Internal Medicine: Open Access

Zhou X, et al., Intern Med 2017, 7:5
DOI: 10.4172/2165-8048.1000253

Research Article Open Access

Co-regulated Intestinal c-kit by Interleukin-17 and Interleukin-10 on Post-infectious Irritable Bowel Syndrome in Mice

Bo Yang[1], **Xuesong Bai**[1], **Yijun Yu**[1], **Honglan Liu**[1], **Cheng Lan**[2], **Dan Liu**[3] **and Xuchun Zhou**[3*]

[1]*Department of Gastroenterology, Dazhou Central Hospital, Dazhou, China.*

[2]*Department of Gastroenterology, Hainan Provincial People's Hospital, Haikou, China.*

[3]Department of Gastroenterology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China.

[*]**Corresponding author:** Dr. Xuchun Zhou, Department of Gastroenterology, The First Affiliated Hospital of Chongqing Medical University, Youyi street, Chongqing, China, Fax: 86-023-68811360; Tel: 8602368811360; E-mail: yblyl9527@126.com

Received date: January 06, 2016; **Accepted date:** September 27, 2017; **Published date:** September 30, 2017

Abstract

Purpose

The aim of this study was to investigate the probable immune mechanisms of PI-IBS.

Methods

C57L/B6 mice, which have been infected by 500 Trichinella larvae for 56 days, were used to PI-IBS model mice, the levels of the intestinal mRNAs and proteins of c-kit, interleukin-10 (IL-10), and IL-17 were measured by RT-PCR and western blotting or ELISA. The locations of c-kit were detected using immunohistochemical staining.

Results

Levels of the c-kit protein and mRNA were up-regulated in the whole PI-IBS mice intestine. Compared to the control bowel segments, IL-17 levels were significantly higher in the duodenum and ileum, and IL-10 levels were lower in jejunum, ileum, and colon. By immunohistochemical staining, the increased signals of the c-kit were mainly detected in the submucosa and myenteron.

Conclusions

These results suggested that increased levels of the c-kit resulted in the changes of the intestinal motility and visceral sensitivity in PI-IBS mouse model induced by Trichinella Spiralis infection, and which may be co-regulated by IL-17 and IL-10 in pathophysiology of PI-IBS.

Keywords: Post-infectious irritable bowel syndrome; C-kit; Interleukin-10; Interleukin-17

Introduction

Irritable bowel syndrome (IBS) is a highly prevalent functional gastrointestinal disorder characterized by abdominal pain and alterations in bowel habits [1]. Caused by Viruses, bacteria, and parasites infection, ranged from 3.7% to 36%, has been commonly referenced to as "PI-IBS" [2]. Low-grade inflammation and chronic alteration of the immune system at the molecular level have been proved to target the mucosal secretory function, smooth muscle and enteric nervous fibers [3-5]. So far, we only know that motility disorder and hypersensitivity are typical characters in PI-IBS patients, however, the mechanism of alterations in the motility and visceral sensitivity is unwell defined in PI-IBS.

Intestinal c-kit is widely distributed in the interstitial Cells of cajal (ICC), termed as c-kit positive cells, and which is the identity marker gene of the ICC [6]. ICC are present throughout the gastrointestinal tract, from the esophagus to the anus, three main functions of that include acting as mechanoreceptors, transmitting enteric neuronal signals to smooth muscle cells, and placing slow waves and regulating their propagation.6 Overexpressed c-kit was required for development and maintenance of ICC, also was necessary for development of coordinated motility patterns and for survival of ICC [7,8] Although some papers have shown c-kit positive cells were changed in PI-IBS patients and animal models [9,10] it is always unclear that the correlation between c-kit and immune regulatory cytokines.

IL-10 and IL-17 are the classical immune regulatory cytokines, because they can regulate inflammatory response. IL-10 is released by T-regulatory cells to reduce inflammation [11-16] IL-17 produced by T-helper 17 cells has a protective role in infections, its main function is to induce the production of multiple pro-inflammatory molecules and subsequent activation of macrophages and neutrophils, [17] meanwhile, it also have a regulatory function limiting accumulation and/or activity of neutrophils during the inflammatory process by attenuating the anti-apoptotic effect of inflammatory cytokines [18] The IL-23/IL-17 signal pathway has been best described, which regulates IL-12/IFN-γ, and drives neutrophil migration to mediate inflammation injury [19-22] However, uncontrolled IL-17 responses

can augment production of inflammatory factors including IL-1, IL-6,IL-8,TNF-α,GM-CSF, and MIP-2 [21,23].

