with asthma and without symptoms of gastrointestinal disease were included in the study. Patients with a familial history of inflammatory bowel disease were excluded from the study. In addition, to exclude coeliac disease, measurements of IgG and IgA antigliadin and antiendomysium Abs were performed and were negative in all patients. There were six women and nine men. The mean age was 44 ± 4.1 yr and the range was from 15 to 64 yr. Asthma was defined according to the criteria of the international consensus report on diagnosis and management of asthma (14). All patients exhibited a history of episodes of dyspnea and wheezes and had a reversible airway obstruction characterized by a 20% increase in forced expiratory volume in 1 s (FEV_1)[1] 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, was evaluated by skin-prick tests. Skin-prick tests were negative for food allergen. Total serum IgE levels (Phadebas paper radioimmunosorbent test, Pharmacia Diagnostics, St. Quentin-Yvelines, France) was determined. Serum-specific IgE was measured by the Phadebas radioallergosorbent test (Pharmacia Diagnostics) in patients with positive skin-prick tests. Seven patients had allergic asthma and eight had nonallergic asthma. None of the patients had clinical signs of eczema at the time of the study. All patients had normal sinus and chest x-ray films. All patients were studied for pulmonary function tests. FEV_1 was determined using a spirometer (Jaeger Hellige, Strasbourg, France). Regular medications were taken by 11 out of 15 patients: seven patients were treated with inhaled steroids and oral steroids at a mean dose of 18.4 ± 3 mg/d at the time of the study, and four received inhaled steroids. All patients also received intermittent inhaled short-acting β2-agonist, taken as needed. The clinical severity of asthma was assessed according to the international report: mild (four cases), moderate (four cases), and severe (seven cases). Characteristics of the patients are summarized in Table 1.

We also studied eight nonasthmatic smoking patients with chronic obstructive pulmonary disease (COPD), and six atopic asymptomatic nonasthmatic healthy subjects, and six nonatopic healthy subjects as control groups. They had no allergic diseases and had never had asthma. All COPD were treated with intermittent inhaled anticholinergics.

Each patient signed an informed consent statement, and the protocol was approved by the Lille CHRU hospital's ethical committee (CP 94/34).

Duodenal Biopsies. Gastroduodenal endoscopy was performed in all subjects. No endoscopic lesion was found. Systematic biopsies were performed in the duodenum. Intestinal fragments were properly oriented to avoid tangential sectioning artifact, fixed immediately in fresh 4% paraformaldehyde/PBS, and further processed for paraffin embedding. Paraffin blocks were sectioned at 4 μm for histochemistry and immunolabeling. Pathological study was performed on hematoxylin–eosin and May–Grünwald–Giemsa stains to assess the gut architecture and the number of mononuclear cells, including lymphocytes, in the epithelium and lamina propria. Indirect immunoenzymatic methods were used to identify eosinophils and mast cells with mAbs directed against the eosinophil peroxidase (Oncogene Science Inc., Uniondale, NY) and the human tryptase (Chemicon International, Inc., Temecula, CA), respectively. Assessment of architectural mucosal abnormalities was graded as normal, partial villous atrophy, and total villous atrophy. The pattern of cellular mucosal change was determined blindly by two different pathologists, without knowledge of the clinical data, on similar areas in the different biopsies. The number of intraepi-

[1] *Abbreviations used in this paper:* COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; GSE, gluten-sensitive enteropathy.

Table 2. *Pathological Abnormalities of the Gut Mucosa in Asthmatics, Patients with COPD, Nonatopic Healthy Controls, and Atopic Healthy Controls*

	Asthmatics	COPD	Nonatopic controls	Atopic controls
Epithelium*				
Intraepithelial lymphocytes	37.3 ± 3.8‡	13.5 ± 0.9	11.4 ± 4	24.5 ± 4.4‡
Intraepithelial eosinophils	2.5 ± 0.8‡	0.2 ± 0.2	0	0.8 ± 0.4‡
Lamina Propria§				
Mononuclear cells	115.6 ± 8‡	52 ± 6.2	51.8 ± 6	99 ± 6.7‡
Lymphocytes	61.4 ± 3.2‡	27.5 ± 0.7	24 ± 0.9	54 ± 5.7‡
Mast cells	3.3 ± 0.6‡	1.5 ± 0.4	1.3 ± 0.3	3.1 ± 0.2‡
Eosinophils	12.1 ± 1.2‡	0.5 ± 0.4	0.5 ± 0.2	11.1 ± 1.8‡

*Results are expressed as the number of inflammatory intraepithelial cells per 100 epithelial cells (mean ± SEM).
‡Significantly different from COPD and nonatopic controls.
§Results are expressed as number of cells per 10^4 μm^2 muscularis mucosae (mean ± SEM).

