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ORIGINAL PAPER

OVERVIEW OF INFLAMMATORY BOWEL DISEASES PATHOMECHANISM

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Abstract. Inflammatory bowel diseases (IBD) including ulcerative colitis (UC) and Crohn's disease (CD) are two genetically related, chronic inflammatory disorders. The main differences between these two phenotype variants consist in the fact that while Crohn's disease is manifested as a deep, transmural, intestinal wall inflammation typically located at the level of small intestine, especially in the ileum part, but can also be found wherever along the gastrointestinal canal, ulcerative colitis is usually located at the level of the large bowel and interests the superficial intestinal wall layers. Concerning the most probable causes and pathomechanism, literature data indicate a combination of internal and external factors leading to the development of a chronic inflammation at the level of bowel tissue as well as a generalized inflammatory status.

Key words: IBD causes and pathomechanism, *Crohn's* disease (CD), ulcerative colitis (UC)

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are two genetically related, chronic inflammatory disorders commonly known as *Inflammatory Bowel Diseases* (IBD). Both phenotype variants are characterized by an increased production of inflammatory cytokines causing chemotaxis, chemokinesis, synthesis of superoxide radicals and the release of the lysosomal enzymes by phagocytes responsible for the alteration of the bowel muscle contractile function,

bowel tissue inflammation boosting, pain and bleeding as well as the installation of the diarrhoeal process leading to water, minerals, and microelements loosing.

Literature data [1 - 6] indicate the following key pathomechanism aspects: the alteration of the colonic muscle contractile function appears to be related with the enhancement of the interleukin-1 beta (IL-1B) level; bowel tissue inflammation boosting has been related with increased levels of proinflammatory cytokines such as tumour necrosis factor (TNF)-alpha, interferon (IFN)-gamma, nuclear factor (NF)-kappaB and many other interleukins such as IL-10, IL-12, IL-23, etc., as well as with increased activity of pro-oxidant enzymes (ciclooxigenase-2/COX-2 and mieloperoxidase/MPO) *versus* (vs.) decreased activity of antioxidant enzymes (superoxiddismutaze/SOD and catalase/CAT); pain, bleeding as well as the diarrhoeal process installation result from the association of the colon muscle spasm activity with the bowel tissue inflammatory secretory phase.

Concerning the aetiology aspect, despite the numerous progresses in the last years, IBD causes still remain incompletely elucidated. At the present, the large majority of the studies and experts agree to IBD multifactorial aetiology and the interplay of some genetic, environmental, and immunological factors, some of the specialists also considering the role of psyhoneuroendocrine dysfunctions.

In the following, the most important factors causing inflammatory bowel diseases
(IBD) development and the most probable mechanism of action will be presented.

1.The main external factors causing IBD development and probable pathomechanism

Smoking and changes in microbial flora, also called microflora, are the main external risk factors scientifically related with IBD development.

The relationship between **cigarette smoke** (CS) and bowel inflammatory lesions have been proven by numerous animal model experiments and clinical trials, studies also showing the value of significant independent risk factor of smoking in IBD pathogenesis. Thus, if Crohn's disease (CD) studies indicated smoking as the most important environmental risk factor, in the specific case of ulcerative colitis (UC) results were contradictory suggesting apparently protective role of smoking in ulcerative colitis (UC) *versus* it's certain damaging role in Crohn's disease (CD) [7]. Accordingly, studies [8] on 102 patients with IBD *versus* an identical number of controls emphasized that the active smokers had a low risk for development of ulcerative colitis (UC), but the lack of history of smoking was not associated with an increase in the risk. The history of quitting smoking prior to the onset of symptoms was related to a significant increase in the risk of developing the disease thus suggesting apparently beneficial effects of smoking on IBD patients explained by *the withdrawal of the immunosuppressive effects of smoking triggers the disease onset in a genetically susceptible individual or*

