Abstract. – The Irritable bowel syndrome (IBS) is a clinical syndrome characterized by chronic abdominal discomfort associated with changes in bowel habits and these symptoms can’t be explained by any biochemical or organic abnormalities. The review summarizes the relevant findings that have emerged in recent years on the pathogenesis of this syndrome.

The most important mechanisms recently implicated in the genesis of IBS symptoms are the abnormal intestinal motility, the incongruous intestinal gas production and the enhanced intestinal nociception. A lot of evidence confirms the presence of dysfunction of the intrinsic enteric nervous system (ENS) as demonstrated by the presence of altered expression of transient receptor potential vanilloid 1 (TRPV1), acid sensing ion channel 3 (ASIC3), putinergic receptor P2X, ligand-gated ion channel 3 (P2X3r), tetrodotoxin-sensitive receptor 2 (TTRX2) and others. There are different assumptions that explain these phenomena, and the impairment of the immune system is one of the most reliable. In IBS subjects it was found that the immune system is altered in both the cellular composition and its activation. Many studies have shown that inflammation and immune dysregulation affect the sensitivity of nerve fibers so it is vital to build on this argument for the development of effective therapies to control the symptoms of this syndrome.

Key Words: IBS, Pathophysiology, Immunity, Cytokine.

Abbreviations

Irritable bowel syndrome (IBS); Constipation predominant IBS (IBS-C); Diarrhea predominant IBS (IBS-D); Mixed bowel pattern IBS (IBS-M); Alternator IBS (IBS-A); Postinfectious IBS (PI-IBS); Inflammatory bowel disease (IBD); Functional gastrointestinal disorders (FGID); Gastroesophageal reflux disease (GERD); Central nervous system (CNS); Migrating motility complex (MMC); Enterochromaffine cells (EC cells); Small intestinal bacterial overgrowth (SIBO); High-amplitude propagating contractions (HAPC); Non-adrenergic non-cholinergic fibers (NANC fibers); Intrinsic enteric nervous system (ENS); Intrinsic primary afferent neuron (IPAN); Calcitonin gene-related peptide (CGRP); Nerve growth factor (NGF); Neurotrophic tyrosine kinase receptor family (Trk receptors); Glial cell-derived neurotrophic factor (GDNF); Adenosine triphosphate (ATP); Acid sensing ion channel (ASIC); Dorsal-root ASIC (DRASIC); Mammalian degenerin (MDEG); Transient Receptor Potential (TRP); Transient receptor potential vanilloid 1 (TRPV1); Purinergic receptor P2X, ligand-gated ion channel 1, 2 and 3 (P2X1, P2X2, P2X3); Voltage-gated sodium channels (VGSC); Tetrodotoxin-sensitive receptors (TTRX); tetrodotoxin-resistant receptors (TTRXr); Proteases activated receptors (PARs); Cannabinoid receptor 1 and 2 (CB1, CB2); Prostaglandin E (PGE); Prostaglandin F2 (PGF2); Prostaglandin I2 (PGI2); Prostaglandin L2 receptor (PL); Prostaglandin E Receptors (EP); Protein kinase A (PKA); Neurokinin 1, 2 and 3 (NK1, NK2, NK3); Cholecystokinin (CCK); Interleukin 1, 1β, 3, 6, 7, 8, 9, 10, 11, 12, 15 (II-1, II-1β, II-3, II-6, II-7, II-8, II-9, II-10, II-11, II-12, II-15); Tumor necrosis factor α (TNF-α); Peripheral blood mononuclear cells (PBMCs); CC-chemokine ligand (CCL); CXC-chemokine ligand 9 (CXCL9); CXC-chemokine ligand 10 (CXCL10); Natural killer cells (NK cells); Interferon γ (IFNγ); Forkhead box P3 (FOXP3); Transforming growth factor β (TGFβ); Granulocyte macrophage colony-stimulating factor (GM-CSF); Granulocyte-colony stimulating factor (G-CSF); Macrophage colony-stimulating factor (M-CSF); Lipopolysaccharide (LPS); Monocyte chemotactic protein-1 (MCP-1); Zonules occludens protein 1 (ZO1); Trans-Epithelial Resistance (TER); Tight junction (TJ); Chemokine receptor 1 and 2 (CXCR1, CXCR2); Neuron Specific Enolase (NSE).

Introduction

The first description of Irritable Bowel Syndrome (IBS) dates back to 1918 by Powell. A
century later was still considered a diagnosis of exclusion, but many experts now believe, however, the IBS diagnosis can be made by clinical criteria and patients with typical symptoms (described in Manning Rome criteria) should not be subjected to routine laboratory tests (blood count, thyroid function studies, stool for ova and parasites and abdominal imaging) or routine colonic imaging, if warning signs are absent (rectal bleeding, anaemia, weight loss, nocturnal symptoms, a family history of colon cancer and/or age of onset more than 50 years, abnormal physical examination, recent antibiotic use and a short history of symptoms). In IBS-D and IBS-M patients serological tests for celiac disease screening should be performed and lactose-breath test should be considered when suspected lactose intolerance.

