

Gastric mucosal mast cells in atopic subjects

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Intragastral allergen provocation under endoscopic control (IPEC) allows direct observation of gastric mucosa reactions after contact with inhalant allergens that reach the stomach. We selected patients with proved atopy to *Parietaria* but without clinical and endoscopic signs of gastric disease, and we tested them with the specific inhalant allergen during IPEC, recording gastric macroscopic reaction and mucosal mast-cell changes in biopsy specimens. All atopic patients showed visible changes in gastric mucosa quantified as IPEC score. Mast-cell numbers detected in atopic patients ($135.4 \pm 102.6/\text{mm}^2$ of stromal area) were significantly higher than in nonatopic subjects ($59.8 \pm 25.4/\text{mm}^2$; $P < 0.03$) and were positively correlated to atopic IPEC score ($P < 0.01$). In addition, 6/12 atopics who had both higher mast-cell counts and IPEC score showed an intraepithelial distribution of gastric mast cells which displayed ultrastructural features of partial degranulation. It is likely that changes observed in our patients with allergy to *Parietaria* reflect a subclinical activation of mast cells in the gastric mucosa.

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Evidence from studies in animals and man indicates that the stomach is a target organ for IgE-mediated reactions to food proteins. In animal models, luminal antigenic challenge with food allergen in sensitized rats leads to an antigen-specific gastric reaction which is histologically characterized by degranulation of mucosal mast cells (3). In man, intragastral allergen provocation under endoscopic control (IPEC) allows direct observation of the gastric mucosa reactions after allergen contact and has been proved to be useful in the diagnosis of food allergy (17–19).

Mast cells, which are known to be effector cells in allergic disease, are numerous in the gastric mucosa of many species (11). *In vitro*, duodenal mast cells from patients with food allergy, when exposed to food extracts, released histamine in a dose-dependent fashion and appeared to be sensitized to specific IgE (15). Gastric biopsies showed that mast-cell counts in the gastric mucosa from patients with food allergy were higher than in nonatopic subjects (17, 18). Furthermore, repeated antigen challenge induces mucosal mast-cell hyperplasia in the rat intestine (13). The involvement of mast cells in allergic reactions has recently been confirmed by studying mucosal distribution and ultrastructural features of these cells in bronchial, nasal, conjunctival, and intestinal mucosa of patients with atopy (1, 4, 5, 8–10, 14, 16).

Since inhaled particles can also reach the stomach

(2), it is likely that gastric mucosa of subjects with respiratory atopic diseases comes into contact with specific inhalant allergens. Interestingly, subjects with gastric ulcers and hypersensitivity to house-dust components showed an immediate reaction of the gastric mucosa after IPEC with *Dermatophagoides* or cockroach antigens (19, 20). In this study, patients with proved atopy to *Parietaria*, but without clinical and endoscopic signs of gastric disease, were tested with IPEC using the specific inhalant allergen, and the gastric macroscopic reaction and mucosal mast-cell changes in biopsy specimens were recorded.

Material and methods

Subjects

Two groups of subjects were studied: A) 12 patients with rhinoconjunctivitis and/or asthma sustained by isolated allergy to *Parietaria* (one man and 11 women; mean age 35.7 years, range 17–60); B) nine non-atopic subjects (one man and eight women; mean age 41.1 years, range 27–60) used as controls. Atopic patients were simultaneously positive to *Parietaria* and negative to other common inhalant allergens (*Dermatophagoides pteronyssinus* and *D. farinae*, mixed grass pollen, *Olea*, *Ambrosia*, *Artemisia*, cat fur, dog hair, and mixture of molds), as proved by skin test (Bayropharm DHS, Milan, Italy) and RAST (Pharmacia Diagnostics, Uppsala, Sweden).

Nonatopic controls were selected from patients attending the endoscopy unit of our department who were found to be normal at the endoscopic examination of the stomach and negative for features of allergic diseases, including skin and RAST tests. Written informed consent was given by all the subjects.