simply unmasks its symptoms. On the other hand, studies [9] on patients with microscopic colitis (MC) face to controls without MC indicated cigarette smoking (CS) as a risk factor for the development of both forms of microscopic colitis, respectively collagenous colitis (CC) and lymphocytic colitis (LC). Since no significant differences between CC and LC and current smoking and the development of microscopic colitis have found, it has been concluded the high risk of tobacco use in patients with microscopic colitis. Yet, recent clinical trials have also revealed this beneficial effect of smoking in case of ulcerative colitis [10], so that there are authors and studies proposing nicotine low doses administration as an effective treatment of ulcerative colitis [11, 12].Concerning the most probable damaging mechanism, studies [13] indicated that the exposure to CS significantly increased pro-oxidant myeloperoxidase (MPO) activity compared to sham-treated controls. Also, studies on 2,4,6-trinitrobenzene sulfonic acid (TNBS) induced colitis in rats have revealed that the treatment with hexamethonium (an antagonist of nicotinic acetylcholine receptor) before being exposed to smoking reversed the effects of the smoke on colitis and improved the intestinal parameters, thus proving the cigarette smoking damaging effects of DNBS-induced colitis in rat and a mechanism involving a neural pathway [14].

Regarding the most probable beneficial mechanism, given that the exposure of rats to cigarette smoking was associated with high expression of alpha7nAChR nicotinic receptor, it was proposed *plausible to consider whether this pathway is activated in the subset of patients that derive benefit from nicotine* [15].

Changes in **microbial flora** and the corresponding breakdown of the gut tolerance represent the second IBD external cause and pathomechanism and, even if the role of the microbial-host interaction in IBD pathogenesis is strongly sustained by
numerous pharmacological data, due to the fact that human microflora is very complex and largely undefined, so far it has not been completely understood if bacteria-host interaction alteration is the cause or the result of the illness.

Accordingly, measuring roughly 200 - 300 m2, the mucosal surface of the human
gastrointestinal (GI) tract host a number of 300 - 500 different microbial species and subspecies, called microflora, consisting in indigenous flora and transient flora. The differences between the two types of microflora consist in the fact that, while the indigenous flora normally colonizes GI tract living in harmony with the host, the transient flora colonizes GI tract only in short or abnormal episodes frequently disturbing GI harmony [16]. Even if the exactly number of the gastrointestinal inhabitants is uncertain (just because more than 50% of the bacterial strains cannot be cultured under laboratory conditions), modern molecular biology techniques such as bacterial 16S ribosomal RNA analysis, polymerase chain reaction, *in situ* hybridization, flow cytometry and DNA microarray studies show that no single microbial agent has been associated with

certainty with the development of IBD so that *a transient break of the mucosal barrier by some transient or normal flora candidates, followed by the initiation of disease that becomes chronic in the susceptible hosts, even though the initiating infection has been cleared* [17] has been proposed as a probable pathomechanism.

On the other hand, studies [18] revealed that patients with IBD presented concentrations of mucosal bacteria five times higher than that of the samples in the control group (50,000 cfu / microL vs. 10,000 cfu / microL) and characteristic inclusions of multiple polymorphic bacteria within solitary enterocytes located next to *lamina propria* without or having no contact with the faecal stream, the identified bacteria being of faecal origin. Not being secondary to the inflammation process, but as a result of the specific host response, these results have been interpreted as *if the healthy mucosa is capable of holding back faecal bacteria, in patients with IBD this function is profoundly disturbed* [19].

Yet, other studies on IBD patients indicated that the dominant faecal microbiota comprises specific discrepancies and unusual bacterial species which differ from those harboured by infectious colitis (IC) and healthy subjects. By comparing faecal samples from active CD patients, CD patients in remission, active UC, UC patients in remission, patients with IC and healthy subjects, it was clearly revealed that phylum *Firmicutes*, particularly *F. prausnitzii* was underrepresented in active IBD patients as well as in infectious colitis patients suggesting that these bacterial species could be crucial for gut homeostasis, the lower counts of *F. prausnitzii* being clearly associated with the reduced protection of gut mucosa [20].

Moreover, the bacterial diversity in the faeces of CD patients was found reduced compared to controls, the occurred dysbiosis being characterized by a high abundance of *Escherichia coli* and *Shigella flexneri versus* the reduced proportion of *Bacterioides* and *Firmicutes*. Furthermore, it has been revealed five bacterial species as defining the microbiota imbalance of the ulcerated mucosa in CD, an increase in *Escherichia coli* face to a decrease in *Faecalibacterium prausnitzii*, *Lactobacillus coleohominis*, *Bacterioides* sp. and *Streptococcus gallolyticus* bacterial communities respectively, also proving the proliferation of opportunistic pathogenic bacteria in Crohn's diseases (CD) compared to control subjects [21].

