

Mucosal mast cells mediate motor response induced by chronic oral exposure to ovalbumin in the rat gastrointestinal tract

E. TRAVER,^{*},[‡] R. TORRES,[†] F. DE MORA[†] & P. VERGARA^{*},[‡]

^{*}Department of Cell Biology, Immunology and Physiology, Universitat Autònoma de Barcelona, Spain

[†]Department of Pharmacology, Universitat Autònoma de Barcelona, Spain

[‡]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Spain

Abstract We previously demonstrated that oral chronic exposure to ovalbumin (OVA) causes intestinal hypermotility in Sprague-Dawley rats. In this study, the objective was to determine the mechanism of action of OVA and the role of mucosal mast cells in the regulation of motor activity in this model. Rats were orally exposed to OVA during 6 weeks. Intestinal mucosal mast cells (IMMCs) were counted and rat mast cell protease II (RMCP II) measured in duodenum, jejunum, ileum and colon. Anti-OVA IgE, IgG, and IL-4 were measured in serum. Eosinophils and IgE⁺ cells were counted in jejunum. In an additional study rats were treated with the mast cell stabilizer ketotifen and mast cell number, RMCP II concentration and motor activity *in vitro* were evaluated. OVA exposed rats showed an increase in mucosal mast cell number and in RMCP II content in small intestine and colon. However, variables of a Th₂ type response were not affected by exposure to OVA: (i) neither OVA specific IgE nor IgG were found; (ii) IL-4 did not increase and, (iii) the number of eosinophils and IgE⁺ cells was identical in the exposed and unexposed groups. These results brought us to hypothesize a possible non-Ig-mediated action of OVA on mast cells. Ketotifen significantly diminished the response to OVA: Ketotifen reduced the number of mast cells and the RMCP II content and blocked increased intestinal contractility. In addition ketotifen modified motor response in both OVA exposed and

unexposed animals giving evidence of the importance of mast cells in intestine motor activity driving.

Keywords capsaicine, food allergy, irritable bowel syndrome, ovalbumin, rat mast cell protease II.

INTRODUCTION

Irritable bowel syndrome (IBS) is a highly prevalent gastrointestinal motility disorder broadly characterized by abdominal pain/discomfort associated with altered bowel habits. The underlying mechanisms causing these symptoms still remain unclear but some patients associate the onset of symptoms with the ingestion of particular foods. However, identification of true food intolerance is still challenging^{1,2} because there is some evidence that response to food might not be always mediated by IgE and IgG mechanisms.^{3,4}

We previously demonstrated that chronic oral exposure to ovalbumin (OVA) in normal responder IgE rats caused an increase of mast cells in the duodenum and an increase of intestinal motor activity *in vivo*. This dysmotility could still be observed several weeks after the end of OVA exposure, and in contrast to other IBS experimental models, no signs of inflammation were observed.⁵ Other authors have shown that mast cell activation could be responsible for chronic changes in gastrointestinal motor activity that could explain some of the clinical signs of IBS.^{6,7}

Mast cells are associated with Th₂ type immune response, i.e. activation of Th₂ cells producing IL4 and leading to the induction of IgE or IgG synthesis and the activation and recruitment of eosinophils.⁸ In fact, mast cells are so widely recognized as effector cells in allergic disorders and other IgE-associated acquired

Address for correspondence

Patri Vergara, PhD, Unitat de Fisiologia (Edifici V), Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain.

Tel: +34-93-5811848; fax: +34-93-5812006;

e-mail: patri.vergara@uab.es

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immune responses, that it is difficult to think of them in any other context, despite the evidence that intestinal mucosal mast cells (IMMCs) can be activated by several non-immunological stimuli including neurotransmitters, neuropeptides, and nerve stimulation.^{5,9,10} In our previous study we observed a lack of systemic response to OVA, i.e., absence of detectable increases of IgE and the lack of systemic response to OVA challenge in animals previously exposed to this protein.⁵

Taking into consideration these previous findings, the objective of this study was to focus on: (i) the specificity of OVA to induce mast cell activation; (ii) the extension of the response in the intestine as well as the characterization of the systemic response in this model and (iii) the implication of mast cells in the development of motor changes. The following parameters were evaluated: (i) anti-OVA IgE and IgG; IL4, IgE⁺ cells and eosinophil count after exposure to OVA; (ii) Intestinal mucosal mast cell count in the whole small intestine and colon after exposure to OVA or to gelatine as well as after mast stabilization with ketotifen; (iii) measurement of mast cell activity by measuring rat mast cell protease II (RMCP^{II}) in the small intestine and colon; (iv) quantification of muscle contractility of jejunum and colon strips from rats exposed to OVA with or without prevention of mast cell degranulation with ketotifen.

MATERIALS AND METHODS

Animals and experimental groups

Five-week-old specific pathogen free (SPF) Sprague-Dawley (SD) rats were purchased from Charles River (Les Oncins, France). Animals had free access to water and a standard pellet diet that did not contain either any trace of OVA, any other egg derivative, or gelatine. Animals were maintained in an environmentally controlled room in groups of four animals in each cage. The night before the experiment, food was removed and each animal was caged individually. All experimental procedures were approved by the Ethical Committee of the Universitat Autònoma de Barcelona.