Intragastral provocation under endoscopic control (IPEC)

IPEC was performed as previously described (18). Briefly, 10 ml of a water solution containing 100 AUR (Activity Units by RAST) (22) of *Parietaria* extract (Bayropharm DHS, Milan Italy) was applied to normal antral mucosa under endoscopic control (Olympus GIF 100, Olympus Optical Co, Tokyo, Japan). The mucosa reaction was monitored for 15 min, and two independent observers scored mucosal changes according to the following scale: 0 = no changes, 1 = mild erythema; 2 = marked erythema plus swelling; 3 = dark red erythema plus petechial bleedings. Tests were performed blind since neither observer knew the patient's allergic status. In addition, biopsy specimens were obtained from the challenged areas before and 15 min after allergen provocation.

Sample processing for light and electron microscopy

The biopsy samples were immediately placed into 2.5% phosphate-buffered glutaraldehyde at pH 7.2 for 2 h and washed in 0.1 M phosphate buffer pH 7.2, for 24 h. The specimens were postfixed in 1% osmium tetroxide for 1 h at 4°C and subsequently dehydrated in a graded series of alcohols and propylene oxide. The resin embedding medium (araldite) was allowed to infiltrate the tissue gradually over a 2-h period, 1:3, 2:2, 3:1 araldite:propylene oxide mixtures being used. Samples were left overnight in flat molds with fresh araldite, and were finally polymerized at 60°C for 48 h.

One-micrometer Giemsa-stained, araldite-embedded sections were studied by light microscopy. The mast-cell count and the measurement of mucosal areas were done blind by one investigator using a program (IM 2500) of a computer-assisted image analysis system (MONO, Immagini e Computer, Rho-Milan, Italy). This system consists of a CCD color camera mounted on a Leitz light microscope (Ernest Leitz, Wetzlar, Germany), a color monitor, a 486-processor-based computer with DOS operating system, an image-processing unit, and a digital cursor. After having calibrated the video image for its spatial dimension, 10 separate fields at 40× magnification were randomly selected for each sample, consecutively captured in the digital memory, and

Table 1. Characteristics of subjects studied

Patient no.	Subject	IPEC* score	Mast-cell count**	
			Before	After
1	Atopic	2	223	220
2	Atopic	3	217	200
3	Atopic	3	146	140
4	Atopic	1	72	76
5	Atopic	3	391	380
6	Atopic	2	74	80
7	Atopic	1	43	40
8	Atopic	2	82	90
9	Atopic	2	168	150
10	Atopic	1	28	34
11	Atopic	2	102	98
12	Atopic	2	79	82
13	Nonatopic	0	11	14
14	Nonatopic	0	60	62
15	Nonatopic	0	40	38
16	Nonatopic	0	40	36
17	Nonatopic	0	68	68
18	Nonatopic	0	94	90
19	Nonatopic	0	75	66
20	Nonatopic	0	80	84
21	Nonatopic	0	70	76

* Gastric mucosal reaction after intragastral provocation under endoscopic control (scored 0 to 3: 0, no changes; 1, mild erythema; 2, marked erythema plus swelling; 3, dark red erythema plus petechial bleeding).

** As mean number of mast cells per square millimeter of stromal area, counted in 10 separate fields at ×40 magnification.

