Journal of Cytology & Histology

Hermanowicz et al., J Cytol Histol 2013, 4:5
DOI: 10.4172/2157-7099.1000201

Research Article Open Access

Mast Cells Connections with Nerve Fibers in Hirschsprung's Disease

Hermanowicz A[1*], Matuszczak E[1], Komarowska M[1], Debek W[1], Chyczewski L[2] and Klek S[3]

[1]*Department of Pediatric Surgery, Medical University, Bialystok, Poland*
[2]*Department of Medical Pathomorphology, Medical University, Bialystok, Poland*
[3]*Stanley Dudrick's Memorial Hospital, General and Oncology Surgery Unit, Skawina, Poland*

Abstract

1.1 Introduction: Hirschsprung's disease (HD) is rare (1/5000 newborns) congenital condition of the bowel nervous system, clinically characterized by dilatation and hypertrophy of colon. There are an increased number of mast cells (MC) in aganglionic bowel segments and in those with intestinal neuronal dysplasia (IND). The exact role of MC in HD is still not known. The aim of this study was to examine the colonic distribution of mast cells (MC) and the relationship between MC and nerve fibers and to estimate connections of these cells with gut nervous system.

1.2 Material and Methods: Distal colon wall specimens were harvested from patients with normal bowel function and with normal ganglion cells, patients with constipations and with normal ganglion cells and a group of patients with Hirschsprung's disease. The age of the patients ranged from 2nd day of live to 12 years. The density of the mast cells was calculated using a light microscope with digital camera. The connections of localization of a MC with nerve fibers and ganglions were taken under consideration and observed under light microscope. Test for the significance of differences between groups were performed using the Kruskal-Wallis test.

1.3 Results: We revealed that there is a greater number of the MC in mucosa, lamina propria, submucosa and muscle layer of large bowel in patients with HD. There was no difference in density of the MC in serosa. Some of the colon MC in HD was located near or they were even attached to the hypertrophic nerve trunks. We found a correlation of MC number with the number of nerve fibers in HD patients.

1.4 Conclusions: Higher number of MC and their localization in close connection with hypertrophied nerve trunks in patients with HD than in patients with constipation or normal bowel function, is a proof of the influence of MC on defected nervous system in aganglionic bowel. MC probably affect hypertrophied nerve trunks. Mobilization and activation of the mast cells which produce and store NGF can be directed to repair defective nervous system. Obviously, MC repairing reaction is not effective in restoring nervous system function of the gut.

Introduction

Hirschsprung's disease (HD) is rare (1/5000 newborns) congenital condition of the bowel nervous system, clinically characterized by dilatation and hypertrophy of colon. The true nature of the disease is a aperistaltic, aganglionic part of a distal colon. The main symptoms of the disease are severe bowel obstruction. Harald Hirschsprung in 1886 for the first time described the disease as a "colon dilatation and hypertrophy". In 1901 Tittel as a first described an absence of nerve ganglions in the colon with Hirschsprung's disease [1-3].

In HD the aganglionic bowel is characterized by the presence of hypertrophic nerve trunks and increased numbers of adrenergic and cholinergic nerve fibers. All symptoms of HD-delayed meconium passage in newborns, intestinal obstruction, constipation and toxic megacolon are consequences of this fact [1-3]. Kobayashi et al. [4] have found an increased number of mast cells (MC) in aganglionic bowel segments and in those with intestinal neuronal dysplasia (IND). MC are connective tissue cells with cytoplasmic metachromatic granules, involved in many functions in human body. Among a great variety of biological active substances, MC synthesize, store and release nerve growth factor (NGF) responsible for the growth and repair of nerve fibers [4-10]. MC are observed in great amount in digestive tract and also in close contact with blood vessels and nerve fibers [9,11-16]. Substances produced by MC may exert an important effect on embryology, growth, differentiation and regeneration of intestinal nervous system. Additionally, MC products modulate inflammation processes thus influencing on the clinical course of HD [4,9,17]. They are also observed in central nervous system in physiology and pathology. The role of MC as a potential agents for promotion of neoplastic transformation is widely discussed in literature. High mast cells concentration is found in neurofibromas, malignant schwannomas, leiomyosarcomas or in other malignancies of the central nervous system [18]. Although there are a couple of studies on MC in HD in the literature, the exact role of MC in HD is still not known [4,9,12,19-21]. The aim of this study was to examine the colonic distribution of mast cells (MC) and the relationship between MC and nerve fibers and to estimate connections of these cells with gut nervous system.

