

ORIGINAL ARTICLE

A pilot study of colonic B cell pattern in irritable bowel syndrome

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Abstract

Objective. Low-grade gastrointestinal inflammation has been reported in patients with irritable bowel syndrome (IBS). However, the colonic B-cell pattern has not been investigated in these patients. Therefore, the aim of this pilot study was to investigate the distribution and isotype of immunoglobulin-producing B cells in the colonic mucosa of IBS patients. **Material and methods.** Patients with IBS ($n = 12$) fulfilling the Rome II criteria were compared with controls ($n = 11$). Immunohistochemical staining of biopsies from the sigmoid and ascending colon was performed. **Results.** The number of IgA⁺ B cells in the ascending colon was lower in IBS patients than in controls ($p = 0.039$). Furthermore, unlike controls, IBS patients had a reduction of IgA⁺ B cells in the ascending colon relative to the sigmoid colon ($p = 0.04$). Neither the IgG⁺, nor the IgM⁺ colonic B-cell numbers differed between IBS patients and controls. Very few colonic IgE⁺ cells were detected and there was no difference between the two subject groups. **Conclusions.** The reduced number of colonic IgA⁺ B cells in IBS patients suggests that the disorder may be associated with a modified gut immune defence. Whether this phenomenon is causally related to symptoms remains unknown and merits further investigation in a larger group of patients.

Key Words: IBS, immunoglobulin, inflammation, irritable bowel syndrome

Introduction

Irritable bowel syndrome (IBS) is common in Western society [1], but the pathophysiology of this disorder is still not fully understood [2]. However, in recent studies it is suggested that a role is played by a limited inflammatory response in the intestinal mucosa, although the underlying mechanisms remain unclear [3–6]. During the past few years several reports have described increased recruitment of T cells to the intestinal mucosa of IBS patients [4,7–9]. It has been suggested that B cell mediated food hypersensitivity reactions may contribute to the gut immune activation, as IBS patients respond favourably to specific elimination diets [10–13]. Alteration in intestinal bacterial composition is also frequently discussed as an important key player in the pathogenesis of IBS [14,15]. Moreover, increased numbers of intestinal mast cells are reported to be associated with IBS symptoms [16,17].

The immunoglobulin isotype produced by B cells reflects the help given by T cells to the B cells at the

time of antigen recognition and activation. The expression of IgM on the cell surface of a B cell indicates that the cell has not encountered its specific antigen, although IgM may also be produced and secreted during the early phase of an adaptive immune response. The T-helper-2 cytokine interleukin-4 induces a B-cell class switch to IgE and IgG4, whereas the T-helper-1-associated cytokines interleukin-12 and interferon- γ induce a class switch to IgG1 [18]. IgA-producing B cells are most frequently found in mucosal tissues where the level of tumour growth factor- β is constitutively high [19]. The IgA produced in the intestinal mucosa is secreted out into the lumen and functions as an “antiseptic paint” in the defence and protection of the intestinal tissue against various pathogens. The large amount of IgA in the intestinal mucosa and in the lumen is regarded as crucial in fighting and eliminating microorganisms without activation of inflammatory mechanisms of the gut immune system.

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Despite the fact that previous studies have demonstrated increased serum levels of food-specific IgG antibodies in IBS patients [11,20], the colonic mucosal B cell pattern has not been investigated in this patient population. Therefore, the aim of this pilot study was to determine the distribution and isotype of immunoglobulin-producing B cells in the ascending and the sigmoid colonic mucosa in a limited number of IBS patients.

Material and methods

Study subjects

This study was approved by the Ethics Committee of the University of Göteborg, and was performed after receiving written informed consent from all subjects. Study subjects were recruited among patients referred for colonoscopy at the Sahlgrenska University Hospital, Göteborg, Sweden. Two biopsies from the ascending (5 cm above the ileocaecal valve) and the sigmoid colon, respectively, were taken from each patient.

