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Gut barrier in health and disease: focus on childhood

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Abstract. – The gut barrier is a functional unit, organized as a multi-layer system, made up of two main components: a physical barrier surface, which prevents bacterial adhesion and regulates paracellular diffusion to the host tissues, and a deep functional barrier, that is able to discriminate between pathogens and commensal microorganisms, organizing the immune tolerance and the immune response to pathogens. Other mechanisms, such as gastric juice and pancreatic enzymes (which both have antibacterial properties) participate in the luminal integrity of the gut barrier. From the outer layer to the inner layer, the physical barrier is composed of gut microbiota (that competes with pathogens to gain space and energy resources, processes the molecules necessary to mucosal integrity and modulates the immunological activity of deep barrier), mucus (which separates the intraluminal content from more internal layers and contains antimicrobial products and secretory IgA), epithelial cells (which form a physical and immunological barrier) and the innate and adaptive immune cells forming the gut-associated lymphoid tissue (which is responsible for antigen sampling and immune responses). Disruption of the gut barrier has been associated with many gastrointestinal diseases, but also with extra-intestinal pathological condition, such as type 1 diabetes mellitus, allergic diseases or autism spectrum disorders. The maintenance of a healthy intestinal barrier is therefore of paramount importance in children, for both health and economic reasons. Many drugs or compounds used in the treatment of gastrointestinal disorders act through the restoration of a normal intestinal permeability. Several studies have highlighted the role of probiotics in the modulation and reduction of intestinal permeability, considering the strong influence of gut microbiota in the modulation of the function and structure of gut barrier, but also on the immune response of the host. To date, available weapons for the maintenance and repair of gut barrier are however few, even if promising. Considerable efforts, including both a better understanding of the gut barrier features and mechanisms in health and disease, and the development of new pharmacological approaches for the modulation of gut barrier components, are needed for the prevention and treatment of gastrointestinal and extraintestinal diseases associated with gut barrier impairment.

Key Words:
Gut barrier, Gut microbiota, Intestinal mucus, MUC2, Epithelial cells, Immune system, IBD, IBS, SIBO, Gastrointestinal diseases, Probiotic, *Bacillus clausii*.

Introduction

The Gut Barrier: General Concepts and Components

Every day, thousands of microorganisms and nutrient compounds come into contact with the gastrointestinal tract. Balancing this interaction requires a complex, multitasking system, involved in several functions, such as protection from pathogen, regulation of absorption of nutrients, interaction between gut microbiota and the mucosal immune system, and many others. In a healthy organism, the gut barrier is able to satisfy such needs.

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nisms, such as gastric juice and pancreatic enzymes (which both have antibacterial properties) participate in the luminal integrity of the gut barrier[1].

From the outer layer to the inner layer, the physical barrier is composed of gut microbiota (that competes with pathogens to gain space and energy resources, processes the molecules necessary to mucosal integrity and modulates the immunological activity of deep barrier), mucus (which separates the intraluminal content from more internal layers and contains antimicrobial products and secretory IgA), epithelial cells (which form a physical and immunological barrier) and the innate and adaptive immune cells forming the gut-associated lymphoid tissue (which is responsible for antigen sampling and immune responses)[2].

Gut Microbiota

The human gut microbiota is a complex ecosystem that includes at least 10^{14} bacteria (about 1.5 Kg), counting up to 2000 species, with predominance of anaerobic bacteria, and also other microbes, such as yeasts[3] and viruses, mainly bacteriophages (viruses that attack and lyse bacteria) which have great influence on biochemical cycles and have been predicted to help maintaining microbial species diversity[4]. It interacts with the human body in a symbiotic manner. In health, gut microbiota plays a paramount main role within our organism, being involved in many functions, such as the metabolism of nutrients and drugs, the regulation of many metabolic pathways, the maintenance of epithelial integrity, the modulation of gastrointestinal motility, the stimulation and maturation of both systemic and mucosal immunity, the production of vitamins and micronutrients[5]. The balance between gut microbiota and host is maintained because of several mechanisms, such as gut secretions (gastric acid, mucus, biliary salts, mucosal immune globulines), mucosal barrier, intestinal motility, mucosal and systemic immunity, interaction among different bacteria strains, and others. Many factors can break such homeostasis, such as ageing, unhealthy diet, drugs (e.g. steroids or proton-pump inhibitors), and several (gastrointestinal, vascular, infectious, neurologic) diseases. Qualitative and quantitative alterations of gut microbiota may, therefore, occur, realizing the so-called dysbiosis and leading to gut microbiota-associated diseases. The alteration of gut microbiota can indeed bring to many digestive and extradigestive diseases such as inflammatory bowel disease (IBD), food allergy and intolerance, cancer, obesity and metabolic syndrome, liver disease, and functional diseases as irritable bowel syndrome (IBS)[6]. Different options of gut microbiota modulation are available to date[6].