thelial lymphocytes was expressed per 100 epithelial cells. The number of inflammatory cells in the lamina propria was expressed for a surface of 10^4 μm^2 of lamina propria. Cell counts were based on an individually defined mucosal tissue unit constituting a 4-μm-thick and 500-μm block of tissue overlying 200 μm of muscularis mucosae (15). The presence of IL-3, IL-5, and GM-CSF proteins was detected with polyclonal rabbit Abs directed against human IL-3, human IL-5, and human GM-CSF (all from Genzyme Corp., Cambridge, MA). These Abs were used at 1/50 for the IL-3 and GM-CSF immunolabeling and at 1/250 for IL-5 detection. After incubation for 45 min at room temperature, slides were briefly washed in TBS. The binding was detected by means of streptavidin complex (LSAB2 Kit, Alkaline Phosphatase; Dako Corp., Copenhagen, Denmark). Enzymatic complex, alkaline phosphatase, anti–alkaline phosphatase, was added at a 1/50 dilution and developed with phosphate substrate and neofuscin (Sigma Chemical Co., St. Louis, MO). For immunochemistry, controls included: (*a*) omission of the first Ab, and (*b*) substitution of the first Ab by an irrelevant Ab of the same isotype but with a different specificity (anti–*Echinococcus granulosus*).

Electron Microscopy. Duodenal biopsies were immediately fixed in 2% glutaraldehyde in cacodylate buffer. Half of the specimens were embedded in epon and processed further for conventional electron microscopy. The other specimens were embedded in Lowicryl K4M (Oxford, Orsay, France) for immunocolloidal gold labeling.

Ultrastructural Immunogold Labelings. Ultrathin sections on nickel grids were incubated for 10 min on a drop of Tris-HCl–buffered saline (20 mmol/liter Tris-HCl, 0–5 mol/liter NaCl) (TBS), pH 7.4, containing 5% (wt/vol) ovalbumin, supplemented with 1% heat-inactivated normal goat serum. This was followed by incubation with polyclonal rabbit Abs to human IL-3, IL-5, and GM-CSF used as primary reagents. The grids were then rinsed with TBS-ovalbumin and incubated on a drop of the 1-nm gold-conjugated goat anti–rabbit IgG (1/50). After 1 hour of incubation at room temperature, sections were thoroughly washed with TBS, postfixed for 10 min in distilled water containing 1% glutaraldehyde and washed again with distilled water. Finally, the sections were subjected to silver enhancement according to a modification of Danscher's silver lactate hydroquinone (Sigma Co., St. Quentin Fallavier, France) physical developer. The specificity of the immunostaining was tested by omitting the first Ab and substituting the specific Abs with the preimmune serum or with unrelated Abs whose labeling had been analyzed previously. To exclude nonspecific binding to proteoglycans, additional control was performed with an Ab directed against a toxoplasmic amylopectin used as primary Ab. No gold deposit was observed. Lowicryl sections were contrasted with uranyl acetate before examination with an electron microscope (EM 10; Carl Zeiss Ltd., Welwyn Garden City, UK).

Statistical Analysis. Statistical analyses were performed with a computer (Macintosh; Apple Co., Cupertino, CA) using the Statview II Software (Statview, Abacus Concepts, Inc., Berkeley, CA). When applicable, data were expressed as mean ± SEM. The Kruskal-Wallis analysis of variance procedure was applied first to the quantitative data of the four groups. When this was significant, each pairing was examined by means of the Mann Whitney U test. Only P values <0.05 were considered significant.

Results

Results of the immunopathological analysis of endoscopically normal areas in patients and controls are summarized in Table 2.

Architectural mucosal abnormalities (partial villous atrophy) were observed in the intestinal mucosa of three patients with asthma but not in the patients from the control groups. Significant cellular mucosal changes were detected in the gut of the patients with asthma and atopic controls when compared with COPD or nonatopic controls. The villous epithelium was infiltrated by a significantly increased number of lymphocytes and eosinophils in asthmatics and atopic controls (Table 2). The lamina propria was infiltrated by a significantly increased number of mononuclear cells, including lymphocytes and mast cells, and an increased number of eosinophils statistically different from control biopsies. No neutrophils were counted on the studied areas. Ultrastructural examination of the duodenal biopsies in asthmatics showed numerous eosinophils and mast cells associated with lymphocytes in the lamina propria. The fine structure of eosinophil granules was altered. Most intracytoplasmic

