From gut microflora imbalance to mycobacteria infection: is there a relationship with chronic intestinal inflammatory diseases?


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The gut of a healthy adult harbours a myriad of different microbial species. It is estimated that approximately $10^{14}$ are present in total bacterial colony forming units (CFU). Each colony colonizes a specific intestinal tract. In healthy adults, the main control of intestinal bacterial colonization occurs through gastric acidity but also other factors can influence the intestinal microenvironment such as pH, temperature, competition among different bacterial strains, peristalsis, drugs, radiotherapy and much more.

Impaired microbial homeostasis leads to an alteration of the permeability of tissue, together with the activation of the intestinal immune system MALT (mucosal associated lymphoid tissue). In this regard we discuss the increasing experimental evidences of the role of commensal microbiota in the activation of specific intestinal immunocompetent cells. The aforementioned micro-environmental changes provide the substrate for the etiopathogenetic outbreak of numerous pathologies of gastro-intestinal tract, such as intestinal chronic inflammation (Crohn’s disease and Ulcerative Colitis), together with a miscellany of extra intestinal disorders.

This article is an overview of the latest scientific findings about the close causal relationship between intestinal microfloral flora and inflammatory bowel diseases or other extra-intestinal diseases; it is also mentioned the possible relationship between mycobacteria and Chron’s disease. Finally we analyse the beneficial role of probiotics.

KEY WORDS: Chronic intestinal diseases, Intestinal microflora Imbalance, Intestinal immune system, Mycobacteria, Probiotics.

Introduction

Ulcerative colitis (UC) and Crohn’s disease (CD) are two major healthcare problems of the digestive tract, commonly known as inflammatory bowel diseases (IBD), characterized by chronic and spontaneous inflammation due to a complex interaction of genetic, microbial, and environmental factors, which results in continuous activation of the mucosal immune system 1. Increasing evidences indicate that changes in gut microbiota, with an increase of pathogenic bacteria and a decrease of health-promoting symbionts, play an important role in promoting and maintaining intestinal inflammation in IBD 2. In this regard it has also been underlined the ability of specific components of the gut microbiota to activate intestinal immunocompetent cells 3,4.

The other side of the coin is represented by probiotics.
Intestinal microbiota and related diseases

ALTERATION OF COMMENSAL MICROBIOTA COMPOSITION AND “GAS-RELATED SYNDROME”

The human gut harbours about 100 trillion bacteria and more than 500 different species are present in the colon, so global bacterial concentration can reach 9x10^{13} units. Until now, it remains a mystery how such a large number of bacteria can lodge and coexist in our intestine without causing damage to the host organism. Mucus seems to play an important role in protecting the intestinal mucosa. It is responsible for the integrity of the mucosa maintaining both a stable solution in the submucosal glands secretions (secretory IgA) and a healthy mucosal tissue tropism itself 7. Also the commensal intestinal flora exerts protective functions infact the complex symbiotic relationship between it and host leads benefits to both parties. This interaction, for example, is the basis of a regular modulation of numerous physiological functions throughout the digestive system 8.

In healthy individuals, the main control of bacterial colonization in the digestive tract occurs through the gastric acidity, because it can neutralize the transit of unwanted bacteria. Among other factors involved in controlling intestinal bacterial flora composition, we recall pH, temperature, interaction between different bacterial strains, peristalsis, secretion of digestive and pancreatic metabolic components expressed by the gut microflora. Endoluminal accumulation of toxic compounds can infact exert a mutagenic action on intestinal mucosa. We then understand that the maintenance of proper homeostasis of microbial saprophytic organisms is essential in order to avoid the onset of inflammatory intestinal diseases, including cancer and extra intestinal diseases15. Infact 1-2% of all colorectal cancers develop from a background of inflammatory bowel diseases such as Crohn’s disease and Ulcerative Colitis 16.