Intestinal Mucus

Mucus is the first physical barrier that bacteria meet in digestive tract. It protects the epithelium from harmful microorganisms and antigens, but also as a lubricating agent for intestinal motility. It is composed of two layers: an inner layer, strongly adhering to the epithelial cells, and an outer layer, that is thicker (100 μm versus 50 μm) looser and less adherent, as measured in animal models[7].

The internal mucosal layer is dense and does not allow bacteria to penetrate, thus making the surface of epithelial cell free from bacteria, while the external mucus layer is the habitat of the gut microbiota. This compartmentalization is essential for intestinal homeostasis in a highly colonized ambient. The highly glycosylated mucin (MUC2) plays a key role in the constitution of the mucus layer. After secretion by goblet cells, MUC2 organizes in a hydrated and expanded network with other secreted proteins, forming an organized mucous layer. The protein composition is similar in the two mucous layers, deriving from a common cell[8]. The importance of the mucosal barrier has been further demonstrated in animals whose genes coding for MUC2 had been deleted: in this model, the bacteria, because of the absence of mucus, are close to the epithelial cells, with consequent increase of intestinal permeability caused by the loss of the barrier of mucus, that triggers an inflammatory reaction which can encourage the development of colon cancer[9]. Furthermore, the mucus goes far beyond its barrier function: its content in glycans, linked to mucin MUC2, not only serves as food for the bacteria, but also as a binding site, and probably is also involved in the selection of specific microbial species, which are essential for the maintenance of the integrity, homeostasis and intestinal function[7].

Epithelial Cells

The intestinal epithelium is organized in a single layer of 20 μm, and consists of 5 different cell types: enterocytes, endocrine cells, M cells, goblet cells and Paneth cells. The enterocytes are the most represented type[10]. They act as a physical barrier, inhibiting the translocation of luminal

contents in the inner tissues. They are connected by intercellular junctions, characterized by transmembrane proteins that interact with near cells and with intracellular proteins associated with the cytoskeleton. Together, these components form a complex and homogeneous network. In the intestinal epithelium there are two main types of junctions: the adherentes junctions (AJs) and the tight junctions (TJs). Adherentes Junctions are mainly formed of cadherins connected to the actin cytoskeleton via a family of catenins, while Tight Junctions are the results of interactions of occludin, claudins and JAM-A connected to the actin cytoskeleton via zonula occludens proteins (ZO-1, ZO-2) and α-catenin[11]. The phosphorylation of myosin and contraction of the actin-myosin complex regulates the strength of such connections, and therefore the permeability of the epithelial barrier[12]. Impairment of intestinal permeability brings to the transit of endoluminal molecules in deeper layers, resulting in the activation of the adaptive immune response and leading to the inflammatory state. Such mechanism is observed mainly in infectious enterocolitis and IBD. In the first condition, entero-hemorrhagic *E. coli* (EIIEC) and entero-pathogenic *E. coli* (EPEC) have the ability to adhere to the intestinal epithelial cells and to break the integrity of the barrier through the alteration of tight junctions[13]. In IBD, a dysfunction of the AJ protein due to a reduction of E-cadherin has been described; this alteration causes the weakening of intercellular connection and the triggering of the inflammatory response[14]. High concentrations of IFN-gamma and TNF-alpha, typical of UC and CD, can modulate the expression of several proteins of the tight junctions as the ZO-1, the JAM-A, occludin, the claudin-1 and claudin-4[15]. Even in this case, such a phenomenon causes an increase of intestinal permeability and the consequent development of disease.

