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Targeted Therapies for Inflammatory Bowel Disease and Colorectal Cancer: An Increasing Need for Microbiota-Intestinal Mutualism

Tomasello Giovanni[1], Tralongo Pietro[2], Jurjus Abdo[3], Matar Michel[3], Angelo Leone [2*]

1. Euro-Mediterranean Institute of Science and Technology, Palermo 90139, Italy;

2. Department of Experimental and Clinical Neurosciences, Section of Histology, University of Palermo, Palermo 90133, Italy;

3. Department of Anatomy, Cell Biology and Physiology, School of Medicine, American University of Beirut, 1107-2020 Beirut, Lebanon

ABSTRACT

The involvement of intestinal microbiota and dysbiosis in the pathogenesis of inflammatory bowel disease (IBD) and colorectal cancer (CRC) is a well-established fact to be taken into real consideration when developing targeted therapies. This review aims to depict how advances in our understanding of the role of intestinal flora in the pathogenesis of IBD and CRC are shaping up the therapeutic protocols of their management. It is demonstrated that there is a circadian regulation of colocyte gene expression in response to microbiota. Dysbiosis leading to a decrease in microbiome biodiversity is also described in IBD patients whereby thick layers of adherent mucosa associated bacteria exist both in ulcerative colitis (UC) and Crohn's disease (CD). Probiotics based approaches using lactobacilli and bibidobacteria improved clinical symptoms of IBD's through the GALT immune modulation. In addition, microbiota transplantation has also been used for IBD treatment. Feacal microbiota transplantation (FMT) consists of transferring gastrointestinal microbiota from a healthy donor to an IBD patient by duodenal infusion of liquid stool suspension to establish microbial homeostasis. The destruction of mucosal integrity facilitates the passage of bacteria in the injured zone to trigger chronic inflammation, eventually leading to CRC development by creating a carcinogenic environment. Actually, a high level of fusobacterium nucleatun and other bacteria are prevalent in CRC patients, thus suggesting a potential role of these organisms in the initiation and progression due to the production of genotoxic metabolites causing a direct damage to DNA integrity. Besides, regular probiotics intake may actively prevent the whole process.

Key words:
Microbiota
Inflammatory bowel disease
Colorectal cancer
Ulcerative colitis
Targeted therapies

Introduction

Microbial involvement in inflammatory bowel disease (IBD) and colorectal cancer (CRC) is now well established. In addition, technological advances in molecular medicine provide a considerable amount of data in support of genetic susceptibility to Crohn's disease (CD), ulcerative colitis (UC) and CRC[1-2]. Actually, the development of newer molecular tools for the

***Corresponding Author:**
Angelo Leone, E-mail: angelo.leone@unipa.it

global assessment of the gut microbiome and identification of nucleotide-binding oligomerization domain-containing protein 2 (NOD2) in 2001, and other susceptibility genes, for CD in particular, have led to a better understanding of the aetiopathogenesis of IBD[3]. Numerous genome-wide association studies have identified further genes involved in gastrointestinal innate immunity to better elucidate the relationship of the local innate immunity with the adjacent luminal bacteria[4]. These microbial studies aim towards a much deeper elucidation of the normal composition of the gut microbiome and its perturbations in the setting of IBD. The condition of "altered" or perturbed microbiome is called "dysbiosis". It represents a key player in the protracted course of inflammation in IBD and, possibly in CRC[2, 5]. Such advances have also spurred the search for specific pathogens, which may have a role in the metamorphosis of the gut microbiome from a symbiotic entity to a putative pathogenic one[2, 6-8]. Here is a review of recent advances in our understanding of microbial involvement in the pathogenesis of IBD and CRC, and such advances are shaping up the therapeutic management of gastrointestinal diseases nowadays and in the coming years.

Microbiota and IBD

Epidemiology and clinical manifestations of IBD

Chronic IBD is an emerging group of pathologies encompassing CD and UC with a significantly augmented prevalence and incidence in industrialized countries[9]. The prevalence and incidence of IBD around the world are summarized in Table 1.

