ABSTRACT: Various commensal enteric and pathogenic bacteria may be involved in the pathogenesis of inflammatory bowel diseases (IBDs), a chronic condition with a pathogenic background that involves both immunogenetic and environmental factors. IBDs comprising of Crohn’s disease, and ulcerative colitis, and paeditis are chronic inflammatory conditions, and known for causing disturbed homeostatic balance among the intestinal immune compartment, gut epithelium and microbiome. An increasing trend of IBDs in incidence, prevalence, and severity has been reported during recent years. Probiotic strains have been reported to manage the IBDs and related pathologies, and hence are current hot topics of research for their potential to manage metabolic diseases as well as various immunopathologies. However, the probiotics industry will need to undergo a transformation, with increased focus on stringent manufacturing guidelines and high-quality clinical trials. This article reviews the present state of art of role of probiotic bacteria in reducing inflammation and strengthening the host immune system with reference to the management of IBDs. We infer that t healthcare will move beyond its prevailing focus on human physiology, and embrace the superorganism as a paradigm to understand and ameliorate IBDs.

KEY WORDS: Gut Microbiome, Immunomodulation, Inflammatory bowel diseases, Probiotics.

INTRODUCTION

The mammalian gut contains 300–500 different bacterial species, and overall concentration of microbial species inhabiting the human gastrointestinal (GI) tract may reach $10^{11}$–$10^{12}$ cells per gram of luminal contents (Guarner and Malagelada, 2003). Under normal circumstances, majority of the GI symbionts are protective and some are neutral, but a few can turn pathogenic if gut physiology become abrupt (Sartor, 2004). This dynamic community of GI microbiome plays a critical role in maintaining intestinal health serving metabolic, digestive, trophic as well as protective functions (Guarner and Malagelada 2003). Probiotics when administered or ingested in specific quantities, exert beneficial physiologic and therapeutic health benefits besides their normal metabolic properties (Sartor 2004; Fioramonti et al., 2003). Examples of probiotic bacteria demonstrated to have beneficial effects include lactic acid bacteria (LAB), Bifidobacterium sp., Escherichia coli Nissle 1917, Streptococcus thermophiles and a non-pathogenic yeast, Saccharomyces boulardii etc. (Fioramonti et al., 2003; Kumar et al., 2009; 2010; 2011; 2012a, b, c; Nagpal et al., 2007; 2010; 2012a, b, c; Nagpal and Kaur, 2011). Potential mechanisms of probiotic action (Fig. 1) include competitive interactions, production of antimicrobial metabolites, detoxification of certain carcinogens reaching GI tract, influences on the epithelium, and immunomodulation (Table 1) (Sartor 2004, 2006).
Gut microbiome in health and gastric diseases

The association between gut microbiota composition and various aspects of host health, including physiological development, metabolism, and immunological responses, is well established (Amaral et al., 2008; Bauer et al., 2006; Nagalingam and Lynch, 2012). Clearly, early development of the mammalian system is highly dependent on microbial colonization. It has been found that specific GI bacterial species activate host immune responses to facilitate their own survival and exclusion of other competing microorganisms. For instance, Bacteriodetes thetaotaomicron induces host production of defensins, presumably to eliminate potential competing organisms (including pathogens) and reduce competition for available space; however, in doing so it also plays a key role in regulation of postnatal angiogenesis (Stappenbeck et al., 2002). On the other hand, certain pathogenic GI microbes such as Salmonella enteritica and Typhimurium induce host inflammation to facilitate their fitness. This species has recently been shown to proliferate under conditions of GI inflammation due to its ability to use the unusual terminal electron acceptor, tetrathionate, generated upon the interaction of reactive oxygen species and luminal thiosulfate (Winter et al., 2010). Ohkusa et al., (2009), has also been postulated that resident bacteria may cause colitis by compromising the colonic epithelial barrier.

