



HAL
open science

COLONIC MAST CELLS IN CONTROLS AND SLOW TRANSIT CONSTIPATION PATIENTS

Gabrio Bassotti, Vincenzo Villanacci, Riccardo Nascimbeni, Moris Cadei,
Stefania Manenti, Giuseppe Sabatino, Christoph A Maurer, Gieri Cathomas,
Bruno Salerni

► **To cite this version:**

Gabrio Bassotti, Vincenzo Villanacci, Riccardo Nascimbeni, Moris Cadei, Stefania Manenti, et al..
COLONIC MAST CELLS IN CONTROLS AND SLOW TRANSIT CONSTIPATION PATIENTS.
Alimentary Pharmacology and Therapeutics, 2011, 34 (1), pp.92. 10.1111/j.1365-2036.2011.04684.x .
hal-00637804

HAL Id: hal-00637804

<https://hal.science/hal-00637804>

Submitted on 3 Nov 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**COLONIC MAST CELLS IN CONTROLS AND SLOW TRANSIT
CONSTIPATION PATIENTS**

Journal:	<i>Alimentary Pharmacology & Therapeutics</i>
Manuscript ID:	APT-0385-2011.R1
Wiley - Manuscript type:	Original Scientific Paper
Date Submitted by the Author:	14-Apr-2011
Complete List of Authors:	Bassotti, Gabrio; Gastroenterology & Hepatology SEction, Clinical & Experimental Medicine Villanacci, Vincenzo Nascimbeni, Riccardo Cadei, Moris Manenti, Stefania Sabatino, Giuseppe Maurer, Christoph Cathomas, Gieri Salerni, Bruno
Keywords:	Large intestine < Organ-based, Constipation < Topics, Histopathology < Topics, Motility < Topics

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

COLONIC MAST CELLS IN CONTROLS AND SLOW TRANSIT CONSTIPATION PATIENTS

*Gabrio Bassotti, °Vincenzo Villanacci, §Riccardo Nascimbeni,
°Moris Cadei, °Stefania Manenti, *Giuseppe Sabatino,
#Christoph A Maurer, ##Gieri Cathomas, §Bruno Salerni

**Gastroenterology & Hepatology Section, Department of Clinical and Experimental
Medicine, University of Perugia; °2nd Pathology Unit, Department of Pathology and
§2nd Surgical Chair, Department of Surgery, University of Brescia, Italy;
#Department of Surgery and ##Cantonal Institute of Pathology, Liestal Hospital,
Switzerland*

Running head: colonic mast cells in constipation

Key words: colon, mast cells, slow transit constipation

CONFLICT OF INTEREST STATEMENT: NONE DECLARED

Address for correspondence:

Professor Gabrio Bassotti

Clinica di Gastroenterologia ed Epatologia

Ospedale Santa Maria della Misericordia

Piazza Menghini, 1

06156 San Sisto (Perugia), Italy

Fax: +39-075-584-7570

Email: gabassot@tin.it

Summary

Background. There is recent evidence that mast cells (MC) may play important roles in the gut, especially concerning visceral hypersensitivity and motor activity. However, most data are only available for clinical conditions characterized by diarrhea, where MC have chiefly investigated in the mucosal layer of the colon, and there is almost no information concerning constipation.

Aim. To investigate MC distribution in all colonic layers in controls and severely constipated patients.

Methods. Full-thickness specimens from colons of patients undergoing surgery for slow transit constipation (n= 29), compared **to controls**, were obtained and the number of MC (evaluated by **specific monoclonal antibodies**) counted as a whole and in single colonic segments (cecum, ascending, transverse, descending, and sigmoid).

Results. Compared to controls, constipated patients revealed significantly higher number of MC, both as overall number and in single colonic segments. The distribution of MC resulted fairly homogeneous in the various segment of the large bowel, in both controls and patients, **and no significant difference in the percentage of degranulated cells was found between groups.**

Conclusions. Colonic MC display a homogeneous distribution within the viscus. This cell population is shown to increase in severely constipated patients, and this might represent a compensatory mechanism trying to vicariate for the impaired propulsive activity of these patients.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Introduction

Considered for many years to be cells specifically participating in allergic reactions, mast cells (MC) **have also relevant** roles in both innate and adaptive immunity and play a role in many different processes, including clearance of enteric pathogens, food allergies, visceral hypersensitivity, and intestinal cancer [1].

In the human gut MC, by interacting with other cell types, may influence motor and sensory functions [2]. **Thus, MC are** increased in the colonic mucosa of patients with irritable bowel syndrome (IBS) [3], **and correlated** with symptoms (especially pain and **diarrhoea**) in these subjects [4-8]. Interestingly, it has been demonstrated that mucosal MC mediators from IBS patients excite nociceptive visceral sensory nerves in experimental animal studies [9], providing some possible explanation **on the mechanisms** of visceral hypersensitivity in these patients.

However, MC could also play some role in other pathological conditions characterized by constipation as expression of an abnormal motility of the colon. For instance, it has been shown that patients with megacolon due to Chagas's disease display an increased number of MC **in the colon** [10-12], and the same is true for patients with Hirschsprung's disease [13-16].

Slow transit constipation (STC) is a subtype of chronic functional constipation

sometimes requiring surgery for symptom relief [17]. Since to date there are no data on the presence and distribution of colonic MC in **constipated patients**, purpose of the present study was to study these cells in STC patients, and compare the result with those obtained in control subjects.