Table 1 Worldwide Prevalence and Incidence of CD and UC*

	Worldwide prevalence	Worldwide incidence
CD	26-199 cases in 100 000 people 201 cases in 100 000 adults	3.1-14.6 cases in 100 000 people
UC	37-246 cases in 100 000 people 238 cases in 100 000 adults	2.2-14.3 cases in 100 000 people

*The Data are obtained from center for disease control and prevention website.

In UC mucosal lesions, the rectum appears and extends to the entire colon with a hyperemic mucosa, in severe cases, which becomes bloody and ulcerated with pseudo-polyps. On the other hand, CD can affect any part of the gastrointestinal tract from the mouth to the anus. These chronic inflammatory pathologies involve systemic clinical manifestations ranging from articular (arthritis) to cardiovascular (endocarditis), endocrinological (thyroiditis), ocular and cutaneous[1, 5, 10-12].

In patients affected by IBD and secondary arthritis, bacterial antigens and genetic materials, often belonging to gram-negative, have been found in synovial fluid. Furthermore, several studies have shown that aberrant migration of intestinal lymphocytes or mononuclear cells is responsible for the onset of joint inflammation[13]. This phenomenon is probably due to the penetration of saprophytic commensal micro-flora through damaged tight mucosal joints with consequent loss of impermeability. Genetic polymorphisms of HLA-B27 and the receptor for interleukin 23 increase both the risk and susceptibility of developing both IBD and arthritis[14-15]. Since among the microorganisms detectable in CD patients, there is *Klebsiella,* infection by this bacterium in the bowel may cause ankylosing spondylitis, through the production of anti-*Klebsiella* antibodies. The latter, can also bind to cross-reactive self-antigens like HLA-B27 and collagen fibers in the joints, with release of further new antigens on the surface of damaged tissue. These new antigens are responsible for prolonged or continuous production of autoantibodies and further damages to the articular tissues with a perpetuation in the disease process. Recurrent *Klebsiella* infections could explain the characteristic trend present in the patients affected by CD and ankylosing spondylitis[16]. Moreover, the elevated levels of heat shock protein (Hsp) 60, Hsp10, Hsp70 and Hsp90 have been found in serum and colonic biopsies derived from IBD patients. The latter presents molecular structures very similar to those detectable in the microbiome counterpart, supporting the hypothesis of an exacerbated gut associated lymphoid tissue (GALT) activation in response to these self-antigens through the phenomenon of molecular mimicry at the base of IBD etiopathogenesis[17-18].

Circadian regulation of colocyte gene expression in response to microbiota

The microbial associated molecular patterns (MAMPs) are responsible for the activation of immune system through interacting with pattern recognition receptors (PRRs) and subsequent triggering of inflammatory processes. Recent evidences support the great importance of sleeping processes to prevent several pathological conditions including inflammation, even CRC. The circadian rhythm switches on/off different genes

in GALT, such as TLR1, TLR5, TLR9 and NOD2. Several homeostatic intestinal processes, such as nutrient absorption, cell proliferation, gut motility and metabolic activities, are known to be regulated in a circadian manner[20]. Components of circadian clock, like BMAL1, are required for the correct functionality. In the mouse small intestine, some TLR genes are expressed in a circadian manner[21]. The gene encoding for NOD2 receptor which belongs to the group of NLR intracellular receptor genes, is the first susceptibility gene to be linked to CD[3]. Moreover, NLR is important for the release of antibacterial compounds, like cryptidins by intestinal Paneth cells. For this reason, the mouse microbiota shows a major intestinal susceptibility to inflammation and colitis[22]. Thus, it is very important to highlight that the dialogue between PRRs expression and bacterial MAMPs is highly regulated. The absence of microbiota precludes PRR-mediated signaling, as well as the function of the clock, thus impairing genic expression in colocytes, dependent on both PRRs and clock components, representing the base for the breakage of the delicate equilibrium involved in regulation of gut innate and adaptive immunity. Furthermore, the expression of several genes involved in gut innate immunity (angiogenin 4, TSLP, and claudin2 and claudin12) is microbiota-vitamin D3 dependent, since a defective vitamin D3 receptor signaling has been shown to increase the susceptibility to IBD[23]. RORα transcription factor seems to be involved in the circadian activation of BMAL1 expression in colonic monocytes[4]. Moreover, the short chain fatty acid receptor FFAR3 (GPR41), involved in intestinal motility control, seems to be regulated by ROR α in a ZT0 > ZT12 circadian manner. In fact, in antibiotic-induced microbiota-depleted mice FFAR3 is significantly decreased. Of note, rhythmic activation of IKKb and JNK represents a very important factor for correct timing of colocyte homeostatic functions dependent on genes activated by AP1 and NF-kB, showing a circadian activation pattern at diurnal times ZT20-ZT4 which correspond to the mouse ''active phase''. Furthermore, the circadian rhythm of IKKb and JNK activation prevents the inappropriate activation of RevErba by PPARα. These mechanisms ensure that, during the same ZT20-ZT4 active phase, the transactivating (RORα and BMAL1/Clock) and transrepressing (RevErba/E4BP4) molecular clock components can adequately control the temporal expression of RORE- and E-box-containing genes encoding colonic homeostatic functions. Importantly, microbiota derived MAMPs maintain the circadian clock through activation of RevErba by PPARα, and also controlling proper repression mediated by E4BP4, thereby allowing the expression of the numerous D-box-containing genes encoding IEC homeostatic functions[25-26]. These conclusions suggest that the dialogue between microbiota and circadian system may have different effects on the development of IBD.