Now that c-kit positive cells were changed in PI-IBS which have low-grade inflammation and chronic alteration of the immune system, we hypothesis IL-10 and IL-17 can co-regulate intestinal c-kit in different bowl segments of PI-IBS mice.

Materials and Methods

Establishment of PI-IBS mice model

The experimental procedure was approved by the Animal Welfare committee of Chongqing Medical University, China. In our published paper, procedures and methods about establishing PI-IBS mice model had been introduced [24]. Briefly, Specific pathogen-free female C57L/B6 mice were infected by 500 Trichinella larvae. When no obvious inflammatory infiltrate was observed, abdominal withdrawal reflex and intestine transportation time were measured to accessed PI-IBS mice model.

Immunohistochemical Staining

Paraffin-embedded tissues sampled at day 56 PI were cut into 5-μm-thick sections. To deparaffinize, the sections were immersed in xylene, at 56°C, two times for 20 min, and hydrated with ethanol (two times with 100%, one times with 95%, and one time with 75% ethanol) for 5 min. Then sections were pretreated with 3% hydrogen peroxide for 20 min at 37°C, and antigen retrieval was achieved by boiling in citrate buffer (0.01 M, pH 6.0) for 20 min. After cooling at room temperature for 1 h, the specimens were treated with 5% bovine serum albumin for 30 min at 37°C followed by overnight incubation with 1: 200 diluted rabbit anti-mouse c-kit polyclonal antibody (Boster, China) at 4°C. After washing in phosphate-buffered saline, the slides were incubated for 60 min at 37°C with the corresponding secondary biotinylated goat anti-rabbit antibody (Boster, China) at a 1: 300 dilution. After washing, slides were incubated with DAB chromogen (Zsbio, China) for 5 min. nuclei counterstain was performed with Mayer's hematoxylin. Slides were with water and then immersed with 75,85,90 and 100% ethanol and finally, 100% xylene. Sealed slides were recorded digitally using Leica Microsystem (Leica, Germany). The immunohistochemical analysis was comprehensively scored as staining intensity and the proportion of positive cells [25]. The scale of staining intensity: 0, not detected; 1, minimal; 2, mild; 3, moderate; 4, marked. The proportion scale of positive cells: 0, no staining, 1, c-kit positive cells were in less than 1% population; 2, c-kit positive cells in 1-10%; 3, c-kit positive cells in 10-50%; 4, c-kit positive cells in more than 50%.

ELISA and Western Blotting for protein assay

For ELISA and western blotting [26], all different bowel segments were fractured using ultrasonic disrupter (Bandelin, Germany) and homogenized in RIPA buffer (Takara, Japan). And then homogenates were centrifugated at 10000 rpm for 20 min, the supernatants were determined protein concentrations with the BCA protein assay kit (Beyotime, China), and were diluted into a 3000μg/ml concentration. Tissue cytokines levels were assayed by using mouse IL-10 and IL-17 ELISA kits (Boster, China) according to the manufacturer′s protocols. Aliquots of 30 μg proteins were subjected to electrophorese on a polyacrylamide gel for c-kit protein. The primary antibodies were c-kit (Boster, China) and β-actin (Boster, China). The β-actin protein acted as an internal standard. The signal was detected using ECL chemiluminescent detection system (Bio-Rad, America). The densities of the bands were assessed by the quantity one software (Bio-Rad, America). The relative protein levels of target protein were obtained by correction with the corresponding internal standard.