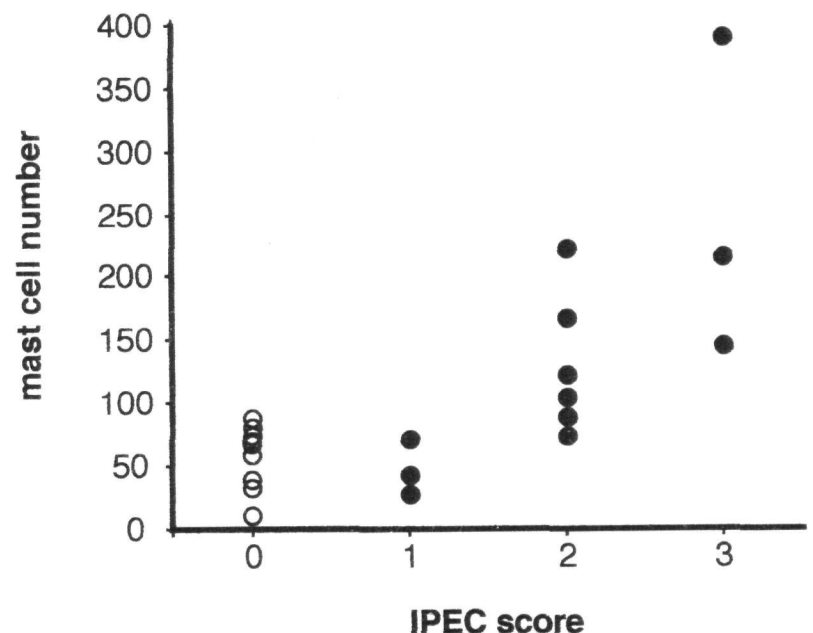


Fig. 1. Correlation between IPEC score and mast-cell number in atopic (●) and nonatopic (○) subjects. Mast-cell number, reported on y-axis, represents mean number of mast cells per square millimeter of stromal area, counted in 10 separate fields at ×40 magnification. Gastric mucosal reaction was reported as IPEC score on x-axis according to following scale: 0, no changes; 1, mild erythema; 2, marked erythema plus swelling; 3, dark red erythema plus petechial bleedings. A significant positive correlation ($P < 0.01$) was found in atopic patients by Spearman's rank correlation test.

thus visualized on the monitor ($110 \times 140 \mu\text{m}$). The area of mucosa available in the biopsy specimens ranged from 1.4 to 3.1 mm^2 . The stromal area lying beneath the epithelium was then outlined each time with the digital cursor, excluding mucosal glands and blood vessels. The count of mast cells present in each area considered was then recorded, and the program automatically furnished the number of elements per square millimeter. The results were expressed, for each sample, as the mean number of mast cells per square millimeter of stromal area.

Thin sections of representative regions were obtained by diamond knives, stained with uranyl acetate and lead citrate, and examined in a Siemens 101 transmission electron microscope.

Statistical analysis

Differences in the mast-cell counts were analyzed by the Mann-Whitney U test. The mast-cell number and IPEC score were correlated by Spearman's rank correlation test.

Results

IPEC

Before allergen provocation, the gastric mucosa was macroscopically normal in all the subjects studied. After allergen challenge, visible changes of the gastric mucosa were observed in all atopic patients. The intensity of reactions varied from mild edema to

massive swelling and petechial bleeding (Table 1). No visible reaction was detected in nonatopic subjects. Macroscopic changes of the gastric mucosa occurred 1–3 min after allergen application and persisted for at least 5 min.

Biopsy findings

Before allergen provocation, minimal signs of focal inflammatory infiltration in the biopsy specimens from both atopic and nonatopic subjects were observed. These findings included the presence of lymphocytes and plasma cells in the lamina propria and rare neutrophils and eosinophils. By contrast, the number of mast cells in gastric mucosa was significantly ($P < 0.03$) higher in allergic patients ($135.4 \pm 102.6/\text{mm}^2$) than nonatopic subjects ($59.8 \pm 25.4/\text{mm}^2$). A positive correlation ($P < 0.01$) was found between mast-cell count and IPEC score in atopic subjects (Fig. 1). After allergen challenge, no changes in the mast-cell counts were observed in either the atopic ($132.5 \pm 97 \text{ mm}^2$) or nonatopic ($59.3 \pm 25/\text{mm}^2$) groups.

In the six atopic patients with higher mast-cell counts and IPEC score (subjects 1, 2, 3, 5, 9, 11; Table 1), mast cells were also localized near and within the foveolar epithelium. In addition, many of these cells exhibited eccentric nuclei and tail-like cytoplasmic projections (Figs. 2 and 3).

Electron microscopy showed that 30% (median value) of mast cells from these six subjects displayed