Dysbiosis and probiotics for IBD

It is now well established that inflammatory pathologies affecting the gastrointestinal tract are narrowly correlated to dysmicrobism and other various factors, such as genetic background and diet. On the basis of dysbiosis, a characterizing condition of IBD, a question remains to be answered: "Is dysbiosis a cause of IBD or just a secondary phenomenon?" Research on IBD onset and development is oriented towards the investigation into molecular mechanisms underlying the instauration and perpetuation of GALT activation. Increasing evidences suggest that the intestinal microbiota play a role in initiating, maintaining and determining the severity of IBD. The precise role of microbiota in the etiology of IBD as linked to a continuous antigenic stimulation that has the potential to activate pathogenic T cells and, subsequently, cause chronic intestinal injury. Together, the above mentioned factors concur to the typical alterations of GALT, associated with IBD[2, 27]. Mutations in genes encoding for PRRs, such as Nod2/CARD15, significantly contribute to loss of immune tolerance[28-29]. Children with altered microbial flora have a higher incidence of developing IBD during adulthood. Approaches based on mucosal bacterial isolation have shown increased concentrations of *Bacteroidesvulgatus* and *Enterobacteriaceae*, especially *E coli,* and decreased concentrations of *Bifidobacteria* species, in subjects affected by CD[30-31]. Mucosal specimens derived from CD patients revealed a highly significant presence of *Mycobacterium avium,* suggesting a potential role of this enteric pathogen in disease causation[32]. Dysbiosis involves the decrease in microbiome biodiversity, with under representation of the phyla *Bacteroidetes* and *Firmicutes* in feces/mucosa-associated among IBD patients[33]. Indeed, Swidsinski group demonstrated thick layers of adherent mucosal associated bacteria in both UC and CD patients with higher bacterial concentrations in CD[34]. Immunological studies conducted on patients with IBD revealed the presence of specific antibodies and T cell subsets in both serum and tissue. In particular, significantly higher systemic antibody responses were found in UC towards *Peptostreptococcusanaerobius*, in parallel with higher recovery rates from the colonic mucosa of this strain[30]. The employment of lactic acid-producing organisms, firstly discovered in the beginning of the 20th century by Metchnikoff, revealed a successful tool for ameliorating the inflammatory background[35]. Indeed, fermented milk contains