IBS patients were defined according to the Rome II criteria [21] and underwent routine diagnostic colonoscopy to exclude organic bowel disease. The patients were defined as non-inflammatory as assessed by routine histology. In particular, no evidence of collagenous or lymphocytic colitis was detected based on standard criteria [22]. Twelve IBS patients were included (7 F, mean age 35.6 ± 13.5 years; body mass index (BMI) 23.1 ± 4.3 kg/m²) and 5 had diarrhoea-predominant IBS (d-IBS) and 7 had alternating bowel habits (a-IBS) [21]. All of these patients had a negative diagnostic work-up, which was determined by the clinical presentation. Food-related symptoms were common in all the patients, even though there was no clinical suspicion of a specific food allergy. In 2 of the IBS patients the symptom duration was less than 1 year, in 7 patients the symptom duration was 1–5 years, while 3 of the patients included in the study reported having IBS symptoms for more than 5 years. Acute onset after supposed infectious diarrhoea was described by two d-IBS patients.

We included 11 healthy volunteers, recruited through an advertisement, as control subjects (6 F, mean age 40 ± 7.9 years; BMI 26.4 ± 4.2 kg/m²). These subjects were free from gastrointestinal symptoms and their colonoscopies were normal, both macroscopically and microscopically.

Sample collection

Biopsies were assessed from the ascending colon and the sigmoid colon, respectively, using traditional forceps. The biopsies were kept in histocon

(Histolab, Göteborg, Sweden) on ice until being embedded in Tissue Tek OCT (Sakura Finetek, Zoeterwoude, The Netherlands) and snap frozen in liquid hydrogen. The embedded biopsies were kept at a temperature of -80°C until further analysis.

Immunohistochemical analysis

For analysis of immunoglobulin production of B cells, 5- μm cryo-cut sections from the colonic biopsies were first fixed in 50% acetone followed by fixation in 100% acetone. The sections were then incubated for 40 min in 35°C phosphate buffered saline (PBS) supplemented with 0.1% glucosylase, 0.36% D-glucose and 0.1% saponin, to block endogenous enzyme activity. This was followed by 30-min incubation with 1% goat or mouse serum in 0.1% bovine serum albumin (BSA)-PBS solution. The sections were then incubated with horse-radish peroxidase (HRP) conjugated monoclonal antibodies in PBS supplemented with 2% BSA and 0.05% Tween for 1 h at room temperature. The antibodies were specific for human IgM⁺ B lymphocytes (goat anti-human IgM), human IgG⁺ B lymphocytes (goat anti-human IgG), human IgA⁺ B lymphocytes (goat anti-human IgA) and human IgE⁺ B lymphocytes (mouse anti-human IgE). All immunoglobulin-specific antibodies were purchased from Southern Biotech (Birmingham, Ala., USA).

Specific antibody reactions were developed by adding an avidin-biotin-HRP complex (Dako, Glostrup, Denmark) according to the manufacturer's instructions. Tissue sections were developed with chromogen 3-amino-9-ethylcarbazole containing H₂O₂ (AEC substrate chromogen) (DakoCytomation) and counterstained with Mayer's haematoxylin (Histolab). All slides were washed in H₂O and mounted with Faramount aqueous mounting medium (Dako). For negative controls, the specific antibody was replaced with PBS and did not give positive staining. Tissue sections were examined by light microscopy and the number of specifically stained cells per mm² was determined by using Leica Qwin software.

The numbers of immunoglobulin-producing B cells per mm² in the colonic mucosa were determined and compared between patients with IBS and healthy control subjects. The number of the respective cell population was measured in the ascending colon as well as in the sigmoid colon, to determine whether any colonic regional differences were present.

Statistical analysis

All statistical evaluations were made using the StatView Software (SAS Institute, Cary, N.C.,

USA). The Wilcoxon signed-paired test or the non-parametric Mann-Whitney test was used to evaluate significance of differences; *p*-values of less than 0.05 were regarded as significant.