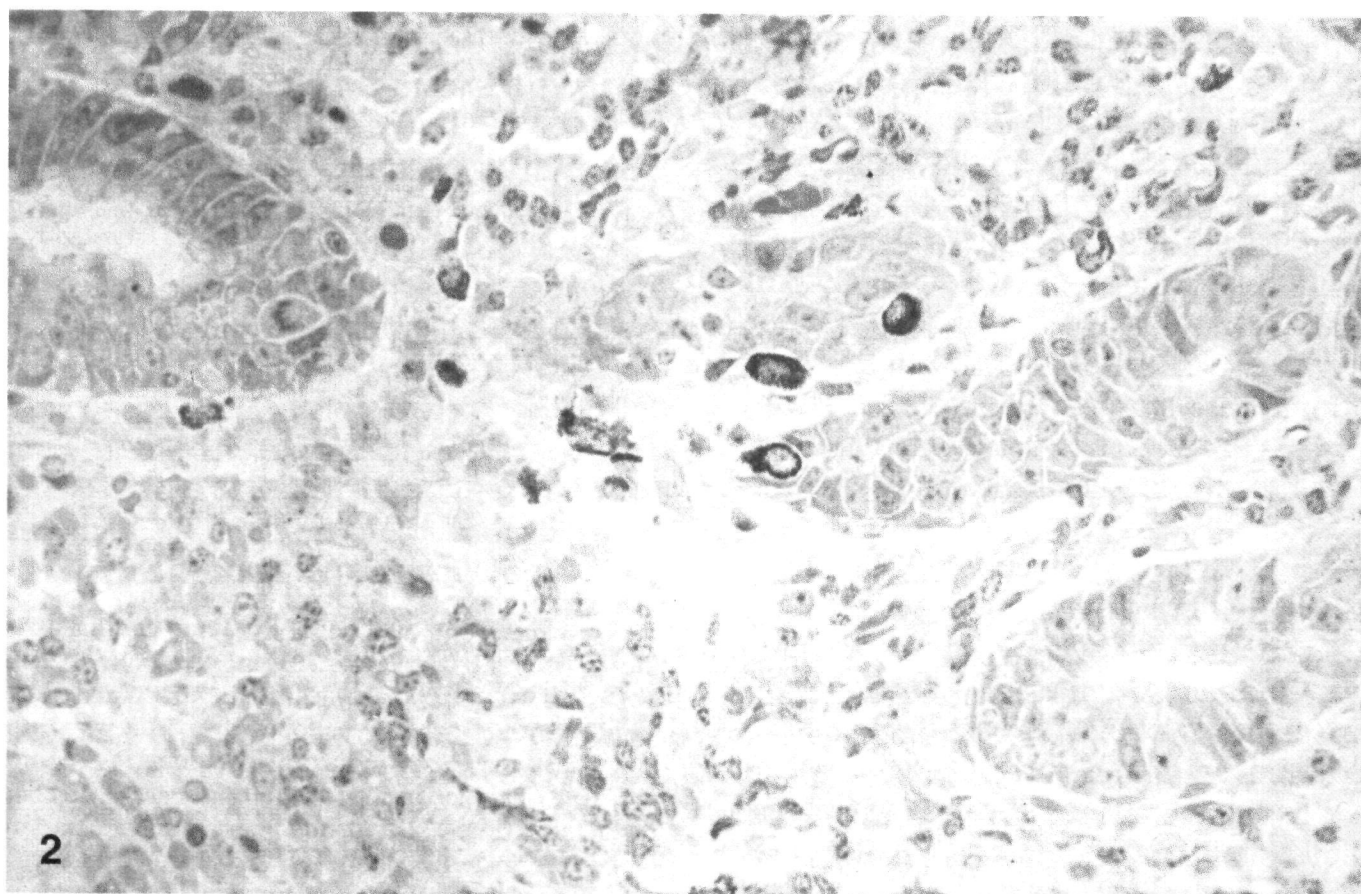


Fig. 2. Gastric mucosa of atopic patient (no. 5). Numerous mast cells are present in lamina propria. Semithin section; Giemsa $\times 400$.

