

Original article

Passive sensitization of human airways induces mast cell degranulation and release of tryptase

Background: This study was designed to examine the effect of passive sensitization (PS) on human bronchial mast cells. PS with asthmatic serum induces a hyper-responsiveness to nonspecific agonists, and immunoglobulin (Ig)E binding mainly on mast cells.

Methods: Bronchi dissected out from 19 lung specimens were incubated in normal or asthmatic serum. Immunohistochemistry was performed using monoclonal antibodies (MoAbs) directed against tryptase, chymase, or *c-kit*. Mast cells were classified as fully granulated (type I), partly (type II) or largely degranulated (type III). Tryptase was measured in supernatant using ELISA. Contractile response was recorded in a separated set of experiments using an organ bath system.

Results: PS decreased both tryptase positive cells (47.9 ± 10.0 vs. 26.7 ± 4.8 cell/mm², $P=0.003$) and chymase positive cells (26.1 ± 3.3 vs. 14.9 ± 1.8 cell/mm², $P=0.01$), but did not alter the number of *c-kit* positive cells. PS decreased the proportion of type I (55.4 vs. 28.9%, $P<0.0001$) and, concomitantly increased that of types II (23.2 vs. 41.0%, $P<0.0001$) and III (21.4 vs. 30.1%, $P=0.04$). Following PS, tryptase concentration significantly increased and the magnitude of histamine response, was correlated with the amount of type II mast cells.

Conclusion: PS of human isolated bronchi induces a mast cell degranulation related to *in vitro* hyper-responsiveness, along with a tryptase release.

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Passive sensitization (PS) of human tissues has been initially used to analyze allergen specific IgE response (1,2). More recently, PS of human isolated bronchi with serum from asthmatic patients containing a high concentration of both specific and total immunoglobulin (Ig)E, has been described as one of the experimental models that best mimics the bronchial hyper-responsiveness observed in asthmatic patients. The specific bronchial contractile response to allergen, that is a key feature of allergic asthma *in vivo* (3) is closely reproduced *in vitro* following PS conditions (4–6). It has also been demonstrated that PS alters the bronchial contractile response *in vitro* to a variety of nonspecific agonists including histamine, KCl, tachykinins, or leukotriene C4 (4,7–10), as it is observed *in vivo* in asthmatic patients (11). The mechanisms of PS-induced allergen-mediated bronchial contraction have been characterized and involve the release of inflammatory

mediators mainly leukotrienes (12). By contrast, the mechanisms by which PS alters the nonspecific mechanical response of isolated bronchi remain largely unknown (13).

There is strong evidence that human monoclonal IgE can mimic PS in terms of contractile response to allergen (5), but its role regarding bronchial responsiveness to nonspecific agonists in the absence of any allergen challenge is less clear (14). Watson et al. have investigated this issue (15,16), and concluded that, although specific IgE determines specific allergen responsiveness, increased contractile response to histamine is not directly related to IgE but to some other factors associated with IgE response including cytokines or proteases, present in the asthmatic serum. Tryptase, a mast cell-derived neutral protease, increases *in vitro* bronchial reactivity to histamine (17) and promotes calcium influx in human airway smooth muscle cells (18). Tryptase is stored in the mast-cell granules and released upon degranulation. *In vivo*, there is strong evidence that mast cells localized to bronchial mucosa from asthmatic patients, have a degranulated aspect when compared to mast cells from nonasthmatic subjects (19,20). Furthermore, this degranulation

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observed in asthmatic tissues can be related to the observed correlation between tryptase concentration in bronchioalveolar lavage fluid and the severity of *in vivo* bronchial hyper-responsiveness (20). Thus, it was our hypothesis that PS could induce a mast-cell degranulation in the absence of allergen stimulation, leading tryptase to modify bronchial reactivity.

The aim of the present study was thus to analyze the effect of PS on mast cells present in the airway wall and to define their degranulation status using an automated system of colour recognition. The results indicate that, PS induces a significant mast-cell degranulation correlated with *in vitro* bronchial hyper-responsiveness, and a concomitant release of tryptase.

Material and methods

Tissue donors

Human lung was obtained at thoracotomy as previously described (21) from 19 patients undergoing resection for pulmonary carcinoma. Morphometric data of the patients whose lung specimens were used in this study, are given in Table 1A. Patients were non-atopic (i.e., absence of specific IgE against common aeroallergens) and did not report any clinical history of asthma.