Materials and Methods

Distal colon wall specimens were harvested from three groups of patients. Group 1: patients with normal bowel function and with normal ganglion cells (n=10), Group 2: patients with severe constipations and with normal ganglion cells (n=12) and Group 3: patients with Hirschsprung's disease (colon aganglionosis n=20). The full thickness samples were taken transrectally 1-2 cm above dental line (group 2 and 3). In group 1 the colon wall was sampled during surgical repairs performed because of anal atresia. The age of the patients ranged from

***Corresponding authors:** Adam Hermanowicz, Department of Pediatric Surgery, Medical University, Bialystok, Poland, Tel: +48857450921; Fax: +48857450920; E-mail: ahermanowicz@wp.pl

Received October 24, 2013; **Accepted** December 02, 2013; **Published** December 04, 2013

Citation: Hermanowicz A, Matuszczak E, Komarowska M, Debek W, Chyczewski L, et al. (2013) Mast Cells Connections with Nerve Fibers in Hirschsprung's Disease. J Cytol Histol 4: 201. doi: 10.4172/2157-7099.1000201

2nd day of live to 12 years (mean age 87 months, 49 months and 6 months respectively for groups). The first diagnosis of HD was based on clinical symptoms, radiological findings and rectomanometric results. In every case the final diagnosis of HD was confirmed histologically. The criteria for the recognition of Hirschsprung disease included: an absence of parasympathetic ganglion cells in the myometric and submucosal plexus of the rectum, also extending to the colon and the absence of ganglion cells and the presence of hypertrophic nerve trunks.

None of the patients presented episodes of enterocolitis during or before biopsy. The patients with intestinal neuronal dysplasia were excluded.

The specimens were fixed in a 10% buffered formalin solution, embedded in paraffin at 56°C, then cut into 5 μm slices and stained with hematoxilin and eosin (H+E). Following deparaffinizion, endogenous peroxidase activity was quenched by 3% hydrogen peroxide in methanol for 30 minutes. The sections were then incubated with the mouse monoclonal antibody for mast cell tryptase (DakoCytomation, Code M 7052) in 1:100 dilutions, for whole night at 4°C temperature and labeled with LSAB plus (DAKO) enzyme reagent and diaminobenzidine (DAB) chromogen for 5 minutes. Sections were counter-stained with hematoxylin. The mast cell tryptase expression is detectible in both resting and activating mast cells.

The density of the mast cells was calculated using a light microscope with digital camera. LUCIA software was used as a morphometric tool. The MC were counted in five random fields in submucosa and muscle layer of the bowel wall using light microscopy (x200). Mucosa and lamina propria were excluded from investigation in this study because of possible impact of a diet on the inflammatory process.

The connections of localization of a MC with nerve fibers and ganglions were taken under consideration and observed under light microscope. Test for the significance of differences between groups were performed using the Kruskal-Wallis test.

The study protocol was approved by local ethics committee.

Results

We have found that MC were localized besides blood vessels and in contact with hypertrophic nerve trunks in aganglionic segment from HD patients. In a bowel specimens from a group 1 the MC were also localized near the nerve fibers and blood vessels but they were also spread in the whole layer (Figures 1 and 2). In the non-HD patients we observed the low concentration of the MC in the colon (Figure 3).

The number of mast cells in the submucosa and in the muscle layer was higher in HD patients in comparison with other groups. The difference was seen but it was not statistically significant ($p>0.05$; $p<0.1$) in submucosa. In muscle layer the difference was statistically significant ($p<0.05$). In the muscle layer cells were located solitary and they were in a close connection with nerve trunks and blood vessels.

We evaluated the nerve trunks in 10 High Power Fields. The number of the nerve trunks ranged from 3 up to 10 nerve trunks. All of them showed lack of the ganglion cells, what was confirmed with synaptophysin staining.

Using parameters obtained by image analysis morphometry we found a correlation of MC number with the number of nerve fibers in HD patients (correlation coefficient=0.472). In the non-HD patients the correlation coefficient of MC with number of nerve fibers was 0.382.