EXPERIMENTAL EVIDENCES FOR A ROLE OF COMMENSAL MICROBIOTA IN THE ACTIVATION OF INTESTINAL IMMUNOCOMPETENT CELLS

The composition of the microbiota has been suggested to influence susceptibility to IBD 17,18, which are mediated by both innate and adaptive arms of the host immune system 19. It is thus possible that distinct members of the commensal microbiota engage specific components of the immune system and in such a way participate in the regulation of intestinal immune homeostasis 20. This ability of specific intestinal microbiota has interesting clinical implications in the cases of SIBO (small intestine bacterial overgrowth) which is found in association to IBD 21 or during PPI (Proton Pump Inhibitor) therapy 22.
The emerging role of Paneth cells in regulating commensal flora composition.

Recently, another important factor has been recognized to be involved in microflora control: the activity of Paneth cells. These particular cellular elements of innate immunity are specialized ileal epithelial cells located at the base of small intestine mucosal invaginations, called crypts of Lieberkühn. Paneth cells regulate the intestinal microbiota composition via secretion of granule contents including antimicrobial peptides – α-defensins and secretory phospholipase A₂ (sPLA₂) – and lysozyme 35. A defect in the autophagy pathway of intestinal epithelium is responsible for the Paneth cell pathology 36. Autophagy is an evolutionarily conserved process, with several forms described to date 37. However, the most studied form is “mac autophagy” whereby cytoplasm and cytoplasmic organelles are encapsulated in double-membrane-bound vesicles (autophagosomes) and delivered to lysosomes, where they are degraded and their constituents recycled 37-40. This macroautophagic process is increased in response to cellular stress, such as starvation or growth factor withdrawal, for which the term “induced autophagy” has been suggested 41. Autophagy is thought to protect the cell by eliminating or limiting the growth of bacterial pathogens, a process termed “xenophagy”; therefore dysfunction of xenophagy might lead to persistent infection 42. Considering CD only, one susceptibility allele is in the predicted autophagy gene ATG16L1 43-46. IRGM and LRKK2 are two additional autophagy genes associated with an increased risk to develop CD 43,47,48. The mutant Paneth cells show defects in the exocytic pathway. They also have degenerating mitochondria and an abnormal endoplasmic reticulum, which may reflect the loss of organelle degradative capacity associated with the autophagy defect, since autophagy plays an important role in removing damaged or dysfunctional organelles. These defects correlate with the absence of lysozyme in the protective mucus layer of the ileum 36,49. In addition to its role in maintaining the granule exocytosis pathway, Arg16L1 is an important brake for the expression of pro-inflammatory genes in Paneth cells and the regulation of endotoxin-induced inflammasome activation 50. An increase in transcripts associated with Peroxisome Proliferator-Activated Receptor (PPAR) signaling, acute phase reactants, adipocytokine signaling and lipid metabolism is present when Arg16L1 is defective. Many of these genes are directly implicated in inflammation, and especially two of these transcripts, leptin and adiponectin, are known to be increased in CD patients 51,52. Saitoh and al.
demonstrated that Atg16L1-deficient macrophages stimulated with the Toll-like receptor 4 (TLR4) ligand lipopolysaccharide (LPS) gave as response an increased secretion of IL-1β, and IL-18 via TRIF (Toll/IL-1 receptor domain-containing adaptor inducing IFN-α)-dependent activation of caspase-1 and that, probably, autophagy is the main controller of inflammasome activation and it limits the production of the inflammatory cytokines IL-1, and IL-18 50. Increased IL-1β, may, among other effects, increase epithelial barrier permeability 53, possibly enhancing microbial product translocation. However, the increased production of cytokines may reflect failure of an autophagic stress response and, as postulated by Kuballa et al., the net effect of ATG16L1 coding variation should depend upon the balance between the high microbial load in the gut and the ability of autophagy to mediate defense against invading pathogens and internalized self and non-self antigens 54. Recent data support a "two-hit hypothesis" wherein host (or potentially environmentally)-mediated alterations in the intestinal microbiota may only induce dysregulated intestinal inflammation characteristic of CD (and IBD) when present together with a tendency to hyperrespond to microbial stimuli 55,56.