Patients

Surgical full-thickness specimens from twenty-nine patients (24 women, 5 men, age range 27-75 yrs) undergoing subtotal colectomy with ileorectostomy for severe intractable constipation were **evaluated**. Twenty of these patients have previously been investigated in a study on enteric nervous system (ENS) abnormalities [18]. Inclusion criteria were: 1) long-standing history of constipation (more than three years); 2) one or fewer evacuations per week; 3) no frequent (more than two episodes per month) or chronic abdominal pain; 4) sensation of incomplete evacuation in >1/4 defecations; 5) negative history for (sub)occlusive episodes; 6) unresponsiveness to appropriate and intensive medical treatment, including high fiber diet, stimulant and osmotic laxatives, and enemas. Intestinal transit time, measured by means of radiopaque markers, was delayed in all patients (up to more than 240 hours). Causes of secondary constipation were excluded by drug history, physical examination and laboratory screening (blood chemistry, thyroid hormones and, where appropriate, oral glucose tolerance test, sex hormone profiles and antinuclear antibodies). To exclude organic diseases or mechanical causes of constipation and megacolon or megarectum, each patient underwent double-contrast barium enema and/or colonoscopy. Absence of Hirschsprung's disease was demonstrated by normal relaxation of the internal anal sphincter at anorectal manometry [19]. No patient had evidence of obstructed defecation, as documented by anorectal manometry and/or defecography.

At the time of surgery all patients were treated with osmotic laxatives (polyethylene glycol); two patients used antihypertensive drugs (lisinopril).

Controls

Since right and left sections of the colon were analyzed in patients, for cecum, ascending and transverse we obtained control samples from 10 patients (8 women, 2 men, age range

1
2
3 38-70 years) undergoing right hemicolectomy, and for descending and sigmoid from 10
4
5 patients (6 women, 4 men, age range 41-78 years) undergoing left hemicolectomy, both
6
7 for non-obstructing cancer. These patients were not constipated and had no colonic
8
9 dilatation. **At the time of surgery the patients were prepared with polyethylene glycol;**
10
11 **three subjects used antihypertensive drugs (two lisinopril and one propranolol).** The
12
13 control specimens were taken at least 3 cm from the resection margin in tumour free
14
15 areas.
16
17
18
19

20 21 22 **Methods**

23
24 Tissues were fixed in 10% neutral-buffered formalin for 24 hours, then full-thickness
25
26 samples **from multiple colonic segments** (cecum, ascending, transverse, descending,
27
28 and sigmoid areas) were taken and transversal sections obtained. **Sections were**
29
30 **processed for both conventional histology (H&E and Trichrome stain) and**
31
32 **immunohistochemistry (IHC).**
33
34

35
36 **MC detection.** A specific antibody targeting MC tryptase [20] (monoclonal mouse clone
37
38 10D11, dilution 1:200, Novocastra, UK) was used. Paraffin sections were dewaxed and
39
40 rehydrated through decreasing alcohol series up to distilled water. Sections were then
41
42 subjected to heat-induced epitope retrieval by immersion in a heat-resistant container filled
43
44 with citrate buffer solution (pH 6.0) placed in a pressure cooker and microwaved for 20
45
46 minutes. Endogenous peroxidase activity was suppressed by incubation with 3% solution
47
48 of H₂O₂ for 5 minutes.
49
50
51

52
53 **In addition, MC were also assessed by an anti-Kit antibody (CD117, rabbit polyclonal**
54
55 **antibody, IgG, dilution 1:50, Dako, Carpinteria, CA); sections were processed as**
56
57 **above and Kit immunostaining was carried out using a peroxidase-based**
58
59 **visualization kit (Dako EnVision™), following the manufacturer's recommendations.**
60

Data analysis

Slides from patients and controls were coded and read in blind **by one pathologist (VV)**.

For each patient, the number of immunopositive cells was calculated and expressed as the mean of cells on 10 well stained and well oriented microscopic fields for each region of interest at x 40 magnification (Olympus BX 40). For each anatomic area (cecum, ascending, transverse, descending, and sigmoid) MC were counted in the mucosa, submucosa, inner muscle layer, outer muscle layer, and serosa. **The percentage of degranulated MC in the mucosa was also calculated for both groups. We categorized MC as intact or degranulated according to previously described criteria [21]. Cells were counted as intact if they had discrete, focal, and well circumscribed immunostaining for tryptase, whereas the degranulated ones had discrete and relatively focal staining for tryptase but did not have well circumscribed immunostaining.**

Moreover, since in previous investigations MC were found closely to nerve fibres [22,23], we evaluated whether these cells were also present at the level of the submucous or myenteric plexus.

Statistical analysis

Data were firstly analyzed with the D'Agostino-Pearson test, and resulted normally distributed; therefore, comparisons were made by the Student's t-test for independent values, the analysis of variance (ANOVA) for repeated measures, **and the test for difference of proportions**. Values of $p < 0.05$ were chosen for rejection of the null hypothesis. Data are presented as median (95% CI).

Ethical considerations

Since this was a retrospective study, no individual patient identification was involved and no study-driven clinical intervention was performed; therefore a simplified IRB approval was obtained.

Results

Conventional histology. No increased amount of fibrosis was found in patients, compared to controls, for both H&E and Trichrome stain.