The first attempts in this direction have been made by a British research group which made the so-called Manning et al criteria in 1978 that identified patients with abdominal pain without an organic cause accompanied by changes in bowel habits and/or bloating. These were reviewed and updated in the light of new clinical evidence in the Rome II, Rome III, and finally Rome III guidelines that define the syndrome and better classify the other symptoms coexisting with abdominal pain. In Rome II criteria IBS subjects were subclassified in Diarrhea-predominant IBS (IBS-D), constipation-predominant IBS (IBS-C) and IBS with mixed bowel pattern (IBS-M) according to the predominant bowel habit. Some patients switched subtype over time and were called “alternators” (IBS-A).

Epidemiology and Natural History

Prevalence

The prevalence of IBS varies considerably depending on criteria used for diagnosis. Most of the studies are based on the Manning et al criteria or the Rome criteria, in particular Rome I and Rome II. Whereas population studies, the IBS prevalence varies according to geographical criteria (see Table I) but is estimated that 7-10% of people have IBS worldwide. IBS is more common in women, in lower socioeconomic groups and in younger than 50 years of age. Despite IBS does not predispose to severe illness, it affects quality of life and patients turn to medical and health resources more often than healthy subjects.

The use of different diagnostic criteria is probably the reason why we don’t know the exact prevalence of each IBS subgroup. IBS-D includes 50% of IBS population in North America, while in European countries the mixed subtype is the most frequent. The Asian population is very heterogeneously distributed according to different studies.

Natural History

IBS symptoms have a characteristic trend: acute periods alternate with periods of relative well-being. In some studies it was found that patients who met the diagnostic criteria for IBS report symptoms that could even disappear after about 2.5 months (in 38%-50% after one year follow-up and 33%-55% after 10 years). After all, it isn’t necessary to become asymptomatic: after a 10 years follow-up, 55% of IBS patients do not meet IBS diagnostic criteria but 44% report other functional gastrointestinal symptoms.

A major concern of patients with IBS is to develop an organic disorder, especially cancer or IBD. Epidemiological studies on IBS patients show that their life expectancy is similar to the

Table I. Prevalence of IBS in different geographic regions based on diagnostic criteria used.

<table>
<thead>
<tr>
<th>Population</th>
<th>Diagnostic criteria</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>North America</td>
<td>Manning</td>
<td>8-20%</td>
</tr>
<tr>
<td></td>
<td>Rome I</td>
<td>7.8-13%</td>
</tr>
<tr>
<td></td>
<td>Rome II</td>
<td>4.7-11.4%</td>
</tr>
<tr>
<td>Western Europe</td>
<td>Manning</td>
<td>2.1-22%</td>
</tr>
<tr>
<td></td>
<td>Rome I</td>
<td>2-13%</td>
</tr>
<tr>
<td></td>
<td>Rome II</td>
<td>1-8%</td>
</tr>
<tr>
<td>Asia</td>
<td>Manning</td>
<td>2.3-11.5%</td>
</tr>
<tr>
<td></td>
<td>Rome I</td>
<td>8.5-10.4%</td>
</tr>
<tr>
<td></td>
<td>Rome II</td>
<td>3.7-22%</td>
</tr>
</tbody>
</table>
Focus on irritable bowel syndrome

general population and there was no increased incidence of organic disorders29. Cancer was subsequently diagnosed in 1% and IBD in 0.6% of IBS patients, but these percentages are similar in healthy subjects30.

Age, Gender and Ethnicity

IBS is more common in women, with a female/male ratio ranging from 1.2 to 3.1 in Western countries and from 0.8 to 2.1 in developing countries31. There aren’t, however, substantial differences in symptoms reported in the two genders31. IBS is common in all age groups, including children and adolescents32.

It is difficult to establish if there is an association between the ethnicity and the development of IBS because all studies consider people living in different geographic areas, so they are not directly comparable. However, studies based on different ethnic groups inhabiting the same geographical area show that there aren’t substantial differences between racial groups33,34.

Comorbidities

People with IBS often report symptoms or diseases not directly related to the intestine, including:

• FGID: IBS often occurs in conjunction with other functional gastrointestinal disorders (FGID). In these subjects is a high prevalence of dyspepsia32, but other functional disorders are also common. Only one third of IBS patients does not show any symptoms such as dyspepsia or gastroesophageal reflux (GERD)35. According to experts, in the natural history of FGID is frequent the switching between different symptoms. A prospective cohort study examined the IBS population and 40% of subjects one year after the initial diagnosis of IBS still fulfill the diagnostic criteria, 20% became asymptomatic and 40% was classified with another functional gastroenteric disorder39.

• GERD: from 30% to 70% of patients with gastroesophageal reflux disease also have IBS, while 17-79% of IBS patients also have GERD36.