Oral exposure to ovalbumin

Following Saavedra and Vergara,⁵ 6-week-old rats ($n = 10$) were orally exposed to OVA (OVA Grade V, Sigma, St Louis, MO, USA) by gavage (1 mg OVA mL⁻¹ saline solution (SS), 1 mL per animal). Gavage dosing was performed daily during 6 weeks. A group of rats ($n = 10$) not exposed to OVA was used as a control (Unexposed) group in which the induction protocol was conducted as described before but replacing OVA by SS. Ten days after the exposure period, half of the exposed animals ($n = 5$) was submitted to saline challenge (0.5 mL of saline solution 0.9% NaCl) and the other half ($n = 5$) to OVA challenge (0.5 mL of 0.2 g mL⁻¹ OVA solution) by gavage. Unexposed animals were also challenged as

described for OVA exposed animals. The objective of this challenge was to evaluate the immediate response to OVA in both unexposed and OVA exposed rats. Animals were killed 1 h after the challenge.

Exposure to gelatine With the objective to determine the specificity of the response to OVA, an additional group ($n = 10$) was exposed to gelatine from cold water fish skin (Sigma), using the same methods as described for OVA. Another group not exposed to gelatine ($n = 10$) was used as a control. The challenge was performed as in OVA but with gelatine solution.

Positive controls As positive control of IgE response, a group ($n = 4$) of male Brown Norway (BN) rats, a high IgE responder breed (Charles River, Les Oncins, France), was orally exposed to OVA in the same way as SD rats. As a positive control of IgG and IL4 production, two male BN rats were sensitized to OVA by the 11 days sensitization protocol (OVA 11d BN) according to¹¹ while another two BN rats ($n = 2$) following the same protocol received saline solution (sham 11d BN).

Animals were killed by an overdose of anaesthesia mixture of ketamine (Imalgene®, Merial, Lyon, France) + xilacine (Rompun, Bayer, Kiel, Germany). Blood samples were taken from the portal vein. Afterwards, tissue samples from duodenum, jejunum, ileum and colon were removed and either fixed in 4% formaldehyde solution or kept frozen at -80°C .

Measurement of anti-OVA IgE in serum

Presence of anti OVA-specific IgE in serum was measured by ELISA as previously described¹² and by Passive Cutaneous Anaphylaxis (PCA) as described previously by Ovary.¹³

Measurement of anti-OVA IgG in serum

For the detection of anti OVA-specific IgG, 96-wells microtitre plates were coated overnight at 4°C with 100 μL per well of a 5 $\mu\text{g mL}^{-1}$ solution of OVA in carbonate buffer, pH 9.6. After 1 h of incubation with PBS/0.02%T20/1% BSA at 37°C , serial dilutions of rat serum in PBS/0.02%T20/1% BSA were added to the wells and incubated for 1 h at 37°C followed by incubation with 100 μL per well peroxidase conjugated goat anti rat IgG (H+L) (diluted 1 : 5000 Zymed, San Francisco, CA, USA.) in PBS/BSA-Tween 20 for 1 h and colour developed with tetramethylbenzidine following a standard ELISA procedure. Negative control sera were measured at a 1 : 4 dilution, providing the reference value taken to determine the titre of the test sera. Each test serum was titrated starting at a 1 : 4 dilution and the reciprocal of the furthest serum dilution giving an extinction >0.1 units above the reference value was read as the titre.

Measurement of IL4 in serum

Interleukin-4 concentration in the portal serum was measured by a commercial kit (BD Biosciences, San Diego, CA, USA).

Protein extraction

Intestinal segments from jejunum and mid colon were frozen in liquid nitrogen and stored immediately at -80°C . For protein extraction, the frozen gut samples were ground up into a powder with a pestle and mortar, and then diluted in extraction buffer

containing a set of protease inhibitors (Complete; Roche Molecular Biochemicals, Indianapolis, IN, USA) 100 mmol L⁻¹ PMSF and 1 mol L⁻¹ HEPES. After an incubation of 20 min in ice, samples were centrifuged at 21913 g (10 min, +4 °C) and supernatants were kept frozen at -30 °C.

Measurement of rat mast cell protease II

Rat mast cell protease II concentration in the intestinal protein extract was measured by ELISA using a commercial kit (Moredun Animal Health, Edinburgh, UK) as previously described.⁹

Rat mast cell protease II immunohistochemistry and intestinal mucosal mast cells count

Immunodetection of RMCPII was carried out on formaldehyde-fixed duodenum, jejunum, ileum and mid colon sections using a monoclonal antibody (Moredun Animal Health) as previously described.⁵ Positively stained cells were counted in three to five sections per animal. Seven to 10 well-oriented villus-crypt units (VCU) were examined per section at ×400 magnification.

IgE immunohistochemistry and IgE⁺ cell count

Immunodetection of IgE was carried out using a monoclonal antibody (Zymed). Sections were incubated with Citrate Buffer (pH = 6) in a steam bath (95 °C) for 20 min to increase antigenicity. Sections were counterstained with haematoxylin and the number of positive cells established as above.

Congo Red staining and eosinophil count

Formaldehyde-fixed sections from small intestine and colon were stained with Congo Red for detection of eosinophils. Sections were counterstained with haematoxylin. Positively stained cells were counted as described for mast cells.