Immunohistochemistry. As a first observation, it was found that the overall number of MC, in both controls and patients, decreased from the mucosal to the serosal surface.

Table 1 shows MC distribution in the mucosa of controls and patients and representative pictures are shown in Figure 1 (A-D).

Overall, MC were significantly increased in patients compared to controls ($p < 0.001$), even in single colonic segments. Interestingly, MC distribution was fairly homogeneous within different colonic segments in controls (ANOVA, $p = 0.79$) and STC patients (ANOVA, $p = 0.38$).

Table 2 shows MC distribution in the submucosa of controls and patients. Again, MC were significantly increased in patients compared to controls in the entire colon ($p = 0.001$), and in the various colonic segments; each separate segment of the viscus displayed homogeneous distribution of this cell population in both controls (ANOVA, $p = 0.32$) and STC patients (ANOVA, $p = 0.61$).

Table 3 shows MC distribution in the inner muscle layer of controls and patients. No significant differences were found between the two groups ($p = 0.09$), the single colonic segments, and the cells' distribution within segments in controls (ANOVA, $p = 0.7$) and patients (ANOVA, $p = 0.52$).

1
2
3 Table 4 shows MC distribution in the outer muscle layer of controls and patients. The
4
5 overall number of cells was significantly increased in patients compared to controls ($p=$
6
7 0.002), whereas comparison of single segments showed no differences between groups in
8
9 the ascending and descending colon. Distribution of MC within different colonic segments
10
11 was slightly but significantly different in controls (ANOVA, $p= 0.0048$), but not in STC
12
13 (ANOVA, $p= 0.5$).
14
15

16
17 Table 5 shows MC distribution in the serosa of controls and patients. Again, no significant
18
19 differences were found between the two groups ($p= 0.36$), the single colonic segments,
20
21 and the cells' distribution within segments in controls (ANOVA, $p= 0.07$) and STC patients
22
23 (ANOVA, $p= 0.50$).
24
25

26
27 **MC numbers obtained by CD117 stain almost overlapped those obtained by trypsinase**
28
29 **stain (data not shown), and the MC were morphologically differentiated by**
30
31 **interstitial cells of Cajal by their different aspect. The percentage of degranulated**
32
33 **MC (Figure 2 A) in the mucosa was not different between controls (1.07%) and**
34
35 **patients (2.5%, $p= 0.13$), and in H&E sections these cells were always associated to**
36
37 **the presence of eosinophils (Figure 2 B). No MC were found contacting neurons**
38
39 **within the submucous or myenteric plexus (Figure 2, C and D).**
40
41
42
43
44

45 46 Discussion

47
48 Gastrointestinal MC are considered as a heterogeneous population of immunocytes that
49
50 may be subdivided in mucosal MC (mainly located in the mucosa) and connective tissue
51
52 MC (distributed in the serosal layer) [24]. There are **only a few** data on the distribution of
53
54 this cell population **within** the gut [25]; studies in experimental animal models showed that
55
56 MC are evenly distributed along the **gastrointestinal tract** [26], **whereas data available**
57
58
59
60

1
2
3 **for** the human colon (but only from the mucosa) reported a decreasing density from the
4
5 cecum to the rectum [3], and an age-related reduction of MC in the rectal mucosa [27].
6
7
8 The present study, conducted on full thickness surgical specimens, **investigated** the
9
10 distribution of colonic MC in normal subjects and patients with STC. We showed that this
11
12 cell population displays a decreasing inward-outward (from mucosa to serosa) gradient,
13
14
15 **that its density** is relatively constant in the different bowel layers of various colonic
16
17 segments in both controls and constipated patients, and that the latter display a significant
18
19 increase of MC, compared to controls, in the colonic layers of all colonic segments, except
20
21 for the inner muscle layer and the serosa.
22
23

24 **We chose to detect MC by IHC for tryptase and CD117, two sensitive and specific**
25
26 **methods [28]. Although tryptase may be considered more specific for MC [29], the**
27
28 **results of tryptase and CD117 stains show a highly significant correlation (as in the**
29
30 **present study) [30,31], so that staining for mast cell tryptase alone apparently is**
31
32 **sufficient.**
33
34

35
36 What is the significance of these findings? MC have been chiefly investigated in the
37
38 **duodenal and** colonic mucosa of IBS patients [32-34], where they are thought to
39
40 represent an important pathogenetic factor and a potential therapeutic target [36-38].
41
42
43 However, **MC** have been less extensively studied in patients complaining of constipation.
44
45
46 Apart from sporadic observations of an increased number of **colonic** mucosal MC in
47
48 constipation-predominant IBS patients [39], the only available studies in constipated
49
50 patients were obtained in chagasic patients [10-12] and in those with Hirschsprung's
51
52 disease [13-16]. Concerning Chagas' disease, **the increase of MC** found in these subjects
53
54 **has** been put in relation to the increased fibrosis found in the colon of these patients [11],
55
56
57 whereas in Hirschsprung's disease **this** increase has been hypothesized as a possible
58
59 mechanism of ENS reparation [15].
60