RT-PCR for mRNA assay

Total RNA in the intestinal mucosa was extracted using trizol solution (Takara, Japan). The gene expressions of cytokines were assayed by RT-PCR. The β-actin mRNA level was determined as an internal reference, and their levels of expression were quantitated by optical densitometry after electrophoresis on agarose gel. All primers were listed in [Table 1]. The reverse-transcription was conducted at 37°C for 15 min, 95°C for 5 sec. The PCR cycling condition was 36 cycles at 94°C for 40 sec, 55°C (IL-10), 57°C (IL-17) or 59°C (β-actin) for 30 sec and 72°C for 35 sec. The PCR end products were electrophoresed on 5% agarose gel and stained with ethidium bromide. The gray values of the bands were calculated using quantity one software (Bio-Rad, America). The relative mRNA expression levels of target genes were normalized to the corresponding internal standard.

Cytokines	Primer sequence
IL-10 (116 bp)	Forward 5′—ACAGCCGGGAAGACAATAAC—3′
	Reverse 5′—CAGCTGGTCCTTTGTTTGAAAG—3′
IL-17 (123 bp)	Forward 5′—CGCAATGAAGACCCTGATAGAT—3′
	Reverse 5′—CTCTTGCTGGATGAGAACAGAA—3′
c-kit (214 bp)	Forward 5′—CGACGCAACTTCCTTATGAT—3′
	Reverse 5′—AGGACCTTCAGTTCCGACAT—3′
β-actin (470 bp)	Forward 5′—AGGCTGTGCTGTCCCTGTATG—3′
	Reverse 5′—GAGGTCTTTACGGATGTCAACG-3′

Table 1: RT-PCR primer sequences.

Citation: Bo Yang, Xuesong Bai, Yijun Yu, Honglan Liu, Cheng Lan, Dan Liu, Xuchun Zhou (2017) Co-regulated Intestinal c-kit by Interleukin-17 and Interleukin-10 on Post-infectious Irritable Bowel Syndrome in Mice. Intern Med 7: 253. doi:10.4172/2165-8048.1000253

Statistical analysis

Statistical analysis was performed using SPSS 19.0 software. Data were expressed as the mean ± standard deviation (SD).The independent sample T-test was used to compare the results between the two groups. P-value<0.05 was considered statistically significant.

Results

Immunohistochemistry staining

Immunolabelling showed signals of the c-kit were mainly detected in the submucosa and myenteron (Figure 1A and 1B) . Staining scores of the whole intestine in PI-IBS group were notably higher than these of control group (P<0.05, Figure 1C).

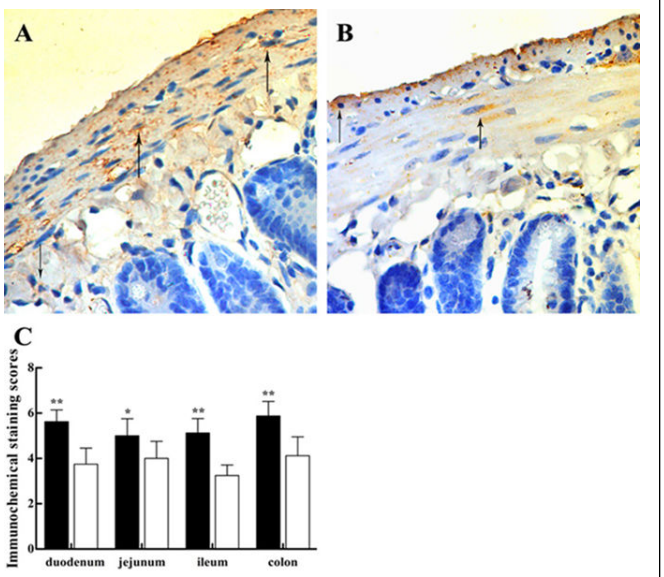

Figure 1: Comparison of the c-kit immunochemical staining between PI-IBS (A) and control (B) groups. Black arrow: C-kit positive cell, original magnification ×400, n=3. C. immunochemical staining scores; Black: PI-IBS group (n=3), white: control group (n=3). Data are presented as mean ± SD. *P<0.05, **P<0.01 versus control group.

ELISA and western blotting

As shown in the Figure 2, Jejunum, ileum, and colon expressed much less IL-10 in PI-IBS group than these in control group (all P<0.05); however, compared to control mice, concentrations of the IL-17 in duodenum and ileum of PI-IBS mice were significantly higher(all P<0.05). C-kit levels in PI-IBS mice were noticeable up-regulated in different bowl segments (all P<0.05) (Figure 2).