Ketotifen treatment

An additional study was carried out with another four groups of SD rats ($n = 6$). Two of them were unexposed ($n = 6$) and two exposed to OVA ($n = 6$). One exposed and one unexposed group were treated with ketotifen fumarate salt, a mast cell membrane stabilizer (Sigma Chemicals). The treatment with ketotifen was performed from 1 day before the beginning of the exposure period until the euthanasia of the animals. Ketotifen was dissolved in drinking water at 0.1 mg mL⁻¹, which allowed dosing of ketotifen at 10 mg kg⁻¹ day⁻¹ as described previously.¹⁴ Water intake was controlled daily. Animals were killed by decapitation ten days after the end of the OVA exposure period. Tissue from jejunum and colon was taken and kept and proceed for IMMC counting and RMCPII measurement as described above.

Muscle contractility studies

Full thickness preparations were obtained from mid colon and were cut 1 cm long and 0.3 cm wide and hung for organ bath study oriented to record circular muscle activity. Longitudinal oriented full thickness jejunum sections 1 cm long were also studied.

Preparations were mounted under 1-g tension in a 10-mL muscle bath containing oxygenated Krebs solution (95% O₂ - 5% CO₂) at 37 ± 1 °C. The composition of Krebs solution was (in mmol L⁻¹): 10.10 glucose, 115.48 NaCl, 21.90 NaHCO₃, 4.61 KCl, 1.14 NaH₂PO₄, 2.50 CaCl₂, and 1.16 MgSO₄ (pH 7.3–7.4). Changes in tension of the strips were measured using isometric force transducers, recorded on a chart recorder (Grass Instruments Co., Quincy, MA, USA.), and digitized (AcqKnowledge MP100; BIOPAC Systems, Inc., Goleta, CA, USA). After 1 h equilibration, spontaneous contractility and response to carbachol (10 µmol L⁻¹, Sigma chemical) were measured. Response to capsaicine (0.1 µmol L⁻¹, Sigma) was also studied. Stock solutions were prepared by dissolving drugs in distilled water (carbachol), or DMSO (capsaicine).

In vitro stimulation of mast cell culture

A murine mast cell culture (C1.MC/57.1) was stimulated *in vitro*. After 10 min of centrifugation at 400 g, supernatants were discarded and pellet was dissolved in PBS/2% fetal bovine serum (FBS). Cell count was performed and viability was assessed. A plaque of 12 wells was used, 5 × 10⁵ cells in 400 µL of PBS/2%FBS was placed per well. In each well was added: (i) 100 µL of saline solution (SS), or (ii) OVA solution (10 and 100 ng mL⁻¹) or (iii) OVA hydrolysed (OVH) solution (10 and 100 ng mL⁻¹) or (iv) IgE-DNP (Dinitrophenol) + DNP-HSA (human serum albumin) (50 ng mL⁻¹). We incubated the plaque for 2 h at 37 °C (CO₂), and the reaction was stopped with ice. After 20 min of centrifugation at 500 g, 400 µL samples from the supernatants were taken. Pellet was dissolved in 200 µL of PBS. Samples were submitted to five cycles of freezing/thawing in order to break mucosal mast cell membranes and to release cellular contents. All the samples (supernatant and pellet) were frozen and stored at -30 °C until assayed for histamine by means of a commercial kit (IBL, Hamburg, Germany).

Statistics

All data are expressed as mean ± SEM. Statistical analysis of the results was performed with one-way ANOVA followed by a *post hoc* Bonferroni test. An unpaired student's *t*-test was also used when indicated. The linear correlation between RMCPII content and motor responses was also estimated. Differences between groups were considered statistically significant at $P < 0.05$.

RESULTS

Ovalbumin-induced changes in intestinal mucosal mast cell count

Oral exposure to OVA significantly increased IMMC numbers in all the areas of the intestine (Table 1): Duodenum showed similar results to those reported before,⁵ but in this case it was shown that the mast cell response to OVA affected the whole intestine. Fig. 1 shows a representative example of the increase of mast cells in the colon).

It is also noteworthy that intestine from rats (both exposed and unexposed) that received an OVA challenge 1 h before taking the samples showed a slightly

	Unexp/sham chal	Unexp/OVA chal	OVAexp/sham chal	OVAexp/OVA chal
IMMC per VCU				
Duodenum	6.12 ± 0.13	6.01 ± 0.43	9.95 ± 0.29***	10.57 ± 0.25***
Jejunum	7.48 ± 0.69	6.45 ± 0.26	11.44 ± 0.60*	10.43 ± 0.27*
Ileum	6.03 ± 1.54	5.00 ± 0.25	9.10 ± 0.74	9.54 ± 10.04*
Colon	2.64 ± 0.27	2.24 ± 0.22	5.33 ± 0.38***	4.54 ± 0.11***†
Eosinophil per VCU				
Duodenum	8.09 ± 0.75	7.20 ± 0.55	7.46 ± 0.58	7.13 ± 0.86
Jejunum	9.76 ± 0.41	9.80 ± 0.17	10.40 ± 0.06	10.87 ± 0.78
Ileum	5.30 ± 0.55	5.00 ± 0.16	5.06 ± 0.31	5.33 ± 0.19
Colon	2.98 ± 0.26	3.20 ± 0.23	3.06 ± 0.33	3.13 ± 0.17

IMMC, intestinal mucosal mast cell; VCU, villus-crypt unit; OVA, ovalbumin.