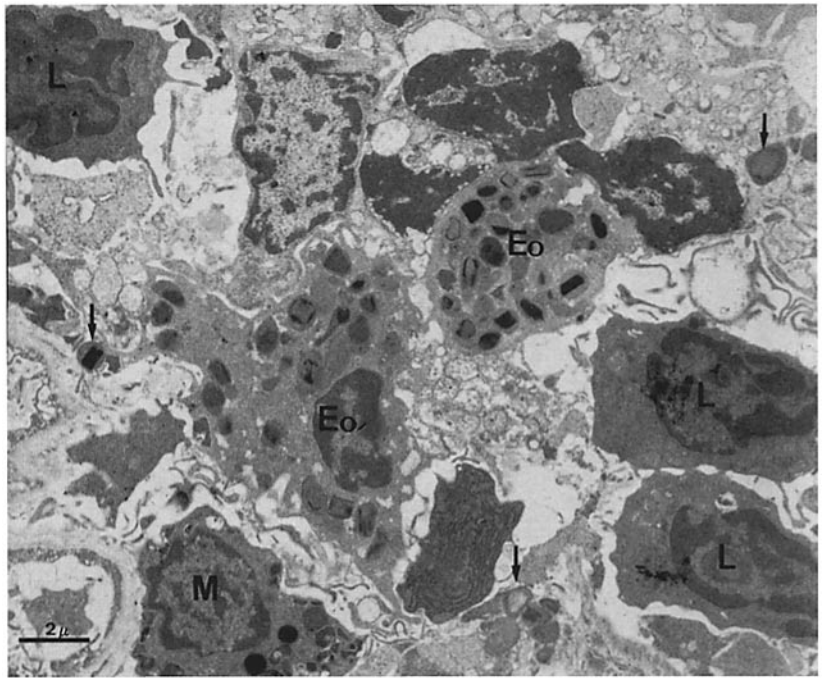

Figure 1. Ultrastructural aspect of the infiltrate of the lamina propria in an asthmatic patient. Eosinophils (*Eo*) and mast cells (*M*) associated with lymphocytes (*L*). The arrows show free extracellular eosinophil granules.