RT-PCR

The result of RT-PCR showed that c-kit mRNA levels were significantly up-regulated in the duodenum, jejunum, ileum and colon of the PI-IBS mice (all P<0.05, Figure 3B). IL-10 mRNA levels were significantly lower in the intestinal segments including jejunum, ileum and colon of PI-IBS group (all P<0.05, Figure 3C), and IL-17 mRNA levels in the duodenum and ileum were notably higher in PI-IBS mice compared to control mice (all P<0.05, Figure 3D) (Figure 3).

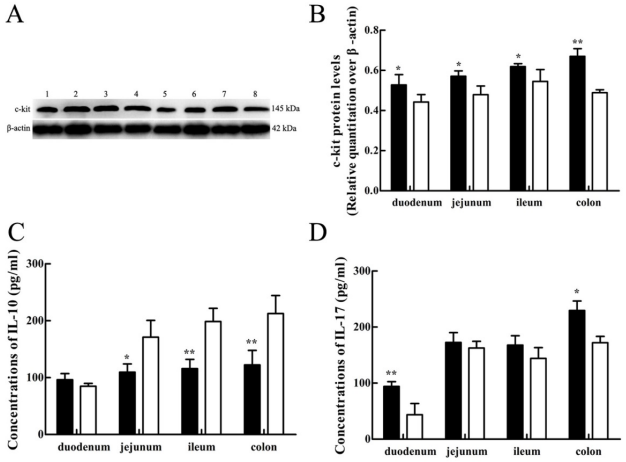

Figure 2: Cytokine protein levels of c-kit (B), IL-10 (C), and IL-17 (D) in the different intestinal segments in the PI-IBS (n=8) and control (n=8) groups. A. Representative graphs of the western blotting assay. Lanes 1-4 are duodenum, jejunum, ileum and colon of PI-IBS group, lanes 5-8 are duodenum, jejunum, ileum and colon of control group. Black: PI-IBS group, white: Control group. Date are presented as mean ± SD. *P<0.05, **P<0.01 versus control group.

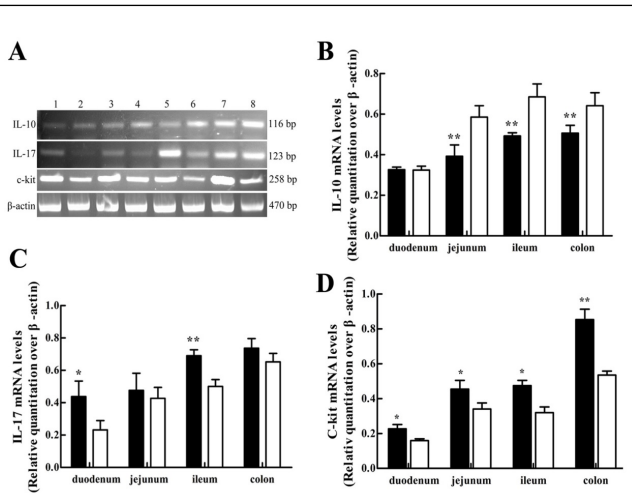

Figure 3: Expression of the cytokine mRNA levels of: IL-10 (B), IL-17 (C), and c-kit (D) in different bowel segments in the PI-IBS (n=8) and control (n=8) groups. A. Representative graphs of the PCR assay. Lane 1,3,5, and 7 are duodenum, jejunum, ileum and colon of PI-IBS group, lane 2,4,6, and 8 are duodenum, jejunum, ileum and colon of control group. Black: PI-IBS group, white: Control group. Date are presented as mean ± SD. *P<0.05, **P<0.01 versus control group

Discussion

PI-IBS has been defined as acute onset IBS symptoms (meeting Rome Ⅲ criteria) that develop after the individual, who has not

previously met the Rome criteria, experiences a gastrointestinal infection with two or more of the following characteristics: Fever, vomiting, diarrhea, or a stool culture positive for an infectious agent [27]. Though the mechanism is unclear, visceral hypersensitivity and the intestinal motor dysfunction have been defined to participate in [28,29]. We used the method of literature to establish PI-IBS mouse model [30]. Infected mice could act as PI-IBS mice when the intestine inflammation could not be examined by pathological examination, AWR scores increased and ITT became short.

