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REVIEW

Gut microbiota imbalance and chaperoning system malfunction are central to ulcerative colitis pathogenesis and can be counteracted with specifically designed probiotics: a working hypothesis

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Abstract In this work, we propose that for further studies of the physiopathology and treatment for inflammatory bowel diseases, an integral view of the conditions, including the triad of microbiota–heat shock proteins (HSPs)–probiotics, ought to be considered. Microbiota is the complex microbial flora that resides in the gut, affecting not only gut functions but also the health status of the whole body. Alteration in the microbiota’s composition has been implicated in a variety of pathological conditions (e.g., ulcerative colitis, UC), involving both gut and extra-intestinal tissues and organs. Some of these pathologies are also associated with an altered expression of HSPs (chaperones) and this is the reason why they may be considered

chaperonopathies. Probiotics, which are live microorganisms able to restore the correct, healthy equilibrium of microbiota composition, can ameliorate symptoms in patients suffering from UC and modulate expression levels of HSPs. However, currently probiotic therapy follows *ex-adiuvantibus* criteria, i.e., treatments with beneficial effects but whose mechanism of action is unknown, which should be changed so the probiotics needed in each case are pre-determined on the basis of the patient’s microbiota. Consequently, efforts are necessary to develop diagnostic tools for elucidating levels and distribution of HSPs and the microbiota composition (microbiota fingerprint) of each subject and, thus, guide specific probiotic therapy, tailored to meet the needs of the patient. Microbiota fingerprinting ought to include molecular biology techniques for

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sequencing highly conserved DNA, e.g., genes encoding 16S RNA, for species identification and, in addition, quantification of each relevant microbe.

Keywords Microbiota · Probiotics · Ulcerative colitis · Heat shock proteins · Molecular chaperones · Inflammation

Introduction

We argue that two parameters are essential in defining, understanding, and diagnosing inflammatory bowel diseases (IBD): the intestinal flora (microbiota) and heat shock proteins (HSP), many of which are molecular chaperones. A third pillar, which pertains to treatment, is probiotics. The combination constitutes a triad that we propose as a blueprint for future clinical and laboratory research.

Consequently, in the first three sections of this overview, we introduce molecular chaperones and their pathologies (chaperonopathies), the microbiota, and probiotics. In the following sections, we discuss ulcerative colitis (UC), as an illustrative example of our proposal, based on our work and that of others in the literature. We hope to provide an alternative standpoint to view UC that might reveal as yet undisclosed aspects of pathogenesis and, thus, open the way for the development of novel treatments, more efficacious than those currently available.

Molecular chaperones and chaperonopathies

Molecular chaperones are a family of ancestral proteins that have been conserved throughout evolution, being present from bacteria and archaea to humans [1]. Many molecular chaperones are heat shock proteins (HSPs) and the terms are often used as synonyms in the literature, although some HSPs are not chaperones and vice versa. The entire complement of chaperones-HSPs of an organism has been called the chaperoning system [2]. It is constituted of chaperones, co-chaperones, and chaperone cofactors. The main groups of chaperones are shown in Table 1. Their typical function is to assist other proteins in folding as well as in re-folding after stress, or in degradation after irreversible misfolding [3]. However, during evolution, some of them have acquired other, extrachaperoning functions (e.g., apoptosis control [4]) and the property to be actively secreted into the extracellular space. The secreted chaperones may function in a paracrine/endocrine fashion, for example, modulating the response of certain immune system cells [5]. A few illustrative examples of reported interactions between the chaperone HSP60 and the immune system are displayed in Table 2. Since HSP-chaperones are important for cell and tissue homeostasis, their structural

Table 1 Main HSP-chaperones classified according to their molecular weight

MW (kDa) range	Classical family
200 or higher	Saccin
100–199	HSP100/I110
81–99	HSP90
65–80	HSP70/DnaK
55–64	HSP60 and CCT subunits (chaperonins of Group I and II, respectively)
35–54	HSP40/DnaJ
34 or less	Small HSP (e.g., crystallins)

Modified from Macario et al. [5]

Table 2 Examples of reported interactions between HSP60 and the immune system

Cells	Receptor(s)	Effect(s)
Adipocytes	Unknown (interacts with HSP60 N-terminus)	Adipocyte inflammation; muscle cell insulin resistance
Endothelial cells, smooth muscle cells, dendritic cells	LOX-1	Cross-presentation on MHC class I molecules (?)
Macrophages	TLR4	TNF-alpha production
Macrophages and dendritic cells	Unknown (TLR4 independent)	IFN-alpha production that leads to antigen-dependent T cell release of IFN-gamma
Myocardium	TLR4	IRAK-1 activation
Neutrophilic granulocytes	Unknown	Production of ROS and release of primary-granule enzymes
Peripheral blood mononuclear cells	Unknown	TNF-alpha secretion
T cells	CD30	IL-10 secretion
T cells	TLR2 and/or TCR	Tregs population enhancement
T cells	CD54 RA+ RO-	Proliferation
T cells	TLR2	Down-regulation of chemokines receptor expression (CXCR4 and CCR7)

Modified from Macario et al. [5] and updated

and/or functional defects can cause diseases of various types affecting diverse tissues and organs; these pathologic conditions are named chaperonopathies [3, 6].

Chaperonopathies, like many other groups of diseases, can be classified in various ways [3, 7]. For instance, taking into consideration the way they manifest themselves and cause pathology, chaperonopathies can be classified into by

excess, by defect, and by mistake or collaborationism. Quantitative variations (i.e., excess or defect) in chaperone levels may be due to various mechanisms, such as gene overexpression, diminished mRNA or protein degradation, and slower trafficking than normal through any given cell compartment [6, 7]. However, some chaperonopathies occur when a normal chaperone functions to favor a pathogenic agent (e.g., virus or bacterium), or a pathologic cell (e.g., a cancer cell), and its consequences, such as inflammation. These chaperonopathies are called by mistake or collaborationism, since they act cooperating “traitorously” with the enemy, the disease-causing agent and the disease itself, rather than with the host, the human body [7].