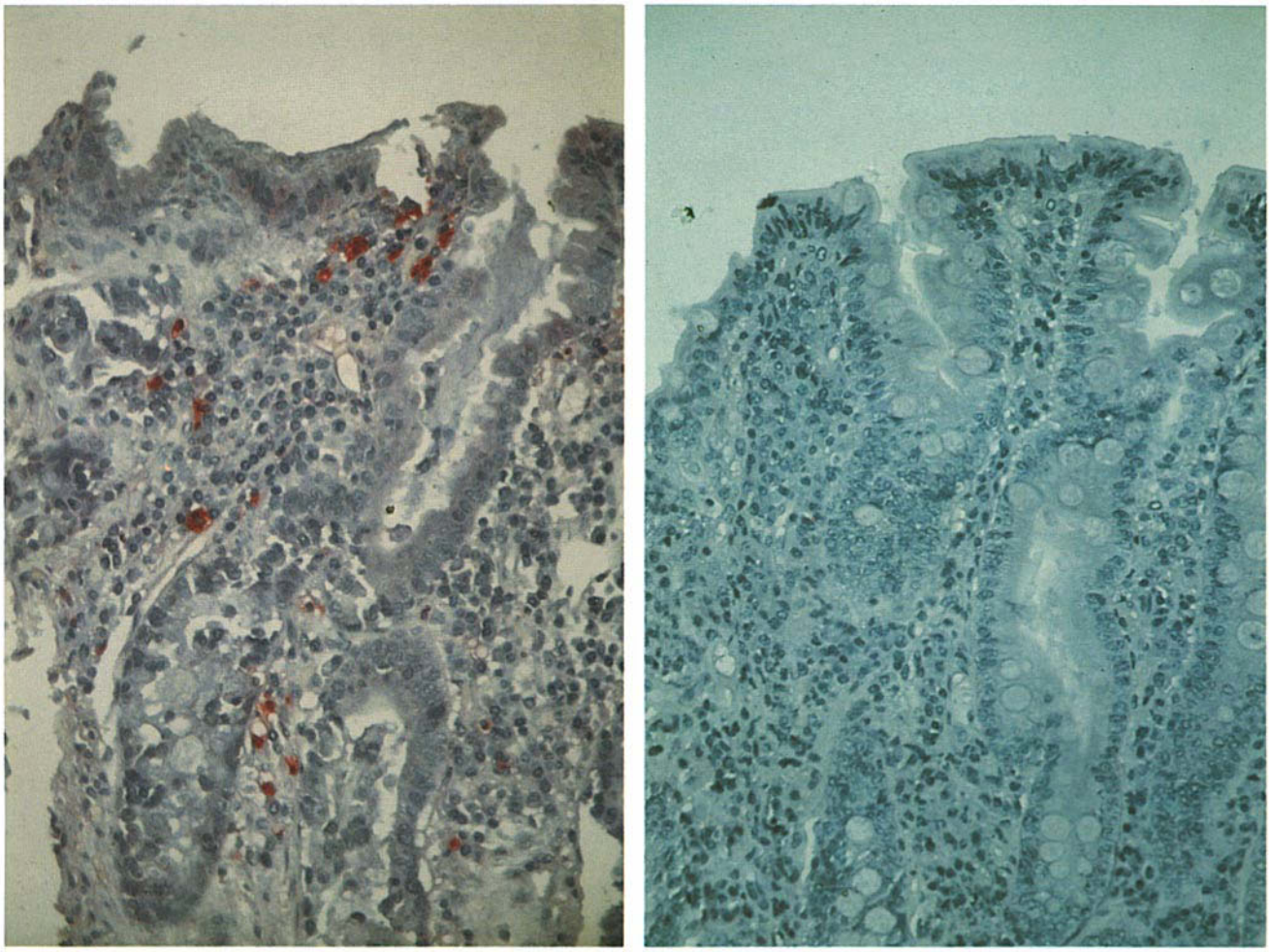

Figure 2. Representative IL-5 immunochemistry of the lamina propria gut mucosa in an asthmatic patient (*left*) and a patient with COPD (*right*). Indirect immunophosphatase ×400.

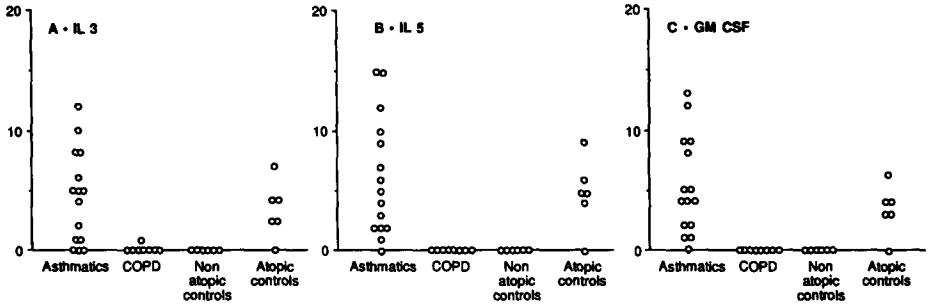

Figure 3. Number of IL-3, IL-5, and GM-CSF immunoreactive cells per 10^4 μm^2 of muscularis mucosae in duodenal biopsies of patients with bronchial asthma, patients with COPD, nonatopic healthy controls, and atopic healthy controls.

granules had an inverted density of their central core (Fig. 1), but numerous free extracellular eosinophil granules showed similar alterations.

By immunohistochemistry, cells stained with the Ab directed against IL-5 (mean 6.2, range 0–15) (Fig. 2) were slightly more numerous than the cells stained with the Ab directed against IL-3 (mean 4.5, range 0–12) and against GM-CSF (mean 5.3, range 0–13) (Fig. 3). This labeling was significantly higher in asthmatics than in COPD and nonatopic controls. Interestingly, cells from atopic controls also stained with the Ab directed against IL-5, IL-3, and GM-CSF. No significant staining was observed in the mucosa of patients with COPD or nonatopic controls. Controls without the first Ab or with an irrelevant Ab were negative.

Combined ultrastructural examination with immunogold stainings showed dense deposits within the eosinophil granules with Abs directed against IL-3, IL-5, and GM-CSF. When the granules were not altered, the dense deposits were located in the granule matrix, around the central core, as illustrated for IL-5 in Fig. 4. Dense deposits were also found within mast cell granules with the three Abs. They were localized over the whole surface of electron-dense granules, as illustrated in Fig. 5 for GM-CSF. No immunolabeling of lymphocytes was found.

Allergic and nonallergic asthmatics had no significant differences in their immunopathological features (Table 3). Lymphocyte and mononuclear cell counts and density of the infiltrate of the lamina propria did not differ according to the treatment. However, patients treated with oral steroid (severe asthma) had a significantly lower number of eosinophils and mast cells than asthmatic patients treated with inhaled steroids (moderate asthma) or untreated patients (mild asthma). Similarly, the number of cells stained with the Ab directed against IL-3, IL-5, and GM-CSF was significantly lower in patients with severe asthma than in patients with moderate or mild asthma.

Discussion

The purpose of this study was to assess the immunoreactivity of the intestinal mucosa in asthmatics and therefore to gain some insight into the common immune mocusal system in humans. Experimental data have clearly provided evidence for extensive migratory patterns to and between mucosal sites that are collectively called the mucosa-associated lymphoid tissue (16, 17). Our results demonstrated significant pathological abnormalities, i.e., airway-like inflammation of gut mucosa in asthmatics and atopic controls free of gastrointestinal symptoms.