Results

Fewer IgA-producing B cells in the ascending colonic mucosa of IBS patients

We determined and compared the number and isotype specificity of B cells in the colonic mucosa of patients with IBS and control subjects. The number of IgA-producing B cells was found to be significantly lower in the ascending colon of IBS patients compared with that in control subjects (Figure 1A, B). The number of IgA-producing B cells in the sigmoid colon did not differ between IBS patients and control subjects (Figure 1A). We also investigated whether there were any regional differences in the distribution of IgA-producing cells in the ascending and the sigmoid colon in IBS patients and in control subjects. The number of IgA⁺ B cells was significantly lower in the ascending colon relative to the sigmoid colon of IBS patients (Figure 1C). This regional difference in the number of colonic IgA⁺ B cells was not seen in control subjects. Thus, IBS patients have a reduced number of IgA⁺ B cells in the ascending colon but not in the sigmoid colon.

Normal levels of IgG-, IgM- and IgE-producing B cells in the colonic mucosa of IBS patients

The number of IgG-producing B cells did not differ between IBS patients and control subjects in either the ascending or the sigmoid colon (Figure 2A). Furthermore, the segmental distribution of IgG-producing B cells in the ascending colon and the sigmoid colon was similar in IBS patients and control subjects (Figure 2A). Also the numbers of IgM⁺ B cells were comparable in both the ascending colon and the sigmoid colon of IBS patients and control subjects (Figure 2B). Furthermore, there was no variation in the number of IgM⁺ B cells in the different regions in the colonic mucosa in either subject group. Very few IgE-producing B cells were detected in the colonic mucosa and there was no difference between IBS patients and control subjects. IgE⁺ B cells were found in the colonic mucosa in 2 out of 11 IBS patients and in 3 out of 11 control subjects (data not shown). In conclusion, IBS patients displayed normal levels of IgG-, IgM- and IgE-producing B cells in the ascending colon as well as in the sigmoid colon.

Discussion

This study was designed to determine the immunoglobulin isotype pattern of the colonic B cells in IBS patients. Our main finding was that the number of IgA⁺ B cells in the ascending colon was lower in IBS patients in relation to that in controls. Accordingly, IBS patients had reduced numbers of IgA⁺ B cells in the ascending colon as compared to the sigmoid colon, which was not seen in our control subjects. The lower number of colonic IgA⁺ B cells could lead to reduced secretion of IgA into the lumen of IBS patients.

Secretory IgA is important in maintaining mucosal tolerance and in protecting mucosal surfaces from pathogens and food antigens, and reduced intestinal IgA production may therefore result in a defective gut immune defence and impaired oral tolerance [18,23,24]. It was recently reported that mice with reduced levels of luminal IgA have a defective tolerance to food antigens [25]. Furthermore, children with multiple food adverse reactions have somewhat lower levels of IgA, at least in the blood [26], which emphasizes the importance of IgA production in maintaining oral tolerance. Thus, the reduced levels of colonic IgA-producing B cells in IBS patients recorded in our study may result in defective oral tolerance, which could be a possible explanation for the increased gut immune activity reported in this patient population.

It could be hypothesized that the reduction in IgA-producing cells in IBS patients in our study was present before the IBS symptoms developed, which may also have increased the susceptibility of these individuals to bacterial and viral infections. This hypothesis is supported by a recent report demonstrating that patients with recurrent *Clostridium difficile*-associated diarrhoea (CDAD) [27], analogous with the IBS patients in our study, have decreased numbers of colonic IgA-producing B cells. We have not ruled out the possibility that the IBS patient group, like patients with coeliac disease [28], may harbour individuals with undiagnosed IgA deficiency. However, as the IBS patients and control subjects in this study had comparable levels of IgA⁺ B cells in the sigmoid colon, it is less likely that IBS is associated with a general IgA deficiency. Nevertheless, it would be of interest to address this question in future studies concerning the presence and function of B cells in IBS patients.