IBDs is an emerging disease that affects 20 out of 100,000 genetically susceptible people in Europe and North America. Approximately 5–20% of the global human population is estimated to suffer from inflammatory bowel diseases (IBDs) (Drossman et al., 2002; Hillilä MT, Färkkilä, 2004), and the situation seems to worsen in future. The major clinical symptoms include abdominal discomfort or pain, diarrhea, constipation, bloating, and flatulence. As the conventional therapies for IBDs are considered to be only moderately effective, new approaches in treatment are being sought.

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Though the pathogenesis of IBDs remains unclear, available evidences suggest that altered gut motility, visceral...
hypersensitivity, and dysregulation of the brain–gut axis are some of the well studied mechanisms (Drossman et al., 2002). Clinical evidences suggest that an imbalanced gut microbiome and enteric bacteria-mediated mucosal inflammation may be associated with IBDs (Linskens et al., 2001; Chadwick et al., 2002). The chronic idiopathic IBDs such as Crohn’s disease (CD), ulcerative colitis (UC), and pouchitis may be caused by a hyper-responsive cell-mediated immune response to intestinal commensal bacteria or microbial aberrancies in susceptible individuals (Podolsky 2002; Sartor 2004). Therefore, it is rational to consider therapeutic approaches that correct the aberrancies in microbiota and eliminate the inflammation-inducing bacteria and adjuvants for the treatment of IBDs in conjunction with anti-inflammatory and immunosuppressant agents (Sartor 2004). Some probiotic strains applied either singly or in combination appeared to be effective in relieving some of the IBDs symptoms, such as constipation, flatulence, and borborygmi. However, the effect and efficacy varies widely between studies and between strains of probiotics.

The relation between a dysregulated bacterial ecosystem and mucosal inflammation in IBDs has been demonstrated in a variety of clinical and basic studies (Tamboli et al., 2004; Mahida and Rolfe 2004). The CD and UC are chronic aggressive disorders with a prevalence of 0.1–0.5%, and the both disorders have distinct features. The UC is characterized by inflammation with superficial ulcerations limited to the mucosa of the colon, and inflammation normally starts in the rectum and continuously spreads throughout the large intestine. Enteric microflora varies considerably between active IBD patients and healthy conditions, and the studies comparing enteric bacteria profiles in active and non-active IBD have shown a decrease in bifidobacteria and LAB in patients with active diseases, but normal in patients with remission (Giaffer et al., 1991). Even though no specific pathogen has been identified as the cause of IBDs, populations of some pathogenic and potentially harmful enteric bacteria are observed to be higher in patients suffering from IBDs (Seksik et al., 2003; Cummings et al., 2003). The pathogenic bacteria trigger intestinal inflammation by secreting enterotoxins that increase epithelial cell permeability (Robertson and Sandler 2001) producing immunosuppressive proteins that dysregulate the mucosal immune system, and may impair the epithelial cellular metabolism.

The distal intestinal epithelia depend on butyrate as the primary energy source (Singh et al., 2008; Kumar et al., 2012a, Macfarlane and Macfarlane, 2012. Interestingly, H$_2$S and sulphate-reducing bacterial counts are found to increase in patients with UC (Pitcher and Cummings 1996), and the block of butyrate metabolism by H$_2$S has detrimental effects on mucosal permeability and healing. In the intestinal lumen of IBD patients, balance between commensal and detrimental seems to be interrupted with secondary harm on immune system activities, though the changes in microbial composition may be transient with a limited applicability (Sartor, 2005; Dotan and Rachmilewitz 2005).