• Psychiatric disorders: the increased frequency of psychiatric disorders in patients with IBS have long been highlighted: 25-30% of patients with major depression met diagnostic criteria for IBS, with an higher prevalence than in healthy subjects37; 25% of IBS subjects present an association with depressive mood disorder and in 30% of these subpopulation overlap anxiety37. Other psychiatric disorders appear to be associated with IBS: eating disorder, panic attack, obsessive-compulsive disorders and posttraumatic stress syndrome38.

• Gynecological disorders: IBS seems to be associated with an increased appearance of gynecological diseases. 60% of women with dysmenorrhea also had IBS-like symptoms, a 20% higher percentage compared to control group. IBS is also more common in women with dyspareunia or genito-urinary diseases such as recurrent cystitis39.

• Asthma: in two population studies the association between asthma and IBS was found40 and in a case-control study patients with asthma had 20% higher prevalence of IBS compared to the control group41.

• Previous surgery: a meta-analysis of Hasler and Schoenfeld41 considered and analyzed several studies about the possible relationship between IBS and surgery. Patients with IBS turn to surgery more often, especially for:
  – hysterectomy;
  – cholecystectomy;
  – appendectomy;
  – pelvic surgery.

Nevertheless, data are often inconsistent: According to an Italian study, a wrong surgical indication may be the cause of the increase in cholecystectomies43 and another interesting theory is that the surgery itself is a risk factor for the development of IBS symptoms. After hysterectomy there is a 10% increase in the frequency of IBS, especially IBS-C subtype, and about 11 years after hysterectomy and 7 years after cholecystectomy there is an increase in the appearance of IBS symptoms45.

Risk Factors

Through population and case-control studies several risk factors for the development of IBS symptoms were identified.

• Familiar aggregation: in twins genetic factors influence the development of IBS for more than 20%-46.

• Early life events: Animal studies have amply demonstrated that IBS can be “triggered” by stressors events occurring during life37. In this context, the main evidence in humans are:
IBS is associated with previous history of chronic abdominal pain in childhood, particularly between 7 and 9 years. The socio-economic class was considered a potential risk factor: three studies show that a low socio-economic class is a negative risk factor for development of IBS while in another report wasn’t the same result. External events occurring in childhood may correlate with a greater likelihood in the development of IBS:
- low birth weight;
- use of nasogastric suction at birth;
- history of sexual abuse in childhood.

Pathophysiology

Gastrointestinal Motility

Small Intestine Motility

IBS patients have a lower pain threshold compared with healthy subjects: various not harmful stimuli (mechanical stimuli caused by intestinal motor activity for example) can elicit a painful sensation in these patients. Over time different methods were developed and various parameters were evaluated to better study this problem. Today it is not yet possible to identify a specific motor pattern in IBS, in fact, reports on MMCs have shown that there is a high variability in the periodicity of MMCs in IBS patients than in healthy subjects, although older studies report a correlation between the prevailing intestinal habit and MMCs frequency (in IBS-D, between two successive MMC cycles there are shorter intervals while they are longer in patients with IBS-C). Also the clustered activity was studied and was more frequent in IBS subjects. Björnsson et al studied the duodenal motor activity and found an increase in duodenal retroperistalsis in IBS patients, especially in postprandial phase.

The small intestine motor disorder was also indirectly demonstrated: the intestinal transit is slower in patients with IBS-C and is faster in patients with IBS-D. Sadik et al developed a procedure to analyze the motility of upper and lower digestive tract and they highlight that patients with other functional gastrointestinal disorders have also an impaired transit of small intestine.

Colorectal Motility

Recent studies on colorectal motility show that in large bowel of IBS subjects are present particular motor patterns: an increased frequency of HAPC, especially in patients with non constipated IBS, an exaggerated and prolonged postprandial motor response of large bowel, the recto-sigmoid muscle has an abnormal muscle tone after a low-caloric meal and the colorectal reflex is attenuated.

Disturbance of Intestinal Gas Production

Swelling and/or abdominal distention are found in the majority of patients with IBS and some Authors speculate that IBS patients have an altered gas transit that may trigger these symptoms. But Lasser et al showed that patients with functional gastrointestinal disorders do not have greater gas volume in the gut than healthy subjects. For this reason, many researchers believe that if an abnormal intestinal gas volume does not explain bloating and abdominal pain, the pivot point could be the disturbance of intestinal motility in association with an abnormal gastrointestinal sensitivity. Other factors such as an ineffective gas propulsion in the small intestine, an abnormal gastrointestinal reflex activity, an altered viscerosomatic reflex activity, the abdominal wall muscular dystonia, the abdominal and phrenic disorganization may be involved in the pathogenesis of abdominal bloating.