* $P < 0.05$ and *** $P < 0.001$ compared with its respective unexposed group; † $P < 0.05$ between sham and OVA challenge in the OVA exposed group (unpaired t -test).

Table 1 Intestinal mucosal mast cell (IMMC) number and eosinophils expressed per villus-crypt unit (VCU) in duodenum, jejunum, ileum and colon. Data expressed as mean ± SEM from five rats

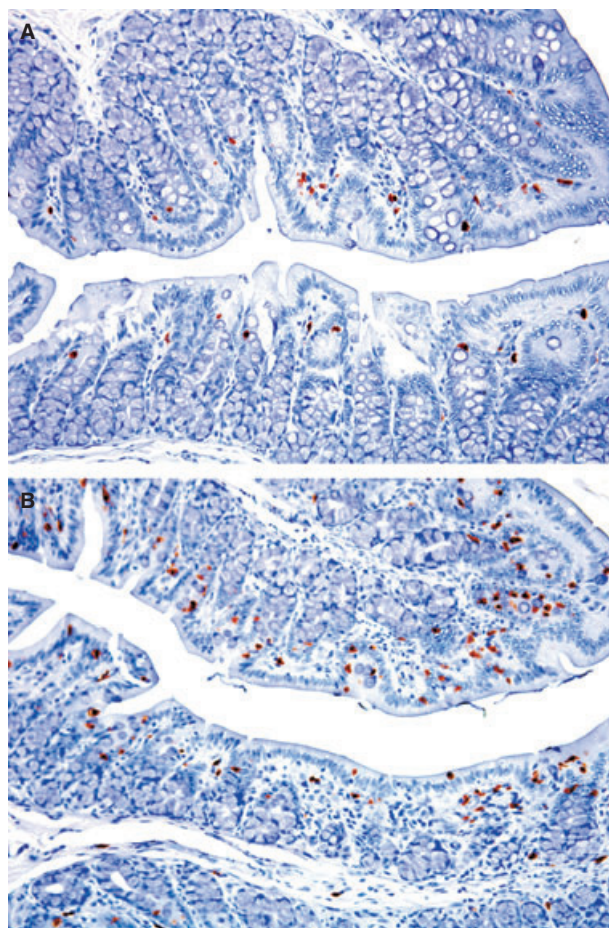


Figure 1 Microphotographs showing rat mast cell protease II (RMCPII) immunopositive cells (mucosal mast cells) in the intestinal mucosa of colon at 200× magnification. (A) Unexposed. (B) Ovalbumin (OVA) exposed.

lower IMMC number in all intestinal segments. This difference was more remarkable in the colon, reaching statistical significance (Table 1).

Characterization of OVA-induced local and systemic reaction

Ovalbumin but not gelatine-induced changes in IMMC count Oral exposure to gelatin (GEL) did not increase IMMC number [(Unexp/shamchal: 8.92 ± 0.52 ($n = 5$); Unexp/GELchal: 8.18 ± 0.78 ($n = 5$); GELexp/shamchal: 8.65 ± 0.54 ($n = 5$); GELexp/GELchal: 7.75 ± 0.25 ($n = 5$) in jejunum]. No difference in the number of MMC between unexposed with or without challenge in the GEL group was observed.

Ovalbumin-induced reaction is not of allergic type No increase in anti OVA-IgE was detected in the portal vein sera of either of the groups. In contrast, the positive control (OVA orally exposed BN rat sera) showed a significant increase in titre compared with the reference value ($\Delta = 2.2 \pm 0.8$ 2Log IgE titre). Furthermore, oral exposure to OVA did not locally increase IgE sensitized cell number in the intestinal mucosa (Table 2) whereas BN rats either unexposed or OVA exposed showed a significantly higher number of IgE⁺ cells (data not shown). A similar result was obtained performing PCA assay: none of the sera caused Blue Evans extravasation at the injection site. In contrast, the positive control sera (OVA orally exposed BN) induced swelling in the corresponding site.

No increase in anti OVA-specific IgG was detected in any of the portal vein sera of either of the groups. However, as in anti OVA-IgE titration, there was an increase in the titre in the positive control (OVA 11d BN rat sera) compared to the reference value ($\Delta = 2.5 \pm 0.2$ 2Log IgG titre).

Oral exposure to OVA did not affect Th₂ response-related parameters such as eosinophil count and IL4 concentration. The number of eosinophil cells at all parts of the intestinal mucosa remained unchanged in the OVA-exposed groups (Table 1). In a similar way,

Table 2 IgE⁺ cell number expressed per villus-crypt unit (VCU) in jejunum and IL4 concentration in portal serum after oral exposure to OVA expressed per pg mL⁻¹. Data expressed as mean $\pm$ SEM from five rats

	Unexp/sham chal	Unexp/OVA chal	OVAexp/sham chal	OVAexp/OVA chal
IgE ⁺ cells per VCU	2.08 $\pm$ 0.33	2.17 $\pm$ 0.24	1.89 $\pm$ 0.13	1.88 $\pm$ 0.37
IL4[pg mL ⁻¹]	1.92 $\pm$ 0.04	1.86 $\pm$ 0.01	1.86 $\pm$ 0.01	1.98 $\pm$ 0.09

OVA, ovalbumin; VCU, villus-crypt unit.