Our group in the past proposed, as a working hypothesis, that some chronic inflammatory and autoimmune diseases could be considered for the purposes of investigation and clinicopathological approach as models of chaperonopathies by mistake. Among the latter, we may cite chronic obstructive pulmonary disease [8], IBD [9], myasthenia gravis [10], and other autoimmune diseases such as those listed in Table 3 [11]. The interplay between the chaperoning and the immune systems of any given organism can in principle occur at least in three ways, involving mechanisms pertaining to innate or adaptive immunity and cell signaling in a hormonal fashion [12–14]. Other chaperonopathies by mistake are some forms of cancer, in which the chaperones are involved in the carcinogenic steps, e.g., by blocking apoptosis and favoring tumor cell growth by various mechanisms [4, 15, 16]. In addition, tumor cells can secrete HSPs, via exosomes and other ways, and the

released molecules most likely interact with specific targets in the immune system [17, 18].

The intestinal microbiota: definition and biological functions in health and disease

The human gut harbors about 100 trillion bacteria, and more than 500 different species are present in the colon, so the total personal bacterial concentration can reach 9×10^{13} numbers. The sum of these bacterial cells, which form a “tissue in the organ”, is termed the intestinal microbiota. It resides in the gut of the host establishing a mutually beneficial relationship and modulating, through its metabolic activities, the host’s health status [19].

The microbiota exerts diverse physiological functions such as inhibition of pathogenic bacteria and short fatty acids synthesis; stimulation of nutrient and mineral absorption, of the intestinal immune system and of gut cellular renewal; synthesis of vitamins and amino acids; and the decomposition of protein compounds [20, 21]. The majority of bacteria composing the microbiota are not pathogenic to the gut, but pathogenic species, for example, Clostridia or Colibacillacea, are also present. A strictly controlled balance between pathogenic and non-pathogenic bacteria is crucial for maintenance of a physiological state [22]. Alteration of the microbiota’s composition (e.g., abnormal expansion of one bacterial species with respect to others) that may be caused by psychophysical, alimentary, or environmental stress is defined as dysbiosis and may lead to pathological conditions [23, 24].

Mounting experimental evidence shows an association between microflora imbalance (i.e., dysbiosis) and IBD. Among the various etiopathogenic hypotheses proposed, one postulates that a change in the saprophytic microbial flora is the *primum movens*, namely the primary stimulus, which causes mucosal damage [25, 26]. Specifically, dysbiosis leads to a modification of intercellular tight junctions responsible for the correct structure of the epithelial layer of intestinal mucosa. This inevitably leads to an increase in, and change of, mucosal permeability [27]. Consequently, penetration of antigens takes place into the intercellular space leading to the activation of the mucosa-associated lymphoid tissue (MALT), with recruitment and activation of the inflammatory cascade (e.g., activation of leukocytes and production of cytokines, such as TNF-alpha) and tissue damage [25]. In addition, recent data provided evidence of the ability of some components of the microbiota to activate Th17 cells in the small intestine lamina propria [28–30].

An altered stimulation of the immune system leading to inflammation may be a link between dysbiosis and metabolic diseases. It has been proposed that in the obese and in

Table 3 Diseases in which HSP60 may act as autoantigen

Tissue/organ	Cell/structure	Pathology
Vessels	Endothelial cells	Vasculitis, atherosclerosis
Heart	Myocardiocytes	Myocarditis, infarct, heart failure
Joints	Synovial cells	Rheumatoid arthritis
Pancreas	Beta cells	Diabetes
Thyroid	Thyroid follicular cells	Hashimoto’s thyroiditis
Liver	Hepatocytes, biliary ducts	Chronic active hepatitis, primary biliary cirrhosis
Adrenal glands	Glomerular zone cells	Addison’s disease
Nervous system	Synapses	Myasthenia gravis
Kidney	Endothelial cells (glomerulus)	Glomerulonephritis
Skin	Keratinocytes, fibroblasts, endothelial cells	Scleroderma, pemphigoid, psoriasis, dermatomyositis

Modified from Cappello et al. [11], Campanella et al. [116]

type 2 diabetes patients, a high-fat diet changes the gut microbiota in a complex way, specifically decreasing *Bifidobacterium* spp. This phenomenon is associated with higher levels of lipopolysaccharides (LPS) in the plasma (metabolic endotoxemia) and with a LPS-dependent secretion of proinflammatory cytokines. High-fat diet and LPS promote low-grade inflammation-induced metabolic disorders (insulin resistance, diabetes, obesity, steatosis, and infiltration of adipose tissue by macrophages) [31, 32].

The connection between microbiota composition and metabolic disorders extends also to cardiovascular diseases (CVD). Administration of a probiotic containing *Lactobacillus plantarum* (Lp299v) and *Bifidobacterium lactis* (Bi-07) reduced myocardial infarction in rats, which was associated with a decrease in circulating leptin levels [33]. This suggests that altering the intestinal microbiota with probiotics to decrease leptin levels in the circulation may mitigate cardiac hypertrophy and enhance cardiac remodeling after myocardial infarction. An explanation of the link between gut microflora composition and CVD could be the ability of microbiota to metabolize dietary phosphatidylcholine (PC) generating trimethylamine that is converted in the liver to trimethylamine N-oxide (TMAO) [34]. Interestingly, associations were found between coronary artery disease, peripheral vascular disease, and history of myocardial infarction with the metabolites choline, TMAO, and betaine [35]. Since an alteration in microbiota composition may determine both intestinal and extra-intestinal pathologic manifestations, it is not surprising that for almost 50 years, the pharmaceutical industry has been proposing probiotics as therapeutic agents for restoring the bacterial homeostasis of the human intestine.