Gastrointestinal Perception

Visceral hypersensitivity is considered an important pathogenic factor in IBS. Many studies emphasize the link between IBS and an increased intestinal sensitivity. The rectal hypersensitivity was identified as a marker of disease and two studies reported that this parameter has a good sensitivity (95-100%) and specificity (71.8-72%) to discriminate IBS from healthy subjects. However, other reports dispute these evidences because only 20-60% of IBS patients showed hypersensitivity and a lower rectal pain threshold was found more frequently in IBS-D patients while the pain was perceived at higher volumes of rectal distension in IBS-C than in controls. Most of these reports have focused the attention on colorectal hypersensitivity but others demonstrate an enhanced sensitivity also in other sites as the esophagus, stomach and small intestine.

Naliboff et al developed an interesting theory to explain the pathogenesis of hypersensitivity in IBS subjects based on the evidence that peripheral and central factors are involved. Much evidences correlates IBS with psychiatric disorders.
as ansia, depression or other and recent studies evidenced an abnormal CNS processing of peripheral stimuli in IBS subjects but is still clear that organic peripheral dysfunctions are present (see above) and are also recently demonstrate with molecular analysis (see below). this is the basis for the development of new drugs that could revolutionize the treatment of IBS.

**Chronic Inflammatory Infiltrate in IBS**

In patients who meet the clinical diagnostic criteria for IBS macroscopically evident specific pathological lesions are not found at colonoscopy, but with in-depth histological investigation many researchers detect the presence of pathological lesions which may be important in order to explain the pathophysiology of IBS. Many experts have shown an increased number of inflammatory cells in colonic mucosa of IBS patients and several studies used quantitative immunohistochemical analysis to uncover these changes that usually are not visible at routine histological evaluation.

In healthy subjects, two weeks after an episode of acute gastroenteritis by *C. jejuni*, the bowel return macroscopically and microscopically normal. However, qualitative histological analysis show a residual inflammation that gradually decreases until it disappears after 3 months and inflammatory changes persist for a year and over in a small subgroup of patients with IBS symptoms.

Both the lamina propria, the epithelial surface and crypts contain an increased number of T lymphocytes. This increase was found in PI-IBS and non-PI-IBS patients and IBS-D is associated with an higher number of mucosal T lymphocytes than IBS-C.

In biopsies from terminal ileum and rectum-sigma, the number of NSE, substance P and serotonin positive nerve fibers was increased in patients with PI-IBS and non PI-IBS compared with healthy subjects and these fibers are more concentrated near mucosal mast cells. The distance between axonal fibers of ENS and inflammatory cells has decreased in IBS patients than in controls.

In PI-IBS patients an increased number of enterochromaffin cells in rectal biopsies was found but this result has not been confirmed by studies on patients with non PI-IBS. The intestinal infection by *C. jejuni* has been widely studied and several works show that after two weeks of infection IBS patients had five times higher sinaptolisyne positive EC cells than in the control group, the number of EC cells gradually decrease 6 and 12 weeks after the infection and in some subjects who remained symptomatic after one year this number was as high as 2 weeks after infection.

**Immunology in IBS Patients**

**Mast-cells**

Many studies showed that the number of tissutal mast cells in the large and small bowel increased in IBS patients (although there are some reports that contrast this evidence) and an increased density of mast cells in close proximity to enteric nerve endings in terminal ileum, cecum and rectum.

**Monocytes and Macrophages**

In IBS subjects there are increased IL-6 and IL-8 plasma levels, proinflammatory cytokines produced primarily by monocytes and macrophages.

According to O’Sullivan et al, the number of tissutal macrophages is normal in IBS patients, while Spiller et al show a 50% reduction but, measuring the calprotectin expression, the number of activated macrophages was increased. Another study indirectly highlighted a lack of tissutal macrophages in IBS patients: the expression of chemokines for the recruitment of macrophages, CCL and CXCL 10, in intestinal biopsy specimens was decreased.

**Natural-killer Cells, Neutrophils and Eosinophils**

Studies investigating the role of innate immunity in IBS are few. Elsenbruch et al reported a low number of NK cells in the blood of IBS patients compared to healthy subjects. In later studies this evidence has not been confirmed, but an increased number of activated NK cells was found. The interpretation of these data is, however, difficult since studies are based on a small number of patients. A Swedish study has found an increased myeloperoxidase production (a mediator of the inflammatory response led by neutrophils) in mucous secretions of IBS subjects, but not of neutrophil gelatinase-associated lipocalin, but in a subsequent study these data were not confirmed measuring the elastase concentration, and in colonic biopsy the tisutal neutrophil population was not different between healthy and IBS patients. Finally, IBS patients have a normal eosinophils count in stool, in duodenal mucosa and in rectal secretions.
Cell-mediated Immune System

The evidence of the involvement of cell-mediated immune system in the pathogenesis of IBS is supported by many studies:

- **CD4+ T Lymphocytes and Treg cells.** Many investigations on mucosa have reported an increased number of CD4+ lymphocytes in the colon of IBS patients\(^{87,88}\) and some markers of activation are elevated, while these cells have a reduced proliferative response in vitro\(^{103}\). This result agrees with the presence of an increased number of mucosal Treg cells\(^{88}\), but the immunosuppressive and regulatory Treg cells population was subsequently studied and was equally\(^{104}\) or less represented\(^{103}\) in IBS compared with healthy subjects.