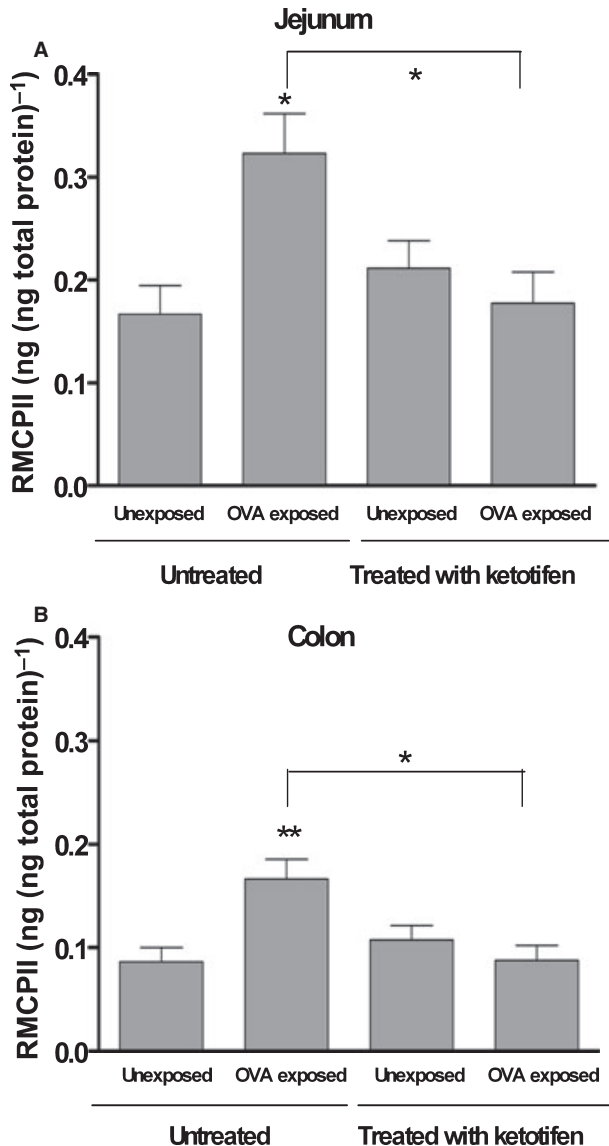


Figure 2 Rat mast cell protease (RMCPII) content in protein extract expressed as ng per ng of total protein in jejunum (A) and colon (B) of Unexposed (Unexp) and OVA exposed (OVAexp) and in similar groups treated with ketotifen (ket). Bars represent mean $\pm$ SEM from six rats. * P < 0.05 and ** P < 0.01 in relation to unexposed group or as indicated.

exposure to OVA did not increase IL4 concentration in portal vein serum (Table 2). By contrast, the positive control (OVA 11d BN) gave a much higher concentra-

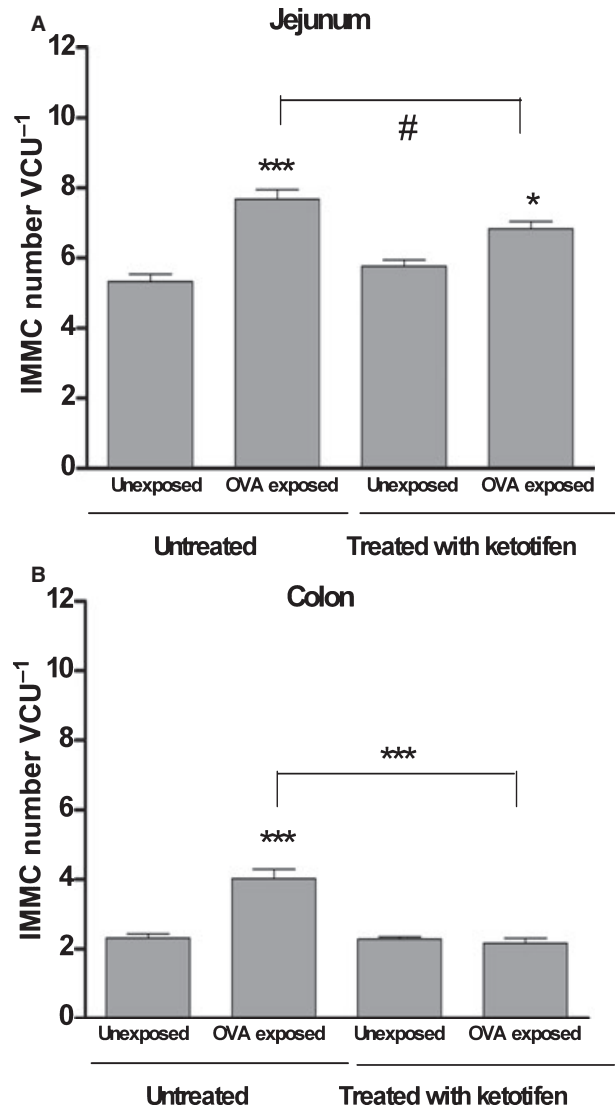


Figure 3 Intestinal mucosal mast cell (IMMC) number expressed per villuscrypt unit (VCU) in jejunum (A) and colon (B) of Unexposed (Unexp) and OVA exposed (OVAexp) and in similar groups treated with ketotifen (ket). Bars represent mean $\pm$ SEM from six rats. * P < 0.05 and *** P < 0.001 in relation to unexposed group or as indicated; P < 0.05 between indicated groups (unpaired t-test).

tion (409.9 pg mL⁻¹) compared to the reference value. Even sham-sensitized BN rat sera (sham 11d BN) showed a higher IL4 concentration (8.2 pg mL⁻¹) than SD, OVA exposed rats.