Tissue preparation

After resection, tissues were immediately transferred to the laboratory in ice-cold oxygenated Krebs-Henseleit solution (22). From a macroscopically tumour-free part of each of the specimens, segments of human bronchus (5th to 7th generation), 0.5–1.5 mm in internal diameter, were carefully dissected from surrounding parenchyma without damaging the airway membrane.

Passive sensitization (PS)

PS was performed as previously described (7,23). Briefly, bronchi were incubated for 12 h at 25°C in sera from a series of atopic asthmatic patients, whose characteristics are reported in Table 1B. Control tissues were incubated in sera from healthy non-atopic subjects. Both sensitized and non-sensitized bronchi were incubated with or without the protease inhibitor benzamidine 100 µM (Sigma, Saint-Quentin-Fallavier, France).

Sample processing and immunohistochemistry

Bronchial rings were then cut into small fragments of 2 × 2 × 2 mm and processed in glycolmethacrylate (GMA) as previously described (22). Briefly, specimens were placed into the embedding resin prepared with benzoyl peroxide, GMA monomers, and N,N-dimethylaniline and polymerized overnight at 4°C.

After inhibiting the endogenous peroxidase using sodium azide and hydrogen peroxide, 2 µm sections were incubated overnight at room temperature with a variety of mouse MoAbs including anti-human tryptase antibody AA1 (24), anti-human Chymase (Chemicon, Souffelweysheim, France), anti-human *kit* receptor YB5.B8 (Becton Dickinson, Pont de Claix, France), anti-human IgE (Immunotech, Marseille, France), anti-human Eosinophils (EG2, Pharmacia, Saint-Quentin-en-Yvelines, France), anti-human Monocytes CD68 (Dako, Trappes, France), anti-human T lymphocytes CD3 (Dako) and anti-human Neutrophil elastase (Dako). Control slides were treated similarly, omitting the primary MoAb or using an unrelated antibody (25). Biotinylated goat anti-mouse F(ab')₂ (Dako) was then applied to the sections, and followed by the

Table 1 (A) Patient characteristics, and (B) Serum characteristics

(A) Patient characteristics	Mean	(Range)
Sex (M/F)	18/1	
Age (years)	64	(22–81)
Smoking (pack/year)	27	(0–60)
FEV1 (%)	91	(62–114)
FEF25–75% (%)	72	(28–141)
TLC (%)	96	(74–136)
PO ₂ (mmHg)	77	(61–95)
(B) Serum characteristics	Healthy serum	Asthmatic serum
Sex (M/F)	5/5	3/5
Age (yr)	35	23
FEV1 (%)	111	102
Eo (cell/mm ²)	82	581
Total IgE (mUI)	33	311
RAST (SU/ml)	0	3
Skin test	-	+

FEV1, forced expiratory volume in 1 second; FEF 25–75, forced expiratory flow between 25% and 75% of forced vital capacity; TLC, total lung capacity; %, percentage of predicted values; PO₂, arterial partial pressure of oxygen; Eo, eosinophil blood concentration; RAST, class 0 < 1.4; class 3: 20–100.

streptavidin-biotin horseradish-peroxidase complex (Dako). Amino-ethyl carbamazole (Sigma) and hydrogen peroxide were used as substrate to develop a peroxide-dependent red colour reaction at 37°C.

Quantification of immunostaining

Light microscopy was performed using an Optiphot microscope (Nikon, Japan). Cells staining positively with each MoAb were automatically counted by Quancoul© software (Bordeaux, France) at a magnification of × 100 in all of the specimens excluding alveoli as previously described (22). The total area examined was calculated by delineating the area of the bronchial section on a video interactive display system (× 40). In a subset of experiments (patients 1–11), a compartmental analysis was also performed delineating bronchial epithelium, submucosa and the smooth muscle layer. In this latter area, we delineated both smooth muscle bundles and surrounding extra-cellular matrix. The cell counts were expressed as number of cells/mm² of tissue, and percentage of cells in each bronchial layer.

