Abstract. Irritable bowel syndrome (IBS) is a common chronic gastrointestinal disorder characterized by abdominal discomfort or pain associated with altered bowel habits, bloating and abdominal distension. In total, 5–20% of the world population has been reported to suffer from IBS (1). The degree and frequency of symptoms vary between patients, ranging from tolerable to severe and from daily symptoms to intermittent symptoms at intervals of weeks/months (2-14).

IBS is not known to be associated with the development of serious disease or with high mortality (15,16). However, IBS considerably reduces quality of life to the same degree of impairment as major chronic diseases, including diabetes, congestive heart failure, renal insufficiency and hepatic cirrhosis (17-21). Besides the increased morbidity caused by IBS, the syndrome is an economic burden to society in varying forms, for example by increasing the incidence of sick leave and the over-consumption of healthcare resources (1).

The pathogenesis of IBS is unknown. Intestinal low-grade inflammation has been proposed as one of the factors contributing to the development of IBS (1). Support of this assumption is observed in histopathological examinations of mucosal biopsies from the ileum, caecum, colon and rectum, mostly from IBS patients with diarrhoea as the predominant symptom (IBS-D) but even from patients with constipation as the predominant symptom (IBS-C), which revealed a mucosal infiltration of mast cells and lymphocytes (22‑28). Low‑grade mucosal inflammation appears to be evident in a subset of IBS, i.e., post-infectious IBS (PI-IBS) (1,24,26), but it is not clear, however, whether low-grade inflammation also occurs in sporadic IBS. The present study was therefore undertaken to examine the possible occurrence of low-grade mucosal inflammation in the rectum as a representative of the large intestine of patients with sporadic IBS.

Introduction

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Patients and methods

Patients and controls. In total, 50 patients with IBS that fulfilled the Rome III Criteria (http://www.romecriteria.org) using the IBS module were included in the study (29). These patients consisted of 42 females and 8 males with an average age of 34 years (range 18-62 years). Of these, 30 patients...
had IBS-D and 20 patients had IBS-C. All patients had their symptoms for numerous years and were not able to connect the onset of the IBS symptoms to any particular events, including gastrointestinal or other infections. All patients underwent a complete physical examination and had the following investigative blood tests: full blood count, electrolytes, calcium, inflammatory markers, liver and thyroid function tests. They underwent a gastroscopy with duodenal biopsies and coeliac disease was excluded.

The controls used in this study consisted of 27 subjects, 19 of which were female and 8 of which were male, with an average age of 53 years (range 20-65 years) that underwent colonoscopy with rectal biopsies. Of these subjects, 20 underwent colonoscopy due to gastrointestinal bleeding, where the source of the bleeding was identified as haemorrhoids (18) or angiodysplasia (2) and 7 subjects were examined due to health worries caused by a diagnosis of colon carcinoma in a relative. All control subjects had no other gastrointestinal complaints or systemic diseases.

The present study was performed in accordance with the Declaration of Helsinki and was approved by the local Committee for Medical Research Ethics. All subjects provided oral and written consent.

Colonoscopy. A standard colonoscopy was performed in the patients and controls, and biopsies were taken from the rectum ~15 cm from the anus. The biopsies were fixed in 4% buffered paraformaldehyde overnight, embedded in paraffin and cut into 5-µm-thick sections.

Histopathology and immunohistochemistry. The sections were stained with haematoxylin and eosin and then immunostained with the avidin-biotin complex (ABC) method using the Vectastain ABC and the 3,3′-diaminobenzidine (DAB) Peroxidase Substrate kits (Vector Laboratories, Burlingame, CA, USA). The primary antibodies used were monoclonal mouse anti-human CD45 (Dako, Carpinteria, CA, USA; code no. M0701), monoclonal mouse anti-human CD47 (Dako; code no. I5647), monoclonal mouse anti-human CD68 (Dako; code no. M0814) and monoclonal mouse anti-human mast cell tryptase (Dako; code no. M7052). CD45 is considered as a leucocyte common antigen and is expressed exclusively on cells of the haematopoietic system and their progenitors. CD57 is expressed by subsets of NK cells and CD8+ lymphocytes and by a small percentage of CD4+CD45RO+ T lymphocytes. CD68 labels human monocytes, macrophages and myeloid cells. Human mast cell tryptases comprise a family of trypsin-like neutral serine proteases that are predominantly expressed in mast cells.

Computerized image analysis. A computerised image analysis was performed using Olympus software: Cell D. When using x40 objectives, the frame (field) on the monitor represented an area of 0.14 mm² of the tissue. The number of intraepithelial leucocytes cells and the area of the epithelial cells were measured in each field. The number of leucocytes, lymphocytes, macrophages and mast cells in the lamina propria were counted per microscopic field. All measurements were performed in 10 randomly chosen fields for each individual. The immunostained sections from the IBS patients and the controls were coded and mixed and the measurements were taken without the knowledge of the section's identity.