Immune System

A complex network of immune cells, known as "gut associated lymphoid tissue" or GALT, represents a functional deep barrier that protect us from external injuries. GALT consists of both isolated and aggregated lymphoid follicles, and contains up to 70% of the immune cells of the whole human body[16]. It drives immunological response to pathogenic microorganisms, bringing to immune tolerance to commensal bacteria, by its physiological relationship with the outer layer and the contact with external environment through specific immune cells, as the dendritic cells and the M-cells within the Peyer's patches. Such cells have the ability to acquire microorganisms and macromolecules and to present antigens to T lymphocytes, which produce cytokines that activate the immune response[17].

Integrity of such structures is required for the maintenance of the physiologic gut homeostasis. The disruption of such equilibrium leads to the transit of luminal contents towards the underlying tissues and thus in the bloodstream, with a consequent activation of the immune response and the induction of a state of inflammation. This mechanism underlies the pathogenesis of many gastroenterological diseases, including infective enterocolitis, inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), small intestinal bacterial overgrowth (SIBO), celiac disease, liver fibrosis, acute pancreatitis food intolerances and even atopic diseases[18,19].

Gut Barrier and Pediatric Diseases

Disruption of the gut barrier has been associated with many gastrointestinal diseases, but also with extra-intestinal pathological condition, such as type 1 diabetes mellitus, allergic diseases or autism spectrum disorders, as is shown in Table I[20].

Gastrointestinal Diseases

Coeliac Disease

The foretype of diseases associated with gut barrier impairment is Coeliac Disease (CD): in celiac subjects, the ingestion of gliadin fraction of wheat gluten and similar alcohol-soluble proteins (prolamines) of barley and rye triggers an immune reaction in genetically susceptible subjects, leading to small bowel inflammation and flattening of duodenal villous pattern, and, consequently, to malabsorption of micro- and macro-nutrients[21].

Indeed, in the early development of CD, tight junction are opened, and gliadin reaches submucosa trough breeches of mucosal epithelium, eliciting the immune adaptive response and consequent chronic inflammation[22]. A disfunction of the angiotensin receptors of the duodenum and jejunum has been suggested to play a main role in the pathogenesis of sprue-like olmesartan enteropathy, a recently discovered malabsorption condition similar to CD[23].

Table I. Gut barrier alterations in gastrointestinal and extraintestinal diseases.

Disease	Gut Barrier alterations
Gastrointestinal Diseases	
Celiac Disease	Opening of the tight junctions (TJ); gliadin in the submucosa eliciting the immune adaptive response
Inflammatory Bowel Diseases	Alterations in sensing the and reacting to gut microbiota; deficiency of antimicrobial peptides resulting in alterations of gut microbiota; deficient or defective mucins
Infectious diarrhea	Alterations of occludin distribution from TJ into the cytosol (Enteropathogeenic Escherichia coli EPEC); re-arrangement of actin and dissociation of occludin, ZO-1, and ZO-2 from the lateral TJ membrane (Clostridium difficile toxins); replacement of infected mature enterocytes by cryptic-derived immature enterocytes (Rotavirus)
Post-infectious Irritable Bowel Syndrome	Long-lasting inflammatory response with an increase in IL-1β mRNA expression, in the number and activation of mast-cells and in the density of nerve fibres closely attached to mast cells
Extraintestinal Diseases	
Type 1 diabetes	Alterations in microbial composition.
Atopic and allergic diseaes	Alteration of TJ permeability; increased intraluminal antigens absorption triggering allergic responses.
Autism spectrum disorders	Impairment of gut microbiota composition; alterations of intestinal permeability and "leaky gut hypothesis"

Inflammatory Bowel Disease

Inflammatory Bowel Disease (IBD) are a complex of almost three condition (Crohn's disease, ulcerative colitis, undetermined colitis) characterized by a chronic inflammation of the gastrointestinal tract resulting from the interaction of environmental (particularly intestinal microbiota) and genetic factors[24]. In IBD, the concentration of intestinal bacteria adhering to enterocytes is higher than in healthy controls and increases progressively with the severity of the disease. In addition, there is a reduction of the bacterial diversity with an increase of the Enterobacteriaceae (including *E. coli*), and a marked decrease in number of Bacteroidetes and Clostridia. Such imbalance condition leads to a reduction in the growth and maturation of enterocytes, with a consequent increase of intestinal permeability[25].