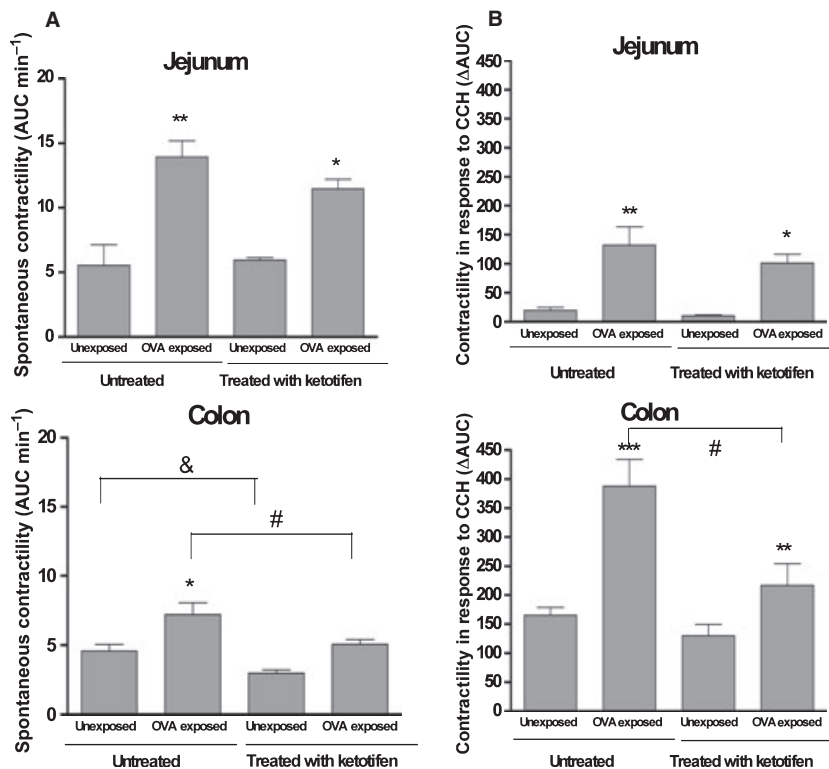


Figure 4 Spontaneous motor activity (A) measured as the area under the curve (AUC) per minute and response to carbachol (B) as the increment of AUC (Δ AUC) in Unexposed (Unexp) and OVA exposed (OVAexp) and in similar groups treated with ketotifen (ket). Bars represent mean $\pm$ SEM from six rats. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ in relation to unexposed group or as indicated; & #, $P < 0.05$ between indicated groups (unpaired *t*-test).

Effect of ketotifen on OVA-induced increase in RMCPII intestinal content and mast cell count

Rat mast cell protease II concentration in the intestinal extract of chronically OVA exposed rats was higher in both the small intestine (jejunum) and colon. Ketotifen did not induce any changes on RMCPII concentration in the intestinal extract both in jejunum and colon (Fig. 2). Similarly, ketotifen in the unexposed group caused no effect in IMMC number. However, the treatment with ketotifen in the OVA exposed group reduced the increase of the IMMC number. This effect was more remarkable in the colon where mast cell number was similar to non-exposed animals (Fig. 3).

Ovalbumin-induced changes in intestinal contractility and ketotifen effect

Chronic oral exposure to OVA caused an increase in spontaneous contractility both in jejunum and colon muscle. Treatment with ketotifen decreased motor activity in the colon of the unexposed group. In addition, ketotifen diminished the response to OVA in both the jejunum and colon (Fig. 4A).

Furthermore, the correlation between spontaneous contractility and RMCPII content was analysed by linear regression using the data from all groups

($n = 24$). In the colon RMCPII content was found to correlate highly with spontaneous contractility ($P < 0.0001$, $r^2 = 0.6071$) whereas in the jejunum the correlation was near to but did not reach statistical significance ($P < 0.0558$, $r^2 = 0.1633$).

Similarly, OVA caused an increase in the response to carbachol both in jejunum and colon. The treatment with ketotifen decreased carbachol response in the OVA exposed group (Fig. 4B). A correlation between the response to carbachol and RMCPII content was found in the colon ($P < 0.0001$, $r^2 = 0.6732$) but not in the jejunum ($P < 0.1451$, $r^2 = 0.0983$).

Capsaicine induced an increase of spontaneous activity in both jejunum and colon of OVA exposed rats. Ketotifen, both in non-exposed and OVA exposed rats, turned the response to capsaicine into an inhibition of the spontaneous activity (Fig. 5).

Ovalbumin-induced mast cell activity in vitro Both OVA and OVH solution significantly increased the percentage of histamine released *in vitro*. However, no correlation was found between the dose and magnitude of response (Table 3).