1
2
3 Although we did not find an increased amount of fibrosis in the colon of our patients, we
4 feel that, since MC are a source of nerve growth factor [40,41], an increased number of
5 these cells could indeed, as hypothesized for congenital megacolon, represent an attempt
6 of the viscus at repairing the damaged neuroenteric circuitries present in these subjects
7 [18,20]. Moreover, the highest number of MC in constipated patients was found in the
8 mucosa and submucosa; in both human beings [4-8] and experimental animal models
9 [42,43] an increased number of mucosal MC has been reported described associated to
10 **diarrhoea**, probably due to the release of a wide variety of bioactive mediators by these
11 cells [44,45]. **The percentage of degranulated MC was higher in patients, although**
12 **not significantly compared to controls; interestingly, the degranulated MC were**
13 **always accompanied by the presence of eosinophils, enforcing the concept of the**
14 **eosinophil-MC axis [46] that is considered to play an important role in functional**
15 **gastrointestinal disorders.**

16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34 Physiological data available on propulsive activity in severely constipated patients (i.e.,
35 those sometimes requiring surgery for refractoriness to other therapeutic options)
36 consistently show that the main events, represented by the high-amplitude propagated
37 contractions [47,48], are consistently reduced or absent [49-54]. However, **there is**
38 **evidence** that compensative mechanisms exist in these **patients, represented** by the
39 preservation of the low-amplitude propagated contractions [55], that try to vicariate the
40 propulsive deficit [56]. Interestingly, these contractions are identical to those recorded in
41 clinical conditions characterized by **diarrhoea** [57-59]. Thus, it could be hypothesized that
42 this increase could be an attempt by a deranged colon to counterbalance an impaired
43 propulsive function due to ENS abnormalities [18,20].

44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
In conclusion, **we** investigated the distribution of MC **in all colonic layers** of controls and
severely constipated patients, **and demonstrated** a significant increase of this cell

1
2
3 population in **patients**, increase that could have a pathophysiologic meaning as an
4 attempt to boost, at least in part, a defective propulsive activity of these patients. Further
5
6
7
8 studies, possibly supported by experimental animal models, are obviously needed to
9
10 confirm **and establish whether** these findings **may have clinical values and therapeutic**
11
12 **implications.**
13
14
15
16
17
18
19

20 **Financial support for this study: none**
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

1. Shea-Donohue T, Stiltz J, Zhao A, Notari L. Mast cells. *Curr Gastroenterol Rep* 2010, 12:349-357
2. Mikkelsen HB. Interstitial cells of Cajal, macrophages and mast cells in the gut musculature: morphology, distribution, spatial and possible functional interactions. *J Cell Mol Med* 2010, 14:818-832
3. O'Sullivan M, Clayton N, Breslin NP, Harman I, Bountra C, McLaren A, O'Morain CA. Increased mast cells in the irritable bowel syndrome. *Neurogastroenterol Motil* 2000, 12:449-457
4. Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, Pasquinelli G, Morselli-Labate AM, Grady EF, Bunnett NW, Collins SM, Corinaldesi R. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* 2004, 126:693-702
5. Dong WZ, Zou DW, Li ZS, Zou XP, Zhu AY, Xu GM, Yin N, Gong YF, Sun ZX, Man XH. Study of visceral hypersensitivity in irritable bowel syndrome. *Chin J Dig Dis* 2004, 5:103-109
6. Park JH, Rhee PL, Kim HS, Lee JH, Kim YH, Kim JJ, Rhee JC. Mucosal mast cell counts correlate with visceral hypersensitivity in patients with diarrhea predominant irritable bowel syndrome. *J Gastroenterol Hepatol* 2006, 21:71-78
7. Piche T, Saint-Paul MC, Dainese R, Marine-Barjoan E, Iannelli A, Montoya ML, Peyron JF, Czerucka D, Cherikh F, Filippi J, Tran A, Hébuterne X. Mast cells and cellularity of the colonic mucosa correlated with fatigue and depression in irritable bowel syndrome. *Gut* 2008, 57:468-473
8. Goral V, Kucukoner M, Buyukbayram H. Mast cells count and serum cytokine levels in patients with irritable bowel syndrome. *Hepatogastroenterology* 2010, 57:751-754
9. Barbara G, Wang B, Stanghellini V, de Giorgio R, Cremon C, Di Nardo G, Trevisani M, Campi B, Geppetti P, Tonini M, Bunnett NW, Grundy D, Corinaldesi R. Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. *Gastroenterology* 2007, 132:26-37
10. Pinheiro SW, Rua AM, Etchebehere RM, Cançado CG, Chica JE, Lopes ER, Adad SJ. Morphometric study of the fibrosis and mast cell count in the circular colon musculature of chronic Chagas patients with and without megacolon. *Rev Soc Bras Med Trop* 2003, 36:461-466
11. da Silveira AB, Adad SJ, Correa-Oliveira R, Furness JB, D'Avila Reis D. Morphometric study of eosinophils, mast cells, macrophages and fibrosis in the