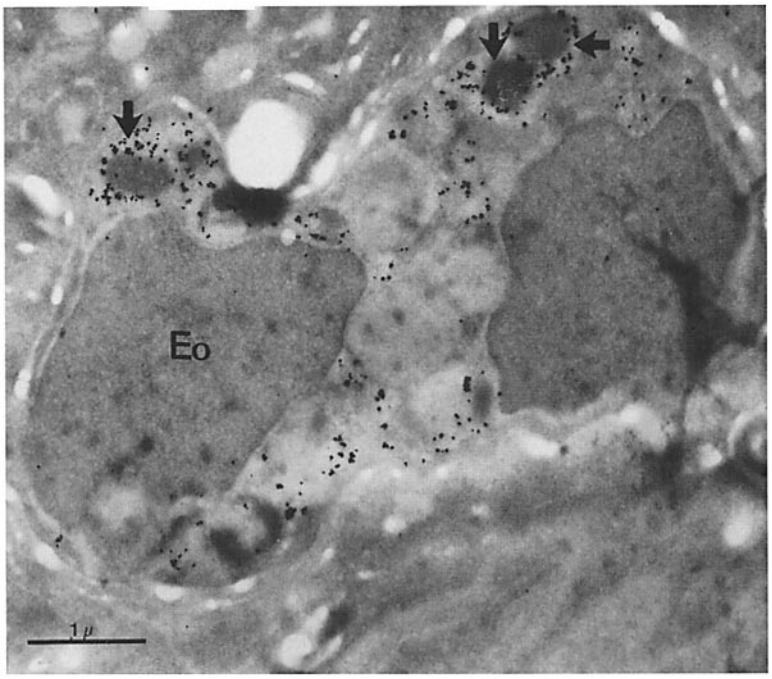

Figure 4. Immunostaining with immunogold-labeled anti-IL-5 Ab. The dark deposits are localized in the matrix of the intact eosinophil granules (*arrows*).

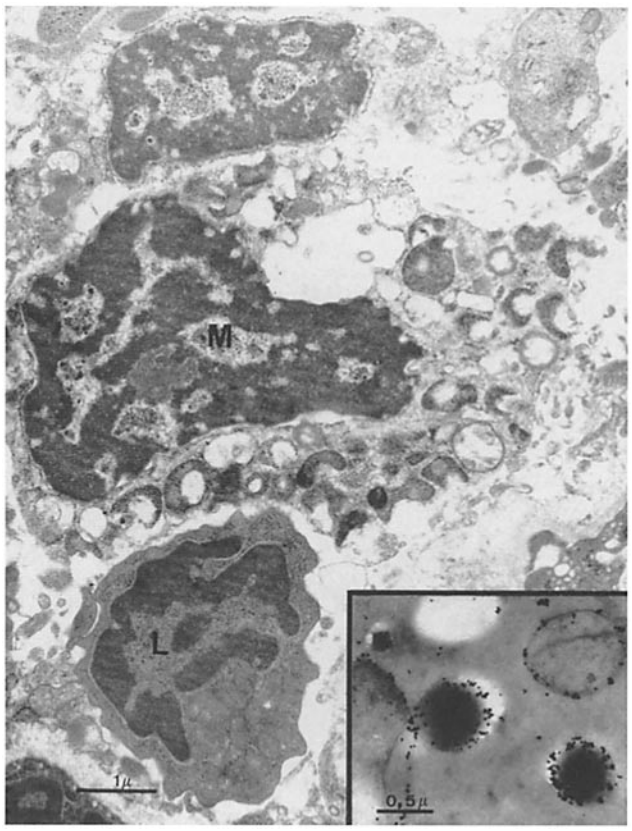

Figure 5. Ultrastructural aspect of mast cell (*M*). Inset: immunostaining with immunogold-labeled anti–GM-CSF Ab. Dark deposits are localized within dense granules and at the periphery of the less dense granules (*arrows*).

The presence of occasional leukocytes in the lamina propria of the proximal duodenum is of doubtful significance. Histological examination of proximal duodenal biopsies obtained from apparently normal duodenam might reveal a slight to moderate increase in lamina propria cell content (18). However, the fact that immunoreactivity for IL-3, IL-5, and GM-CSF was not detected in COPD and nonatopic controls strongly supports the reality of the inflammatory process of gut mucosa in asthmatics. In addition, biopsies of the proximal duodenum accurately reflect the jejunal and distal duodenum in both health and disease (19). This suggests that abnormalities of gut mucosa in asthma are not restricted to small duodenal areas.