- **CD8+ cytotoxic T lymphocytes.** CD8+ cells are able to kill infected or dysfunctional cells and represent the majority of intraepithelial T cells. There are few data on this subpopulation in IBS, but an increased percentage of CD8+ T cells that express the αβ integrin, a homing molecule, in peripheral blood samples was found\(^{105}\) and CD8+ cells in the lamina propria are increased in IBS patients\(^{87,105}\).

- **B lymphocytes and antibody production.** In two studies on IBS subjects, B cells were unchanged\(^{106}\), while in a pilot study the IgA-producing B cells were decreased\(^{107}\).

An increased number of IgG-producing B lymphocytes was found in patients with IBS\(^{108}\) and in PI-IBS patients there are higher levels of specific antibodies for bacterial antigens compared to non PI-IBS patients\(^{109}\).

Cytokine Production

Many researches show that patients who meet diagnostic criteria for IBS have an abnormal cytokine production profile. One of the most studied cytokine is II-6, a mediator of the innate immunity: increased plasma levels have been shown in several reports, often in combination with an increased production of II-8, CXCL-9 and MCP-1 and cytokines (TNF-α, II-6, II-1β). This report is based on a previous study where the levels of II-2, II-6 and II-10 were reduced in a subpopulation of IBS-D subjects\(^{114}\). Moreover, II-1β is increased in rectal mucosa of patients with PI-IBS\(^{115}\).

The growing interest for the immune problem in IBS subjects led experts to seek the presence of specific cytokine genotypes.

A study of Gonsalkorale et al\(^{116}\), shows that in IBS patients the IL-10 high-producer genotype is less frequent compared to healthy subjects, while there are no differences in the TGF-β genotype. This evidence was confirmed by a subsequent research where not only the IL-10 low-producer genotype but also the TNF-α high-producer genotype was more frequent in IBS patients\(^{117}\).

Recently, were studied some polymorphic genes coding for T helper1, T helper2, and T-regulatory cytokines in IBS patients and it was found that there are genotypes that occur more frequently\(^{118}\). In an investigation by Aerssen et al\(^{119}\) in samples of sigmoid mucosa the expression of genes coding for different immune mediators was studied: many changes emerged compared with healthy subjects, but above all the overexpression of a not characterized gene was significant: DKFZP564O0823.

The results of major studies on cytokine production in IBS subjects are summarized in Table II.

The Intestinal Microbiota

The intestinal microbiota plays an important role in food digestion, detoxifying and metabolizing drugs and toxic compounds, producing essential vitamins, preventing colonization of pathogens and the presence of an abnormal gut flora seems to be crucial in the pathogenesis of IBS\(^{120}\).
Focus on irritable bowel syndrome

The Luminal Microbiota

Numerous reports examine the luminal bacterial flora in IBS subjects but results are not always concordant. IBS is very heterogeneous population and the majority of studies did not divide patients into subgroups according to Rome II criteria but some recent reports identified relevant difference between IBS-D and IBS-C subjects (see below). In addition, the study methods used are different, so results are not directly comparable.

Earlier studies were cultured-based but results are contradictory and not exhaustive because the majority of intestinal bacterial species are not cultivable. A decrease of Lactobacilli, Bifidobacteria and Coliforms and an increase of Enterobacteriaceae in the feces of IBS subjects was found but other Authors disconfirm these findings because reported an increase in Clostridia and the number of units was only slightly higher than in the control group. Later results are still preliminary. Swidsinski et al found differences in mucosal but not in fecal communities in IBS-D subjects compared to controls. The bacterial pathogenicity determine whether dendritic cells stimulate an antiinflammatory (through the secretion of inflammatory cytokines such as IL-10 or TGFβ) or an inflammatory response. Bifidobacteria and lactobacilli stimulate IL-10 and TGFβ secretion and inhibit the proinflammatory cytokines production. The microbiota adhering to the mucosa was examined in IBS patients but results are still preliminary. Swidsinski et al found that the bacterial population was composed primarily of Clostridia and the number of units was only slightly higher than in the control group. Later reports showed that Bifidobacterium catenulatum population was decreased in the duodenal mucosa and Pseudomonas aeruginosa was increased in feces and in small intestine of IBS subjects than in control group.

Codling et al found no significant differences in the variability of fecal and mucosal microbiota in IBS-non subtyped subjects and Carrol et al found differences in mucosal but not in fecal communities in IBS-D subjects compared to controls.

The Adherent to the Mucosa Microbiota

While the luminal microbiota is excreted in feces, the mucosa-associated microbiota requires to be studied an endoscopic examination of the intestinal tract to obtain mucosal samples. Bacteria are embedded in the mucus layer that dominates the intestinal epithelium were there are specific binding sites. This is an important defensive mechanism for minimize the epithelial adhesion of pathogens. Few bacteria are able to have a direct contact with the intestinal epithelium and subsequently they are presented to dendritic cells and tissutal macrophages. Many receptors for bacterial components are expressed on the epithelium and on dendritic cells, such as toll-like receptors (TLRs), and each receptor is specific for a ligand (e.g. LPS).