Concerning **the breakdown of the gut tolerance**, even against the commensally microflora, two possibilities were designed: an acquired immunity system weakness involving dendritic cells (DC) function and the occurrence of a defect of the innate immunity involving toll-like receptor (TLR) and nucleotide-binding oligomerisation domain (NOD) receptor, both of them able to disturb the syntheses of defensins and/or cathelicidins antimicrobial peptides.

As proof, aiming to decipher the failure of dendritic cells (DC), some studies [22]

using myeloid blood cells and mucosal dendritic cells isolated from (76) patients with CD or UC in remission *versus* (76) healthy or non-IBD controls have revealed an aberrant lipopolysaccharide (LPS) response of DC at IBD patients.

Furthermore, starting from the fact that the gut tolerance as well as the gut intolerance is based on a number of recognition receptors (including TLRs and NODs receptors known as responsible for the host resistance to microbial pathogens), the bacterial binding to the host cell receptors resulting in the production of various molecules such as cytokines, eicosanoids and antimicrobial peptides, studies [23] demonstrated significant alterations in TLR4 expression and signalling associated with IBD, patients with active IBD showing an up-regulation of the mucosal TLR4 expression. Similarly, studies [24, 25] on NOD receptors evidenced a lack of Paneth cell beta-defensins HD-5 and HD-6 in ileal Crohn's disease in the absence and in the presence of a pattern recognition receptor NOD2 mutation; this lack of defensins has been shown as independent from concurrent active inflammation and resulting in a diminished antibacterial killing by the mucosa. Also, a lack in the killing of different *Escherichia coli, Staphylococcus aureus* and other anaerobic microorganisms of CD mucosa were evidenced.

Based on these findings, Crohn's disease (CD) mucosal immunopathology was explained by *simply taking advantage of bacteria in a niche formed by defensins deficiency added or not to some defects in cytoplasmic NOD-like receptors, and by the failure to induce autophagy leading to lack of bacterial clearance and subsequently to mucosal immunopathology* [25].

2. The main internal factors causing IBD development and probable pathomechanism

Genetic susceptibility and host immune impairments are considered the main internal risk factors for IBD.

In support of **genetic aetiology**, nine susceptibility genes polymorphisms, known as IBD1 – IBD9 loci, have been established. Data indicates chromosome regions 16q (IBD1), 12q (IBD2), 6p (IBD3), 14q (IBD4), 5q (IBD5), 19p (IBD6), 1p (IBD7),19q (IBD8) and 3p (IBD9) as well as many other, less studied, gene polymorphisms situated on chromosomes 2q, 3q, 4q, 7, 11p and Xp.

Briefly, IBD1 locus is situated on chromosome 16q interesting NOD2/CARD15 gene known as playing an important role in the immune response of IBD by recognizing the bacterial molecules (muramyl dipeptide moiety respectively) and activating nuclear factor (NF)-kappaB protein also shown to be responsible for the activation of more than 500 genes. Regarding NOD2/CARD15 genes mutation, there were confirmed three major coding region polymorphisms, R702W, G908R, and 1007fs mutations respectively, all three allele’s variants being translated in a deficit of NF - kappaB activation in response to bacterial components [26].

Moreover, it has been established that *this activating function is regulated by the*
carboxy-terminal leucine-rich repeat domain, which has an inhibitory role and also acts as an intracellular receptor for components of microbial pathogens leading to susceptibility to Crohn's disease by altering the recognition of these components and/or by over-activating NF-kB in monocytes [27]. The relationship between CARD15/NOD2 gene mutation and the protein involved in bacterial recognition by monocytes has also been confirmed in a study comprising 67 sequence variations indicating 9 alleles with a frequency higher than 5% in patients with CD, three of them (R702W, G908R and 1007fs) confirmed as independently associated with CD susceptibility [28].