**Controversial etiopathogenetic role of Mycobacterium paratuberculosis in Crohn’s Disease**

It is generally accepted that Crohn’s disease (CD) results from deregulation of immune responses to luminal antigens in susceptible individuals, but the precise etiology of this inflammatory bowel disease is unknown 57. Several bacteria have been suggested to be involved in CD pathogenesis 58 including *Escherichia coli* and *Mycobacterium avium* subspecies paratuberculosis (MAP). Invasive *E. coli* have been found in higher frequencies in ileal tracts of CD patients 59. The data on the presence of MAP are not uniform, but two meta-analysis of several published studies have concluded that MAP is more often present in CD patients than patients with UC (Ulcerative Colitis) and non-inflammatory bowel disease (non-IBD) 60,61. Also another study described MAP detection using PCR techniques in patients with CD who underwent biopsy and patients with CD whose samples were obtained during surgical resection 62. It has been also postulated 63 that infection of adipocytes or endothelial cells by MAP, rather than epithelial intestinal cells, could determine the unique pathologic features of Crohn's disease. The abnormal proliferation of endothelial cells, for example, could cause a vessel obstruction increasing propensity to granuloma formation.

On the basis of the aforementioned studies we can then conclude that an association between MAP and CD have been derived, until now, from studies that employed molecular, serologic, and immunocytochemical techniques to determine the presence of MAP in patients with CD 60; however little is known about the ability of the bacterium to contribute to the inflammatory response during Crohn's disease.

A decade ago Duchman et al 65 showed that both CD and ulcerative colitis (UC) patients had T cells with reactivity to various commensal bacteria, including *E. coli*, however no differences were found between the two groups. To get information about the relative importance of various bacteria in the ability to elicit an inflammatory T cell response, Olsen et al 66 chose to characterize the specificity of intestinal T cells from CD patients. They isolated T cells from intestinal biopsies of CD, UC and non-IBD patients and detected responses to some tested bacteria. CD patients had a higher frequency of MAP reactive T cells than the UC patients and also a higher frequency of response to MAP compared to other bacterial antigens. Furthermore these T cells produced inflammatory cytokines like IFN-gamma and IL-17. These data suggest a possible role of mycobacteria in CD immunopathology.

In this regard Ren et al 67 found significant higher levels of interleukin IL-4 and IL-2 in MAP positive CD patients compared to MAP negative ones. In particular IL-4 secretion was correlated with IL-2 production in blood cultures in CD, consistent with a Th2 immune response. Also these data provide the evidence of altered T cell function linked to MAP infection in CD and stimulate a debate about the putative role of this bacterium in the onset of the inflammatory intestinal disease. In this regard two other studies showed, respectively, the ability of MPA to invade human small-intestinal goblet cells and elicit inflammation 68 and to cause early phase morphological lesion of bovine ileum 69. In particular Golan and coworkers 68 provided, for the first time, evidence of deleterious effect of MPA infection in a so-called “humanized-mouse model”, which is the only ethically acceptable experimental model in such a field of research, being the alternative the “infectious studies in children”. In particular they transplanted human fetal small intestine or colon at a gestational age 12–16 weeks subcutaneously onto the backs of SCID (severe combined immunodeficiency) mice and infected by an intraluminal inoculation of MPA bacteria. Then, 3 days after infection, mice were euthanized, and the grafts were removed for histologic and immunohistochemical analyses and for detection of inflammatory mediators. In particular they found an increase in tissue levels of IL-6, IL-1β, and TNFα which has also been reported in CD 70. Interesting findings, finally, regard the association between the risk of developing CD and polymorphisms in several genes that are involved in interaction with bacteria. In particular, NOD2 71, which is an intracellular sensor of bacteria, and ATG16L1 46 and IRGM 72, which are involved in autophagy, are believed genetic factors for CD. However currently it is unclear whether the CD associated variants of NOD2, ATG16L1 and IRGM influence the host response to particular bacteria or whether they have more general effects to a wide range of gut bacteria.
Towards a new era in the treatment of intestinal chronic diseases: the probiotics

The correct and thorough knowledge of the mechanisms of microbial homeostasis could be the prelude to unexpected therapeutic or prevention scenarios of many diseases. In this regard numerous clinical trials have already shown the preventive and therapeutic action of probiotics in the treatment of digestive tract diseases. For a long time the so-called “official medicine” ignored the therapeutic potential of probiotics preferring the use of intestinal disinfectants, antibiotics and anti-inflammatory drugs specific for the digestive tract. We know today that the indiscriminate use of broad spectrum antibiotics, immunosuppressive therapy and radiotherapy, eventually cause important changes in bacterial intestinal microflora, which often lead to a procession of symptoms particularly severe.