- 1
2
3 colon of chronic chagasic patients with and without megacolon. *Parasitology* 2007,
4 134:789-796
5
6
7 12. Iantorno G, Bassotti G, Kogan Z, Lumi CM, Cabanne AM, Fisogni S, Varrica LM,
8 Bilder CR, Munoz JP, Liserre B, Morelli A, Villanacci V. The enteric nervous system
9 in chagasic and idiopathic megacolon. *Am J Surg Pathol* 2007, 31:460-468
10
11 13. Kobayashi H, Yamataka A, Fujimoto T, Lane GJ, Miyano T. Mast cells and gut
12 nerve development: implications for Hirschsprung's disease and intestinal neuronal
13 dysplasia. *J Pediatr Surg* 1999, 34:543-548
14
15 14. Demirbilek S, Ozardali HI, Aydm G. Mast-cells distribution and colonic mucin
16 composition in Hirschsprung's disease and intestinal neuronal dysplasia. *Pediatr*
17 *Surg Int* 2001, 17:136-139
18
19 15. Hermanowicz A, Debek W, Dzienis-Koronkiewicz E, Chyczewski L. Topography and
20 morphometry of intestinal mast cells in children with Hirschsprung's disease. *Folia*
21 *Histochem Cytobiol* 2008, 46:65-68
22
23 16. Yadav AK, Mishra K, Mohta A, Agarwal S. Hirschsprung's disease: is there a
24 relationship between mast cells and nerve fibers? *World J Gastroenterol* 2009,
25 15:1493-1498
26
27 17. Lembo A, Camilleri M. Chronic constipation. *N Engl J Med* 2003, 349:1360-1368
28
29 18. Bassotti G, Villanacci V, Maurer CA, Fisogni S, Di Fabio F, Cadei M, Morelli A,
30 Panagiotis T, Cathomas G, Salerni B. The role of glial cells and apoptosis of enteric
31 neurones in the neuropathology of intractable slow transit constipation. *Gut* 2006,
32 55:41-46
33
34 19. de Lorijn F, Kremer LC, Reitsma JB, Benninga MA. Diagnostic tests in Hirschsprung
35 disease: a systematic review. *J Pediatr Gastroenterol Nutr* 2006, 42:496-505
36
37 20. Walls AF, Jones DB, Williams JH, Church MK, Holgate ST. Immunohistochemical
38 identification of mast cells in formaldehyde-fixed tissue using monoclonal antibodies
39 specific for tryptase. *J Pathol* 1990, 162:119-126
40
41 21. **Abonia JP, Blanchard C, Butz BB, Rainey HF, Collins MH, Stringer K, Putnam**
42 **PE, Rothenberg ME. Involvement of mast cells in eosinophilic esophagitis. *J***
43 **Allergy Clin Immunol** 2010, 126:140-149
44
45 22. **Stead RH, Dixon MF, Bramwell NH, Riddell RH, Bienenstock J. Mast cells are**
46 **closely apposed to nerves in the human gastrointestinal mucosa. *Gastroenterology***
47 **1989, 97:575-585**
48
49 23. **Stead RH, Franks AJ, Goldsmith CH, Bienenstock J, Dixon MF. Mast cells,**
50 **nerves and fibrosis in the appendix: a morphological assessment. *J Pathol***
51 **1990, 161:209-219**
52
53
54
55
56
57
58
59
60

- 1
2
3 24. Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev* 1997; 77:1033-1039
4
5
6 25. Barbara G, Stanghellini V, De Giorgio R, Corinaldesi R. Functional gastrointestinal
7 disorders and mast cells: implications for therapy. *Neurogastroenterol Motil* 2006,
8 18:6-17
9
10 26. Noviana D, Mamba K, Makimura S, Horii Y. Distribution, histochemical and enzyme
11 histochemical characterization of mast cells in dogs. *J Mol Histol* 2004, 35:123-132
12
13
14 27. Dunlop SP, Jenkins D, Spiller RC. Age-related decline in rectal mucosal
15 lymphocytes and mast cells. *Eur J Gastroenterol Hepatol* 2004, 16:1011-1015
16
17 28. Li CY, Dry SM. **Are c-kit and mast cell tryptase more effective at identifying**
18 **mucosal mast cells in GI biopsies?** *Mod Pathol* 2004, 17(suppl. 1):121A
19
20 29. Walls AF, Jones DB, Williams JH, Church MK, Holgate ST.
21 **Immunohistochemical identification of mast cells in formaldehyde-fixed**
22 **tissue using monoclonal antibodies specific for tryptase.** *J Pathol* 1990,
23 162:119-126
24
25
26 30. Minnei F, Wetzels C, De Hertogh G, Van Eyken P, Ectors N, Ambu R, Faa G,
27 Kochuyt AM, Geboes K. **Chronic urticaria is associated with mast cell**
28 **infiltration in the gastroduodenal mucosa.** *Virchows Arch* 2006, 448:262-268
29
30 31. Siegert SI, Diebold J, Ludolph-Hauser D, Lohrs U. **Are gastrointestinal**
31 **mucosal mast cells increased in patients with systemic mastocytosis?** *Am J*
32 **Clin Pathol** 2004, 122:560-565
33
34 32. Cremon C, Gargano L, Morselli-Labate AM, Santini D, Cogliandro RF, De Giorgio
35 R, Stanghellini V, Corinaldesi R, Barbara G. Mucosal immune activation in irritable
36 bowel syndrome: gender-dependence and association with digestive symptoms.
37 *Am J Gastroenterol* 2009, 104:392-400
38
39 33. Walker MM, Talley NJ, Prabhakar M, Pennaneac'h CJ, Aro P, Ronkainen
40 J, Storskrubb T, Harmsen WS, Zinsmeister AR, Agreus L. **Duodenal**
41 **mastocytosis, eosinophilia and intraepithelial lymphocytosis as possible**
42 **disease markers in the irritable bowel syndrome and functional dyspepsia.**
43 **Aliment Pharmacol Ther** 2009, 29:765-773
44
45 34. Ortiz-Lucas M, Saz-Peiró P, Sebastián-Domingo JJ. Irritable bowel syndrome
46 immune hypothesis. Part one: the role of lymphocytes and mast cells. *Rev Esp*
47 *Enferm Dig* 2010, 102:637-647
48
49 35. Ohman L, Simrén M. Pathogenesis of IBS: role of inflammation, immunity and
50 neuroimmune interactions. *Nat Rev Gastroenterol Hepatol* 2010, 7:163-173
51
52 36. Corinaldesi R, Stanghellini V, Cremon C, Gargano L, Cogliandro RF, De
53 Giorgio R, Bartesaghi G, Canovi B, Barbara G. **Effect of mesalazine on**
54
55
56
57
58
59
60