We report for the first time histological changes and cytokine production in the duodenum of patients with asthma similar to those described in bronchial mucosa. Development of fiberoptic bronchoscopy has provided an opportunity to obtain bronchial mucosal biopsy specimens from patients with asthma and to characterize features of airway mucosal inflammation (1). Previous observations indicated that the bronchial epithelium and lamina propria of asthmatics are infiltrated with increased numbers of intraepithelial lymphocytes, mast cells, eosinophils, and T lymphocytes (20–22). In this paper, we described a similar inflammatory process of gut mucosa in asthma. In addition, mimicking

Table 3. *Pathological Abnormalities of the Gut Mucosa in Asthmatic Patients According to the Etiology of the Disease or to the Treatment and Disease Severity*

	Epithelium		Lamina propria						
	Lymphocytes	Eosinophils	Mononuclear cells	Lymphocytes	Mast cells	Eosinophils	$IL\text{-}3^+$ cells	$IL\text{-}5^+$ cells	$GM\text{-}CSF^+$ cells
Allergic asthma	39.3 ± 6	2.4 ± 1.2	118 ± 6.1	61 ± 3.4	3.4 ± 1.6	11.1 ± 2.1	4.3 ± 1	6.7 ± 2.2	5.5 ± 1.6
Nonallergic asthma	35.5 ± 4	2.6 ± 0.3	112.8 ± 9.6	62 ± 4	4.2 ± 1.5	13 ± 1.8	4.6 ± 1.2	5.7 ± 2.1	5 ± 1.3
Oral steroids									
(severe asthma)	35.3 ± 4	2.1 ± 1.3	117 ± 5.9	61 ± 3.8	2.2 ± 0.4*	8.7 ± 1.4*	1.9 ± 0.6*	3 ± 0.9*	2.6 ± 0.2*
No oral steroids	39 ± 3.4	2.8 ± 1.2	113 ± 6.2	61.7 ± 4	5.4 ± 0.6	15.1 ± 1.3	6.7 ± 1.2	9 ± 1.7	7.6 ± 1.3
Mild asthma	38.5 ± 2.1	4 ± 0.9	117 ± 2.4	66 ± 1.4	5.25 ± 0.6	15 ± 0.4	6 ± 0.4	7.2 ± 0.8	6.5 ± 0.6
Moderate asthma	39.5 ± 4.7	1.7 ± 0.2	109 ± 10	56.7 ± 4.4	5.5 ± 0.3	15.3 ± 1.3	7.5 ± 0.3	10.8 ± 1.7	8.7 ± 1.4

*Significantly different from patients without oral steroids.

the inflammatory process described in the bronchi of asthmatics, immunoreactivity of gut mucosa for IL-3, IL-5, and GM-CSF was demonstrated in asthmatics. Duodenal biopsies in COPD and nonatopic controls did not express immunoreactivity for any of the cytokines tested. More than one gut lamina propria cell population expressed immunoreactivity for IL-3, IL-5, and GM-CSF. Combined immunogold staining and ultrastructural examination showed that the three tested cytokines were localized in the matrix of eosinophil granules and in cytoplasmic granules of mast cells. Interestingly, ultrastructural studies showed that immunogold labeled five times more eosinophils than mast cells and that no immunolabeling of lymphocytes was found. It is well known that eosinophils and mast cells express cytokines. However, previous in situ hybridization studies in bronchial biopsies demonstrated that both eosinophils and lymphocytes expressed these cytokines (2, 3). The fact that gut mucosa lymphocytes did not express immunoreactivity of IL-3, IL-5, and GM-CSF is not clear. This discrepancy of expression of lymphokines by lymphocytes in bronchial and gut mucosa might explain the different clinical expression of mucosal reactivity in asthma.

The role of these cytokines in the gut mucosa of asthmatics has not been fully elucidated. IL-3, IL-5, and GM-CSF are important regulators of eosinophil survival, proliferation, and effector function. Particularly, IL-5 is the main mediator for eosinophil recruitment and activation and supports the proliferation and terminal differentiation of eosinophil precursors as well as the prolonged survival of eosinophils in vitro. However, these cytokines might also play a role in modulating the immune response at mucosal sites. IL-5 up-regulates IL-2 receptors and may potentiate the secretion of IgA and other immunoglobulins (23).

Strikingly, the histological and immunohistochemical findings of the gut mucosa in asthma are reminiscent of those previously observed in the duodenal mucosa of patients with coeliac disease (6, 24) and more generally in gluten-sensitive enteropathy (GSE), classically characterized by a lymphoid infiltration of the epithelium and a lamina propria edema with increase in lymphocytes, plasma cells, mast cells, and eosinophils (25). Moreover, we have recently shown that eosinophils are the main source of IL-5 synthesis in the mucosa of patients with GSE (6). Polanco et al. have also shown that bronchial asthma is associated with coeliac disease as frequently as with dermatitis herpetiformis (26). However, asthmatic patients in the present study had no clinical symptoms of coeliac disease, no or few architectural gut abnormalities, and absence of abnormal Ab titers against gliadin or endomysium, which are sensitive and specific markers of GSE. Mucosal lesions in GSE are mainly ascribed to T cells. Similar mucosal changes are also found in other diseases in which T cell–mediated activity toward environmental antigens is likely, such as food allergy. As bronchial asthma is also consistent with a T cell–mediated disease, we hypothesized that the mechanisms responsible for the gut lesions in patients with asthma are similar to those involved in GSE.