DISCUSSION

This study demonstrates that oral exposure to OVA increases IMMC number *in vivo* in all areas of the

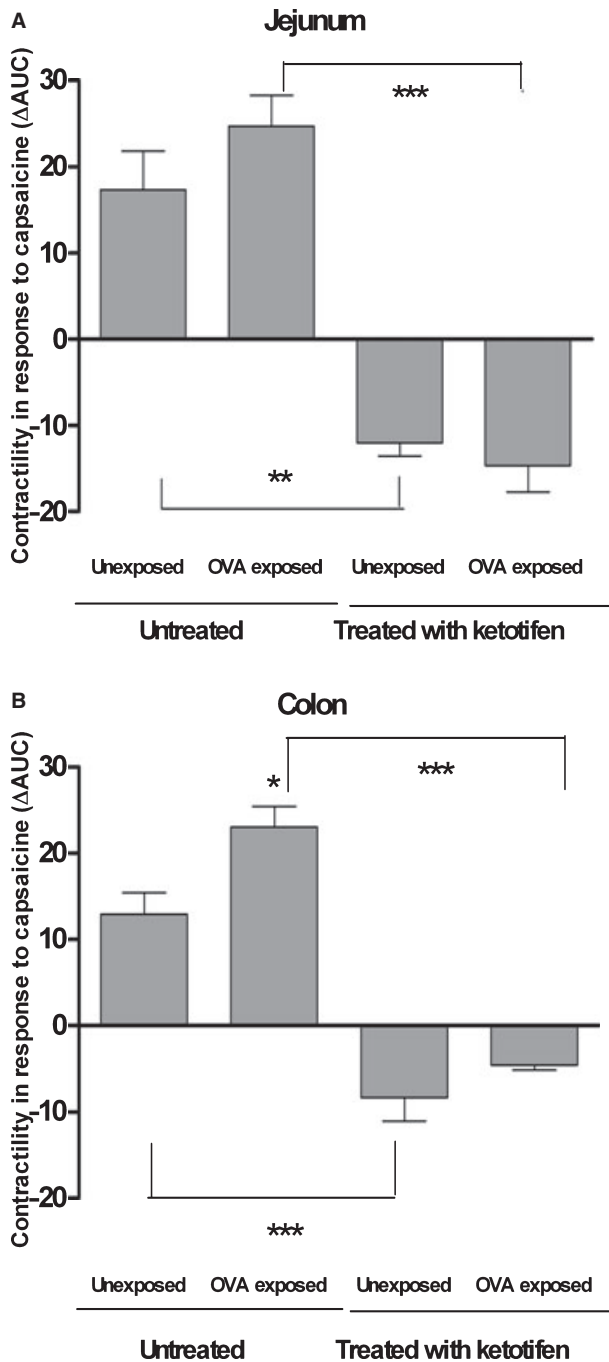


Figure 5 Motor response to capsaicin measured as the increment of AUC (ΔAUC) in jejunum (A) and colon (B) of Unexposed (Unexp) and OVA exposed (OVAexp) and in similar groups treated with ketotifen (ket). Bars represent mean $\pm$ SEM from six rats. ** $P < 0.01$ and *** $P < 0.001$ in relation to unexposed group or as indicated.

intestine. This response was not mediated by either anti-OVA specific IgE or IgG, eosinophil or IL4. In addition, we demonstrate that mast cells are responsi-

ble for the motor changes observed after exposure to OVA. A possible direct effect of OVA, or its digested fragments, multiplied by a positive feedback of activated mast cells is suggested by the results.

We had previously observed that OVA oral exposure increased IMMC number in the duodenum.⁵ In the present study the results demonstrate that the response to OVA affects the entire small intestine and the colon, as shown by the significant increase in the IMMC number in all intestinal sections of OVA exposed rats. In fact, the duodenum seems to be the least affected area indicating that an enzymatic hydrolysis of OVA may be necessary to induce a response. By contrast, the colon was the section where the effect of OVA was stronger; showing that hypersensitivity to some foods could be a cause of IBS.^{15,16} In parallel to the increase of mast cell, the RMCPII increase observed in the small intestine and in the colon indicates an enhanced mast cell activity under chronic exposure to OVA.

An increase in the number of mast cells has been found recently in diarrhoea-predominant IBS patients with no significant increase of tryptase either in serum or intestinal lumen.¹⁷ This result suggests that mast cells release their mediators in a piecemeal way or that the continuous exposure to the antigen causes a continuous response. In the present study, the small but significant degranulation observed after OVA challenge further supports this hypothesis.