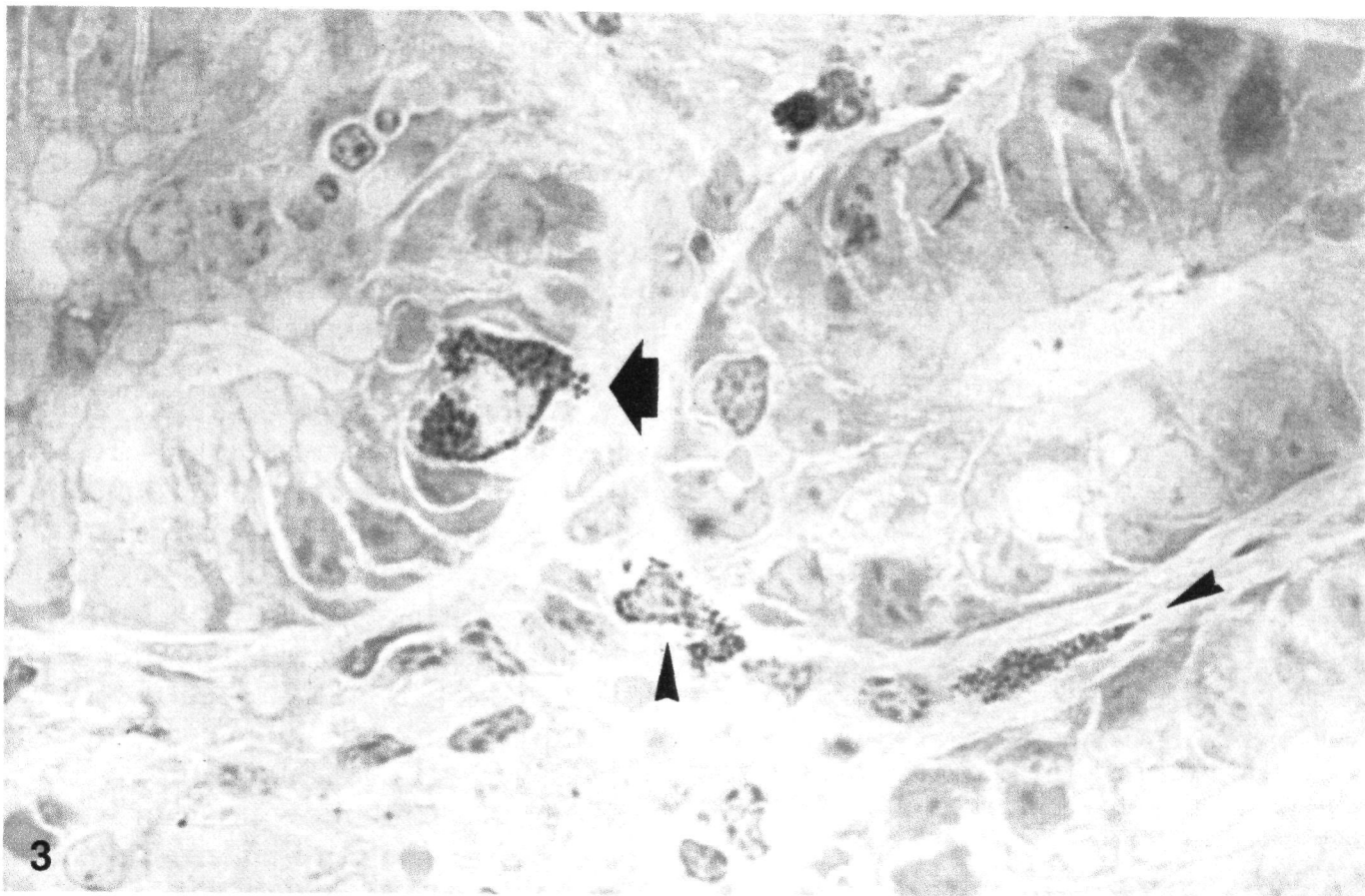


Fig. 3. Gastric mucosa of atopic patient. Mast cell containing metachromatic granules is located in foveolar epithelium (large arrow). Mucosal mast cells showed eccentric nuclei and tail-like cytoplasmic projections (thin arrowheads). Semithin section; Giemsa $\times 800$.

ultrastructural changes characteristic of partial degranulation before allergen provocation. Secretory features included the formation of intracytoplasmic channels containing altered intragranular materials (Fig. 4), and the extracellular release of granule contents through openings in the surface membrane. The externalization of the degranulation channel led to a more complex membrane surface architecture with numerous narrow processes (Fig. 5). Some of the surface processes and membranes were discontinuous with the cell surface (Fig. 5). Cytoplasmic filaments appeared to be prominent, particularly in

the paranuclear region. The remaining cytoplasmic granules showed no signs of swelling and matrix pattern alteration. No evidence of increased mast-cell degranulation was observed after allergen challenge. None of the remaining six atopic subjects and none of the nonatopic patients showed ultrastructural signs of mast-cell degranulation either before or after challenge.