In order to evaluate mast-cell degranulation, we used two different approaches. The first approach was based on the comparison of a *kit*-receptor expression (i.e. a surface marker specific for mast cells) with that of tryptase and/or chymase (i.e. proteases stored in non-degranulated mast cells). The variation of both expressions in sensitized and non-sensitized specimens was used to determine the amount of degranulated mast cells in such conditions. The second approach was based on the morphological analysis of bronchial mast cells, using a method derived from that previously described by Pesci and coworkers (26). The proportion of degranulated mast cells was estimated by classifying the AA1 positive cells at a × 400 magnification in 3 classes: I, fully granulated; II, partly degranulated, and III, largely degranulated. Mast cells were assigned to one of the 3 classes on objective criterion based on an automated method of colour analysis of each cell and determination of the distribution of three imaging parameters i.e., optic density, hue and hue density, as previously described (22).

Mechanical recordings

Eight lung specimens (patients 12–19) were prepared for both functional and morphological study. Bronchial rings were mounted

in an organ bath system in duplicate (EMKA Technologies, Paris, France) filled Krebs-Henseleit solution at pH 7.4, bubbled with 95% O₂ in CO₂ gas mixture and maintained at 37°C, as described previously (7). At the beginning of each experiment, resting tensions were adjusted to 1.5 g in airways, a passive load that stretches this type of preparation to its optimal length (7), and a supramaximal bolus of acetylcholine (Sigma) (ACh 10⁻³ M, final concentration in the bath) was administered to each ring to determine the maximal ACh contractile force. After washing, the contraction of bronchial rings in response to histamine (10⁻⁸ – 10⁻³ M) (Sigma) was assessed and cumulative concentration-response curves (CCRC) to histamine were then constructed. At the end of each experiment, and after washing, all of the bronchial rings were challenged with 50 µl (500 U) of *Dermatophagoides pteronyssinus* (*D. pter*) (Institut Pasteur, Paris, France) to verify that none of the lung tissues was naturally sensitized, and that PS was successful.

Quantification of tryptase concentration

For the detection of both α- and β-tryptase, we used the ELISA previously developed (27). Briefly, microtitre plates (Nunc Maxisorp, Life Technologies, Paisley, UK) were coated overnight at 4°C with a 1/3000 dilution of ammonium sulfate-precipitated rabbit polyclonal capture antibody against human tryptase. All subsequent steps took place at room temperature and were separated by four washes with phosphate-buffered saline (PBS, Life Technologies) containing 0.1% Tween 20 (PBS-T). Non-specific protein binding sites were blocked with 3% bovine serum albumin (BSA, Life Technologies) in PBS-T for 1 h. Fifty µl of serum (healthy or asthmatic, before or after incubation with a bronchial ring), diluted in PBS-T or tryptase standard were applied to wells and assayed in triplicate. Biotinylated detecting mouse monoclonal antibody AA5 (Promega, Madison, WI, USA) against human tryptase was applied at 3 µg/ml for 90 min. Extravidin-horseradish peroxidase conjugate (Sigma) was then added for 30 min. The colour reaction was developed with 0.3 mg/ml *o*-phenylene diphosphate (Sigma) dissolved in citrate/phosphate buffer, pH 5.5 containing 0.005% H₂O₂. After 7 min, the reaction was stopped with 3 M H₂SO₄ and the plates were read at 490 nm. For the standard curve, purified human lung tryptase was diluted in normal rabbit serum (Sigma) from 64 ng/ml to 1 ng/ml.

Statistical analysis

Because the cell counts were not normally distributed, results were analyzed by means of the Wilcoxon's paired rank test to compare the

number of cells in each experimental condition. The χ^2 test was used to compare the percentage of each experimental condition in each type of mast-cell granulation. In order to evaluate the relationship between the mast-cell degranulation and bronchial responsiveness, a Spearman's correlation matrix was constructed using morphological parameters (i.e. the percentage of type I, II and III) and functional parameters (i.e. the plateau of the CCRC to histamine and maximal response to *D. pter*).