The term “probiotics” has been used for the first time in 1965 by Lilly and Stilwell. They reported the observation that certain substances contained in intestinal segments, if placed in vitro with organic tissue, stimulated their growth. Subsequently, further studies better defined the role of these substances, identifying them as commensal intestinal bacteria. Today we tend to identify with the term of probiotics microorganisms (usually bacteria) producing beneficial effects on the host. These are part of the normal intestinal microbial flora together with the commensal. This feature distinguish them from pathogenic bacteria, both exogenous (Salmonella, etc.) and residents (Bacilli, Clostridia, Klebsiella, Proteus, etc.). These aren’t part of the normal flora and, when present, they are usually about 0.02% of total. We know that administration of certain live bacteria can have beneficial effects thanks to the ability of restoring microflora intestinal balance. Today pharmaceutical industry, sensing the safe therapeutic potential of probiotics, has studied and marketed preparations of probiotics, has studied and marketed preparation of probiotics and systemic immunity in early life. Another evidence of beneficial effects of probiotics is provided by Schmidt and coworkers who showed a role of Rhamnosus GG in colon cancer. In particular this action was carried out lowering the levels of some enzymes as beta-glucosidase, beta-glucuronodase and urease which are considered carcinogenic factors in colorectal cancer.

Interaction between probiotics and intestinal immune system.

In regard of colonic diseases, it has been shown that Lactobacillus Rhamnosus GG also interacts with intestinal immune system (MALT). For example it is able to increase the number of IgA and other immunoglobulins secreted by the intestinal glands. In particular it modulates the antigen recognition by the intestinal lymphoid tissue at the level of Peyer's patches and it reduces the levels of proinflammatory cytokine TNF-α. Further studies showed a role of Rhamnosus also in improving the permeability of intestinal barrier. Recently another study has provided evidence, for the first time, of a new ability of a mixture of two probiotics (Lactobacillus acidophilus and Bifidobacterium longum) to induce the expansion of a subtype of intraepithelial lymphocytes, but not of lamina propria lymphocytes; interestingly administration of the aforementioned probiotics was able to prevent the onset of a chemically-induced colitis in mouse. Dong and colleagues also showed, in a murine experimental model, an unexpected role of intestinal bifidobacteria in promoting the maturation of dendritic cells and expression of IL-12 locally in the gut, in influencing the development of T cells in the thymus and in favoring the development of T-helper cell type 1 response. In addition, these bacteria enhanced antibodies synthesis by PBMCs (peripheral blood mononuclear cells), thereby affecting the development of both the gut and systemic immunity in early life. Another evidence of beneficial effects of probiotics is provided by Schmidt and coworkers who showed a role of Lactobacillus acidophilus NCFM, Bifidobacterium bifidum BI-98 and BI-504 were able to improve the gut-associated inflammation which usually occurs in IBD. In particular these microorganisms could enhance the suppressive effect of APC (Antigen Presenting Cells) on regulatory T cells (Treg cells).

Riassunto

Nell’intestino di un individuo sano esistono miliardi di batteri e la loro presenza è condizionata da una serie di fattori quali l’acidità gastrica, la temperatura, la competizione tra i vari ceppi, la peristalsi, l’uso di antibiotici o i trattamenti radioterapici.
Alterazioni della composizione della flora intestinale possono condurre all'attivazione del sistema immunitario intestinale e conseguentemente all'insorgenza di malattie infiammatorie croniche quali il morbo di Crohn e la colite ulcerativa.

In questo articolo, partendo da un'attenta analisi dei più recenti dati presenti in letteratura, sarà messo in evidenza lo stretto nesso causale tra la disbiosi e le malattie infiammatorie croniche intestinali ed extraintestinali; inoltre si farà cenno alle evidenze sperimentali riguardanti la possibile, ma dibattuta, relazione tra micobatteri e morbo di Crohn. Infine saranno sottolineate le potenzialità terapeutiche dei probiotici.

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