IBD patients may exhibit an excessive response to some components of the gut microbiota, predominantly because of a weakness of mucosal barrier. NOD2, the first susceptibility gene for Crohn's disease, encodes for an intracellular receptor, important in sensing the microbiota and in the regulation of the release of antimicrobial peptides (AMPs) by Paneth cells (PC). Deficiency of antimicrobial peptide production results in a qualitative and quantitative alterations of gut microbiota that can trigger immune adaptive response and intestinal inflammation[26]. In patients with ileal Crohn's disease there is a reduction in the expression of PC alpha-defensins, but no change or an increase in the expression of other PC products than healthy controls. This modification leads to a reduced total antimicrobial activity against *E. coli* and S. aureus corresponding to the reduction in human defensin 5 (HD5) expression, as shown in ileal mucosal samples[27]. This specific decrease in HD-5 has been more recently observed also in pediatric CD patients[28].

In mice with a reduced expression of HD5, moreover, the observed modification of gut microbiota from small Bacilli and Cocci to predominant fusiform bacterial species, suggests that HD5 expression can modulate in a dose-dependent manner the composition of the intestinal microbiota, whose alterations may promote bacterial invasion of the mucosa and predispose to chronic inflammation[27].

A role for antimicrobial peptides has been presumed also in ulcerative colitis: since the defensin response seems to be apparently adequate, the barrier dysfunction may result from deficient or defective mucins, thus affecting their role in binding defensins and other AMPs to their proper site of action[29].

Infectious Diarrhea and Post-infectious Irritable Bowel Syndrome

Intestinal permeability, measured with [51Cr]EDTA testing, is significantly higher in patients with infectious diarrhea associated with invasive pathogens or intestinal inflammation in comparison with normal controls[30], thus demonstrating that infectious gastroenteritis impairs the intestinal barrier. Enteric pathogens can alter the equilibrium of gut barrier in different ways. Many virulence factors can cause the disruption of Tight Junctions of epithelial cells, altering occludin distribution from TJ into the cytosol (Enteropathogenic *Escherichia coli* – EPEC) or through a re-arrangement of actin and dissociation of occludin, ZO-1, and ZO-2 from the lateral TJ membrane (Clostridium difficile infection)[31].

In rotavirus infection, the main mechanism underlying diarrhea is the malabsorption of nutrients induced by replacement of infected mature enterocytes by cryptic-derived immature enterocytes, with reduced membrane enzymatic activities. Recently, other pathogenic mechanism have been identified. A non-structural protein (NSP4), produced by infected enterocytes, causes a rearrangement of actin and intercellular junctions, as well as gut barrier dysfunction[32].

Post-infectious irritable bowel syndrome (PI-IBS) is a common disorder wherein symptoms of IBS begin after an episode of acute gastroenteritis. Published studies have reported incidence of PI-IBS to range between 5% and 32%. The mechanisms underlying the development of PI-IBS are not fully understood, but are believed to include persistent sub-clinical inflammation, changes in intestinal permeability and alteration of gut flora[33]. The pathogenesis of PI-IBS lays in the disruption of barrier function by an enteric pathogen, which triggers an enhanced and long-lasting inflammatory response. Wang et al. noticed an increase in IL-1β mRNA expression, in the number and activation of mast-cells and in the density of nervous fibers closely attached to mast cells in the intestinal mucosa of patients with PI-IBS, thus reflecting an enhancement of the immune response to previous inflammation. IL-1 can inhibit intestinal transport of water, causing diarrhea, and is a potent hyperalgesic agent which may be responsible for PI-IBS symptoms[34]. The urinary Lactulose:Mannitol Excretion Ratio (the most common test for the assessment of gut permeability) remains elevated in individuals with post-infectious Irritable Bowel Syndrome 4 months to 4 years after their initial infection, as well as the number of inflammatory cells, particularly T lymphocytes and macrophages[35].