The bacterial pathogenicity determine whether dendritic cells stimulate an antinflammatory (through the secretion of inflammatory cytokines such as IL-10 or TGFβ) or an inflammatory response. Bifidobacteria and lactobacilli stimulate IL-10 and TGFβ secretion and inhibit the proinflammatory cytokines production. The microbiota adhering to the mucosa was examined in IBS patients but results are still preliminary. Swidsinski et al found that the bacterial population was composed primarily of Clostridia and the number of units was only slightly higher than in the control group. Later reports showed that Bifidobacterium catenulatum population was decreased in the duodenal mucosa and Pseudomonas aeruginosa was increased in feces and in small intestine of IBS subjects than in control group.

SIBO

The small intestinal overgrowth syndrome (SIBO) is characterized by a qualitative and/or quantitative modification of small bowel microbiota. Normally

<p>| Table II. Summary of the main reports on cytokine production in patients with IBS. |
|---------------------------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Blood</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>$\Delta_{110,112}$</td>
<td>$=98_{111}$</td>
</tr>
<tr>
<td>IL-6</td>
<td>$\Delta_{112}$</td>
<td>$=111_{114}$</td>
</tr>
<tr>
<td>IL-8</td>
<td>$\Delta_{95,112}$</td>
<td>$=111_{115}$</td>
</tr>
<tr>
<td>IL-1β</td>
<td>$\Delta_{104,110,112}$</td>
<td>$=111_{114}$</td>
</tr>
<tr>
<td>IL-12</td>
<td>$\Delta_{111}$</td>
<td>$=114$</td>
</tr>
<tr>
<td>IL-10</td>
<td>$\Delta_{93}$</td>
<td>$=111$</td>
</tr>
<tr>
<td>IL-13</td>
<td>$\Delta_{111}$</td>
<td>$=111$</td>
</tr>
<tr>
<td>IL-5</td>
<td>$\Delta_{111}$</td>
<td>$=111$</td>
</tr>
</tbody>
</table>

The small intestinal overgrowth syndrome (SIBO) is characterized by a qualitative and/or quantitative modification of small bowel microbiota. Normally
in duodenum and in proximal jejunum is present a small number of bacteria compared with other lower intestinal tracts\textsuperscript{143} but in subjects presenting SIBO the microbial investigation of jejunal aspirate evidences a microbial population $\geq 10^5$ CFU (colony forming unit) per mL were the normal value is $\leq 10^4$ CFU per mL\textsuperscript{144}. Clinically the SIBO could be asymptomatic, produce non-specific symptoms typical of IBS as abdominal pain, bloating, diarrhea, abdominal discomfort, flatulence or present sign of malabsorption, skin manifestation, liver lesion, deficiency syndrome (by nutrient depletion as iron, calcium, vit. D, vit. B$_2$), etc\textsuperscript{142}.

SIBO is frequently found in individuals with a previous diagnosis of IBS (30-85%)\textsuperscript{145,146} and symptoms overlap those of IBS, so an etiopathogenetic correlation has been proposed. There are several hypotheses about: IBS may cause or be caused by SIBO. The main pathogenetic factors of IBS are the motor disorder, the visceral hypersensitivity and associated psychosocial factors, these would lead to a change of the intestinal microbiota causing SIBO\textsuperscript{147}. Other Authors instead believe that SIBO, as in the IBS-P, is the cause of IBS: the inflammatory-immune dysregulation secondarily trigger motor disorders and visceral hypersensitivity\textsuperscript{148,149}. A final theory is that the two syndromes should be understood as two completely separate realities\textsuperscript{150}.

**The Permeability of Epithelial Barrier**

*In vivo* and in vitro studies suggest that the immune activation in IBS patients may be the result of an increased exposure to local antigens, associated with an increased permeability of the epithelial barrier. Patients with postinfectious IBS have an increased permeability in the small intestine compared to healthy subjects especially in patients with non PI-IBS\textsuperscript{151}.

Analysis of colonic biopsies in IBS subjects showed that there is an increased paracellular permeability and a decreased expression of a tight-junctions structural protein, the zonulin (ZO1)\textsuperscript{152}. Moreover, the permeability worsen in mice when the intestinal mucosa is exposed to the fecal supernatant of IBS-D subjects but this effect is absent in PAR-2 knock-out mice (see below).

The production of mucous is also altered, in IBS there is an overexpression of MUC20, a gene involved in the mucin production\textsuperscript{153}.

**Neuroimmune Interactions**

The number of nerve fibers expressing TRPV1\textsuperscript{151}, and the number of mast cells and lymphocytes is increased in IBS patients\textsuperscript{90,93}; this is an important confirmation for the role of neuroimmune interactions in IBS. Further evidence is given by different studies.