Results

Effect of passive sensitization on mast cells

The morphological aspect of bronchial tissue was similar in sensitized and nonsensitized specimens, including integrity of epithelium. As previously observed (21), PS increased the number of IgE-bearing cells (Fig. 1A, $P=0.006$). Concomitantly, we observed a significant decrease in the number of both tryptase (Fig. 1B, $P=0.003$) and chymase positive cells (Fig. 1C, $P=0.01$) within the whole bronchial wall. In contrast, the YB5-B8 positive cell number was not significantly altered by the PS procedure (Fig. 1D). Compartmental analysis showed that, in terms of absolute number of cells, the AA1 positive cells decreased in the epithelial, submucosal and smooth muscle layers, but the difference only reached significance within epithelium, and submucosa (Table 2). Regarding the other phenotypes of inflammatory cells, we did not observe any significant alteration in the number of lymphocytes, neutrophils, activated eosinophils, or monocytes-macrophages following PS (data not shown).

The morphological study allowed us to detect the previously defined three classes of mast cells in all of the bronchial specimens. Figure 2A shows a typical aspect of the different stages of mast-cell degranulation observed under light microscopy. These morphological classes of mast cells corresponded to different distributions of optic density, hue density and hue as illustrated

Table 2. Number of tryptase positive cells in passively sensitized and nonsensitized tissues according to the different compartments of the airway wall

Patient No.	Epithelium cells/mm ² (%)		Submucosa cells/mm ² (%)		Smooth muscle layer cells/mm ² (%)	
	N	A	N	A	N	A
1	0.00 (0.0)	0.00 (0.0)	25.81 (82.6)	4.17 (81.8)	26.54 (17.4)	6.86 (18.2)
2	19.89 (2.1)	0.00 (0.0)	38.82 (82.9)	25.57 (56.5)	48.74 (15.0)	41.49 (43.5)
3	20.54 (3.5)	0.00 (0.0)	41.05 (81.2)	26.04 (80.7)	43.79 (15.3)	36.15 (19.3)
4	54.96 (3.0)	4.84 (3.7)	20.09 (69.7)	4.83 (66.7)	38.16 (27.3)	21.72 (29.6)
5	116.19 (23.3)	19.66 (6.5)	36.55 (67.9)	23.91 (81.8)	61.32 (8.80)	42.71 (11.7)
6	13.92 (7.7)	30.27 (3.3)	37.47 (81.9)	29.74 (76.7)	50.20 (10.4)	166.45 (20.0)
7	136.23 (13.9)	84.37 (36.1)	35.36 (80.3)	12.01 (57.4)	62.67 (5.80)	16.65 (6.60)
8	61.74 (3.8)	8.75 (0.9)	33.92 (77.2)	31.24 (69.9)	104.37 (19.0)	103.52 (29.2)
9	311.36 (4.4)	28.14 (3.0)	138.23 (89.1)	35.96 (76.0)	116.89 (6.50)	38.57 (21.0)
10	164.51 (20.4)	55.95 (3.4)	60.21 (69.9)	55.01 (84.5)	83.83 (9.70)	191.77 (12.1)
11	145.90 (8.0)	0.00 (0.0)	24.38 (80.0)	16.65 (81.5)	68.15 (12.0)	294.12 (18.5)
Median	61.70 (4.4)	8.80 (3.0)	36.60 (80.3)	25.60 (76.7)	61.30 (12.0)	41.50 (19.3)
Wilcoxon P		0.005		0.003		

Definition of abbreviations: Tissues were incubated with normal (N) or asthmatic (A) serum. Numbers in parentheses are percentage of total number of tryptase positive cells. p is the probability of the non parametric Wilcoxon paired rank test when N is compared to A tissue. A: Incubation in serum alone