We haven't counted the number of MC connected with a blood vessels but it was also seen that MC were located in perivessel area (Figure 4). This fact if well known in the literature [5,7].

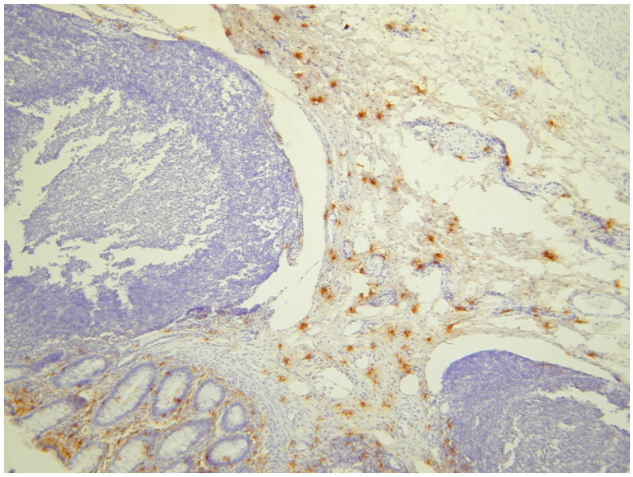

Figure 1: Hirschsprung's disease. Cross-section of the aganglionic colon. Numerous mast cells. Hypertrophic nerve fibers with MC around. (mag 200x).

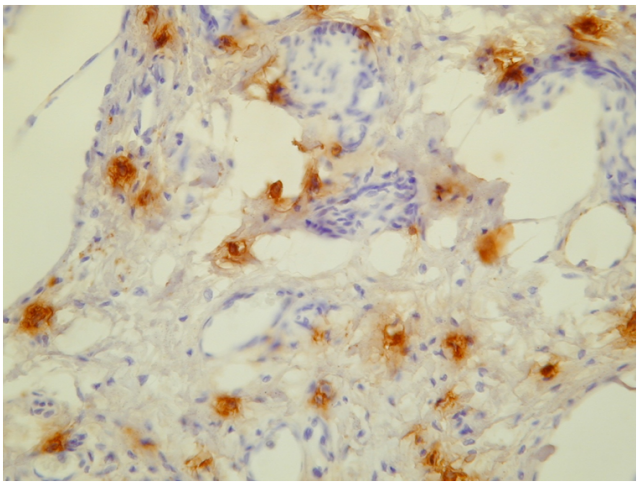

Figure 2: Hirschsprung's disease. Hypertrophic nerve fibers and mast cells localized around (200x mag).

In the non-HD patients the concentration of the MC was lower. The results are in Table 1.

Discussion

The neural hypertrophy in the colonic submucosa associated with aganglionosis is a marker of HD. The thickness of submucosal nerve fibers has been measured by various authors in previous studies [4,16,20]. In this study we analyzed the location and a count of a MC in HD and non-HD cases. MC regulate many different physiological processes. MC through neuronal growth factor (NGF) influence also nervous system [4,9,10,16,21-25]. Some authors, Kobayashi et al., Demirbilek et al., Furgal et al., Puri et al. [4,19,20,25] showed elevated content of NGF and increased number of MC in the aganglionic colon wall in HD in children. In the present study a significant increase was also noticed in MC in HD. We revealed that there is a greater number of the MC in mucosa, lamina propria, submucosa and muscle layer of large bowel in patients with HD. There was no difference in density

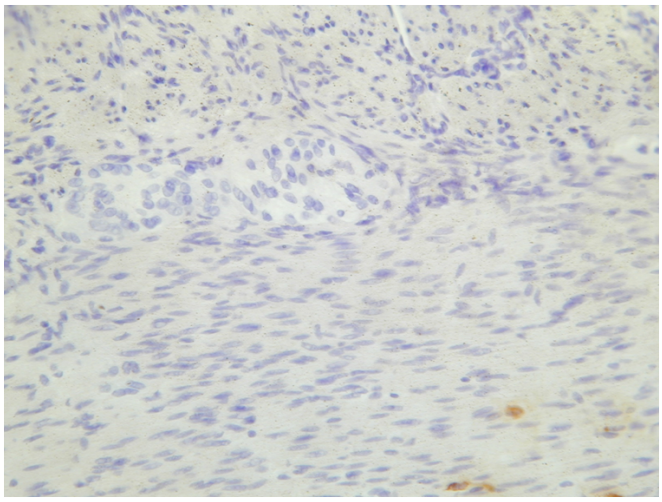

Figure 3: The non HD patient. Border of the two layers of longitudinal and transverse muscles. Single mast cells. normal ganglia.