Regarding the IBD2 region, the chromosome band 12q13-14 situated near the deoxyribonucleic acid marker D12S83 has been proposed as potential susceptibility loci, and the AVIL gene, known as encoding the advillin protein involved in morphogenesis of microvilli, as potential susceptibility gene; moreover, it has been established the major contribution of IBD2 to UC susceptibility and only relatively minor effect in CD [29].

Situated on chromosome 6p, IBD3 region regards 6p21.1-23 band, known as encompassing the HLA gene(s) complex [30].

IBD4 region is hosted on chromosome 14q, the linkage studies indicating 14q11-12 band as being a Crohn's disease susceptibility locus. The affected locus appears to influence the expression of interleukin (IL)-25 genes, known to contribute at the imbalance of Th1/Th2 cytokine responses [31]. Also, there are new data suggesting a possible association between 14q11-12 band and host sensibility to environment factors, including smoking interaction [32].

IBD5 region is situated on chromosome 5q and 5q31 band was confirmed as most probable susceptibility locus. The affected gene(s) involves the organic cation transporter (OCTN) gene cluster, SLC22A4 and SLC22A5 allele variants being associated with CD, but also UC and CD development [33].

IBD6 region was placed on 19p13 chromosome band and two candidate genes within the IBD6 locus have been proposed: DDXL gene known as part of human major histocompatibility complex (MHC) class III and ICAM-1 gene, part of immunoglobulin superfamily [34].

IBD7 region was placed on 1p36 chromosome band and the most probable affected gene is a tumour suppressor gene involved in carcinomas namely RUNX3; proving that, RUNX3 mRNA expression was associated with inflamed colonic mucosa in UC patients [35].

Summing all, until nowadays, more than 150 genes and loci involved in the regulation of innate or acquired immune response, as well as in the intestinal mucosa homeostasis have been analyzed (including gene alleles of IL-2, IL-10, IL-6, IL-23, IL-12B, IL-1B and IL-1 receptor antagonist/ IL-1Ra, tumour necrosis

factor/TNF-alpha, ATG16L1, STAT3, JAK2, etc.) but, despite of this tremendous work, some recent meta-analyses studies [36, 37] have reported that while Crohn's disease was mostly associated with NOD2/CARD15 and ATG16L1 autophagy gene allele mutations, both affecting the intracellular processing of bacterial components, in ulcerative colitis the predominant association signal was in the major histocompatibility complex region, HLA class II genes, the corresponding genes mediating the epithelial defence function.

Concerning the role of **immune response in IBD,** even though it is still unclear whether the immune disturbance in IBD is either the cause or the result of the disease, profound alterations in the mucosal immunity have been demonstrated.

Thus, since the 70s, pharmacological studies revealed that the lymphocytes taken from peripheral blood or intestinal mucosa of patients with colitis had the effect of disintegration of colon epithelial cells *in vitro*, this cytotoxic effect being lost after the colectomy or disease remission [38]. An increased production of immunoglobulin within the mucosa, and complement activation during the exacerbation of disease and the increase of T lymphocytes population within the mucosa, but no change in the ratio of T helper/T suppressor cells, have also demonstrated [39].

Subsequent immunohistological studies on patients with UC and CD had to reveal a considerable increase in the number of IgG immunocytes in the *lamina propria* of the mucous membrane, especially in infiltrations around ulcers. Moreover, in the specific case of a highly active inflammatory process associated with a pronounced destruction of the epithelium, the complement fractions C3, C4, and C9 were also present in the epithelium of crypts and in blood vessels of the mucous membrane and submucosa, one-third of the patients emphasizing a decreased number of crypts with secretory and serum IgA [40]. Furthermore, in UC patients the number of IgG-containing cells was three times that of the controls, as the number of IgE positive cells; differently, the numbers of IgA- and IgM-containing cells were not different from controls suggesting that the *lamina propria* of large bowel mucosa is the place of primary immunological processes in UC [41]. Accordingly, studies indicated that within and around lymphoid follicles most T cells expressed the restricted common leukocyte antigen (CD45R), while most *lamina propria* T cells were negative for CD45R finally concluding that *T and B cells alter their surface antigen expression as they emerge from follicles and enter into the inflamed lamina propria, the marked infiltration by CD45R+lymphoid cells that did not coexpress T- or B-cell surface antigens being a characteristic feature of UC and CD* [42].