The rationale behind their potential effect is linked to the presumed dysregulation of immune responses to commensal bacteria in IBD patients. Furthermore, the risk of IBDs among first-degree relatives is known to be higher (Abraham and Cho, 2009). Environmental triggers, such as stress (Konturek et al., 2011), air pollution (Beamish et al., 2011), and smoking are considered to have an impact on the disease. This is evidenced by a recent report that patients with CD who smoke have a more complicated disease course (Leung et al., 2012). Evidences are there that stress may have a profound effect on bacterial gut microbes leading to increased adhesion and translocation of bacteria due to increased barrier permeability. Hence, the gut microbes that play a major role in overall health of host, will play altered role under stressful milieu. But the one area where research has greatly evolved is in the concept of gut immunity. The studies on interleukin-10 (IL-10) gene-deficient mouse have revealed the development of colitis when they were exposed to commensal bacteria, while no colitis was observed in germ-free mice (Kuhn et al., 1993). These findings support the role of an abnormal or dysregulated immune response to commensal bacteria in the pathogenesis of IBDs. In humans, the colon and the distal ileum are the regions containing very high microbial densities and, these are areas mostly affected in IBDs.

**TYPES OF GUT INFLAMMATORY DISEASES**

**Crohn's Disease**

CD is a common idiopathic IBDs of unknown etiology that affects the GI tract, and is believed to develop as a result of an aberrant immune response to intestinal microbes in a genetically susceptible host. The pathogenesis of CD is multifactorial, and several factors have been implicated in its development including genetic and environmental ones (Lakatos et al., 2006). In adult CD, most studies have failed to show any beneficial effect of probiotics both in induction or maintenance therapy. In terms of induction therapy, only two placebo-controlled studies are available (Malchow et al., 1997; Schultz et al., 2004). Both studies found no significant difference in the time needed to induce therapy in the two groups. As for studies in maintenance therapy, one small-randomized trial with *Saccharomyces boulardii* showed a significant reduction in the number of patients suffering from relapse in the combined mesalamine–probiotic group (Guslandi et al., 2000). However, these promising findings have not been confirmed by other trials, and there is no evidence to suggest that probiotics are beneficial for the maintenance of remission in CD (Rolfe et al., 2006). Gionchetti et al., (2003) performed a randomized trial to evaluate the efficacy of a combination of rifaximin and the probiotic preparation VSL#3 (Vivomixx in Europe and Visbiome in USA) in the prevention of postoperative recurrence of CD. Rifaximin (1.8g/day) for three months followed by VSL#3 (6g/day; 1.800 billion bacteria) for nine months was compared with mesalazine (4g/day) for 12 months in 40 patients after curative resection for CD.

**Ulcerative colitis**

Reports are available that highlight the protective role of probiotics in UC. Venturi et al. (1999) showed that VSL#3,
a special probiotic preparation containing $5 \times 10^{11}$/g of viable lyophilized bifidobacteria (B. infantis, B. longum and B. breve), four strains of lactobacilli (L. acidophilus, L. delbrueckii subsp. bulgaricus, L. plantarum and L. casei) and one strain of Streptococcus salivarius subsp. thermophilus significantly increased fecal populations of probiotic bacteria as long as patients were taking the above preparation. A randomized controlled trial demonstrated equivalence of non-pathogenic Escherichia coli to mesalazine for maintenance of remission in UC (Rembacken et al., 1999). In 2001, E. coli Nissle 1917 was shown in a multi-center, double-blind, mesalazine-controlled trial to be equivalent to mesalazine for maintenance of remission in UC. In this study, 327 patients with UC in remission were followed over a 12-month period. Both clinical and endoscopic estimates of disease severity were performed using the Crohn’s activity index and endoscopic activity index (Kruis et al., 2001). Relapse rates were 45.1% for the E. coli group and 36.4% for the mesalazine group by intention to treat analysis. Fedorak et al., (2003) studied 30 patients with mild to moderately active UC in a small, open-label trial of VSL#3 for efficacy of induction of remission or response. Clinical symptoms and sigmoidoscopic ratings were performed at baseline and at week 6, where 19 of 30 (63%) patients achieved remission, 7 of 19 (23%) achieved a response, and 4 of 19 (13%) had no response (Fedorak et al., 2003). An uncontrolled trial of S. boulardii was suggestive of benefit in maintaining clinical remission (using Rachmilewitz’s activity index) in patients with mild to moderate UC in combination with a stable dose of mesalazine 1 g daily. Kruis et al., (2012) have concluded that probiotic E. coli Nissle 1917 shows effects in IBDs, especially in patients with altered enteric microflora, e.g. after gastroenterocolitis or administration of antibiotics. In addition, high vegetable intake reduces the risk of UC, whereas increased fruit and/or dietary fiber intake appears protective against CD. Lacking or reduced availability of certain micronutrients, especially vitamin D, may increase the risk of both diseases (Gentschew and Ferguson, 2012).