No correlation was found between gastric mast-cell number, clinical signs of atopy, and RAST classes in any of the patients studied.

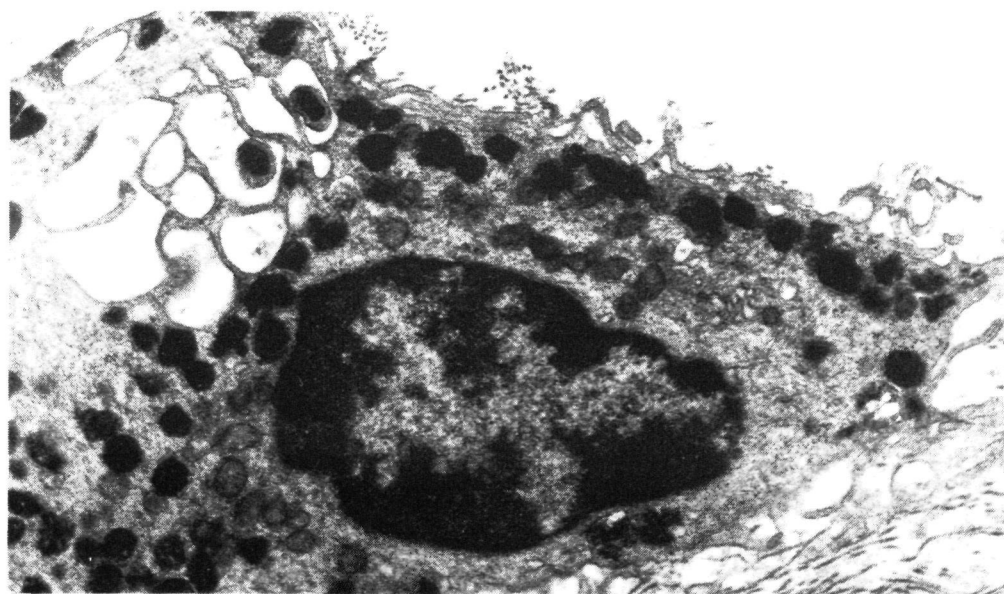


Fig. 4. Mast cell in gastric mucosa of atopic patient. Mast cell shows focal degranulation changes. Note peripheral disposition of residual unaltered granules containing scrolls and crystal patterns. Transmission electron microscopy; $\times 30\,000$.

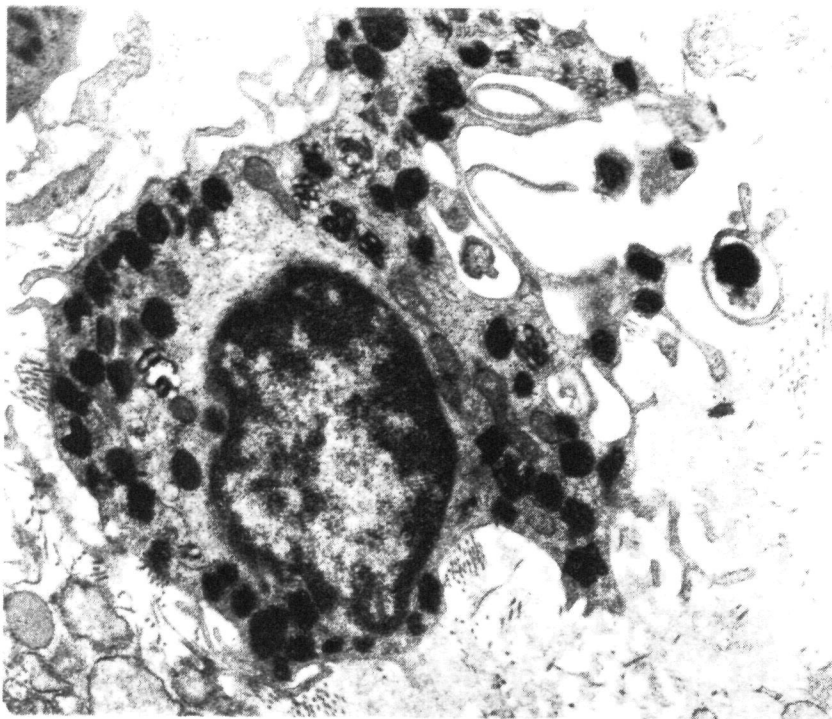


Fig. 5. Mast cell in gastric mucosa of atopic patient. Elongated cell-surface projections and empty intracellular channels constitute prominent features of this degranulating mast cell. Transmission electron microscopy; $\times 30\,000$.