mucosal immune biomarkers in irritable bowel syndrome: a randomized controlled proof-of-concept study. Aliment Pharmacol Ther 2009, 30:245-252

37. Klooker TK, Braak B, Koopman KE, Welting O, Wouters MM, van der Heide S, Schemann M, Bischoff SC, van den Wijngaard RM, Boeckxstaens GE. The mast cell stabiliser ketotifen decreases visceral hypersensitivity and improves intestinal symptoms in patients with irritable bowel syndrome. *Gut* 2010, 59:1213-1221
38. Frieling T, Meis K, Kolck UW, Homann J, Hülsdonk A, Haars U, Hertfelder HJ, Oldenburg J, Seidel H, Molderings GJ. Evidence for mast cell activation in patients with therapy-resistant irritable bowel syndrome. *Z Gastroenterol* 2011, 49:191-194
39. Chadwick VS, Chen W, Shu D, Paulus B, Bethwaite P, Tie A, Wilson I. Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology* 2002, 122:1778-1783
40. Leon A, Buriani A, Dal Toso R, Fabris M, Romanello S, Aloe L, Levi-Montalcini R. Mast cells synthesize, store, and release nerve growth factor. *Proc Natl Acad Sci U S A* 1994, 91:3739-3743
41. Nilsson G, Forsberg-Nilsson K, Xiang Z, Hallböök F, Nilsson K, Metcalfe DD. Human mast cells express functional TrkA and are a source of nerve growth factor. *Eur J Immunol* 1997, 27:2295-2301
42. Feng BS, He SH, Zheng PY, Wu L, Yang PC. Mast cells play a crucial role in *Staphylococcus aureus* peptidoglycan-induced diarrhea. *Am J Pathol* 2007, 171:537-547
43. Perrier C, Thierry AC, Mercenier A, Corthésy B. Allergen-specific antibody and cytokine responses, mast cell reactivity and intestinal permeability upon oral challenge of sensitized and tolerized mice. *Clin Exp Allergy* 2010 40:153-162
44. Jakate S, Demeo M, John R, Tobin M, Keshavarzian A. Mastocytic enterocolitis: increased mucosal mast cells in chronic intractable diarrhea. *Arch Pathol Lab Med* 2006, 130:362-367
45. Söderholm JD. Mast cells and mastocytosis. *Dig Dis* 2009 27 (Suppl 1):129-136
46. **Powell N, Walker MM, Talley NJ. Gastrointestinal eosinophils in health, disease and functional disorders. *Nat Rev Gastroenterol Hepatol* 2010, 7:148-156**
47. Narducci F, Bassotti G, Gaburri M, Morelli A. Twenty four hour manometric recording of colonic motor activity in healthy man. *Gut* 1987, 28:17-25
48. Bassotti G, Gaburri M. Manometric investigation of high-amplitude propagated contractile activity of the human colon. *Am J Physiol* 1988, 255:G660-G664