Also, the presence of specific bacterial strains or the composition of the intestinal flora may be important in the development of IBS symptoms. The gut flora of IBS patients has recently been suggested to differ quantitatively from that of healthy controls, according to real-time polymerase chain

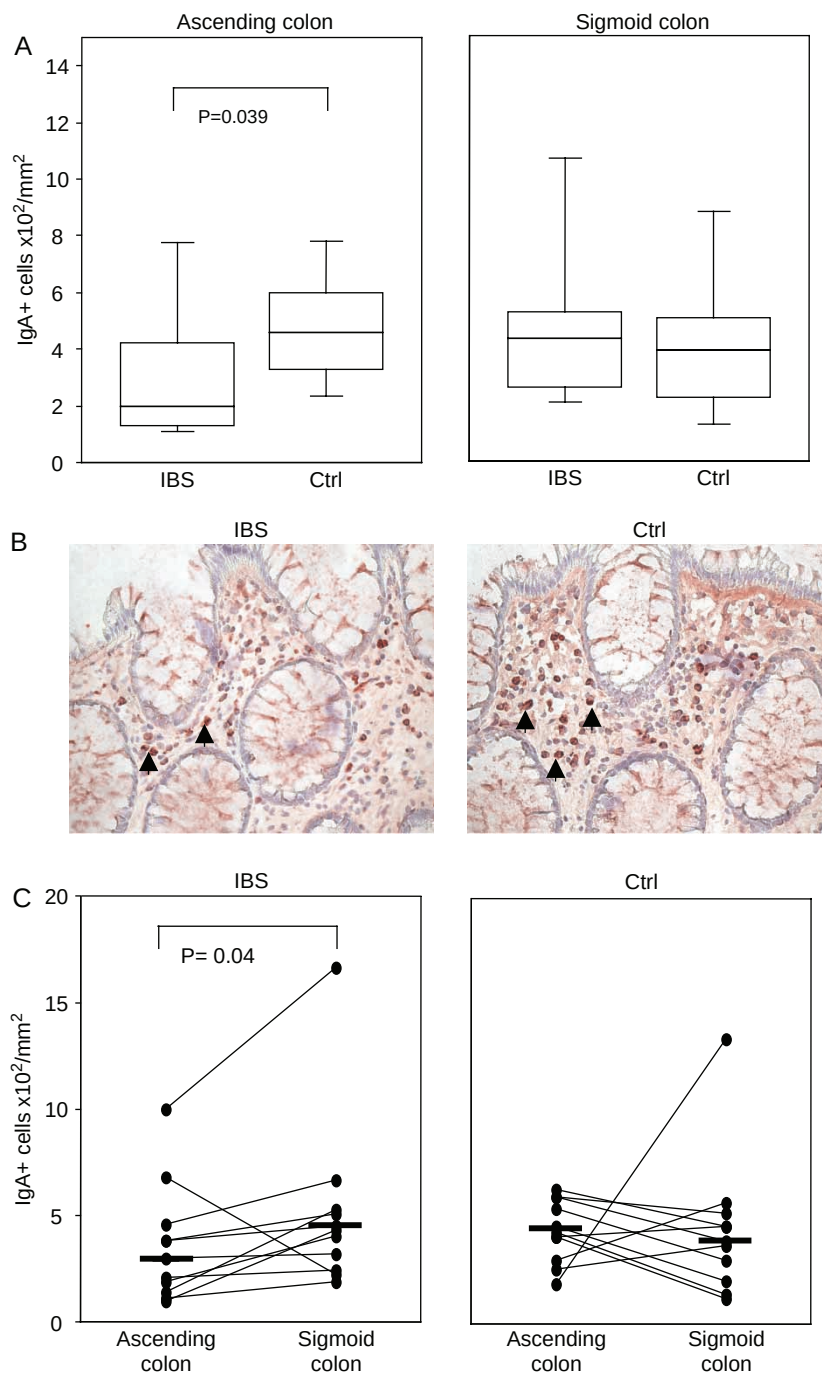


Figure 1. IgA producing B cells in the colonic mucosa of patients with irritable bowel syndrome (IBS). A. Comparison of the number of IgA producing B cells per mm^2 in the ascending colon and the sigmoid colon from patients with IBS and healthy controls (Ctrl); IBS $n = 12/11$ and Ctrl = 11/11. B. Representative photomicrographs showing IgA⁺ B cells in the ascending colonic mucosa of an IBS patient and a control subject. C. Comparison of IgA-producing B cells per mm^2 in the ascending colon relative to the sigmoid colon in IBS patients and control subjects, respectively. Connecting lines show values from paired samples taken from the same individual. Median values are indicated by horizontal bars. IBS, $n = 11$; Ctrl, $n = 11$.

reaction (PCR) analysis of bacterial DNA from faeces [29]. Thus, it is possible that IBS patients have increased intestinal reactivity to gut microbiota and pathogens which, at least theoretically, could result from impaired IgA production and luminal secretion in the ascending colon. However, the limited number of subjects included in the study