The present finding showed that the c-kit protein level increased by 23.66, 21.97, 17.93, and 32.09 percent in duodenum, jejunum, ileum, and colon of PI-IBS mice over these in control mice. The decreased percent of IL-10 were 35.94%, 41.68%, and 42.45% in jejunum, ileum, and colon of PI-IBS mice. The increased percent of IL-17 were 90.12% and 48.46% in duodenum and colon of PI-IBS mice. According to the above data, we found that the increased percent of c-kit only came with decreased IL-10 or increased IL-17 in the duodenum, jejunum, and ileum is lower than that accompanied by decreased IL-10 and increased IL-17 in colon. We hypothesized that IL-10 and IL-17 could co-regulate the expression of intestinal c-kit in PI-IBS, and which may maintain a low-grade inflammation but no tissue injury to perform regulatory processes. The below mechanisms may support our hypothesis.

At the acute mucosal inflammation phase, immune system will down-regulate IL-10 level and up-regulate IL-17 level so that immune cells can produce more pro-inflammatory cytokines to defense invader and remove damaged cells [12-14,19-21] IL-17 has a protective role in infections, its main function improve inflammatory effects by activating NF-κB, MAPKs and C/EBP cascades to induce the production of multiple pro-inflammatory molecules and subsequent activation of macrophages and neutrophils [17], meanwhile, it also have a regulatory function limiting accumulation and/or activity of neutrophils during the inflammatory process by attenuating the anti-apoptotic effect of inflammatory cytokines [18] The IL-23/IL-17 signal pathway has been best described, which regulates IL-12/IFN-γ, and drives neutrophil migration to mediate inflammation injury [19-21]. Uncontrolled IL-17 responses can augment production of inflammatory factors including IL-1,IL-6,IL-8,TNF-α,GM-CSF, and MIP-2 [21,23], these cytokines were reported to be increased in the peripheral blood and intestinal mucosa of PI-IBS patients [22], and which would alter the gut physiology and host immunity and cause clinical symptoms [22,31]. IL-10, as a classical anti-inflammatory cytokines, reduces inflammation reaction through numbers of mechanisms: It can diminish the production of inflammatory mediators including IL-1β and IFN-γ in T cells and activated macrophages [12-14]; it also can reduce the antigen-presenting cells surface expression of major histocompatibility complex class II molecules, co-stimulating and adhesion molecules [15,16]. Decreased IL-10 levels and uncontrolled IL-17 in this study may hold a low-grade inflammation mode undetected by pathological examination [22,31]. Meanwhile, mucosal acute inflammation of the gut resulted in loss of ICC function because of structural injury and loss of c-kit positivity [32]. When the low-grade inflammation was not enough to cause tissue damage but could result in mild cell injury, inflammatory cytokines can act as stimulating factors to improve protein synthesizing just like inflammatory hyperplasia and autoimmunity disease, in this moment, the rough endoplasmic reticulum and Golgi apparatus of the injury ICC need to synthesize proteins (including c-kit) to form new plasma membrane and to recover structure and functions [32]. Overexpressed c-kit is required for development and maintenance of ICC, also is necessary for development of coordinated motility patterns and for survival of ICC [7,8]. In the local intestine of PI-IBS, decreased IL-10 levels also induce mast cell hyperplasia [33,34]. High concentration of 5-Hydroxytryptamine (5-HT), produced by mast cells, were detected in PI-IBS patients, which can combine with 5-HT2B receptors expressed on the plasma membrane of ICC and promote proliferation of ICC [35,36], hyperplasia ICC cells would synthesize more c-kit under a low-grade inflammation condition.

Another important finding in this study was that c-kit positive signals were mainly detected in the submucosa and myenteron where the ICC mainly pace slow waves [37]. More c-kit positive cells can accelerate the intestinal muscular contraction and peristaltic propagation [38,39]. In addition, an increased in the number of ICC can shorten the intervals between the migratory motor complex cycles and decrease the bowel transmitting time [40].