- 1
2
3 49. Bassotti G, Gaburri M, Imbimbo BP, Rossi L, Farroni F, Pelli MA, Morelli A. Colonic
4 mass movements in idiopathic chronic constipation. *Gut* 1988, 29:1173-1179
5
6
7 50. Bassotti G, Chiarioni G, Vantini I, Betti C, Fusaro C, Pelli MA, Morelli A. Anorectal
8 manometric abnormalities and colonic propulsive impairment in patients with severe
9 chronic idiopathic constipation. *Dig Dis Sci* 1994, 39:1558-1564
10
11 51. Bassotti G, Chiarioni G, Germani U, Battaglia E, Vantini I, Morelli A. Endoluminal
12 instillation of bisacodyl in patients with severe (slow transit type) constipation is
13 useful to test residual colonic propulsive activity. *Digestion* 1999, 60:69-73
14
15
16 52. Hervé S, Savoye G, Behbahani A, Leroi AM, Denis P, Ducrotté P. Results of 24-h
17 manometric recording of colonic motor activity with endoluminal instillation of
18 bisacodyl in patients with severe chronic slow transit constipation.
19 *Neurogastroenterol Motil* 2004, 16:397-402
20
21
22 53. Rao SS, Sadeghi P, Beaty J, Kavlock R. Ambulatory 24-hour colonic manometry in
23 slow-transit constipation. *Am J Gastroenterol* 2004, 99:2405-2416
24
25
26 54. Dinning PG, Zarate N, Hunt LM, Fuentealba SE, Mohammed SD, Szczesniak MM,
27 Lubowski DZ, Preston SL, Fairclough PD, Lunniss PJ, Scott SM, Cook IJ.
28 Pancolonic spatiotemporal mapping reveals regional deficiencies in, and
29 disorganization of colonic propagating pressure waves in severe constipation.
30 *Neurogastroenterol Motil* 2010, 22:e340-e349
31
32
33 55. Bassotti G, Clementi M, Antonelli E, Pelli MA, Tonini M. Low-amplitude propagated
34 contractile waves: a relevant propulsive mechanism of human colon. *Dig Liver Dis*
35 2001, 33:36-40
36
37
38 56. Bassotti G, Chistolini F, Nzepa FS, Morelli A. Colonic propulsive impairment in
39 intractable slow-transit constipation. *Arch Surg* 2003, 138:1302-1304
40
41
42 57. Bazzocchi G, Ellis J, Villanueva-Meyer J, Reddy SN, Mena I, Snape WJ Jr. Effect of
43 eating on colonic motility and transit in patients with functional diarrhea.
44 Simultaneous scintigraphic and manometric evaluations. *Gastroenterology* 1991,
45 101:1298-1306
46
47
48 58. Bassotti G, de Roberto G, Chistolini F, Sietchiping-Nzepa F, Morelli O, Morelli A.
49 Twenty-four-hour manometric study of colonic propulsive activity in patients with
50 diarrhea due to inflammatory (ulcerative colitis) and non-inflammatory (irritable
51 bowel syndrome) conditions. *Int J Colorectal Dis* 2004, 19:493-497
52
53
54 59. Bassotti G, Villanacci V, Mazzocchi A, Castellani D, Giuliano V, Corsi S, Morelli A.
55 Colonic propulsive and postprandial motor activity in patients with ulcerative colitis
56 in remission. *Eur J Gastroenterol Hepatol* 2006, 18:507-510
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1. MC distribution (median (95% CI)) in the mucosa of controls and patients. Data are expressed as median (95% CI)

	C	STC	p
Total	35 (29-41)	88 (86-92)*	*0.001
Cecum	7.5 (5.5-8.5)	19 (18-20)*	*0.0001
Ascending	7 (5-9)	17 (16-19)*	*0.0001
Transverse	8 (6-10)	19 (15-19)*	*0.0001
Descending	8.5 (4.5-11.5)	18 (16-20)*	*0.0001
Sigmoid	7 (6-8)	18 (16-19)*	*0.0001

Abbreviations: C= controls; MC= mast cells; STC= slow transit constipation

Table 2. MC distribution in the submucosa of controls and patients. Data are expressed as median (95% CI)

	C	STC	p
Total	26 (22-30)	48 (43-51)*	*0.0001
Cecum	5 (4-7)	9 (8-9)*	*0.0004
Ascending	6 (4-7)	10 (9-10)*	*0.0004
Transverse	3 (2-7)	9 (8-12)*	*0.0002
Descending	5 (4-7)	9 (8-10)*	*0.0002
Sigmoid	6 (3-7)	9 (8-11)*	*0.0003

Abbreviations: C= controls; MC= mast cells; STC= slow transit constipation

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 3. MC distribution in the inner muscle layer of controls and patients. Data are expressed as median (95% CI)

	C	STC	p
Total	23 (21-234)	24(23-26)*	*0.07
Cecum	5 (2-6)	5 (4-7)*	*0.56
Ascending	5 (3-6)	4 (4-5)*	*0.85
Transverse	3 (3-5)	5 (4-6)*	*0.08
Descending	5 (4-5)	5 (4-6)*	*0.87
Sigmoid	5 (4-6)	5 (5-6)*	*0.52 vs C

Abbreviations: C= controls; MC= mast cells; STC= slow transit constipation

Table 4. MC distribution in the outer muscle layer of controls and patients. Data are expressed as median (95% CI)

	C	STC	p
Total	19 (18-21)	28(25-29)*	*<0.0001
Cecum	3 (2-4)	6 (5-6)*	*0.05
Ascending	4 (4-6)	5 (4-7)*	*0.13
Transverse	2 (2-5)	5 (4-6)*	*0.001
Descending	5 (4-5)	6 (5-7)*	*0.09
Sigmoid	3 (3-5)	5 (5-6)*	*0.0006

Abbreviations: C= controls; MC= mast cells; STC= slow transit constipation

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 5. MC distribution in the serosa of controls and patients. Data are expressed as median (95% CI)

	C	STC	p
Total	12 (12-15)	12 (12-14)*	*0.64
Cecum	2 (1-3)	2 (2-3)*	*0.4
Ascending	3 (2-4)	3 (2-3)*	*0.08
Transverse	2 (1-3)	2 (1-4)*	*0.6
Descending	3 (1-4)	3 (3-3)*	*0.7
Sigmoid	2 (2-3)	3 (2-3)*	*0.9

Abbreviations: C= controls; MC= mast cells; STC= slow transit Constipation

Figure legend

Figure 1. Representative images of MC in STC. A. Mucosal MC (tryptase, original magnification x 20). B. Submucosal MC (tryptase, original magnification x 40). C. Presence of MC in the inner muscle layer (tryptase, original magnification x 20). D. Serosal MC (tryptase, original magnification x 40).