Probiotics: from definition to therapeutic use

The term probiotics was used for the first time in 1965 [36]. It was reported that certain “substances” obtained from intestinal segments, if placed in vitro with organic tissue, stimulated the growth of the latter tissue. Subsequently, further studies defined better the role of these “substances” identifying them as commensal intestinal bacteria. Today, we tend to associate the term probiotics with microorganisms, usually bacteria, producing beneficial effects on the host. These bacteria are part of the normal intestinal microbial flora acting as a commensal population, which distinguish them from pathogenic bacteria, both exogenous (e.g., *Salmonella*, *Shigella*, *Yersinia enterocolitica*, etc.) and residents (e.g., Bacilli, Clostridia, *Klebsiella*, *Proteus*, etc.) [20]. These latter pathogenic organisms are not part of the normal flora or, if present, they are only about 0.02 % of the total [37].

We know that administration of certain live bacteria can have beneficial effects thanks to the ability of restoring the

intestinal microflora's balance. Today, the pharmaceutical industry, sensing the safe therapeutic potential of probiotics, studies and markets preparations of synergistic bacteria (Table 4). These probiotics generally include various types of bacteria, such as *Lactobacilli*, *Bifidobacteria*, and *Enterococci*, and are offered for therapeutic use in intestinal and extra-intestinal pathologies [36]. In addition, probiotic bacteria might find a use in the prevention of cancers of the digestive tract [27, 36, 37]. Indeed, beneficial effects of probiotics in the gastrointestinal tract have been observed in experimental models of colon cancer. The relationship between probiotics and cancer seems to be based mainly on bacterial metabolism of various dietary constituents leading to production of many compounds, some of which may be carcinogenic and some anticarcinogenic [38, 39].

One of the best recognized uses of probiotics is for diarrheal diseases, particularly in those cases of viral etiology [38, 40]. In addition, probiotics are used as adjuvant therapy in necrotizing enterocolitis [41], *Clostridium difficile* colitis [42], *Rotavirus* enteritis [43], gastric infection by *Helicobacter pylori* [44], infection of urogenital apparatus by *Candida albicans* [43], and in chronic inflammatory diseases [45]. In chronic juvenile arthritis, gut defense mechanisms are disturbed, and orally administered *Lactobacillus* GG has potential to reinforce the mucosal barrier [38, 46].

Probiotic bacteria have been shown to downregulate inflammation associated with hypersensitivity reactions in patients with atopic eczema and food allergy [38, 47]. Another unexpected benefit of probiotics is serum-lipid reduction through their ability to take up cholesterol in the presence of bile and in the absence of oxygen, both conditions present in the intestinal tract [38, 48].

In view of all the observations listed above, we can conclude that probiotics are versatile therapeutic tools with promising potential to treat a variety of human diseases. Probiotics are currently used mainly as *ex-adiuvantibus* treatments, i.e., treatments with beneficial effects but whose mechanism of action is unknown. Nevertheless, we believe that if precise knowledge of the interactions between probiotic bacteria and the cells of the human intestinal mucosa becomes available, the efficaciousness of probiotics to treat some specific conditions will increase. A solid scientific basis of probiotic actions and interactions will no doubt encourage their use in specific cases for which they might appear indicated. An example of this application may be inflammatory bowel diseases (IBD), regarding which information has recently been gathered [49, 50] indicating a close association between clinical status, intestinal mucosa lesions, and immunological parameters with levels of chaperones, such as HSP60, as discussed below.

Table 4 Effects of probiotics

Microflora	Associated effects
<i>Bacteroides</i> species	Chronic colitis, gastritis, arthritis (increased bacterial urease activity in chronic juvenile arthritis)
<i>Bifidobacterium animalis</i>	Decreases <i>Candida albicans</i> systemic dissemination in euthymic or athymic beige mice
<i>Bifidobacteria</i> species	Reduced incidence of neonatal necrotizing enterocolitis
<i>Enterococcus faecium</i> <i>Escherichia faecium</i>	a. Decreased duration of acute diarrhea from gastroenteritis; b. No benefit in diarrhea due to <i>Vibrio cholerae</i> and <i>Escherichia coli</i>
<i>Escherichia coli</i> non-pathogenic strain (serotype O6:D5:H1)	As effective as mesalamine in maintaining remission of ulcerative colitis
<i>Lactobacillus</i> strains	a. Administration of multiple organisms, predominantly <i>Lactobacillus</i> strains shown to be effective in ameliorating pouchitis; b. Lactose digestion improved, decreased diarrhea and symptoms of intolerance in lactose intolerant individuals, children with diarrhea, and in individuals with short-bowel syndrome; c. Microbial interference therapy—the use of non-pathogenic bacteria to eliminate pathogens and as an adjunct to antibiotics; d. Improved mucosal immune function, mucin secretion, and prevention of disease
<i>Lactobacillus acidophilus</i>	a. Significant decrease in diarrhea in patients receiving pelvic irradiation; b. Decreased <i>Candida albicans</i> systemic dissemination in euthymic or athymic beige mice; c. Decreased polyps, adenomas, and colon cancer in experimental animals; d. Prevented urogenital infection with subsequent exposure to three uropathogens <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , and <i>Pseudomonas aeruginosa</i> ; e. Lowered serum cholesterol levels
<i>Lactobacillus rhamnosus</i>	a. Reduced duration and/or risk of rotavirus diarrhea; b. Decreased polyps, adenomas, and colon cancer in experimental animals; c. Reduced duration of acute diarrhea most often caused by gastroenteritis; d. Reduced diarrheal illness in formula-fed toddlers; e. Reduced occurrence of <i>Clostridium difficile</i> diarrhea; f. When co-administered with antibiotics in children, non- <i>Clostridium difficile</i> antibiotic-associated diarrhea is reduced; g. Reduced risk of traveler's diarrhea; h. Reduced severity of pneumonia in children with cystic fibrosis; i. Decreased <i>Candida albicans</i> systemic dissemination in euthymic; or athymic beige mice; j. Increased IgA-specific antibody secreting cells to rotavirus and reduced the duration of diarrhea; k. Increased IgA secretion in Crohn's disease; l. Prevented recurrent, chronic vaginitis; m. <i>Lactobacillus</i> vaginal suppositories effective in reducing the incidence of recurrent urinary tract infections; n. Reduced food allergies and atopic dermatitis; o. Increased interleukin-10 production in atopic children
<i>Lactobacillus fermentum</i> strain KLD	Traveler's diarrhea: no effect
<i>Lactobacillus plantarum</i>	Produced and preserved key nutrients, vitamins, and antioxidants; eliminated toxic components from food; protected food from decay; eradicated pathogens such as <i>Enterobacteriaceae</i> , <i>Staphylococcus aureus</i> and <i>Enterococci</i>
<i>Lactobacillus plantarum</i> (299v and DSM 9843)	a. Reduced incidence of diarrhea in daycare centers when administered to only half of the children; b. Especially effective in reducing inflammation in inflammatory bowel, e.g., enterocolitis in rats, small bowel bacterial overgrowth in children, pouchitis; c. Reduced pain and constipation of irritable bowel syndrome; d. Reduced bloating, flatulence, and pain in irritable bowel syndrome in controlled trial; e. Positive effect on immunity in HIV+ children
<i>Lactobacillus reuteri</i>	a. Shortened the duration of acute gastroenteritis; b. Decreased <i>Candida albicans</i> systemic dissemination in euthymic or athymic beige mice; c. Prevented development of methotrexate-induced and acetic acid-induced colitis in rats; d. Shortened acute diarrhea
<i>Lactobacillus rhamnosus</i> (HN001)	Enhanced cellular immunity in healthy adults in controlled trial
<i>Lactobacillus salivarius</i>	Suppressed and eradicated <i>Helicobacter pylori</i> in tissue cultures and animal models by lactic acid secretion
<i>Saccharomyces boulardii</i>	a. Reduced recurrence of <i>Clostridium difficile</i> diarrhea; b. Effects on <i>Clostridium difficile</i> and <i>Klebsiella oxytoca</i> resulted in decreased risk and/or shortened duration of antibiotic-associated diarrhea; c. Shortened the duration of acute gastroenteritis; d. Decreased only functional diarrhea, but not other symptoms of irritable bowel syndrome; e. Decreased duration of diarrhea induced by tube feedings; f. Ineffective for small intestinal bacterial overgrowth; g. May reduce HIV-related chronic diarrhea; h. Childhood diarrhea; i. Extends remission time of Crohn's disease; j. Increased IgA anti-toxin A responses in pretreated mice
<i>Saccharomyces cerevisiae</i>	Enhanced digestion of sucrose load was shown in infants with sucrase deficiency