The significance of the pathological abnormalities of the gut mucosa in bronchial asthma can be discussed. The characteristics of the inflammatory process of the gut mucosa did not differ between allergic and nonallergic asthma. The changes observed in gut mucosa of patients with severe asthma are more likely due to the systematic treatment with oral steroids than to the severity of the disease, since glucocorticoids are known to inhibit cytokine-mediated eosinophil survival (27).

Our results raise several hypotheses that are not mutually exclusive. First, there is some evidence that, when an antigen is inhaled, a large proportion of it is rapidly translocated into the gastrointestinal tract. In this context, it might be responsible for the development of an inflammatory response of the gut mucosa. In animal experiments, it has been demonstrated that, in response to an inhaled antigen sensitization, induction of IgE-secreting cells was observed in both lung and gut (28, 29). Second, the bronchus-associated lymphoid tissue generates both T and B lymphocyte immunity in response to luminal antigen locally, but also at distinct mucosal sites (30). Thus, inflammation of gut mucosa might also result from a "bronchus to gut" flow of inflammatory cells. Third, one cannot exclude that pathological abnormalities are, at least in part, related to a primary dysfunction of the mucosal immune system characteristic of bronchial asthma. The fact that both asthmatics and atopic healthy controls exhibited the same pathological abnormalities supports the hypothesis that atopy alone is sufficient to elicit the inflammatory changes in intestinal mucosa and that this inflammatory process relates to trafficking of cells rather than to the ingestion of allergen from various routes. Development of obvious clinical symptoms then depends on the local triggering by specific antigens. Whatever the mechanism, our result supports the hypothesis that the common immune mucosal system is involved, as a cause or as a consequence, in bronchial asthma.

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Address correspondence to Benoit Wallaert, M.D., Service de Pneumologie et Immunoallergologie, Hôpital Calmette, Bld. du Pr. J. Leclerc, 59037 Lille Cedex, France.