Thus, the theory of the imbalanced T-cell subpopulations in the intestinal *lamina propria* playing a key role in the pathogenesis of CD has been started [43].

Given these, further studies were focused on specific T cells (eg., Th1, Th2, and Th17 cells) and corresponding key cytokines and modulators (such as TNF-alpha, IFN-gamma and interleukins IL-1, IL-6, IL-8, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23 in the case of CD pathway, and IL-13, IL-33, as IL-10 in the specific case of UC pathway), known to have important regulatory role in gut functions.

Accordingly, studies on Th1 cell mediators have revealed not only a stimulation of the local cytokine production in gut, but also their presence in blood, precisely the synthesis of the cytokines IL-1, IL-6 and TNF-alpha by the peripheral blood mononuclear cells, particularly in CD [44]. Similarly, studies on IL-8, a powerful neutrophil chemoattractant and activator, indicated that the affected colonic mucosa contained significantly raised levels of IL-8 in patients with active disease face to patients with inactive disease, patients with other types of colitis, or normal subjects. Colonic IL-8 levels were significantly correlated with the macroscopic grade of the local inflammation, especially in patients with UC, with the neutrophilic level in mucosal tissue, with colonic IL-1 beta and TNF-alpha levels in both CD and UC [45]. Moreover, studies on IL-1 family cytokines including IL-1 receptor antagonist (IL-1Ra), IL-1 soluble receptor Type I (IL-1sRI), IL-1 soluble receptor Type II (IL-1sRII) and IL-1 receptor accessory protein (AcP) demonstrated their key role in IBD development, the proinflammatory moiety of the IL-1sRI being designed as a systemic marker of inflammation and activity in CD [46]. Also, recent studies indicating an increased expression of IL-23 and IL-27 transcripts in CD confirmed their important role in this phenotype variant [47].

Concerning Th2 cells mediators, studies on peripheral blood mononuclear cells indicated that, unlike IFN-gamma that have shown lower in CD and UC vs. controls and normalized after stimulation with phytohemagglutinin, the level of cytokines IL-10 and IL-13 were significantly higher in patients with CD than in patients with UC and controls, before and after stimulation with phytohemagglutinin thus suggesting that the imbalance between the production of proinflammatory Th1 and anti-inflammatory Th2 cytokines persists even during IBD remission, the disturbances of the immune homeostasis being significantly more expressed in CD than in UC [48]. Differently, other studies on colonic biopsies taken from inflamed and noninflamed areas of UC patients and cultured *in vitro* in order to condition dendritic cells (DCs) demonstrated levels of IL-13 cytokine below the detection limit in most studied cases inquiring the relevance of IL-13 in UC aetiology [49]. Also, even if IL-10 knockout in mice was established as being a genetic model for IBD, recent results on humans are controversial [50] also inquiring the theory of IBD as a results of excessive inflammation.

Given these, the specialist concluded that *there are more and more evidences suggesting that Crohn's disease is rather the consequence of the reduced release of the pro-inflammatory cytokines and an impaired acute inflammatory response*,

supporting that IBD might be an immunodeficiency rather than an excessive inflammatory disease [51].

Besides, being well known that chronic inflammation is always associated with the enhancement of the reactive oxygen/nitrogen species (ROS/RNS) and intermediaries (ROIs, POPs, 8-OHdG), the important role of the oxidative stress is also expected in IBD pathomechanism.

Accordingly, studies on inflamed intestinal mucosal biopsies indicated that while Crohn's disease is associated with increased levels of ROIs, POPs, 8-OHdG, and iron face to decreased levels of copper and Cu-Zn SOD activity, ulcerative colitis was associated with high levels of ROIs, POPs, and iron and decreased levels of zinc and copper trace metals [52]. The tissue NO level was correlated with oxidative damages (precisely with actin cytoarchitecture disruption), the NO level and all measures of oxidative damage in tissue and cytoskeleton proteins in mucosa being correlated with IBD severity [53]. Furthermore, studies on CD mucosa indicated that the epithelial apoptosis levels were strongly associated with the expression of xantine oxidase (XO), although its levels were unaffected by intestinal inflammation and were comparable to those in control mucosa.