It is generally accepted that responses to food allergens are mediated by IgE or IgG.^{12,18,19} However, in our study neither anti-OVA IgE nor IgG were found in portal serum from SD rats exposed to OVA. Similarly, oral exposure to OVA did not increase IgE⁺ cells in the intestinal mucosa. In contrast, an increase in all these parameters was observed in the BN rats used as a positive control indicating that our methodology was able to detect an immune response in accordance with other studies.¹⁰ Our results could look unexpected given that OVA-exposed SD rats have been widely used as a model of OVA allergic response. However, in most protocols of sensitization, OVA exposure is parenteral and systemic by injection while the challenge is given orally only after the animal has been sensitized.¹¹

To check if the response to OVA could have a Th₂ component despite no IgE or undetectable amounts as previously reported,²⁰ both eosinophil count and IL4 concentration were measured in our study. The results showed that there was no Th₂ component. This fact seems to reassert that response to oral OVA in SD rats is not of an allergic nature. Interestingly, in a study of oral sensitization to food proteins where cholera toxin

Table 3 Percentage of histamine release from mast cell C57 after stimulation with saline solution (SS, 0.9% NaCl), IgE-DNP + DNP-HSA (50 ng mL⁻¹) OVA solution (10, 100 ng mL⁻¹) and OVH solution (10, 100 ng mL⁻¹), expressed as mean $\pm$ SEM from five wells

Histamine release	SS (0.9% NaCl)	IgE-DNP + DNP-HSA (50 ng mL ⁻¹)	OVA (10 ng mL ⁻¹)	OVA (100 ng mL ⁻¹)	OVH (10 ng mL ⁻¹)	OVH (100 ng mL ⁻¹)
Percentage (%)	9.87 $\pm$ 0.67***	89.22 $\pm$ 3.19	33.22 $\pm$ 10.23	18.38 $\pm$ 2.03*	16.42 $\pm$ 1.76*	12.83 $\pm$ 3.04

SS, saline solution; NaCl, sodium chloride; DNP, dinitrophenol; HSA, human serum albumin; OVA, ovalbumin; OVH, OVA hydrolysed.

* $P < 0.05$ and *** $P < 0.001$ compared with its respective control (unpaired *t*-test).

was used as adjuvant a systemic Th₂ response was observed.²¹ This indicates that the adjuvant was necessary to induce immune sensitization to food proteins when given orally and further supporting our hypothesis that without the use of an adjuvant response to proteins will be not immune mediated in normal IgE responder subjects.

The fact that a slight decrease in the mast cell number, principally in the colon, was observed after a single exposure to OVA suggests together with the response of the cultivated mast cells to OVA and OVH a possible direct effect on mast cells. Although the results observed with the mast cell line need to be taken with precaution, they are an indication that OVA, or more probably OVA digested fragments, are able to stimulate mast cells. One argument against our results could be that OVA stimulation of mast cells might have been influenced by lipopolysaccharides (LPS) present in the OVA preparation since it is well established that antigen preparations usually contain bacterial LPS and these have been suggested to contribute to spontaneous allergen-induced response in patients.²² However, the intestine contains high amounts of LPS due to gut microbiota and, moreover, an immune response was not observed that could indicate that LPS could be acting as an adjuvant. Furthermore, it has been previously demonstrated that OVH is able to stimulate IMMC by endocrine mechanisms.²³ Other authors have observed that OVA stimulation on mast cells increases the expression of cytokines *in vitro* and induces TNF- α production *in vivo* in an IgE-independent manner.²⁴

The results using ketotifen show two interesting results: (i) that mast cell numbers and mast cell activity are positively driven by mast cells and (ii) that mast cell activity is implicated in motor alterations induced by OVA exposure. We previously demonstrated the existence of a positive feed back both in mast cell numbers and RMCPII release in a postinfectious model of IBS.¹⁴ The current study corroborates this positive feedback even when the mast cell

response is more subtle and not influenced by inflammation.

Mast cell mediators have been demonstrated to be crucial in the development of increased motor activity to expel intestine parasites.^{14,25} Muscle activity results in our study demonstrate: (i) that OVA induced an increase of muscle activity measurable *in vitro* in a similar way as was demonstrated *in vivo*;⁵ (ii) there is a linear correlation between RMCPII in the colon and muscle contractility in the intestine; (iii) stabilization of mast cells by ketotifen blocks increased contractility. Moreover, our study shows that especially in the colon, mast cells drive muscle contractility even in physiological conditions. It is interesting to remark that, for all parameters studied, there was a more remarkable effect in the colon, while the total amount of OVA or OVA fragments reaching the colon must necessarily be very small. Although our results do not provide any information on the mechanism(s) that makes this possible, it has been reported²⁶ that the distribution of mast cell phenotype in the colon differs from that of the small intestine. Our finding also provides an indication of why the colon is the target organ for IBS.

Another important point of our results is that chronic treatment with ketotifen changed the type of response to capsaicin. Capsaicin, agonist of the transient receptor potential vanilloid type-1 (TRPV1) receptor in sensitive fibres, has already been reported to modify sensitivity of intrinsic reflexes in the rat intestine.²⁷ Mast cells are in close proximity to vagal nerves and modify both intrinsic and extrinsic reflexes.²⁸ The fact that ketotifen modifies the response to capsaicin so dramatically indicates that mast cell regulation of gut reflexes is complex and involves sensitive afferents. Furthermore, a correlation between mast cell, substance P fibres and capsaicin receptor TRPV1-expressing sensory fibres in IBS has been reported.²⁹

In conclusion, we have demonstrated that mast cells are involved in the response to OVA *in vivo* despite no other signs of allergic inflammation being demonstrated. Our results suggest that the sustained

stimulation upon mast cells could be responsible for chronic changes in the intestine leading to dysmotility. Our study shows that exacerbated mast cell activity, although moderate in its magnitude, could be induced as a response to some food components. This altered mast cell activity is the cause of altered motor responses, mainly in the colon, and in consequence, our study shows a link between food components and IBS.

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