Acknowledgement

This work was supported by the National Natural Science Foundation of China (Grant No. 81160057).The authors thank Prof. Bao-Quan Fu (Chinese Academy of Agricultural Sciences) for offering Trichinella spiralis. We thank Prof. Yu Li and Mr. Bing Liu (Department of Anatomic Pathology, Chongqing Medical University, China) for the technical assistance.

References

1. Choung RS, Locke GR (2011) Epidemiology of IBS. Gastroenterol Clin North Am 40: 1-10.
2. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, et al. (2006) Functional bowel disorders. Gastroenterology 130: 1480-1491.
3. Spiller R, Garsed K (2009) Postinfectious irritable bowel syndrome. Gastroenterology 136: 1979-1988.
4. Elsenbruch S, Holtmann G, Oezcan D, Lysson A, Janssen O, et al. (2004) Are there alterations of neuroendocrine and cellular immune responses to nutrients in women with irritable bowel syndrome? Am J Gastroenterol 99: 703-710.
5. Liebregts T, Adam B, Bredack C, Roth A, Heinzel S, et al. (2007) Immune activation in patients with irritable bowel syndrome. Gastroenterology 132: 913-920.
6. Sarna SK (2008) Are interstitial cells of cajal plurifunction cells in the gut? Am J Physiol Gastrointest Liver Physiol 294: G372-G390.
7. Rich A, Gordon S, Brown C, Gibbons SJ, Schaefer K, et al. (2013) Kit signaling is required for development of coordinated motility patterns in zebrafish gastrointestinal tract. Zebrafish 10: 154-160.
8. Ghaith O, El-Halabi M, Hashash JG, Sharara AI (2010) Investigational agents for the irritable bowel syndrome. Expert Opin Investig Drugs 19: 1161-1178.
9. Jee SR, Morales W, Low K, Chang C, Zhu A, et al. (2010) ICC density predicts bacterial overgrowth in a rat model of post-infectious IBS. World J Gastroenterol 16: 3680-3686.
10. Pokkunuri V, Pimentel M, Morales W, Jee SR, Alpern J, et al. (2012) Role of cytolethal distending toxin in altered stool form and bowel phenotypes in a rat model of post-infectious irritable bowel syndrome. J Neurogastroenterol Motil 18: 434-42.
11. Gasche C, Bakos S, Dejaco C, Tillinger W, Zakeri S, et al. (2000) IL-10 secretion and sensitivity in normal human intestine and inflammatory bowel disease. J Clin Immunol 20: 362-70.
12. Sabat R, Grutz G, Warszawska K, Kirsch S, Witte E, et al. (2010) Biology of interleukin-10. Cytokine Growth Factor Rev 21: 331-344.

13. Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A (1991) IL-10 inhibits cytokine production by activated macrophages. J Immunol 147: 3815-3822.
14. D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, et al. (1993) Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J Exp Med 178: 1041-1048.
15. Malefyt R, Haanen J, Spits H, Roncarolo MG, te Velde A, et al. (1991) Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. J Exp Med 174: 915-24.
16. Willems F, Marchant A, Delville JP, Gérard C, Delvaux A, et al. (1994) Interleukin-10 inhibits B7 and intercellular adhesion molecule-1 expression on human monocytes. Eur J Immunol 24: 1007-1009.
17. Zhu S, Qian Y (2012) IL-17/IL-17 receptor system in autoimmune disease: Mechanisms and therapeutic potential. Clin Sci 122: 487-511.
18. Costa VS, Mattana TC, da Silva ME (2010) Unregulated IL-23/IL-17 immune response in autoimmune diseases. Diabetes Res Clin Pract 88: 222-226.
19. Li L, Huang L, Vergis AL, Ye H, Bajwa A, et al. (2010) IL-17 produced by neutrophils regulates IFN-gamma-mediated neutrophil migration in mouse kidney ischemia-reperfusion injury. J Clin Invest 120: 331-142.
20. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, et al. (2005) IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 201: 233-240.
21. Iwakura Y, Ishigame H, Saijo S, Nakae S (2011) Functional specialization of interleukin-17 family members. Immunity 34: 149-162.
22. Bashashati M, Rezaei N, Andrews CN, Chen CQ, Daryani NE, et al. (2012) Cytokines and irritable bowel syndrome: where do we stand? Cytokine 57: 201-209.
23. Fossiez F, Djossou O, Chomarat P, Flores-Romo L, Ait-Yahia S, et al. (1996) T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J Exp Med 183: 2593-2603.
24. Yang B, Zhou XC, Lan C (2015) Impact of the alterations in the interstitial cells of Cajal on intestinal motility in post-infection irritable bowel syndrome. Mol Med Rep 11: 2735-2740.
25. Zagzag D, Zhong H, Scalzitti JM, Laughner E, Simons JW, et al. (2000) Expression of hypoxia-inducible factor 1alpha in brain tumors: Association with angiogenesis, invasion, and progression. Cancer 88: 2606-2618.
26. Micili SC, Goker A, Sayin O, Akokay P, Ergur BU (2013) The effect of lipoic acid on wound healing in a full thickness uterine injury model in rats. J Mol Histol 44: 339-345.
27. Ghoshal UC, Ranjan P (2011) Post-infectious irritable bowel syndrome: The past, the present and the future. J Gastroenterol Hepatol 26 Suppl 3: 94-101.
28. Nozu T, Okumura T (2011) Visceral sensation and irritable bowel syndrome; With special reference to comparison with functional abdominal pain syndrome. J Gastroenterol Hepatol 26: 122-127.
29. Barbara G, Cremon C, De Giorgio R, Dothel G, Zecchi L, et al. (2011) Mechanisms underlying visceral hypersensitivity in irritable bowel syndrome. Curr Gastroenterol Rep 13: 308-315.
30. Bercik P, Wang L, Verdu EF, Mao YK, Blennerhassett P, et al. (2004) Visceral hyperalgesia and intestinal dysmotility in a mouse model of postinfective gut dysfunction. Gastroenterology 127: 179-187.
31. Akiho H, Ihara E, Nakamura K (2010) Low-grade inflammation plays a pivotal role in gastrointestinal dysfunction in irritable bowel syndrome. World J Gastrointest Pathophysiol 1: 97-105.
32. Wang XY, Vannucchi MG, Nieuwmeyer F, Ye J, Faussone-Pellegrini MS, et al. (2005) Changes in interstitial cells of Cajal at the deep muscular plexus are associated with loss of distention-induced burst-type muscle activity in mice infected by Trichinella spiralis. Am J Pathol 167: 437-453.
33. Speiran K, Bailey DP, Fernando J, Macey M, Barnstein B, et al. (2009) Endogenous suppression of mast cell development and survival by IL-4 and IL-10. J Leukoc Biol 85: 826-836.
34. Kim HS, Lim JH, Park H, Lee SI (2010) Increased immunoendocrine cells in intestinal mucosa of postinfectious irritable bowel syndrome patients 3 years after acute shigella infection-an observation in a small case control study. Yonsei Med J 51: 45-51.
35. Chun L, Bai Y (2011) Experimental research on modifying liver and spleen decoction for diarrhea-predominant IBD. NUTCM 2: 019.
36. Wouters MM, Roeder JL, Tharayil VS, Stanich JE, Strege PR, et al. (2009) Protein kinase c-gamma-mediates regulation of proliferation by the serotonin 5-hydroxytryptamine receptor 2B. J Biol Chem 284: 21177-21184.
37. Ward SM, Harney SC, Bayguinov JR, McLaren GJ, Sanders KM (1997) Development of electrical rhythmicity in the murine gastrointestinal tract is specifically encoded in the tunica muscularis. J Physiol 505: 241-258.
38. Patterson LM, Zheng H, Ward SM, Berthoud HR (2001) Immunohistochemical identification of cholecystokinin A receptors on interstitial cells of cajal, smooth muscle, and enteric neurons in rat pylorus. Cell Tissue Res 305: 11-23.
39. Dizdar V, Spiller R, Singh G, Hanevik K, Gilja OH, El-Salhy M, et al. (2010) Relative importance of abnormalities of CCK and 5-HT (serotonin) in Giardia-induced post-infectious irritable bowel syndrome and functional dyspepsia. Aliment Pharm Therap 31: 883-91.
40. Powell AK, Bywater RA (2003) Murine intestinal migrating motor complexes: Longitudinal components. J Neurogastroenterol Motil 15: 245-56.