Modified from Drisko et al. [38]

Ulcerative colitis: a bowel disease with extra-intestinal manifestations

Ulcerative colitis (UC) and Crohn's disease (CD) are part of the so-called IBD group [26] and constitute two major healthcare problems pertinent to the digestive tract [51]. Mucosal inflammation may involve either all layers of intestinal wall, as in CD, or only the mucosal and sub-mucosal layers, as in UC [52]. In the intestinal mucosa of patients with UC, the lamina propria is infiltrated by abundant inflammatory cells, while the epithelium undergoes cycles of destruction and repair by regeneration of basal cells [49]. This aspect characterizes the clinical course and the occurrence of complications of UC, differentiating it from CD.

The etiology of IBD is still under investigation [53]. Inflammation of the intestinal wall is chronic and is due probably to a complex interaction of genetic, microbial, and environmental factors, which result in continuous activation of gut-associated lymphoid tissue (GALT) [20]. Typical macroscopic lesions visible by endoscopy are mucosal ulcerations with immune cell infiltration usually accompanied by cryptic abscesses demonstrable by microscopy in biopsies [20].

UC is an inflammatory disease characterized by a chronic, recurrent clinical evolution [54]. Symptoms of UC are bloody diarrhea and abdominal pain that may be complicated by toxic megacolon [55]. Extra-intestinal manifestations (EIM) of UC (Table 5) appear at least once in 46.6 % of the patients and their prevalence may vary depending on the geographic area in which the patient resides [56]. The pathology of the joints manifested as peripheral arthritis and arthralgia is the most common EIM

in UC [56]. Dermatological complications (e.g., psoriasis, alopecia, and pyoderma gangrenosum) are present in 3.8 % of UC patients [57]. Rare complications are glomerulonephritis, autoimmune hemolytic anemia, and celiac disease [57]. Recto- or ano-vaginal fistulae complicating ulcerative colitis [58] are rare but may occur in the patients with severe rectal inflammation, and they can be managed by restorative proctocolectomy with an ileal pouch anus or anal canal anastomosis [59].

A protracted disease with persistent inflammation such as the case in IBD may predispose to the development of colorectal cancer [54]. The molecular mechanisms by which inflammation promotes cancer development are still incompletely understood and could differ from those involved in non-colitis-associated forms of colorectal cancer. In this regard, a recent work has reported on the role of distinct immune cells, cytokines, and other immune mediators in virtually all steps of colon tumorigenesis, including initiation, promotion, progression, and metastasis [60].

Ulcerative colitis as a model of chaperonopathy: evidences for and against it

HSPs have been found increased in UC, both in tissue and serum [9, 61–63]. Pathological effects of HSPs in UC could be due to one or more of the following mechanisms (Fig. 1):

1. Non-disease-specific response to mucosal stress. The inflamed colon tissue would react with a higher rate of protein synthesis, and consequently, it would have a pressing need for the folding of new polypeptides in the face of protein destruction due to the pathological process linked to inflammation. Self-HSPs would be increased as a response to a situation in which the cell needs more of them, but this situation would not necessarily be typical of IBD since it can also occur in other pathologies with inflammation and tissue destruction [64–67]. Elevated levels of self-HSPs would reflect an anti-pathogenic mechanism, and they could be used as indicators of disease and, thus, could have some value as diagnostic markers, and in assessing prognosis and response to treatment.
2. The self-HSPs would form antigen–antibody complexes because they are recognized by the patient's immune system as foreign, i.e., the HSPs have become autoantigens due to, for instance, pathological post-translational modifications that make the human molecules immunogenic with regard to its own immune system [5]. The same consequences would occur if a human (self) HSP shares antigenic epitopes and immunologically cross-reacts with a foreign HSP