An increased incidence of Irritable Bowel Syndrome has been demonstrated also in a cohort of children exposed to *Escherichia coli* 0157:H7 and Campylobacter species as a result of a contamination of municipal water in Walkerton, Ontario[36].

Extraintestinal Diseases

Type 1 Diabetes

Type 1 diabetes (T1D) has been recently considered a consequence of unbalanced immune responses in genetically predisposed individuals. Similarly to other autoimmune diseases, the rapid increase in the incidence of T1D in developed countries points to the role of environmental factors in this disease, especially various microbial and food components encountered at mucosal surfaces as well as gut barrier function anomalies[37]. Multiple studies both in patients with T1D and animal models reveal a strong association with Gut mucosal alterations (probably due to gut microbiota imbalance) that often precede clinical signs of disease[38]. Furthermore, recently, a metagenomic analysis of gut microbiome has been performed in 16 T1D children, revealing significant differences than healthy ones[39].

Atopic and Allergic Diseases

A healthy development of the mucosal barrier is fundamental to avoid penetration of bacteria, toxins and antigens, which may result in a variety of pathological conditions. Selective IgA deficiency, pre-term delivery, intestinal infections and type of feeding during the neonatal period may influence intraluminal antigens absorption and result in the triggering of allergic responses[40]. Increase of intestinal permeability has indeed been demonstrated in preterm infants, since the intestinal barrier gradually develops with fetal maturation[41].

Several studies have established the association between abnormal intestinal permeability and food allergy, even if there is no evidence that impaired intestinal permeability represents the initial event triggering antigen sensitization. However, the alteration of gut permeability has a well-established role in perpetuating the antigen exposure of activated mast cells[42].

Pre-treatment with oral disodium cromoglycate has shown efficacy in reducing the changes of intestinal permeability, the antigen exposition as well as symptoms during food challenge in atopic subjects[43].

Food antigens are transported across the intestinal epithelium to the underlying mucosa, and the consequent stimulation of the immune system leads to the release of cytokines and inflammatory mediators, causing the up-regulation of both transport and processing of food proteins, as well as the alteration of Tight Junction permeability: such changes result in a vicious circle[44].

Autism Spectrum Disorders

Children with autism have often been reported to suffer from gastrointestinal diseases, that are more frequent and more severe than in children from the general population[45].

Such association has been hypothesized because of several evidences. First, a substantial number of children with autism spectrum disorders (ASD) complain of gastrointestinal symptoms[46]. Additionally, activation of the mucosal immune response and an impairment of gut microbiota composition[47] have been repeatedly observed in such population.

ASD children, indeed, show a higher level of Clostridium species than healthy controls[48]. Moreover, an altered intestinal permeability is more frequent among patients with autism (36.7%) and their relatives (21.2%) than normal subjects (4.8%), and in autistic patients on unrestricted diet compared with patients on a gluten-casein-free diet; alterations found in first-degree relatives suggest that an hereditary factor, maybe related to gut barrier structure and epithelial junctions, is involved in the pathogenesis of autism[49]. In a randomized controlled trial, a gluten-and casein-free diet showed efficacy in ameliorating autistic symptoms[50].

Such data may support the "leaky gut hypothesis", which suggests that digestion products of natural foods (such as cow's milk and bread) are able to enter the blood through the leaky gut barrier and to interfere directly with the central nervous system[51].

Pharmacological Modulation of Gut Barrier

Despite the clear evidence, the measurement of intestinal permeability is still far from becoming a laboratory parameter able to drive everyday clinical practice. However, many drugs or compounds used in the treatment of gastrointestinal disorders are able to modify the permeability of the intestinal barrier[52]. Some of the most commonly used substances in gastroenterology are steroids, aminosalicylates, biologics (especially anti-TNF-alpha), probiotics and mucosal protectors (such as proton pump inhibitors). While corticosteroids, salicylates and anti-TNF-alpha act in the modulation of intestinal permeability by reducing intestinal inflammation, probiotics appear to act through packaging the production of mucins and strengthening connection of the epithelial junctions[53]. Corticosteroids are the mainstay therapy for the induction of clinical remission in moderate to severe Crohn's disease[54]. Among them, the prednisone has proved able to reduce intestinal permeability in 50% of patients based on the lactulose/mannitol ratio. Even therapy with prednisolone is able to reduce the permeability in children and adolescents with active Crohn's disease and ulcerative colitis. This effect is due to the anti-inflammatory properties of corticosteroids, including the ability to inhibit the expression of pro-inflammatory cytokines such as TNF-alpha and NF-kB, although high concentrations of steroids (prednisolone and budesonide) are related to a reduction of mucosal healing[55].