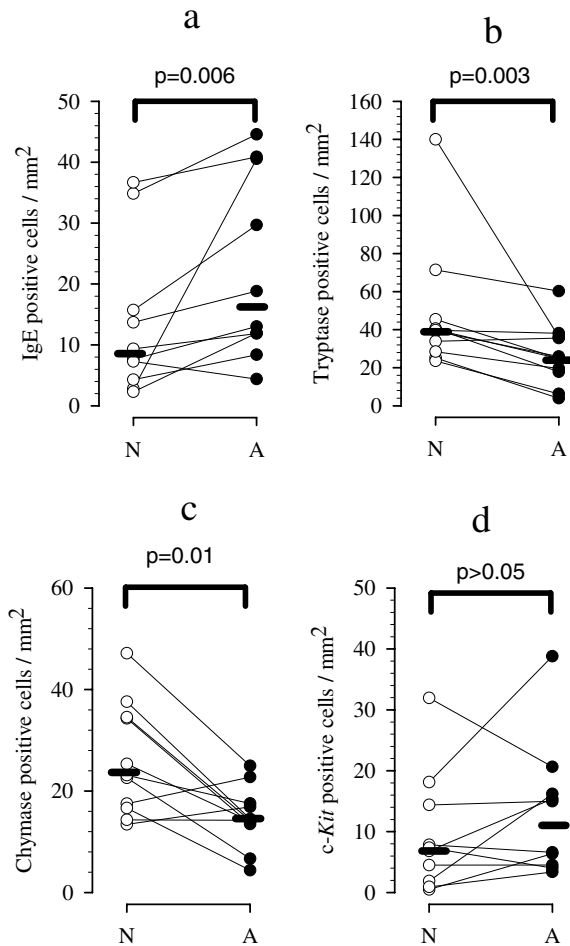


Figure 1. Number of cells within the wall of bronchi incubated with normal (N, open circles) or asthmatic serum (A, closed circles). Cells are stained with (a) anti-IgE, (b) anti-tryptase (c) anti-chymase or anti-c-kit receptor and (d) monoclonal antibodies. Bars represent medians.

in Figure 2B. PS altered the proportion of the different classes of mast cells. We detected a significant decrease in the percentage of fully granulated mast cells (type I) and a concomitant increase in partly (type II) and

Table 3. Proportions of degranulated mast cells in passively sensitized and nonsensitized bronchial specimens

Nonsensitized cells (%)			Passively sensitized cells (%)			<i>P</i>
A: Incubation in serum alone						
I	311	55.4	72	28.9	0.0001	
II	130	23.2	102	41.0	0.0001	
III	120	21.4	75	30.1	0.04	
Total	561		249			
B: Incubation in serum with benzamidine						
I	75	49.7	46	29.3	0.001	
II	61	40.4	92	58.6	0.01	
III	15	9.9	19	12.1	> 0.05	
Total	151		157			

Definition of abbreviations: I (fully granulated mast cells); II (partially degranulated mast cells); and III (completely degranulated mast cells). P is the probability of the c2 test when comparing nonsensitized and sensitized tissues.

largely degranulated mast cells (type III) (Table 3A). This phenomenon occurred in the different compartments of the bronchial wall.

Effect of passive sensitization on bronchial responsiveness

In another set of experiments, PS increased the maximal contractile response to histamine (112.5 ± 17.3 vs. 85.4 ± 10.6) and to *D. pter* (98.1 ± 16.0 vs. 0.0 ± 0.0). The percentage of the different types of degranulated mast cells was plotted against the amplitude of the contractile response to specific and nonspecific agonists in the different experimental conditions. The amount of partially degranulated mast cells (type II) following PS was positively correlated with the maximal contractile response to the non specific agonist, histamine (Fig. 3A). Similarly, the amount of largely degranulated mast cells (type III) was significantly correlated with the maximal contractile response to the specific antigen *D. pter* (Fig. 3B).

Effect of benzamidine on passive sensitization

The difference in the number of tryptase or chymase positive cells between passively sensitized and non-sensitized tissues was abolished when benzamidine was added to the asthmatic sera throughout the incubation time (Fig. 4A,B). However, benzamidine, *per se*, changed neither the number of mast cells as detected by the expression of *kit* receptor nor that of IgE bearing cells (figure 4c, d). Nevertheless, in terms of morphological analysis, PS performed in the additional presence of benzamidine, still significantly decreased the percentage of fully granulated mast cells and concomitantly increased partially degranulated mast cells (Table 3B). However, under these experimental conditions, the proportion of largely degranulated mast cells was similar in sensitized and nonsensitized specimens.

Effect of passive sensitization on tryptase release

Tryptase concentration measured in the supernatant of asthmatic sera following overnight incubation of human bronchi was significantly higher than that measured before (Fig. 5). When benzamidine was added to the incubating asthmatic sera throughout the sensitization protocol, it did not abolish the increase in tryptase concentration (Fig. 5, $P > 0.05$).