Table 5 Extra-intestinal manifestations of ulcerative colitis

Organ systems affected by inflammation	Clinicopathological manifestations
Musculoskeletal system	Peripheral arthritis Sacroiliitis Ankylosing spondylitis Osteoporosis
Skin	Erythema nodosum Pyoderma gangrenosum Aphthous stomatitis
Eye	Uveitis Scleritis Episcleritis
Hepatobiliary system	Primary sclerosing cholangitis
Vascular system	Thromboembolic events
Renal system	Nephrolithiasis

Modified from Evans and Pardi [117]

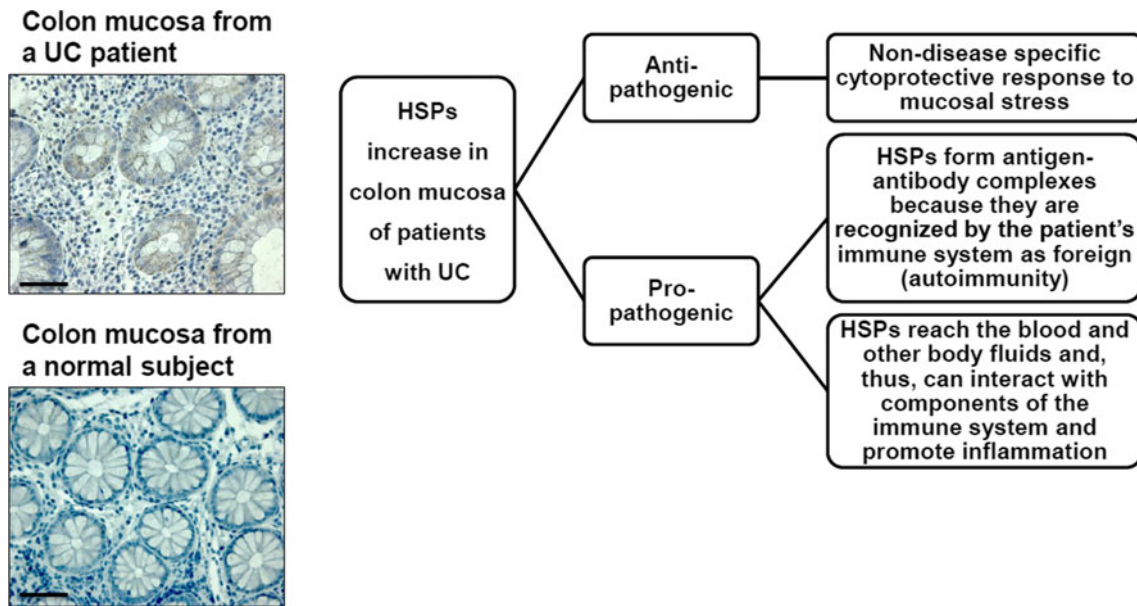


Fig. 1 Proposed pathogenic mechanisms in ulcerative colitis (UC) in which the chaperoning system plays a central role along with imbalances in the gut microbiota. HSP60 is increased in the UC mucosa (*top left* image) as compared with normal mucosa (*bottom left* image) and may play a role against disease progression or, alternatively, contribute to pathogenesis in at least two ways, as depicted within the frames to the *right*. The photographs to the *left*

were taken from immunohistochemical preparations of colon mucosa biopsies obtained from a patient with UC (*top*) and a normal control (*bottom*). Primary antibody: anti-HSP60 (SIGMA, Milan, Italy); clone: H4149; source: mouse; dilution: 1:300. Immunostaining kit: avidin–biotin complex kit (LSAB2, DAKO, Carpinteria, CA). Chromogen: 3,30-diaminobenzidine (DAB chromogen solution, DAKO). Nuclear counterstaining: hematoxylin (DAKO). Bar 100 μ m

ortholog from a bacterium present in the intestinal, or respiratory, or genitourinary tract, or in the skin [11, 68, 69]. Accumulation of antigen–antibody complexes in the intestinal mucosa would trigger inflammation and tissue destruction [70–72]. In this situation, self-HSPs would have a pathogenic effect, contrary to cytoprotection. From the diagnostic viewpoint, measurement of anti-HSP antibodies would help in determining the factors involved in pathogenesis, and this, in turn, will suggest therapeutic strategies, such as blockage of the immune system.

3. It is now known that, normally, self-HSPs reach the blood and other body fluids and, thus, can interact with components of the immune system [73]. Consequently, macrophages, dendritic cells, and neutrophils are activated and produce cytokines and chemokines, eliciting and maintaining inflammation. Likewise, the adaptive immune response can be activated. In both situations, self-HSPs are agents of inflammation and immune response.

In conclusion, taking into account the preceding alternatives 2 and 3, self-HSPs could, indeed, be the etiologic agent in UC or at least one of the primary etiologic factors. The facts that HSP60 levels in the mucosa of UC patients are increased, compared to the mucosa of healthy individuals and significantly correlate with severity of

clinical manifestations support the notion that the chaperonin is involved in UC pathogenesis [9, 49, 50]. Furthermore, UC treatment showed that the more clinically efficacious is the treatment, the more marked is the decrease in HSP60 (as well as in other inflammatory markers) levels in the colon mucosa, to the point that in complete remission the chaperonin levels are those typical of the normal intestine [50]. Finally, also the number of macrophages positive for HSP60 was reduced significantly after therapy [50], the latter indicating that HSP60 may actively participate in triggering and/or maintaining inflammation via macrophage stimulation, since these cells bear HSP60-specific receptors [74, 75]. Therefore, we postulated that UC can be regarded as a chaperonopathy, i.e., a disease in which chaperones are etiologic-pathogenic factors [3, 7]. If self-HSPs in UC were structurally no different from the normal molecules but just increased due to dysregulation of their genes, UC would be a pure dysregulatory chaperonopathy. However, changes in gene expression levels are probably not the only manifestation of disease since structural alterations of the chaperone molecules themselves, for instance, due to pathological post-translational modifications, might also occur. To view UC from the standpoint afforded by the chaperonopathy concept, as a working hypothesis, offers an alternative to think on aspects of pathogenesis

not much explored yet and, thus, opens the road for developing novel tools for diagnosis, assessing prognosis and response to treatment, and for exploring new therapeutic agents (for instance, compounds with the ability to block the pro-inflammatory action of HSP60).