The use of derivatives of 5-aminosalicylic acid (5-ASA) is one of the main treatments in the therapy of mild to moderate uncomplicated IBD. These substances, first of all mesalazine, promote the rapid recovery of mucosal integrity, through signal cascades mediated TGF, thereby reducing the intestinal permeability[56].

Biological drugs, including anti-TNF-alpha (infliximab and adalimumab), have been recently introduced in the treatment of IBD; they act by blocking the action of TNF, reducing inflammation and renovating mucosal integrity[57]. ^{51}CrEDTA testing has shown a significant reduction in intestinal mucosal permeability after treatment with infliximab[58]; moreover, in such experience the reduction of intestinal permeability was proportional to the activity of the disease and to the mucosal healing.

Probiotics are defined as live microorganisms that confer health benefits on the host when administered in adequate amounts[59]. Probiotics are frequently used in clinical practice, and have recently been tested and exploited for the treatment of IBD, SIBO and infectious diarrhea[60]. The most diffused probiotic species are *Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium*

infantis, Bacillus clausii, Lactobacillus acidophilus, Lactobacillus GG, *Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus bulgaricus, Streptococcus thermophiles* in VSL#3, *Escherichia coli* (*E. coli*) Nissle 1917 and yeasts like *Saccharomyces boulardii*. All of them have shown beneficial effects on several gastrointestinal diseases[61].

Many studies have highlighted the role of probiotics in the modulation and reduction of intestinal permeability, considering the strong influence of gut microbiota in the modulation of the function and structure of gut barrier, but also on the immune response of the host.

First of all, probiotics may have a role against pathogenic bacteria, by creating a restrictive luminal environment (as for example lowering luminal pH), inhibiting bacterial adherence and translocation, producing antibacterial substances (bacteriocins) or inducing the expression of human defensins[62]. Probiotics have also a role in the regulation of the function of gut barrier, by altering mucus or chloride secretion but also directly preventing the rearrangement of tight junction proteins after exposure to pathogenic bacteria[63], or restoring a disrupted epithelial barrier[64].

Probiotics can also attenuate proinflammatory responses induced by pathogenic bacteria, enhance epithelial cell regeneration or prevent cellular apoptosis[65].

Moreover, probiotics modulate immune response to potentially harmful antigens, directly affecting the lymphocytes or indirectly interfering in the function of antigen-presenting cells or macrophages of the gut[62], thus maintaining the correct equilibrium between necessary and excessive defense mechanisms.

Conclusions

More than a simple anatomical structure, the gut barrier represents a functional unit which plays a key role in maintenance of intestinal homeostasis and prevention of several gastrointestinal and extraintestinal diseases. Furthermore, the gut barrier concept should be considered as a dynamic idea, since some components, such as gut microbiota, evolve and change, together with our body, during our growth. At birth, our gut is sterile, and is rapidly colonized by the maternal microbiota; both route of delivery (vaginal versus caesarean delivery) and feeding (breastfeeding versus formula feeding) may influence early composition of gut microbiota[66]. Genetic as well as environmental factors contribute to the further development of gut microbiota composition[67]. Disruption of gut microbiota and/or of other components of the gut barrier may lead to dysbiosis and consequently to the development of several gastrointestinal and extraintestinal diseases. The maintenance of a healthy intestinal barrier is therefore of paramount importance in children, for both health and economic reasons. To date, available weapons for the maintenance and repair of gut barrier are few, even if promising. Considerable efforts, including both a better understanding of the gut barrier features and mechanisms in health and disease, and the development of new pharmacological approaches for the modulation of gut barrier components, are needed for the prevention and treatment of gastrointestinal and extraintestinal diseases associated with gut barrier impairment.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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