Discussion

The present results indicate that PS of human isolated bronchi with serum from asthmatic patients, induces, in the absence of antigen, a mast-cell degranulation associated with a tryptase release. In addition, this degranulation is correlated with PS-induced bronchial hyper-responsiveness.

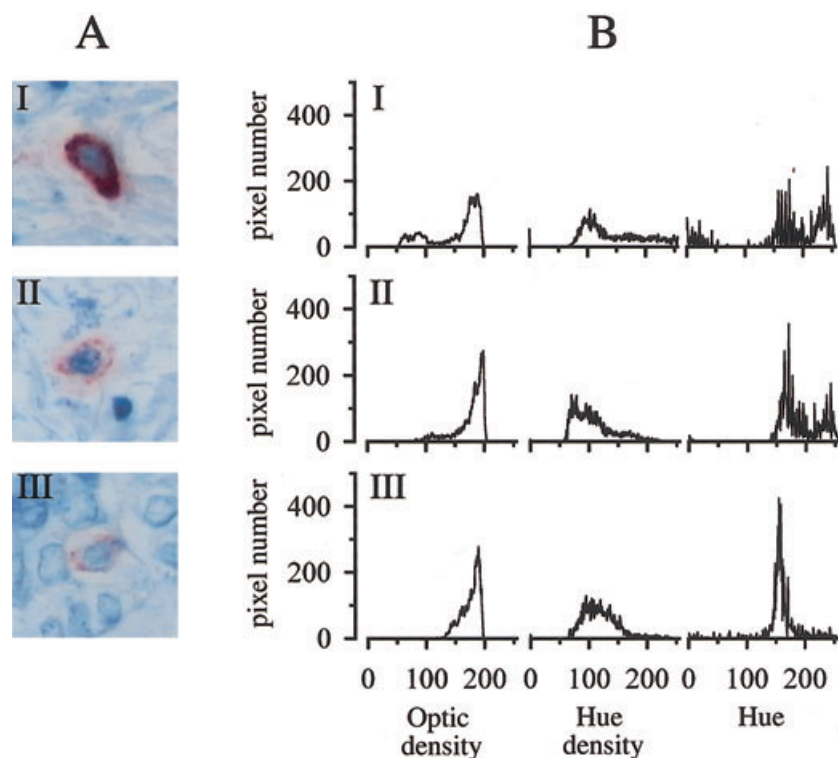


Figure 2. (A) Optic microscopic views (magnification $\times 400$) and (B) distribution histograms of isolated bronchi stained with anti-human tryptase. Mast cells are classified as fully granulated (I), partly degranulated (II), or largely degranulated (III).

PS of human isolated airways alters the mechanical response of human isolated airways to a variety of nonspecific agonists (4,7,8,9,23,28). Such a sensitization can be mimicked using monoclonal IgE (5), and *in vitro* bronchial hyper-responsiveness is closely related to the total IgE concentration in the 'sensitizing' serum (15). However, results obtained from experiments conducted in the presence of a monoclonal anti-IgE or after immunological removal of IgE indicate that the IgE molecule may not be directly responsible for the alteration in the contractile response (16,29). On the other hand, there is a growing body of evidence indicating that tryptase increases the contractile response of airways to nonspecific agonists in a variety of species including guinea pig (30), dog (31) and human (17,32). Because mast cells represent the main phenotype bearing IgE during PS (21), and are significantly increased in the smooth muscle layer in sensitized subjects (33), it was our hypothesis that PS in the absence of antigen could induce a mast-cell degranulation with a concomitant release of tryptase.

In the present work, we thus paid special attention to mast-cell markers in passively sensitized bronchi. For this purpose we used antibodies directed against proteases stored in cytosol granules (i.e. tryptase, chymase) and an antibody directed against a mast cell-surface receptor (i.e. CD117). Our results show that PS decreases the number of tryptase and chymase