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specific compounds and microorganisms, known as probiotics, beneficial to human health. Probiotics counteract the activation of Nf-κB, maintaining it bound to IκB in the cytoplasm, thus inhibiting pro-inflammatory cytokines production. Hegazy group investigated the effect of *Lactobacillus delbruekii* and Lactobacillus fermentum administration on 30 patients with mild to moderate UC, evaluating their potential immune-modulating effects. Results derived from this study revealed that 8-week administration significantly ameliorated the inflammation by decreasing the colonic concentration of IL-6, expression of TNF-α and NF-kB, p65, leukocyte recruitment, as demonstrated by a decrease in colonic MPO activity, and the level of fecal calprotectin compared to sulfasalazine group and the control[36]. Moreover, *Lactobacillus plantarum* has been shown to inhibit the degradation of IκB, consequently leasing to the activity of NF-κB in vitro[37]. Decreased amount of *Faecalibacterium prausnitzii* has been shown to predict a high risk for early reactivation of ideal CD[6]. Probiotics moved to improve the clinical symptoms of IBD through GALT immune modulation based approaches using *Lactobacilli* and *Bifidobacteria*. In particular, probiotics are able to induce T_{reg} cells through an immune-regulatory response involving IL-10 and TGF-β[38]. The study of T cell subsets in IBD patients revealed a predominance of T helper 17 cells (Th17). In particular, IL-17A and IL-17F are abundantly found in inflamed IBD mucosa, suggesting their pivotal role in IBD[39]. Interestingly, a subpopulation of Th17 (supTh17) cells exhibits immune suppressive properties because it expresses high levels of both CD39 and FOXP3 and consequently produces extracellular adenosine. Longhi group reported reduced levels of the above mentioned lymphocyte population in IBD patients[40]. Administration of *Lactobacilluscasei* and *Bifidobacteriumlactis* in mice with TNBS to induce colitis led to a significant reduction of inflammation in the colonic mucosa, reversing malignant changes and exerting a potential role in cancer prevention. Beneficial effects of probiotic treatment have been observed with the restoration of normal goblet cells number. The action of probiotics consists in preventing other luminal bacteria from reaching the lamina propria. Moreover, probiotics modulate the expression of genes encoding junction proteins in colocytes and stimulate the mucosal immune system in the patient's intestinal tract to secrete protective immunoglobulins, such as secretory IgA and protective defensins in the colonic lumen[41].

Microbiota transplantation for IBD

The role of gastrointestinal microbiota in driving chronic inflammation in IBD is well documented, thus the treatments based on microbiota manipulation are paid great interest in clinical practice, with variable evidence for their efficacy. An additional alternative treatment for IBD management is represented by faecal microbiota transplantation (FMT). The principle of FMT indication is based on the concept that antibiotic therapy disrupts the normal microbial homeostasis, allowing pathogen colonization. At this stage, FMT is indicated. It consists in the transfer of gastrointestinal microbiota from a healthy donor to IBD patients by duodenal infusion of liquid stool suspension. In rodent models, FMT not only offers an investigational tool to study the role of microbes in disease development and treatment response, but also a new therapeutic intervention. The gained credibility in the clinical world on FMT is subsequent to the first publication on the effectiveness of this treatment for antibiotic-resistant C difficile-induced diarrhea[42]. Re-establishment of microbial homeostasis has been demonstrated by significant increase in *Bacteroidetes* and *Clostridium* as well as a decrease in *Proteobacteria* according to healthy donor profiles. Recently, Suskind and coworkers enrolled 9 patients, aged 12-19 years with mild-to-moderate CD, to undergo FMT by nasogastric tube. Follow-up was conducted at 2, 6 and 12 weeks, and the results showed an improvement at 2 weeks of (6.4 ± 6.6) and 6 weeks of (8.6 ± 4.9). Thus, 7 of 9 patients were in remission at 2 weeks, 5 of 9 and patients who did not receive additional medical therapy were in remission at 6 and 12 weeks[43].

Microbiota and CRC

Studies on initiation and promotion of colorectal carcinogenesis revealed the crucial role of the rupture in the physiological equilibrium between commensal bacteria inhabiting colonic mucosa. Indeed, some bacterial strains may "drive" initial pathological changes in colocyte behavior and immune system responses. Physiologically, the mutualistic relationships between commensal bacteria and epithelium, promote colonic health, counteract pathogen infections and creation of favorable conditions for developing CRC. Although more than 80% of intestinal bacteria cannot be cultured, identification of all bacteria has become possible by high technology to perform whole DNA genome sequencing. With the evolvement of phylogenetic analysis on bacterial 16 S rRNA genes, this goal has been achieved. In fact, *Firmicutes, Bacteroidetes* and *Proteobacteria* were reported as the most dominant phyla in bacteria adherent to precancerous adenomatous polyps[44]. Thus, once mucosal integrity is destroyed, other bacteria can pass in the injured zone and support CRC development. The creation

of a carcinogenic environment may be caused by a decrease in levels of butyrate-producing species, such as *Ruminococcus* and *Roseburia* species compared with the controls[45].