restricts the number of conclusions that can be drawn and the generalization of our findings remains to be proven in larger series.

Food-related symptoms in IBS patients are common [30]. Previous studies have demonstrated increased levels of food-specific IgG antibodies [10,20,31] in the serum of IBS patients, although

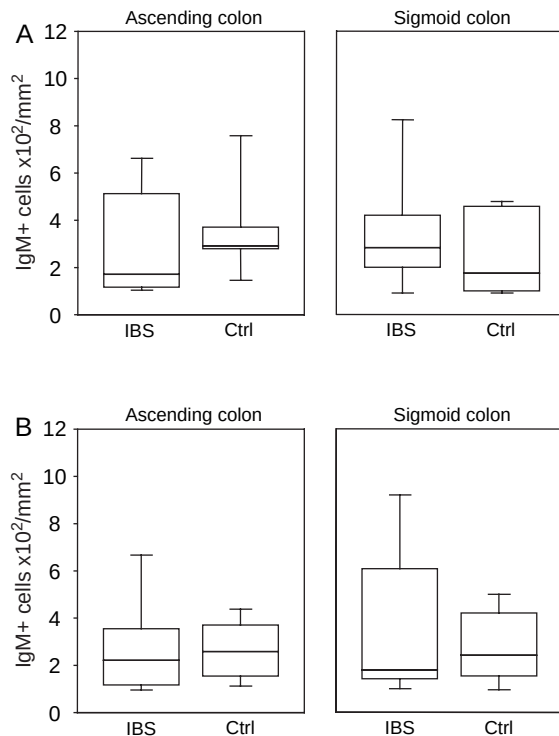


Figure 2. IgG- and IgM-producing B cells in the colonic mucosa of patients with irritable bowel syndrome (IBS). Comparison of IgG- (A) and IgM- (B) producing B cells per mm² in the ascending colon and the sigmoid colon in patients with IBS and healthy controls (Ctrl). IBS, $n = 12$; Ctrl, $n = 11$.

the relevance of food-specific IgE serum antibodies is not altogether clear [31–33]. However, neither the number of colonic IgG, nor IgE-producing B cells differed between IBS patients and controls in our study. In fact, very few colonic IgE⁺ B cells were detected at all and there was no difference between the two subject groups. Despite the normal level of total colonic IgG⁺ B cell counts found in IBS patients in the present study, it cannot be ruled out that there could be an increased production of certain unidentified antigen-specific IgG antibodies. Taken together, the comparable number of mucosal total IgE- and IgG-producing B cells in IBS patients and controls implies that the IBS symptoms in our patient group were not associated with IgE- or IgG-mediated reactions in the colonic mucosa.