Effects of probiotics on UC remission and pathogenesis

In the last 15 years, a number of publications have reported that probiotics can induce remission of UC symptoms but their precise role in maintenance of remission is a matter of debate [76]. In one study, for example, it was found that the combination of balsalazide with a high-potency probiotic mixture was the most effective treatment compared to balsalazide alone and to mesalamine [77]. This study considered several parameters, such as clinical symptoms, endoscopy results, and histologic observations in the evaluation of the three treatment modalities, all of which tend to indicate that the data are comprehensive and reliable. Likewise, another study provided evidence of a dose-dependent effect of probiotics administration in inducing remission of the disease [78].

Probiotics produce their beneficial effects by various mechanisms (Table 6). Firstly, they compete with other luminal bacteria and prevent them from reaching the lamina propria and stimulating the mucosal immune system. Probiotics can also modulate intestinal barrier properties, regulating expression of genes encoding adherence junction proteins [79–81]. Secondly, probiotics enhance mucous production and, thus, protect the mucosa against invasive bacteria.

Thirdly, probiotics stimulate the mucosal immune system in the patient's intestinal tract to secrete protective

immunoglobulins (Ig) such as secretory IgA and protective defensins, and they produce bacteriocins into the lumen [82]. It has also been shown that *Lactobacillus brevis* and *Streptococcus thermophilus* selectively stimulate apoptosis of mucosal immune cells counteracting the excessive accumulation of these cells in the gut [83]. In this regard, it is worth noting that excessive accumulation of mucosal immune cells is believed to play a central role in IBD [84]. Fifthly, probiotics alter the function of the mucosal immune system to make it more anti-inflammatory and less pro-inflammatory [22, 26]. Along these lines, it has been reported that an as yet elusive molecular mechanism triggered by *Lactobacillus plantarum* prevents nuclear factor kappa B (NFκB) binding in murine epithelial gut cells and blocks the proteasome-dependent degradation of the NFκB inhibitor IκBα [85].

Another set of data suggest also a role of probiotics in regulating intestinal cell survival and growth. For instance, proteins produced by *Lactobacillus rhamnosus* GG stimulated protein kinase B (PKB/Akt) activation, promoted cell growth, and inhibited tumor necrosis factor (TNF)-induced epithelial cell apoptosis in cultured cells and in ex vivo colon organ culture models [86]. It is pertinent to mention here that a relationship does exist between microbiota and intestinal cell turnover [21, 87] and that indigenous bacteria, in *Drosophila melanogaster*, can stimulate intestinal stem cells to differentiate [88].

Effects of probiotics on HSPs levels in intestinal mucosa of patients with UC

To the best of our knowledge, there are very few reports describing the effects of probiotics on levels and

Table 6 Effects and possible mechanisms of probiotic treatment on IBD pathogenesis and remission: illustrative reports

Probiotics	Molecular mechanism of action	Therapeutic effects	References
<i>Streptococcus thermophilus</i> and <i>Lactobacillus acidophilus</i>	Activation of ERK isoforms	Improvement of epithelial barrier and transport properties	[79]
<i>Lactobacillus acidophilus</i> , <i>Lactobacillus fermentum</i> , <i>Lactobacillus gasseri</i> , and <i>Lactobacillus rhamnosus</i>	Regulation of gene encoding E-cadherin and β-catenin	Improvement of epithelial barrier function	[85]
<i>Lactobacillus brevis</i> and <i>Staphylococcus thermophiles</i>	Induction of mucosal immune cell apoptosis	No excessive T cell accumulation in the gut	[83]
<i>Lactobacillus plantarum</i>	Inhibition of NFκB pathways	Attenuation of inflammation	[85]
<i>Lactobacillus rhamnosus</i> GG	Regulation of antigen recognition by the intestinal lymphoid tissue. Akt activation, inhibition of cytokine-induced epithelial cells apoptosis	Attenuation of inflammation. Improvement of epithelial response to injury	[27, 86]
<i>Lactobacillus salivarius</i> plus <i>Lactobacillus acidophilus</i> plus <i>Bifidobacterium bifidum</i> BGN4	Reduction in levels of HSP10, HSP60, HSP70, and HSP90 in inflamed mucosa. Reduction in levels of CD68+/HSP60+ cells in lamina propria	Amelioration of symptoms and reduction in inflammation; enhancing the effect of 5-ASA	[49, 50]