Pouchitis

Proctocolectomy with ileal pouch-anal anastomosis may be required in some UC patients because their disease is medically intractable or they develop secondary dysplasia or cancer. Pouchitis or inflammation of the ileal reservoir created during the procedure may develop in 15 to 50% patients (Mac, 2011). Clinically, pouchitis is characterized by variable symptoms, including increased stool frequency and fluidity, abdominal cramping, pelvic discomfort, bleeding, tenesmus, fever and weight loss, and extra-intestinal manifestations in more severe cases (Madden et al., 1990). It is the most common complication of the surgery and although the exact etiology is not clear, host genetic factors, local pouch issues and the microbiota contained within the pouch are thought to be involved (Elahi et al., 2008; Pardi et al., 2009).

Trials for treating mild/ moderate pouchitis are few with small number of adult patient trials. For an unequivocal diagnosis, endoscopic examination and histologic investigation are mandatory (Shen et al., 2001). Pouchitis disease activity index (PDAI) is the most commonly used diagnostic instrument and represents an objective and reproducible scoring system for pouchitis (Sandborn et al., 1994). Active pouchitis is defined as a score $\geq 7$ and remission is defined as a score $< 7$. Studies have suggested that altering the microbiota in the pouch by administering probiotic bacteria can be effective in maintaining remission and reducing the incidence of flare-ups in chronic pouchitis (Gionchetti et al., 2000, Mimura et al., 2004). Moreover, the efficacy of probiotic therapy as prophylaxis to delay the first onset of pouchitis after pouch-surgery has been demonstrated (Gionchetti et al., 2003; Gosselink et al., 2004). Mimura et al. (2004) proved the efficacy of VSL#3 in maintaining remission in antibiotic-sensible pouchitis. Therefore, a strong body of evidence supports the use of probiotics as a therapeutic option in adult pouch. As information is currently scarce on this aspect in infants, studies are warranted to advocate role of probiotics in managing pouchitis in infants.

A molecular overview of IBDs- involvement of genes identified

The precise molecular mechanisms behind the development of IBDs are still not clearly understood. Genome-wide association studies are considerably improving our understanding of genetic susceptibility to IBDs (Budarf

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td><strong>Crohn's Disease</strong></td>
<td></td>
</tr>
<tr>
<td>CARD</td>
<td>NFκB activation and/or regulation, killing of intracellular pathogens, Paneth-cell function, (α-defensin production) (Hugot et al., 2001; Ogura et al., 2001)</td>
</tr>
<tr>
<td>SLC22A4 and SLC22A5</td>
<td>Organic cation, carnitine transporters, possibly transport xenobiotic substances (Peltikova et al., 2004; Torok et al., 2005)</td>
</tr>
<tr>
<td>DLG5</td>
<td>Epithelial scaffolding protein</td>
</tr>
<tr>
<td>PPARG</td>
<td>Intracellular scaffolding protein</td>
</tr>
<tr>
<td><strong>Ulcerative colitis</strong></td>
<td></td>
</tr>
<tr>
<td>MDR1</td>
<td>Efflux transporter for drugs and, possibly, xenobiotic compounds (Panwala, 1998; Maroo and Farrell, 2006)</td>
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et al., 2009). IBDs, including CD and UC, result from an exaggerated mucosal immune response to bacterial antigens in genetically susceptible individuals (Sartor et al., 2006; Xavier and Podolsky, 2007), and consequently there are many genes which are involved therein (Table 2).