The focus of our study was on the colonic B cell production of total immunoglobulin, in contrast to previous studies which determined immunoglobulin levels in serum. Our choice of method has its limitations, since the results obtained only reflect the immunoglobulin production in a limited intestinal region. The obvious advantage of analysing the cell populations in intestinal biopsies with immunohistochemistry, instead of immunoglobulin levels in serum, is that it is possible to detect local reactions in the gut. Results based on serum analysis may not

always be a proper reflection of subtle regional immunological activity alterations in the intestinal mucosa. By basing our studies on biopsies from both the ascending and the sigmoid colon, we found a delicate but distinct colonic segmental variation in colonic IgA-producing B cells in IBS patients, but not in controls. These data correlate well with a previous study by our group in which we reported that gut immune activity present in IBS patients was more pronounced in the ascending colon [8]. Furthermore, Chadwick et al. have shown that the influx of immune cells is more prominent in the ascending colon than in the left-sided colon of IBS patients [4]. Taken together, these findings suggest that there are regional alterations in the immune system of the colonic mucosa in IBS patients, which may be important in the understanding of the increased gut immune activity in this patient population.

In conclusion, the segmental reduction in IgA-producing B cells found in IBS patients suggests that the disorder might be associated with a modified gut immune defence. Whether this phenomenon is causally related to symptoms remains unknown and should be investigated further.

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References

- [1] Agreus L, Svärdsudd K, Nyren O, Tibblin G. Irritable bowel syndrome and dyspepsia in the general population: overlap and lack of stability over time. *Gastroenterology* 1995;109: 671–80.
- [2] Mayer EA, Collins SM. Evolving pathophysiologic models of functional gastrointestinal disorders. *Gastroenterology* 2002; 122:2032–48.
- [3] Barbara G, De Giorgio R, Stanghellini V, Cremon C, Corinaldesi R. A role for inflammation in irritable bowel syndrome? *Gut*;51;Suppl 1:i41–4.
- [4] Chadwick VS, Chen W, Shu D, Paulus B, Bethwaite P, Tie A, et al. Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology* 2002;122:1778–83.
- [5] Törnblom H, Abrahamsson H, Barbara G, Hellström PM, Lindberg G, Nyhlin H, et al. Inflammation as a cause of