positive cells, whereas the number of *kit* receptor positive cells remained unchanged. This observation strongly suggests that mast-cell degranulation occurred because in bronchial tissue, the *c-kit* expression is only ascribed to mast cells. Morphological analysis by optical microscopy confirmed this finding. Using a score previously reported by Pesci et al. (26) we found a significantly higher percentage of degranulated mast cells (i.e. types II and III) in passively sensitized specimens. However, whereas Pesci's method is based on toluidine blue staining (i.e. degranulation of metachromatic cells), we assessed the release of tryptase from mast-cell granules using a specific anti-tryptase MoAb to establish the degranulation score. In addition, in order to characterize the different profiles of mast-cell degranulation, we applied a computerized system of colour recognition able to provide for each pattern the distribution of optic density, hue density and hue, as previously described (22). Using such parameters, the different states of degranulation were clearly identified. This approach for mast-cell counting and identification may explain some discrepancies with previous studies using a traditional analysis (33). Experiments carried out in the presence of benzamidine showed that, in terms of mast-cell morphology, the inhibition of tryptase activity partially diminished the cell degranulation suggesting a mast cell membrane stabilizing effect for benzamidine. Such stabilizing activity has already

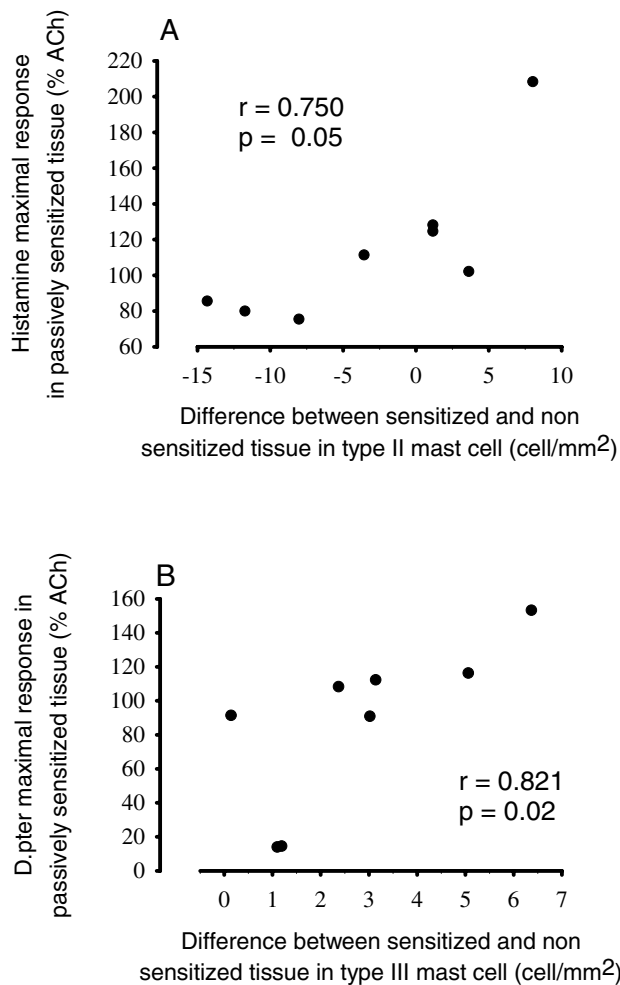


Figure 3. Correlation between (A) istamine- or *D. pter*-induced maximal contractile response (B) in passively sensitized tissue and mast-cell degranulation following PS.

been demonstrated for protease inhibitors in isolated mast cells and may be explained by the fact that tryptase could act as an amplification signal for mast-cell activation (34).

We also sought to demonstrate a concomitant release of tryptase following PS. Using an ELISA, we found that the concentration of tryptase significantly increased in the asthmatic serum used for bronchi sensitization. This finding is consistent with mast-cell degranulation observed in the present study and supports the idea that hyper-responsiveness induced by PS may well be related to tryptase (14,17). By contrast, benzamidine did not alter the tryptase concentration measured in serum following the sensitization procedure. This is consistent with morphological results because specimens treated with benzamidine demonstrated a lower proportion of largely degranulated mast cells but a higher proportion of partly degranulated ones.