Considering epidemiological, genetic and immunological data, it is concluded that the IBDs are heterogeneous disorders of multifactorial etiology in which hereditability and environment interact to produce the disease. It is probable that patients have a genetic predisposition for the development of the disease coupled with disturbances in immunoregulation (Tsianos et al., 2012). At the moment, several genes have been so far related to the diagnosis of CD. The first gene to be identified with CD was CARD15 (caspase recruitment domain family member 15, previously known as NOD2) (Hugot et al., 2001; Ogura et al., 2001). There are three mutations (causing amino-acid substitutions Arg702Trp and Gly908Arg and the frameshift 10076a) found within the region of CARD15 that encodes a leucine-rich repeat, which is responsible for bacterial recognition. At least one of these mutations is present in 25–35% of CD patients of European ancestry, but not in Asian or African American CD patients.

A number of studies have identified several other CD susceptibility genes such as SLC22A4 and SLC22A5 in the IBDs locus (Peltekova et al., 2004; Török et al., 2005). Two functional variants of the organic cation transporters OCTN1 and OCTN2 have been associated with CD in association with CARD15 mutations (Peltekova et al., 2004). Mutations in the transcribed region of SLC22A4, which encodes OCTN1, and the promoter region of SLC22A5, which encodes OCTN2, affect the transcription and function of these carnitine and organic cation transporters. These variants are most actively expressed in the intestinal epithelium, macrophages and T-cells, and cause decreased carnitine transport. Two haplotypes of DLG5, which encodes a scaffolding protein that helps to maintain epithelial integrity, have been associated with CD and combined UC and CD populations (Stoll et al., 2004). Like the OCTN1 and OCTN2 variants, the 113G>A substitution in DLG5 is associated with CARD15 mutations in patients with CD. P-glycoprotein 170 might also function as a ‘flipase’ that moves amphipathic substrates from the inner to the outer leaflet of the cell membrane. MDRI variants have been associated with UC and CD (Brant et al., 2003). MDRI is of particular interest, because it has been associated with treatment refractory IBDs (Maroo and Farrell, 2006), and because mice in which Mdrl has been deleted develop colitis (Panwala et al., 1998).

PPARG (peroxisome proliferative-activated receptor γ) variants have been linked with susceptibility in the SAMPI/YitFc mouse model of spontaneous chronic ileitis, and rare PPARG polymorphisms were found to be associated with human CD (Sugawara et al., 2005). PPARGγ is a nuclear receptor that inhibits NFκB activity: its expression is decreased in patients with active UC (Dubuquoy et al., 2003) and its expression is upregulated by 5-aminosalicylic acid (Rousseaux et al., 2005). In addition to a potential role in protecting against intestinal inflammation, treatment with the PPARγ ligand rosiglitazone was effective in an open-label trial involving UC patients (Lewis et al., 2001) as well as in mouse experimental colitis.

Large-scale genome-wide association studies have become technically feasible given the introduction of high-throughput sequencing, and technologies and development of gene chip-based technologies, development of software to manage data leading to the detection of several additional CD susceptibility genes including IL23R (Duerr et al., 2006; Glas et al., 2007), ATG16L1 (Hampe et al., 2007; Glas et al., 2007), NELL1 (Franke et al., 2007) and IRGM (Parke et al., 2007) as well as an intergenic region on 5p13.1 that modulates PTGER4 expression (Libioulle et al., 2007). In addition, there are a large number of genes modifying the CD phenotype such as variants in the genes of the TLR-4 (Brand et al., 2005), the fractalkine receptor (CX3CR1) (Brand et al., 2006), the macrophage migration inhibitory factor (MIF) (Dambacher et al., 2007) and C-reactive protein (CRP) (Thalmaier et al., 2006). While most of these genes modify only susceptibility and phenotype of CD, the IL23R gene has been shown to also be associated with UC, although this association is far less pronounced than that with CD (Duerr et al., 2006; Glas et al., 2007).