- functional bowel disorders. *Scand J Gastroenterol* 2005;40:1140–8.
- [6] Öhman L, Simrén M. New insights into the pathogenesis and pathophysiology of irritable bowel syndrome. *Dig Liver Dis* 2007;39:201–15.
 - [7] Dunlop SP, Jenkins D, Spiller RC. Distinctive clinical, psychological, and histological features of postinfective irritable bowel syndrome. *Am J Gastroenterol* 2003;98:1578–83.
 - [8] Öhman L, Isaksson S, Lundgren A, Simrén M, Sjövall H. A controlled study of colonic immune activity and beta7+ blood T lymphocytes in patients with irritable bowel syndrome. *Clin Gastroenterol Hepatol* 2005;3:980–6.
 - [9] Spiller RC, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M, et al. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute *Campylobacter* enteritis and in post-dysenteric irritable bowel syndrome. *Gut* 2000;47:804–11.
 - [10] Atkinson W, Sheldon TA, Shaath N, Whorwell PJ. Food elimination based on IgG antibodies in irritable bowel syndrome: a randomised controlled trial. *Gut* 2004;53:1459–64.
 - [11] Zar S, Mincher L, Benson MJ, Kumar D. Food-specific IgG4 antibody-guided exclusion diet improves symptoms and rectal compliance in irritable bowel syndrome. *Scand J Gastroenterol* 2005;40:800–7.
 - [12] Jones VA, McLaughlan P, Shorthouse M, Workman E, Hunter JO. Food intolerance: a major factor in the pathogenesis of irritable bowel syndrome. *Lancet* 1982;2:1115–7.
 - [13] Nanda R, James R, Smith H, Dudley CR, Jewell DP. Food intolerance and the irritable bowel syndrome. *Gut* 1989;30:1099–104.
 - [14] Quigley EM. Bacteria: a new player in gastrointestinal motility disorders: infections, bacterial overgrowth, and probiotics. *Gastroenterol Clin North Am* 2007;36:735–48.
 - [15] Pimentel M, Lezcano S. Irritable bowel syndrome: bacterial overgrowth: what's known and what to do. *Curr Treat Options Gastroenterol* 2007;10:328–37.
 - [16] O'Sullivan M, Clayton N, Breslin NP, Harman I, Bountra C, McLaren A, et al. Increased mast cells in the irritable bowel syndrome. *Neurogastroenterol Motil* 2000;12:449–57.
 - [17] Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* 2004;126:693–702.
 - [18] Fujihashi K, Kato H, van Ginkel FW, Koga T, Boyaka PN, Jackson RJ, et al. A revisit of mucosal IgA immunity and oral tolerance. *Acta Odontol Scand* 2001;59:301–8.
 - [19] Fagarasan S, Honjo T. Regulation of IgA synthesis at mucosal surfaces. *Curr Opin Immunol* 2004;16:277–83.
 - [20] Zar S, Benson MJ, Kumar D. Food-specific serum IgG4 and IgE titers to common food antigens in irritable bowel syndrome. *Am J Gastroenterol* 2005;100:1550–7.
 - [21] Thompson WG, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Muller-Lissner SA. Functional bowel disorders and functional abdominal pain. *Gut* 1999;45 (Suppl 2):II43–7.
 - [22] Bogomoletz WV. Collagenous, microscopic and lymphocytic colitis. An evolving concept. *Virchows Arch* 1994;424:573–9.
 - [23] Hanson LÅ, Ahlstedt S, Andersson B, Carlsson B, Fallström SP, Mellander L, et al. Protective factors in milk and the development of the immune system. *Pediatrics* 1985;75:172–6.
 - [24] Russel GW KM, Lamm ME. Biological activities of IgA. In: Ogra MJ PL, Lamm ME, Strober W, Bienenstock J, McGhee JR, editors. *Mucosal immunology*. San Diego: Academic Press; 2004. pp 225–40.
 - [25] Frossard CP, Hauser C, Eigenmann PA. Antigen-specific secretory IgA antibodies in the gut are decreased in a mouse model of food allergy. *J Allergy Clin Immunol* 2004;114:377–82.
 - [26] Latcham F, Merino F, Lang A, Garvey J, Thomson MA, Walker-Smith JA, et al. A consistent pattern of minor immunodeficiency and subtle enteropathy in children with multiple food allergy. *J Pediatr* 2003;143:39–47.
 - [27] Johal SS, Lambert CP, Hammond J, James PD, Borriello SP, Mahida YR. Colonic IgA producing cells and macrophages are reduced in recurrent and non-recurrent *Clostridium difficile* associated diarrhoea. *J Clin Pathol* 2004;57:973–9.
 - [28] McGowan KE, Lyon ME, Butzner JD. Celiac disease and IgA deficiency: complications of serological testing approaches encountered in the clinic. *Clin Chem* 2008;54:1203–9.
 - [29] Malinen E, Rinttilä T, Kajander K, Matto J, Kassinen A, Krogus L, et al. Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. *Am J Gastroenterol* 2005;100:373–82.
 - [30] Simrén M, Månsson A, Langkilde AM, Svedlund J, Abrahamsson H, Bengtsson U, et al. Food-related gastrointestinal symptoms in the irritable bowel syndrome. *Digestion* 2001;63:108–15.
 - [31] Zuo XL, Li YQ, Li WJ, Guo YT, Lu XF, Li JM, et al. Alterations of food antigen-specific serum immunoglobulins G and E antibodies in patients with irritable bowel syndrome and functional dyspepsia. *Clin Exp Allergy* 2007;37:823–30.
 - [32] Crowe SE, Perdue MH. Gastrointestinal food hypersensitivity: basic mechanisms of pathophysiology. *Gastroenterology* 1992;103:1075–95.
 - [33] Zar S, Kumar D, Benson MJ. Food hypersensitivity and irritable bowel syndrome. *Aliment Pharmacol Ther* 2001;15:439–49.