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References

1. Djukanovic, R., W.R. Roche, J.W. Wilson, C.R.W. Beasley, O.P. Twenfyman, P.H. Howarth, and S.T. Holgate. 1990. Mucosal inflammation in asthma. *Am. Rev. Respir. Dis.* 142:434–457.
2. Broide, D.H., M.M. Paine, and G.S. Firestein. 1992. Eosinophils express interleukin 5 and granulocyte macrophage–colony-stimulating factor mRNA at sites of allergic inflammation in asthmatics. *J. Clin. Invest.* 90:1414–1424.
3. Hamid, Q., M. Azzawi, S. Ying, R. Moqbel, A.J. Wardlaw, C.J. Corrigan, B. Bradley, S.R. Durham, J.V. Collins, P.K. Jeffery, et al. 1991. Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. *J. Clin. Invest.* 87: 1541–1546.
4. Bousquet, J., P. Chanez, J.Y. Lacoste, G. Barneon, N. Ghavanian, I. Enander, P. Venge, S. Ahlstedt, J. Simony-Lafontaine, P. Godard, and F.B. Michel. 1990. Eosinophilic inflammation in asthma. *N. Engl. J. Med.* 323:1033–1039.
5. Woodnar-Filipowicz, A., C. Hensser, and C. Moroni. 1989. Production of the haematopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation. *Nature (Lond.).* 339:150–152.
6. Desreumaux, P., A. Janin, J.F. Colombel, L. Prin, J. Plumas, D. Emilie, G. Torpier, A. Capron, and M. Capron. 1992. Interleukin 5 messenger RNA expression by eosinophils in the intestinal mucosa of patients with coeliac disease. *J. Exp. Med.* 175:293–296.
7. Kita, H., T. Ohnishi, Y. Okubo, D. Weiler, J.S. Abrams, and G. Gleich. 1991. Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. *J. Exp. Med.* 174:745–748.
8. Moqbel, R., Q. Hamid, S. Ying, J. Barkans, A. Hartnell, A. Tsicopoulos, A.J. Wardlaw, and A.B. Kay. 1991. Expression of mRNA and immunoreactivity for the granulocyte/macrophage colony-stimulating factor in activated human eosinophils. *J. Exp. Med.* 174:749–752.
9. Bradding, P., J.A. Roberts, K.M. Britten, S. Montefort, R. Diukanovic, R. Mueller, C.H. Heusser, P.H. Howarth, and S.T. Holgate. 1994. Interleukin-4, -5, and -6 tumor necrosis factor α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am. J. Respir. Cell Mol. Biol.* 10:471–480.
10. Ogra, P.L., M.E. Lamm, J.R. McGhee, J. Mestecky, W. Strober, and J. Bienenstock. 1994. Handbook of Mucosal Immunology. Academic Press, Inc., San Diego. 766 pp.
11. Mestecky, J., J.R. McGhee, S.M. Michalek, R.R. Arnold, S.S. Crago, and J.L. Babb. 1978. Concept of the local and common mucosal immune response. *Adv. Exp. Med. Biol.* 107:185–192.
12. Rudzik, R., R.M. Clancy, P.Y.F. Perey, R.P. Day, and J. Bienenstock. 1975. Repopulation with IgA-containing cells of bronchial and intestinal lamina propria after the transfer of homologous Peyer's patch and bronchial lymphocytes. *J. Immunol.* 114:1599–1604.
13. Wallaert, B., A. Janin, Ph. Lassalle, M.C. Copin, L. Devisme, Ph. Gosset, B. Gosselin, and A.B. Tonnel. 1994. Airway-like inflammation of minor salivary gland in bronchial asthma. *Am. Rev. Crit. Care Respir. Med.* 150:802–817.
14. U.S. Department of Health and Human Services. 1992. International Consensus Report on Diagnosis and Management of Asthma. Publication 92-3091. National Institutes of Health, Bethesda, MD. 72 pp.
15. Brandtzaeg, P., and K. Baklien. 1976. Immunohistochemical studies of the formation and epithelial transport of immunoglobulins in normal and diseased human intestinal mucosa. *Scand. J. Gastroenterol.* 11(Suppl. 36):1–45.
16. Bienenstock, J., D. Befus, M. McDermott, S. Mirski, and K. Rosenthal. 1983. Regulation of lymphoblast traffic and localization in mucosal tissues, with emphasis on IgA. *Fed. Proc.* 42:3213–3217.
17. Strober, W., and W.R. Brown. 1988. The mucosal immune system. *In* Immunological Diseases. 4th ed. M. Samter, D.W. Talmage, M.M. Frank, K.F. Austen, and H.N. Claman, editors. Little Brown and Company, Boston/Toronto. 79–139.
18. Gear, E.V., Jr., and W.O. Dobbins, III. 1969. The histologic spectrum of proximal duodenal biopsy in man. *Am. J. Med. Sci.* 57:90–99.
19. Korn, E.R., and P. Forrozan. 1974. Endoscopic biopsies of normal duodenal mucosa. *Gastrointest. Endosc.* 21:51–54.
20. Beasley, R., W. Roche, J.A. Roberts, and S.T. Holgate. 1989. Cellular events in the bronchi in mild asthma and after provocation. *Am. Rev. Respir. Dis.* 139:806–817.
21. Azzawi, M., B. Bradley, P.K. Jeffery, A.J. Frew, A.J. Wardlaw, G. Knowles, B. Assoufi, J.V. Collins, S.R. Durham, and A.B. Kay. 1990. Identification of activated T-lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am. Rev. Respir. Dis.* 142:1407–1413.
22. Frew, A.J., and A.B. Kay. 1988. The relationship between infiltrating $CD4^+$ lymphocytes, activated eosinophils, and the magnitude of the allergen-induced late phase cutaneous reaction in man. *J. Immunol.* 141:4158–4164.
23. Coffman, R.L., B. Shrader, J. Carty, T.R. Mosmann, and M.W. Bond. 1987. A mouse T cell product that preferentially enhances IgA production. I. Biologic characterization. *J. Immunol.* 139:3685–3690.
24. Colombel, J.F., G. Torpier, A. Janin, O. Klein, A. Cortot, and M. Capron. 1992. Activated eosinophils in adult coeliac disease: evidence for a local release of major basic protein (MBP). *Gut.* 33:1190–1194.
25. Marsh, M.N. 1992. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunologic approach to the spectrum of gluten sensitivity ('Coeliac Sprue'). *Gastroenterology.* 102:330–354.
26. Polanco, I., G. Prieto, R. Lama, S. Carrasco, R. Cadaceo, and J. Larrauri. 1991. Associated diseases in children with coeliac disease. *In* Coeliac Disease. M.L. Mearin and C.J.J. Mulder, editors. Klumer Academic Publishers, Netherlands. 123–129.
27. Wallen, N., H. Kita, D. Weiler, and G.J. Gleich. 1991. Glucocorticoids inhibit cytokine-mediated eosinophil survival. *J. Immunol.* 147:3490–3495.
28. Sedgwick, J.D., and P.G. Holt. 1983. Induction of IgE-isotype specific tolerance by passive antigenic stimulation of the respiratory mucosa. *Immunology.* 50:625–630.
29. Sedgwick, J.D., and P.G. Holt. 1985. Induction of IgE-secreting cells and IgE isotype-specific suppressor T cells in the respiratory lymph nodes of rats in response to antigen inhalation. *Cell. Immunol.* 94:182–194.
30. Berman, J.S. 1990. Lymphocytes in the lung: should we continue to exalt only BALT? *Am. J. Respir. Cell Mol. Biol.* 3: 101–102.