Figure 2. A. Representative image showing a degranulated mast cell (arrow) compared to intact ones (tryptase stain, original magnification x40). B. H&E stain showing the presence of a degranulated mast cell (thin arrow) associated to the presence of eosinophils (thick arrow). Original magnification x100. C. Tryptase stain showing two mast cells (arrows) well outside a myenteric ganglion (original magnification, x40). D. CD117 stain showing interstitial cells of Cajal (arrows) near a myenteric ganglion, but no mast cells near or within the ganglion (original magnification, x40).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

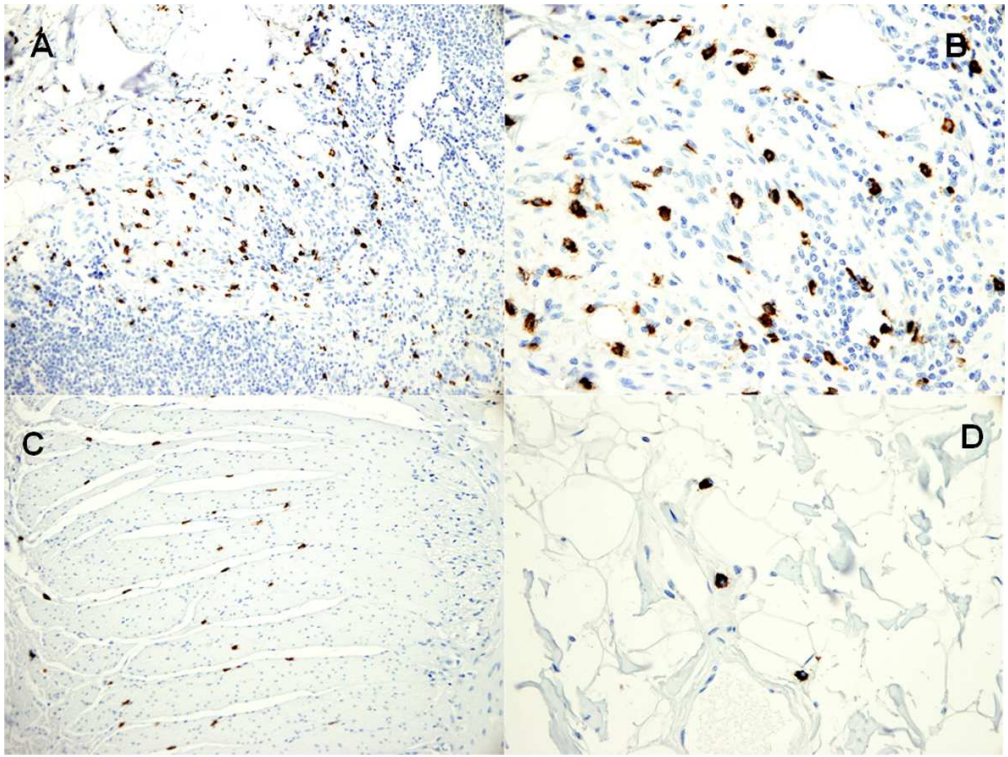


figure 1
99x75mm (300 x 300 DPI)

Review

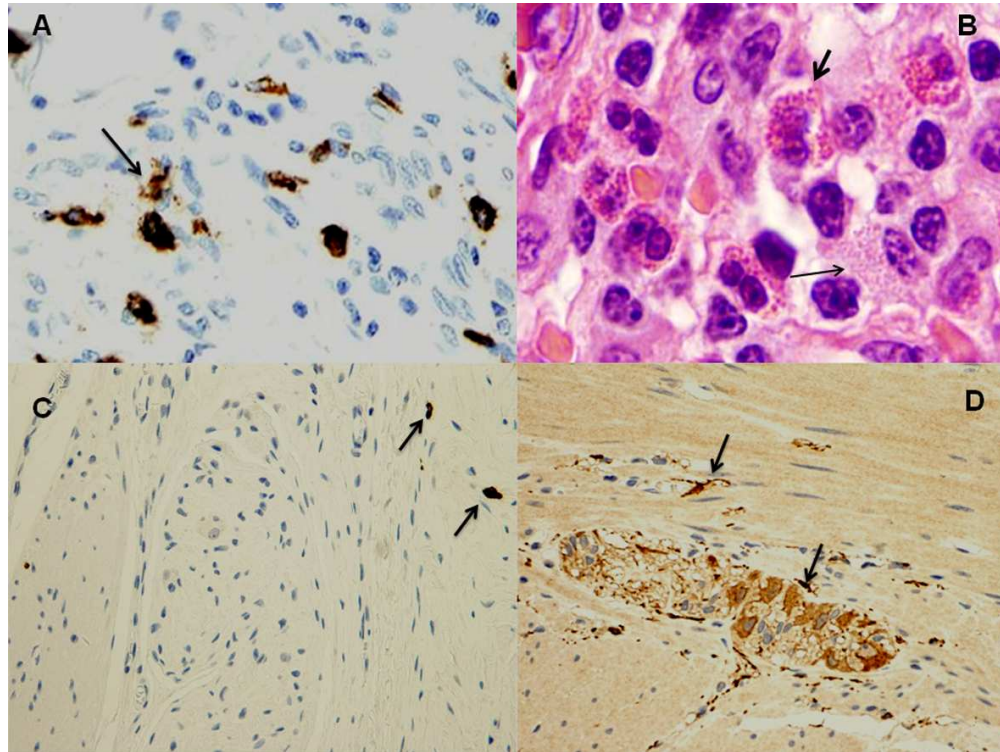


figure 2
99x75mm (300 x 300 DPI)

Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60