expression of HSPs in intestinal mucosa of UC patients. HSPs are important cytoprotectants when expressed intracellularly in the epithelial cells of normal colonic mucosa [64, 66]. For instance, HSP60 is specifically induced by increments of colonic motility, 5-hydroxytryptamine administration, thyrotropin-releasing hormone, and water-immersion stress [89]. In addition, we and others collected data by various techniques suggesting that HSPs might be involved in UC pathogenesis [9, 61] and in colorectal carcinogenesis as well [90–93]. We recently reported that probiotics in combination with standard antibiotic therapy significantly reduced the levels of HSP10, HSP60, HSP70, and HSP90 in the mucosa of UC patients [49, 50]. This reduction was more marked than that caused by the antibiotic alone (Fig. 2). These results were in accordance with other studies that found increased HSP60 levels both in mucosa and in sera of patients with UC [94–97]. In addition, we found that probiotic administration reduced significantly the number of macrophages (CD68 + cells) positive for HSP60 in the lamina propria [50]. This result was in agreement with data from other groups showing that human HSP60 is elevated in mononuclear cells in the mucosa of patients with UC [94] and that some HSP60-derived peptides can stimulate an inflammatory response via production of proinflammatory cytokines in the intestinal mucosa of patients with CD [96]. Since it is known that CD68-positive macrophages have receptors for HSP60 [97, 98] we hypothesize that HSP60 is taken up by CD68-positive cells via those receptors, although we cannot exclude that HSP60 may also be produced by the macrophages themselves. In case there was binding of HSP60 via surface receptors, the expected consequence would be activation of CD68-positive cells followed by an inflammatory response, as shown in *in vitro* models [98]. Finally, probiotic therapy induced reduction in HSP60 down to normal levels, or nearly so, in the cytosol of epithelial cells, in which it increases in pathological situations, perhaps as a prerequisite for its secretion [18]. Current studies aim to elucidate the molecular links between probiotic administration and reduction in HSP levels through gene expression and post-translational modifications. A possible explanation could be that probiotics lower NF κ B expression in intestinal cells [85] and in so doing lowering expression of the *hsp60* gene, a possibility suggested by the finding that NF κ B can bind to the *hsp60* promoter and thereby induce gene expression [8, 99].

Beneficial systemic effects of probiotics beyond the bowel in UC

Probiotics, as well as commensal bacteria of a normal bowel, can induce cytoprotective HSP expression by

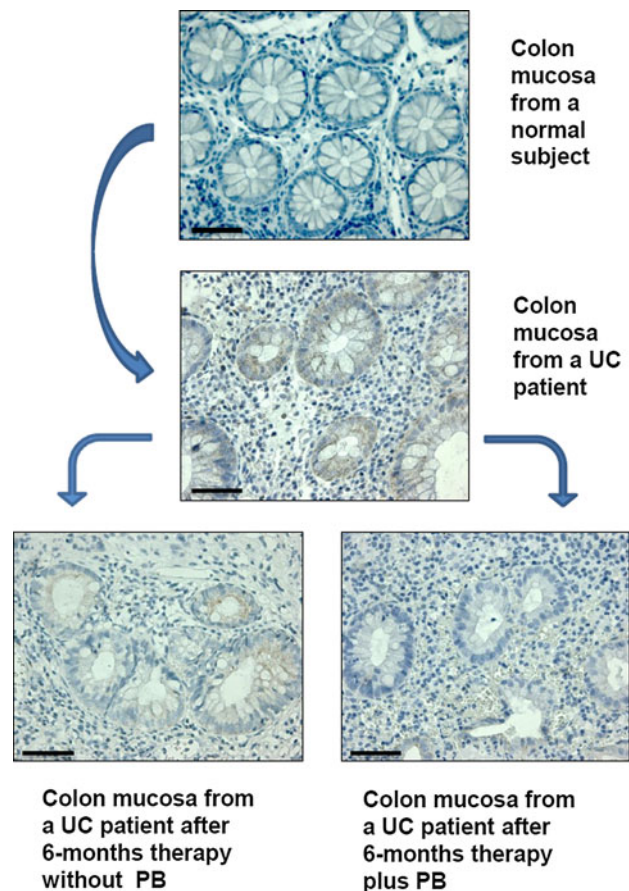


Fig. 2 The levels of HSP60 detected by immunohistochemistry and other methods in the colon mucosa of patients with UC are considerably increased by comparison with those in the colon mucosa from healthy controls but they decrease with treatment and clinical improvement. Illustrative images (immunohistochemistry) pertaining to a patient and a control are shown in the two *top panels*. Treatment with mesalazine (*bottom left*) or with mesalazine and probiotics (PB) (*bottom right*) decreased the levels of HSP60, more markedly in the latter. This reduction correlated with clinical improvement (Mayo score), which was also more pronounced with combined therapy. Analogous results were obtained for HSP10, HSP70, and HSP90 (not shown). For more details see [49, 50]. For technical details see legend for Fig. 1. Bar 100 μ m

secretion of polypeptides or other molecules that interact with epithelial cells [100, 101]. In this way, HSPs participate in the normal anti-stress response of a healthy bowel. However, when overexpressed in an inflamed mucosa, for instance, during relapsing UC, human HSPs may be released into the interstitium and thereby reach the bloodstream in larger amounts than in healthy individuals. In turn, this increase in circulating HSPs above the levels that are tolerated by the immune system might elicit an autoimmune response, thus being involved also in extra-intestinal complications of UC.

In addition, invasion of the patient's circulation by HSPs released by the bacteria colonizing the colon mucosa (these bacterial HSPs share antigenic epitopes with the human

counterparts) is very likely to add to the antigenic burden and raise it above the tolerogenic threshold, which results in autoimmunity.

As described before, patients with UC may develop systemic comorbidities and complications. Some of these are autoimmune comorbidities that have been found associated with increased levels of HSPs. For example, increased levels of HSP70 and HSP90 have been implicated in the onset of autoimmune arthritis [102–105], while HSP70 has been linked to the development of autoimmune hepatitis [106]. Clinical trials have been performed that demonstrated the usefulness of HSP10 in reducing inflammation in some autoimmune processes, such as multiple sclerosis [107], severe plaque psoriasis [108], and rheumatoid arthritis [109]. In addition, HSP90 inhibitors have been proposed for the treatment of autoimmune diseases involving inflammation and activation of the adaptive immune response [105, 110].

Likewise, probiotics have been proposed for treating extra-intestinal complications of IBD [111–114]. We believe that the reduction in HSPs levels in the colonic mucosa of patients with UC by probiotics reflects, at least

in part, the efficacy that probiotics might have in the treatment for systemic complications. Microbiota exerts important functions pertaining to the regulation of homeostasis not only of the colon but also of the whole organism, and adequate balance between the various microbes is necessary for a healthy status. Furthermore, certain non-pathogenic bacteria may favor development of pathologic conditions, e.g., obesity, by providing metabolites to other microbes in complex metabolic interactions [115]. This new paradigm of pathogenicity ought to be borne in mind in medical practice. Microbiota alterations can occur during a number of pathologic, inflammatory conditions including IBD. Treatment with probiotics may contribute to the healing of an inflamed bowel by, for instance, restoring the normal commensal flora. The latter may have positive systemic effects by reducing the levels of circulating pro-inflammatory molecules, including HSPs. IBD are only an example of diseases in which therapy with probiotics may lead to general benefits for the human body.