ROLE OF CYTOKINES IN IBDs

Cytokines and chemokines are small (4–15 kDa), inducible, pro-inflammatory and immune-regulatory proteins that play a central role in the development and homeostasis of immune system. Both CD and UC patients have activated innate (macrophage, neutrophil) and acquired (T and B cell) immune responses and are more susceptible to enteric pathogens (Mow et al., 2004). Tolerance in normal hosts is mediated by regulatory T cells, B lymphocytes, natural killer T cells and dendritic cells that secrete transforming growth factor (TGF)-β, interleukin (IL)-10, interferon (IFN)-α/β and prostaglandin J2. Antibody-neutralization studies have implicated tumor necrosis factor (TNF) and IL-12 p40 in the pathogenesis of CD (Targan et al., 1997; Mannon et al., 2004); while T-cells have been linked to UC by the effectiveness of T-cell-ablative therapies (Sawada K et al., 2005), cyclosporine and tacrolimus (Lichtiger et al., 1994).

A network of cytokines has been extensively investigated in IBDs, and an imbalance of pro-inflammatory and counter-regulatory molecules has been demonstrated in both CD and UC tissues. The pro-inflammatory cytokines IL-1β, IL-6, IL-8, IL-18, IFN-γ and TNF-α are produced in excess (Fiocchi, 1998) while the immuno-suppressive IL-10 is reduced and the inhibitory activity of TGF-β1 signaling is paradoxically inefficient. Both the excess of proinflammatory cytokines and the relative inefficiency of counter-regulatory molecules are required for maintaining, amplifying and perpetuating chronic inflammation in IBDs (MacDonald and Monteolive, 2005). Different secretory patterns and pathways have been identified.
within the cytokines network in IBDs. A general consensus has been reached for a predominant role of T-helper1 (Th1) cytokines in CD.

Evidence also indicates that in the gut mucosa the IL-12-induced Th1 polarization can be expanded by other cytokines, such as IL-15 and IL-18. IL-21, a newly T-cell-derived cytokine of the IL-2 family, has also been found to associate with IBDs. The expression of this molecule is enhanced in IBDs, in direct correlation with disease activity, and a functional evaluation suggests that IL-21 contributes to stabilize the Th1 phenotype at the site of inflammation (Monteleone et al., 2005).

Mucosal immune response in UC differs from that occurring in CD. Several observations indicate a predominant humoral immunity characterized by a high level of IgG released by mucosal plasma cells. T-cells isolated from colonic mucosa of UC patients were found to release high IL-5 and IFN-γ while IL-4 is relatively low. The Th1 cytokine profile, which includes IFN-γ and IL-12 p40, is dominant in patients with CD. Traditional Th1 responses are mediated by IFN-γ, the production of which is stimulated by IL-12, produced by antigen-presenting cells (APCs). Most experimental colitis models also have a predominant Th1 response (Sartor 2004), although in several models, Th1 responses can change into Th2 (type-2 T-helper lymphocyte) responses as the inflammatory process matures (Spencer et al., 2002; Bamias et al., 2005); whereas in UC, the T-cell profile is not properly understood.

In Innate immune responses, macrophages and dendritic cells in the lamina propria are increased in number and have an activated phenotype in both forms of IBDs, but have been studied in greater detail in CD. Production of proinflammatory cytokines and chemokines is enhanced in IBDs (Table 3), and expression of adhesion molecules and co-stimulatory molecules is increased (Sartor and Hoentjen 2005). Cells involved in innate immune responses are activated and the expression of most proinflammatory cytokines and chemokines is upregulated in both CD and UC. Th1 and Th1-related cytokines involved in innate immunity (e.g. IL-12, IL-23 and IL-27) are, however, selectively activated in CD.