The gram-negative bacterium *Fusobacterium nucleatum* binds to E-cadherin through its membrane protein FadA, activating β-catenin signaling, triggering inflammatory and oncogenic responses[46]. Moreover, high levels of *Fusobacteriumn ucleatum* are prevalent in stool derived from subjects affected by CRC, suggesting a potential role of this microrganism in the initiation and progression processes[47]. Increased levels of *Akkermansia muciniphila* and *Citrobacter farmer* have been reported in CRC cases and depletion of the first mentioned strain is associated with IBD progression. On the other hand, depletion of *Bifidobacterium longan, Clostridium clostridioforme* and *Ruminococcus* species has been reported in CRC cases[45]. Thus, bacterial metabolites evoke an immune response characterized by increased levels of IL-17, supporting cancer progression[48]. On the other hand, innate immunity activated by bacterial PAMPs has been reported as a very important factor for tumor progression in murine models. Indeed, TLR2 and TLR4 also play a crucial role in tumor formation, especially in the presence of specific human genetic polymorphisms, such as TLR4 299Gly[49]. In colitis-associated CRC, TLR signaling activates epiregulin, responsible for ERK activation and actively supporting tumor growth[50]. Studies on murine models reveal that dysbiosis "alone" is able to induce CRC formation in presence of polymorphisms responsible for reduced activity of NOD2[51]. Molecular dynamics at the base of dysbiosis encompass the production of genotoxic metabolites from different bacterial strains, such as escherichia coli, enterococcus faecalis and B. fragilis. In particular, cyclomodulins, produced by groups B2 and D of escherichia coli, exert detrimental effects on the mechanisms responsible for cellular differentiation, apoptosis and proliferation control[52]. A direct damage of DNA integrity is caused by B. fragilis toxin through a mechanism involving the polyamine catabolism[53]. The great importance of colonic microbiome in CRC is substantiated by experiments of stool transfer from individuals with colon cancer and healthy germ-free mice. 6-week follow-up results revealed that the composition of microbiota in the stools of mice was of human type and remained stable over time. However, cell proliferation and aberrant crypt foci increased in the colons of mice given the cancerous stools[54]. Regular probiotics intake may actively prevent the initiation and development of CRC. In fact, Hatakka group reported a significant decrease in putative pro-carcinogenic enzymatic activities[55]. Recently, derived from vaginal secretions of adolescent and young adult women, a particular bacterial strain has been tested on HI-29 (colon tumor cell line). The isolated strain exhibited probiotic properties, including low pH and antimicrobial activity against some pathogenic bacteria. Moreover, *Lactobacillus plantarum 5BL* strain also exhibited a remarkable anti-tumor activity against tested human cancer cell line, showing a favorable potential effect as a bioactive therapeutic agent.

Conclusion

Futher clinical investigations into the mutualistic relationship between microbiota and colonic mucosa are useful to clarify the physiological, biochemical and immune-regulatory dynamics. Based on the targeted therapies, treatment of IBD with probiotics may significantly improve the quality of life and reduce the risk of progression towards the onset of CRC. It is very interesting to note that T lymphocytes producing IL-17 have been usually considered as detrimental for the immune homeostasis in colonic mucosa. The oriented therapies towards regaining intestinal microbial equilibrium may represent the key strategy to switch off chronic inflammatory processes, thus preventing the onset of CRC at the same time. This review could prompt the research on IBD and CRC to deepen the knowledge about the exact action mechanisms of intestinal microbiota, consequently discovering potential biological weapons useful to switch off chronic inflammation and actively preventing carcinogenesis. In conclusion, the mutualistic relationship between microbiota and colonic mucosa is proved useful in depicting some dynamics in the initiation and development of IBD and CRC. The oriented therapies towards establishing equilibrium of intestinal microbiota may represent a crucial strategy to switch off chronic inflammatory processes, leading to prevention of CRC onset.

Declaration

The authors of this manuscript declare that they have no conflict of interest.

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