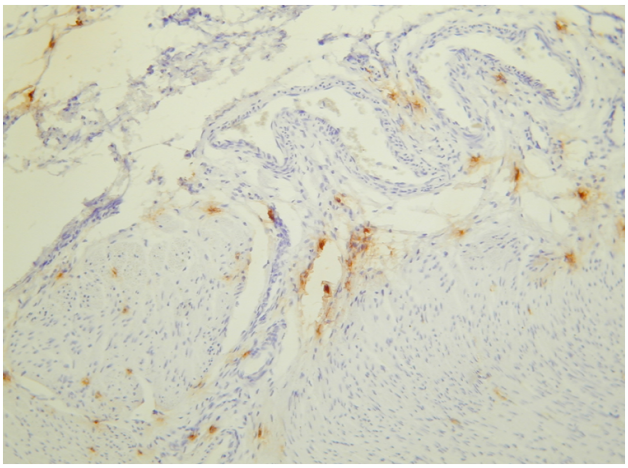

Figure 4: Hirschsprung's disease. MC's around the vessels penetrating in the muscle layer.

	Control group	Constipations	Hirschsprung's disease
Submucosa	95.31 ±37.41	100.34 ± 74.3	121.32 ± 24.21
Muscle layer	35.16 ± 29.53	24.53 ± 19.17	51.67 ± 36.2

Table 1: Mean number of MC ± standard deviation per mm^2 of specimen in submucosa and muscle layer of the large bowel (cells/mm^2).

of the MC in serosa. Furthermore, we also noticed that some of the colon MC in HD were located near or they were even attached to the hypertrophic nerve trunks. We found a correlation of MC number with the number of nerve fibers in HD patients. This is another proof of connection between defected nervous system in aganglionic bowel and MC. MC probably affect hypertrophied nerve trunks. Mobilization and activation of the mast cells can be directed to repair defective nervous system. Obviously, MC repairing reaction is not effective in restoring nervous system function of the gut. Although in HD the MC are probably activated and they are probably producing larger amount of NGF, we still have defective nervous system and all symptoms related with this. So this is certain that NGF produced in MC it is not single substance responsible for repairing a defective nervous gut system [4,9,10,16,25].

Conclusions

Higher number of MC and their localization in close connection with hypertrophied nerve trunks in patients with HD than in patients with constipation or normal bowel function, is a proof of the influence of MC on defected nervous system in aganglionic bowel. MC probably affect hypertrophied nerve trunks. Mobilization and activation of the mast cells which produce and store NGF can be directed to repair defective nervous system. Obviously, MC repairing reaction is not effective in restoring nervous system function of the gut.

We confirm that all authors have read and approved the submission of the manuscript, the manuscript has not been published and is not being considered for publication elsewhere, in whole or in part, in any language, except as an abstract. We also declare no conflict of interest, no financial relationships with any industry (through investments, employment, consultancies, stock ownership, honoraria).