Statistical analysis. A Mann-Whitney U test was performed and P<0.05 was considered to indicate a statistically significant result.

Results

Colonoscopy, histopathology and immunohistochemistry. The colon and rectum of the patients and control subjects were macroscopically normal. Histopathological examination of the colon and rectum biopsies from the patients and controls revealed a normal histology.

Computerised image analysis

Leucocytes. The number of intraepithelial leucocytes observed in the controls, total IBS patients and the IBS-D and IBS-C patients was 95.2±48.4, 102.1±16.2, 98.8±20.6 and 108±29/mm² epithelium (mean ± SE), respectively (Figs. 1 and 2). There was no statistically significant difference between the controls and the total IBS, IBS-D or IBS-C patients (P=0.97, 0.96 and 0.77, respectively).
The number of leucocytes in the lamina propria was 97.1±7.2/field in the controls and 85.8±1.9/field in the total IBS patients. The corresponding figures in the IBS-D and IBS-C patients were 84.1±2.4 and 88.3±2.9/field, respectively (Figs. 2 and 3). There was no significant difference between the controls and the total IBS, IBS-D or IBS-C patients (P=0.17, 0.13 and 0.48, respectively).

**Mast cells.** The number of mast cells in the lamina propria of the controls, total IBS patients and the IBS-D and IBS-C patients was 10.4±0.7, 11.7±0.4, 11.5±0.5 and 12.1±0.8/field, respectively (Figs. 4 and 5). There was no significant difference between the controls and the total IBS patients (P=0.11). Nor was any significant difference observed between the controls and the IBS-D or IBS-C patients (P=0.01 and 0.32).

**Lymphocytes, macrophages and monocytes.** A few lymphocytes were identified intraepithelially and in the lamina propria of the controls and the patients (Fig. 6). Macrophages and monocytes were seldomly encountered in the lamina propria of the controls or patients. These three cell types were sparse in the biopsy material examined which made it difficult to perform a reliable quantification.
Discussion

In unselected cohorts of IBS patients, an increased mucosal cell density of mast cells was observed in the ileum, caecum and colon (22,23,25,27), however, this increase did not occur in all IBS patients that were examined (22,25,27). A previous study reported that numbers of plasma cells, lymphocytes, eosinophils, neutrophils and macrophages were unchanged in a cohort of unselected IBS patients (23). However, a second study of another cohort of unselected IBS patients identified an increase of lymphocytes in 50% of the patients (27). It is conceivable to conclude, therefore, that the increased infiltration of immune cells in the terminal ileum, colon and rectum occurs only in a subset of IBS patients.

PI-IBS is a subset of IBS that is defined as a sudden onset of IBS symptoms following gastroenteritis in individuals who previously have not had any gastrointestinal complaints (30). PI-IBS however, has also been reported following non-gastrointestinal infections, including respiratory, urinary tract and skin infections (31). Sporadic IBS, however, may be defined as a long duration of IBS symptoms in individuals without any connection to a previous gastrointestinal infection. In total, 6-17% of patients with IBS believe that their symptoms began with an infective illness (32). Furthermore, 7-31% of patients who suffer an acute episode of infectious gastroenteritis develop PI-IBS despite clearance of the inciting pathogen (33).

The intestinal mucosa of patients with PI-IBS, as well as of the animal models for PI-IBS, show a low-grade inflammation. Thus, in patients with chronic giardiasis (patients with Giardia infection despite antibiotic treatment), as well as in patients with PI-IBS following Giardia infection, an increased intraepithelial infiltration of lymphocytes has been observed in the duodenal mucosa. The lymphocyte infiltration in chronic giardiasis was reported to be much more prominent than in PI-IBS (28). Similarly, an increased infiltration of T lymphocytes, as well as mast cells, has been reported in the duodenal and jejunal mucosa of a animal model for PI-IBS (34). In the terminal ileum of PI-IBS patients following Shigella infection, an increase in the number of mast cells was also observed (35). The density of T lymphocytes and mast cells was increased in the lamina propria of the rectum in patients with PI-IBS (24,34,35). Similarly, rectal biopsies taken from patients following Campylobacter enteritis showed an increase in the density of CD3, CD4 and CD8 lymphocytes in the intraepithelium and in the lamina propria, which persisted for >1 year subsequent to infection (26,36).

The present study showed that the mucosal density of leucocytes as a whole and lymphocytes, monocytes, macrophages and mast cells in the rectum in the sporadic IBS patients did not differ from that of the controls. These findings oppose low-grade inflammation as a pathogenic factor in sporadic IBS. From the data presented above, low-grade inflammation may play a role in the pathogenesis of a subset of IBS, namely PI-IBS.

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References