Finally, we observed that mast-cell degranulation was significantly correlated with *in vitro* hyper-respon-

Passive sensitization mast cell degranulation

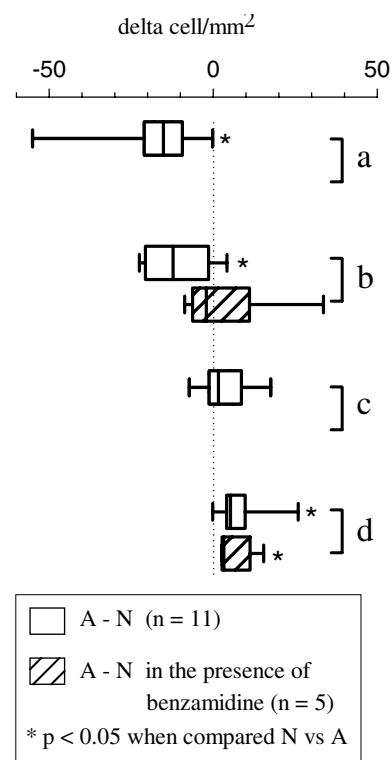


Figure 4. Differences in the number of bronchial cells between specimens incubated with asthmatic serum (A) and paired specimens incubated with normal serum (N). Box plots represent median with 25% and 75% interquartiles; the error bars represent the 5th and 95th percentiles. Cells are stained with (a) anti-tryptase, (b) anti-Chymase, (c) anti-*kit* receptor or (d) anti-IgE.

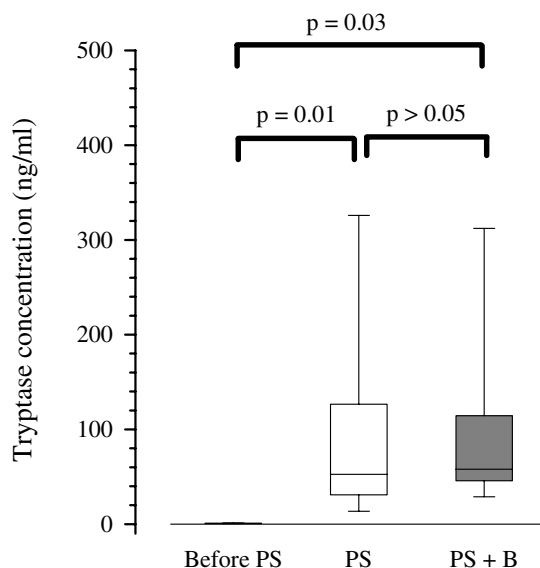


Figure 5. Tryptase concentration measured by ELISA in asthmatic sera, before and after PS, in the absence (white box) or in the presence (hatched box, PS+B) of benzamidine. Box plots represent median with 25% and 75% interquartiles; the error bars represent the 5th and 95th percentiles.

siveness in terms of both maximal response to histamine and to the specific antigen. These results are in agreement with those reported *in vivo*. It is well known that bronchial hyper-responsiveness is correlated with both mast-cell degranulation and an increase in the tryptase concentration in bronchoalveolar (BAL) fluid from asthmatics (20). In addition, a mast-cell degranulation has also been observed in bronchial mucosa from asthmatic patients particularly during periods of exacerbation (19). Therefore, there is evidence that mast-cell alteration is related to bronchial hyper-reactivity. However, all of the mast-cell alterations observed in the present work occurred in the absence of the antigen required for IgE receptor cross linking. The mechanism responsible for such alteration remains unknown. On the one hand, it could be ascribed to nonIgE-related triggers since the mast cell express several receptors specific for activating mediators (e.g., SCF, peptides). In addition, other components such as CD81 may also regulate mast-cell degranulation independently of FcεRI transduction (i.e. tyrosine phosphorylation, calcium mobilization, or leukotriene synthesis) (35). On the other hand, it has recently been demonstrated that monomeric IgE could stimulate signalling pathways in mast cells leading to an important cytokine production including interleukin

(IL)-4, tumour necrosis factor (TNF)-α, IL-13, and IL-6. An effect of monomeric IgE has previously been observed in other inflammatory cells (36,37) and may provide an explanation for the effect of IgE rich serum on human isolated airways.

In conclusion, we have shown that PS induces a mast-cell degranulation with a concomitant release of tryptase. These findings provide good evidence that hyper-responsiveness induced *in vitro* by the incubation of human bronchi with asthmatic serum is, at least in part, owing to mast-cell tryptase and is to some extent correlated with mast-cell activation even in the absence of a specific antigen. This experimental observation is in agreement with the *in vivo* situation during which antigen is absent for long periods of time although symptoms persist. The understanding of IgE receptor signalling in the presence of IgE alone requires studies that may prove to be clinically relevant.

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