Barbara et al\textsuperscript{92} show that interactions between mast cells and nerves may be relevant to the genesis of IBS symptoms, especially in the perception of pain. In a rat model the same group also showed that mediators of colonic mast cells in patients with IBS, but not in healthy individuals, stimulate the visceral sensory neurons\textsuperscript{154}.

The importance of mediators derived from mast cells has also been highlighted by Bühner et al\textsuperscript{155} who reported that supernatants of colonic mucosa of IBS but not those of healthy patients activate the enteric intramucous neurons. It is interesting that the mast cells activation is not associated with any subtype of IBS.

Moreover, supernatants of PBMCs of PI-IBS patients activate pelvic and lumbar splanchnic nerves in mice\textsuperscript{156}.

**Molecular Basis of Altered Pain Modulation in IBS Subjects**

- **TRPV1.** In IBS patients TRPV1 is more expressed in rectum-sigma biopsies compared to healthy subjects and this expression correlates with the gravity of symptom. Moreover, these nerve fibers also showed an increased expression of GDNF and trk A\textsuperscript{157}. In a subsequent report, IBS patients showed an increased number of TRPV1-positive nerve fibers compared to controls\textsuperscript{93}.

- **Acid-sensitive ion channels.** These channels probably play a role in the visceral nociception and hypersensitivity, but scientific evidence in humans is currently lacking. Knock-out mice for ASIC3 gene have a reduced visceral sensitivity\textsuperscript{158}. In another study symptoms-like IBS was induced in mice with the use of zymogen and showed that TRPV1 and ASIC3 play an important role in the development of hypersensitivity\textsuperscript{159}.

- **ATP-gated ion channels.** Animals with IBS-like status induced by exposure to acetic acid showed an increased concentration of P2X3 receptors\textsuperscript{160}. The competitive antagonist A-317491 for P2X3 receptor control induced inflammatory hyperalgesia development in rats\textsuperscript{161}.

- **Voltage-gated sodium channels.** In humans, the evidence supporting the role of sodium channels in the development of pain is increasing. Verne et al\textsuperscript{162} shown a clear reduction in rectal
sensitivity and abdominal pain in patients with IBS treated with intrarectal administration of lidocaine. Yiangou\textsuperscript{163} notes that in intestinal biopsies of patients with rectal hypersensitivity the immunoreactive to sodium channel Nav 1.7 (a TTXr subunit) nerve fibers are significantly increased compared with healthy subjects.

- **Proteases activated receptors.** In IBS patients the presence of mucosal mast cells infiltration is known. During inflammation the mast cells degranulation causes the serine proteases and tryptase release that act on PARs. Animal studies have highlighted the role of PAR receptors in the development of visceral hypersensitivity\textsuperscript{164}. In a recent study by Cenac et al\textsuperscript{165}, an increased proteolytic activity of intestinal supernatants collected from IBS subjects was found and was also shown that a possible way to stimulate the hyperalgesia passes through the PAR-2 activation by proteases. Moreover, these effects were absent in PAR2-deficient mice and were inhibited by serine protease inhibitors and a PAR2 antagonist.

- **Serotonin receptors.** Serotonin is an important neurotransmitter in brain-gut axis, 80\% of the whole body serotonin is found in the gastrointestinal tract\textsuperscript{166}. Serotonin is released from enterochromaffin cells and platelets and stimulates primary afferent neurons, such as IPANs\textsuperscript{167}, through the activation of specific sodium channels\textsuperscript{168}, triggering a intestinal motor response. The activation of 5-HT4 presynaptic receptors intensify the strength of the intestinal muscle contraction\textsuperscript{167}. Serotonin antagonists (Tegaserod) are used as a prokinetic, while agonists for serotonin can alleviate IBS symptoms, especially abdominal pain (Ondansetron)\textsuperscript{169}.

- **Tachykinin receptors.** In animal models NK1 receptor antagonists reduce hyperalgesia\textsuperscript{169} while there are no data for NK2 receptors. In a study of Dénes et al\textsuperscript{170} an antagonist for neurokinin (NK1 and NK2) receptors partially block the serotonin response, but the NK3 receptor antagonist had no effect in animals. In humans the use of Talnetant (a NK3 receptor antagonist) produces negative results in IBS patients and also in healthy individuals\textsuperscript{171}. Substance P is a neurotransmitter of the excitatory neuro-neuronal communication, it is a vasodilator, a mediator of inflammatory processes and is involved in the development of visceral hyperalgesia in animal models of artificially induced colitis\textsuperscript{172}. Both substance P and CGRP are abundantly expressed by sensory fibers of the gastrointestinal tract, and some researches show their role in the development of visceral pain\textsuperscript{173}.

The most important inflammatory stimuli, mediators and receptors implicated in the development of visceral hypersensitivity are summarized in Figure 1.