References

1. Gershon MD, Ratcliffe EM (2004) Developmental biology of the enteric nervous system: pathogenesis of Hirschsprung's disease and other congenital dysmotilities. Semin Pediatr Surg 13: 224-235.
2. Heitz PU, Komminoth P (1990) Biopsy diagnosis of Hirschsprung's disease and related disorders. Curr Top Pathol 81: 257-275.
3. Swenson O (2002) Hirschsprung's disease: a review. Pediatrics 109: 914-918.
4. Kobayashi H, Yamataka A, Fujimoto T, Lane GJ, Miyano T (1999) Mast cells and gut nerve development: implications for Hirschsprung's disease and intestinal neuronal dysplasia. J Pediatr Surg 34: 543-548.
5. Barczyk M, Debek W, Chyczewski L (1995) Mast cells in the gastrointestinal tract. Rocz Akad Med Bialymst 40: 36-57.
6. Bonini S, Rasi G, Bracci-Laudiero ML, Procoli A, Aloe L (2003) Nerve growth factor: neurotrophin or cytokine? Int Arch Allergy Immunol 131: 80-84.
7. Dvorak AM (1997) New aspects of mast cell biology. Int Arch Allergy Immunol 114: 1-9.
8. Dvorak AM, McLeod RS, Onderdonk AB, Monahan-Earley RA, Cullen JB, et al. (1992) Human gut mucosal mast cells: ultrastructural observations and anatomic variation in mast cell-nerve associations in vivo. Int Arch Allergy Immunol 98: 158-168.
9. Hermanowicz A, Debek W, Dzienis-Koronkiewicz E, Chyczewski L (2008) Topography and morphometry of intestinal mast cells in children with Hirschsprung's disease. Folia Histochem Cytobiol 46: 65-68.
10. Leon A, Buriani A, Dal Toso R, Fabris M, Romanello S, et al. (1994) Mast cells synthesize, store and release nerve growth factor. Proc Natl Acad Sci USA 91: 3739-3743.
11. Blennerhassett MG, Tomioka M, Bienenstock J (1991) Formation of contacts between mast cells and sympathetic neurons in vitro. Cell Tissue Res 265: 121-128.
12. Dines KC, Powell HC (1997) Mast cell interactions with the nervous system: relationship to mechanisms of disease. J Neuropathol Exp Neurol 56: 627-640.
13. Hermanowicz A, Debek W, Oksiuta M, Matuszczak E, Chyczewski L, et al. (2010) Mast cells in peritoneal fluid in rats with experimentally induced peritoneal adhesions. Folia Histochem Cytobiol 48: 153-156.
14. Chéret J, Lebonvallet N, Carré JL, Misery L, Le Gall-Ianotto C (2013) Role of neuropeptides, neurotrophins, and neurohormones in skin wound healing. Wound Repair Regen.
15. Roy A, Ganesh G, Sippola H, Bolin S, Sawesi O, et al. (2013) Mast Cell Chymase Degrades the Alarmins Heat Shock Protein 70, Biglycan, HMGB1, and IL-33 and Limits Danger-Induced Inflammation. J Biol Chem .
16. Yadav AK, Mishra K, Mohta A, Agarwal S (2009) Hirschsprung's disease: is there a relationship between mast cells and nerve fibers? World J Gastroenterol 15: 1493-1498.
17. Caughey GH (1994) Serine proteinases of mast cell and leukocyte granules. A league of their own. Am J Respir Crit Care Med 150: S138-142.

18. Reszec J, Hermanowicz A, Kochanowicz J, Turek G, Mariak Z, et al. (2012) Mast cells evaluation in meningioma of various grades. Folia Histochem Cytobiol 50: 542-546.

19. Demirbilek S, Ozardali HI, Aydm G (2001) Mast-cells distribution and colonic mucin composition in Hirschsprung's disease and intestinal neuronal dysplasia. Pediatr Surg Int 17: 136-139.

20. Furgal A, Litwin J (1998) Distribution of mast cells along and across successive segments of the rat digestive tract: a quantitative study. Folia Histochem Cytobiol 36: 19-27.

21. Guy-Grand D, Dy M, Luffau G, Vassalli P (1984) Gut mucosal mast cells. Origin, traffic, and differentiation. J Exp Med 160: 12-28.

22. Coskun N, Sindel M, Elpek GO (2002) Mast cell density, neuronal hypertrophy and nerve growth factor expression in patients with acute appendicitis. Folia Morphol (Warsz) 61: 237-243.

23. Enerbäck L (1966) Mast cells in rat gastrointestinal mucosa. 3. Reactivity towards compound 48/80. Acta Pathol Microbiol Scand 66: 313-322.

24. Enerback L, Befus A, Bienenstock J, Denburg J (1986) Mast cell heterogeneity: the evolution of the concept of a specific mucosal mast cells. Mast cell differentiation and heterogeneity, Raven Press, New York, USA, 1-21.

25. Puri P, Shinkai T (2004) Pathogenesis of Hirschsprung's disease and its variants: recent progress. Semin Pediatr Surg 13: 18-24.

Citation: Hermanowicz A, Matuszczak E, Komarowska M, Debek W, Chyczewski L, et al. (2013) Mast Cells Connections with Nerve Fibers in Hirschsprung's Disease. J Cytol Histol 4: 201. doi: 10.4172/2157-7099.1000201