Peroxynitrite-mediated protein nitration (3-NT) immunoreactivity was substantially increased in luminal crypt cells, neutrophils, and mononuclear cells in the inflamed mucosa of ulcerative colitis patients and the inflamed IBD luminal epithelium contained increased amounts of nitric oxide synthase (NOS), 3-NT and NOS being significantly higher in UC than in CD [54].

Concerning the changes of activity of anti/pro-oxidant enzymes, studies on rectal biopsy specimens taken from patients with either active or quiescent UC, and patients with CD vs. controls have revealed that if in specific case of catalase (of peroxisome), neutral alpha-glucosidase (of endoplasmic reticulum), alkaline phosphatase (of plasma membrane), malate dehydrogenase (of mitochondria), and lactate dehydrogenase (of cytosol) only modest changes of enzymes activity were noticed, in case of lysosomes hydrolases (such as beta-glucuronidase, N-acetylbeta-glucosaminidase and acid phosphatase) enzymatic activity was pronounced in ulcerative colitis, particularly in active disease, but not in Crohn's disease [55].

Also, it was evidenced that while superoxiddismutase and glutathione peroxidase activities were increased during active disease and returned to normal in remission phases, catalase activity remained constantly inhibited suggesting that catalase is not a redox-sensytive enzyme, but a regulator of cellular processes. Due to the fact that the resulting reactive oxygen/nitrogen species can also be stimulated by different cytokines, ROS/RNS were also proposed as potential aetiological and/or a triggering factor in IBD and, consequently, oxidative stress as a possible mechanism underlying the pathophysiology of IBD [56, 57].

The present paper is based on the first author's monographic study entitled *IBD pathomechanism at the basis of herbal-based anticolitis products* [58].

Conclusions

IBD is nowadays seen as a chronic immune dysregulation leading to the loosing of the gut mucosal homeostasis, most probably due to the defective interaction between cytokines and gut commensally, and resulting from the combination of some genetic susceptibilities with a number of environmental triggers. Also, gut mucosal homeostasis has been shown to be a balancing act of effectors and regulatory T cells (such as Th1, Th2, and Th17), an increase in the effector's cell population with excessive inflammatory responses, or a decreased function of regulatory cells able to generate IBD expansion. Whatever the causes, this major immune dysregulation induces an increased formation of inflammatory cytokines causing chemotaxis, chemokinesis, superoxide radicals' synthesis, and the release of the lysosomal enzymes by phagocytes further responsible for the alteration of the bowel muscle contractile function, the emergence of pain, bleeding and diarrhoeal processes ending with a generalized inflammatory status. Concerning IBD incidence, a systematic review study [49] using MEDLINE (1950-2010; 8103 citations) and EMBASE (1980-2010; 4975 citations) databases indicated that the highest annual incidence of UC was 24.3 *per* 100,000 personyears in Europe, 6.3 *per* 100,000 person-years in Asia and the Middle East, and 19.2 *per* 100,000 person-years in North America. The highest annual incidence of CD was 12.7 *per* 100,000 person-years in Europe, 5.0 person-years in Asia and the Middle East, and 20.2 *per* 100,000 person-years in North America. The highest reported prevalence values for IBD were in Europe (UC, 505 *per* 100,000 persons; CD, 322 *per* 100,000 persons) and North America (UC, 249 *per* 100,000 persons; CD, 319 *per* 100,000 persons). Moreover, in time-trend analyses, it was demonstrated that 75% of CD studies and 60% of UC studies had a statistical significance ($P < 0.05$) thus concluding that *the incidence and prevalence of IBD are increasing with time and in different regions around the world, indicating its emergence as a global disease* [49].

Finally, it is well known that developing a chronic inflammatory process, UC as well as CD comprise an increased risk of tumour initiation indicating the important role of sustained allopath and adjuvant treatment in IBD. In this way, it must be mentioned therapeutically potential of plant compounds and herbal medicines exploited for millennia in traditional and folk medicine in order to treat acute and chronic intestinal ailments, the advanced studies during the latest decades revealing their effectiveness in both, preclinical and clinical tests [58].

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