### Symptoms and Immune Activity

To determine whether the mild inflammation and moderate immunological changes seen in IBS have a role in the generation of IBS symptoms, we have to demonstrate an association between the severity or type of reported symptoms and the immunological data. Most convincing data supporting this hypothesis include the evidence of a strong association between the number of mast cells near enteric nerves, as observed in biopsy specimens, with severity and frequency of abdominal pain (coefficient of correlation (r) = 0.70-0.75)\textsuperscript{92}. Other investigations have focused their attention on mast cells population in colonic biopsies and found a weaker but still significant association with gastrointestinal symptoms\textsuperscript{93}. Other studies, however, show no association\textsuperscript{97}. The importance of neuroimmune interactions in the generation of IBS symptoms is also alleged by the relationship between the severity and pattern of gastrointestinal symptoms and the presence of Treg cells in peripheral blood\textsuperscript{103}, the release of proinflammatory cytokines by PBMCs\textsuperscript{110}, the protease activity\textsuperscript{138} and intestinal permeability\textsuperscript{151}. Some reports have also shown a correlation between immune function in peripheral blood and/or bowel and psychological symptoms of IBS, which emphasizes the importance of activity of the brain-gut axis. It is likely that there is an abnormal interaction between the hypothalamic-pituitary-adrenal axis and the immune system in IBS patients and this may be important in genesis of symptoms\textsuperscript{100,110,111}.

### Conclusions

IBS affects many people and severely impacts their quality of life. To date, treatment regimens used very often give a limited benefit to patients. Research is turning its attention to several pathophysiological aspects of IBS to develop more ef-

[1163]
effective and specific drugs. Growing interest is addressing to the interaction between visceral hypersensitivity, inflammation and intestinal microbiota. In IBS is a subtle chronic immune activation, in fact there is an increased number of mast cells also abnormally activated, a cell-mediated immune system dysregulation, an increased T lymphocyte population, a Treg cells imbalance, etc. Most likely the inflammation causes (or is caused by) changes in motility and

**Figure 1.** Neuronal receptors involved in the visceral hypersensitivity: Kinins exert their biological effects through the activation of two transmembrane G-protein-coupled receptors, named NK1, NK2 and NK3 receptors. These short-lived peptides, including bradykinin, kallidin and T-kinin, are generated during tissue injury and by noxious stimulation and they act in CNS as neuromediators in the control of the nociceptive information. The 5-HT receptors are a group of G protein-coupled receptors and ligand-gated ion channels found in the central and peripheral nervous systems. Serotonin is released by enterochromaffin cells and platelets and activates primary afferents to trigger enteric motor responses. Activation of pre-synaptic 5-HT4 receptors increases the power of the bowel muscle contraction. VGSC are Na+ channels essential for the initiation and propagation of action potentials in neuronal excitability. These receptors can be divided into two subgroups: the first group is sensitive to the puffer fish tetrodotoxin (TTX) and the second group is insensitive to the same toxin (TTXr). PGE2, serotonin and adenosine sensitize TTXr channels increasing their activation and inactivation and decreasing their activation threshold. PARs are a subfamily of G protein-coupled receptors expressed in neurons, endothelial cells, myocytes and platelets. They are activated by the action of serine proteases. PAR-2 receptors are activated by mast-cell tryptase. PAR-1 is activated by thrombin, trypsin and other proteases, and is expressed by enteric cells, neurons and immune cells in the GI tract. ATP-gated ion channels: two types of ATP-gated ion channels have been characterized in animal gastrointestinal sensory neurons: ATP gated P2X receptors and G-protein coupled P2Y receptor. The cell damage and sympathetic and extrinsic sensory neurons activation lead to ATP release and so ATP-related ion channels activation. TRPV1 is a receptor activated by capsaicin and its analogues, endocannabinoid as anandamide and other lipidic molecules such as resiferotoxin, thermal stimuli (above 43°C) or exposure to an increased concentrations in protons. Many inflammatory mediators are able to lower his activation threshold, making the receptor elicitable even at room temperature: NGF, Bradykinin, Arachidonic acid metabolites, lipoxygenase products, Leukotriene B4, prostaglandins, adenosine and ATP. His activation evoke a burning pain sensation and release of the neuropeptides substance P and CRGP. ASICs are sodium selective channels of the degenerin/epithelial sodium (Deg/ENaC) superfamily, are activated by extracellular protons and are activated once the pH falls below 7.0.
sensitivity regulatory mechanisms in the GI system. This hypothesis is supported by many molecular analysis that say there is an increased expression of TRPV1, ASIC3, P2X3, TTXr, an increased release of protease activating PARs and a favorable action in symptoms controlling of NK1r, and 5-HT antagonists.

So we can say that IBS is a true “irritable” colon, but unfortunately it is not clear whether this is a precondition or a consequence of inflammation.

To get a definitive answer we need a more thorough and accurate research and one of the main points is the study of quantitative and qualitative pattern of blood and especially tissutal cytokine production. In fact, it is an indispensable index to understand and quantify the chronic inflammation locally before that systemically in IBS subjects.

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