Discussion

In this study, atopic patients with hypersensitivity to *Parietaria*, but without clinical and endoscopic signs of gastric disease, showed visible changes of the gastric mucosa during IPEC with the specific inhalant allergen. The intensity of reaction was positively correlated to the increased number of mast cells in the gastric mucosa. In addition, 6/12 atopic patients, who had both higher mast-cell counts and IPEC score, showed an intraepithelial (foveolar) distribution of gastric mast cells which displayed ultrastructural features of partial degranulation.

Increased mast-cell counts have been reported in the bronchial mucosa of atopic asthmatic patients (12), in the nasal mucosa of symptomatic patients with grass-pollen rhinitis (9), in the tarsal conjunctiva of subjects with pollinosis (14), and in the gastric mucosa of patients with food allergy (17, 18), thus suggesting an involvement of these cells in allergic reactions. Similarly, the increased number of mast cells detected in gastric biopsies could be responsible for the immediate gastric reaction observed in our atopic patients. In fact, this phenomenon seems to be independent of the presence of focal inflammatory infiltrates since they were observed in the lamina propria of gastric mucosa from both atopic and nonatopic subjects. The intraepithelial distribution of mast cells observed in our atopic patients with higher mast-cell counts supports their involvement. In fact, intrafoveolar mast cells were an unusual finding in normal subjects (11), while these cells have been frequently detected in the epi-

thelium of conjunctival and nasal mucosa from patients with pollinosis (9, 14). Furthermore, a migration of nasal mast cells toward the mucosal surface was described during exposure to natural allergen in subjects with allergy to pollens (8, 9).

Morphologic signs of secretory activity are supposed to be a characteristic feature of mast-cell activation. Bronchial and nasal biopsies showed that most mucosal mast cells from patients with inhalant atopy were partially or completely degranulated. By contrast, degranulation was rarely observed in mast cells from nonatopic controls (1, 4, 16,). We detected ultrastructural signs of focal degranulation in gastric mast cells from 6/12 atopic patients, and these changes were similar to those observed in spontaneously degranulated mast cells from the nasal and bronchial mucosa of patients with atopic asthma and rhinitis (4, 21). Furthermore, IgE-mediated degranulation of isolated human lung mast cells *in vitro* showed comparable features (6, 7).

In our study, changes in gastric mast cells were found before allergen challenge and did not vary after IPEC, even if a mucosal reaction occurred. In previous reports, both unchanged and decreased mast-cell number after IPEC was found, while tissue histamine concentrations significantly fell after contact with allergen (17, 18). Therefore, it is likely that changes of tissue histamine content were responsible for the gastric mucosal reaction occurring in our atopic patients after allergen provocation.

On the other hand, it is noteworthy that degranulated mast cells have been detected in bronchial mucosa from asthmatic patients who have not experienced symptoms of asthma for several years (1). The presence of degranulated mast cells irrespective of allergen challenge has been reported to be a feature of atopic asthma, and this could reflect a subclinical mast cell activation in the inactive forms of the diseases (4). In our atopic patients, despite the visible changes of gastric mucosa during IPEC, no gastrointestinal signs of allergy were recorded. It is likely that changes observed in our patients with allergy to *Parietaria* reflect a subclinical activation of mast cells in the gastric mucosa.

The pathophysiologic relevance of this mast-cell hyperactivity is unclear. Patients with gastric ulcers and hypersensitivity to house dust showed an immediate reaction of the gastric mucosa after IPEC with house-dust components, and it has been suggested that inhaled allergens may be involved in the development or progression of gastric mucosal damage in predisposed subjects (19, 20). In this context, the endoscopic and histologic changes observed in our patients with hypersensitivity to *Parietaria*, but without signs of gastric disease, are in line with this hypothesis.

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