It can be postulated that the use of these natural remedies in medical therapy in lieu of, or in addition to,

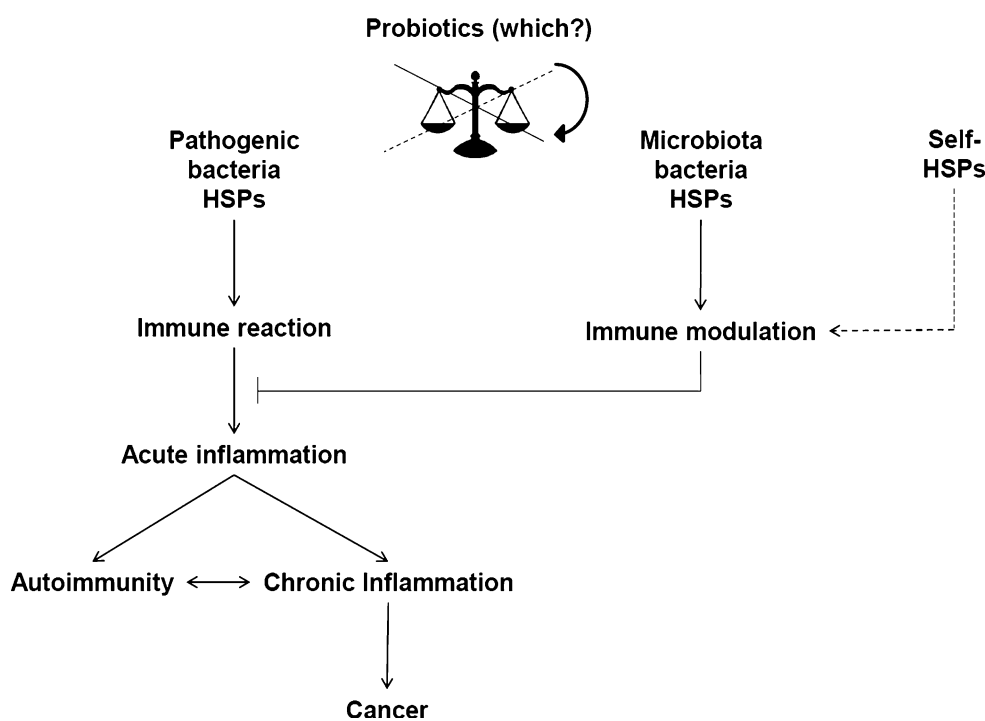


Fig. 3 Working model for the triad probiotics–microbiota–chaperoning system, the latter represented by HSPs. The microbiota composition must be balanced within the physiological range for any given individual and pathogenic bacteria must be absent or at least low and under control, as shown on *top* by the continuous line indicating what should be the position of the scale arms leaning toward the normal microbiota in healthy individuals. Overgrowth of one or more bacterial populations, especially if they are pathogenic, at the expense of the others with overproduction of HSPs may incline

the balance toward disease development, as indicated by the discontinuous line. On the contrary, HSPs from the normal microbiota (microbiota bacteria HSPs) and from the host (self-HSPs) would have a beneficial regulatory effect on the immune system (immune modulation) that would prevent deviation toward inflammation or autoimmunity. If imbalances occur toward the pathogenic side, they can be corrected with probiotics tailored to the specific needs of the individual as shown on *top* by the *curved arrow*

conventional drugs (e.g., antibiotics) deserves intensive investigation by the scientific community in the forthcoming years. For example, an unsolved point is to establish which probiotic bacteria should be administered in relation to which pathologic status in any given patient, considering also the composition of his/her microbiota. A great effort should be made in the future to find simple and economic methods to assess the microbiota of each subject during the various life periods, since we expect changes during puberty, adulthood, and normal aging. A tailored therapy with specifically targeted probiotics may be seen as a novel approach to improving the ability to fight daily stress and to reducing the probability of onset of a variety of diseases, including chaperonopathies.

Conclusions

The current re-evaluation of the intestinal microbiota and its importance in health and disease has also brought up to light the potential of probiotics for its maintenance within healthy limits. It is becoming clear that imbalances in the subpopulations of the microbiota can lead to disease, so there is a need to develop means for restoring the balance. The wise use of probiotics might be one of those means. It is also increasingly clear that the microbiota interacts with the host, particularly with the intestinal mucosa, its cells, and molecules. Among the latter are the components of the chaperoning system, the HSP-chaperones, which have been implicated as etiologic-pathogenic factors in inflammation and autoimmunity. In addition, HSPs produced and released into the host's tissues and circulatory system by pathogenic bacteria, HSPs that immunologically cross-react with the human counterparts (self-HSPs), may be immunogenic, determining acute and, in turn, chronic inflammation as well as, in prone subjects, autoimmunity and cancer development. HSPs produced and released by microbiota, and by human cells (self-HSPs), might modulate immune reactions (e.g., by activating regulatory T cells, Tregs) and arrest inflammation. In this way, probiotics may help to restore a healthy physiology of the immune system, at least in the bowel interface between external and internal environments (Fig. 3).

An integral view of the physiology and pathology of immune disorders, such as IBD, and their treatment ought to include the triad of microbiota–probiotics–HSPs. In summary, physicians and pathologists as well as laboratory professionals devoted to the study, management, and treatment for diseases ought to become aware of this newly defined group of pathological conditions, the chaperonopathies. This awareness will certainly hone their ability to diagnose them correctly, which in turn will lead to better patient management and disease control.

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