GUT MICROBIAL METABOLOME IN GUT DISEASES

The colonic microbiome plays an important role in digestive physiology and makes a significant contribution to homeostasis in the large bowel (Macfarlane and Macfarlane, 2012). The therapeutically important end products of microbial fermentation of undigested hydrocarbons reaching the colon include short chain fatty acids (SCFAs), principally acetate, propionate and butyrate and gases such as carbon dioxide, hydrogen and methane (Samuel et al., 2008). Mounting evidence suggests an important role for the intestinal microbiota in the chronic mucosal inflammation that occurs in IBDs, and novel molecular approaches have further identified a dysbiosis in these patients. Several mechanisms of action of probiotic products that may interfere with possible etiological factors in IBDs have been postulated (Jonkers et al., 2012; Siciliano and Mazzeo, 2012). Probiotics and their metabolic products have been proposed as food supplements for a healthier intestinal homeostasis, and also as therapeutic aids in IBDs with, however, very little clinical benefit. This may be due to the lack of reliable preclinical models for testing the efficacy of different strains (Tsilingiri et al., 2012). The mechanisms by which intestinal bacteria achieve their associated health benefits can be complex and multi-faceted. The gut symbionts participate in the barrier effect by developing antimicrobial activity.

### TABLE 3. Cytokines associated with IBDs.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Crohn’s Disease</th>
<th>Ulcerative colitis</th>
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<tr>
<td><strong>Non-specific immune responses</strong></td>
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<tr>
<td>IL-1β</td>
<td>Elevate; Source macrophages</td>
<td>Elevate; Source macrophages</td>
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<tr>
<td>TNF</td>
<td>Elevate; Source macrophages</td>
<td>Elevate; Source macrophages</td>
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<tr>
<td>IL-6</td>
<td>Elevate; Source macrophages</td>
<td>Elevate; Source macrophages</td>
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<tr>
<td>IL-8</td>
<td>Elevate; Source macrophages</td>
<td>Elevate; Source Epithelial cells</td>
</tr>
<tr>
<td>IL-12</td>
<td>Elevate; Source macrophages</td>
<td>Normal; Source Macrophages</td>
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<tr>
<td>IL-18</td>
<td>Elevate; Source macrophages</td>
<td>Elevate; Source APCs</td>
</tr>
<tr>
<td>IL-23</td>
<td>Elevate; Source macrophages and T Helper cell involved in innate immunity</td>
<td>Normal; Source T Helper cell involved in innate immunity</td>
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<tr>
<td>IL-27</td>
<td>Elevate; Source T Helper cell involved in innate immunity</td>
<td>Normal; Source T Helper cell involved in innate immunity</td>
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<tr>
<td><strong>T-cell responses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Elevate; antigen-presenting cells (APCs)</td>
<td>Normal; antigen-presenting cells (APCs)</td>
</tr>
<tr>
<td>IL-5</td>
<td>Normal; CD4⁺ Lymphocytes</td>
<td>Elevate; CD4⁺ Lymphocytes</td>
</tr>
<tr>
<td>IL-13</td>
<td>Normal; NK T cells</td>
<td>Elevate; NK T cells</td>
</tr>
<tr>
<td>IL-17</td>
<td>Elevate; CD4⁺ Lymphocytes</td>
<td>Normal; CD4⁺ Lymphocytes</td>
</tr>
<tr>
<td>IL-21</td>
<td>Elevate</td>
<td>Normal</td>
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against enterovirulent bacteria and the antimicrobial activity is related to a number of bioactive biomolecules including antimicrobial peptides (Hassan et al., 2012; Nagpal et al., 2012; Siciliano and Mazzeo, 2012) organic acids, hydrogen peroxide, diacetyl and bacteriocins (Singh et al., 2008; O’Shea et al., 2011, 2012).

Vero-cytotoxin produced by *E. coli* O157:H7 is associated with hemorrhagic colitis and hemolytic uremic syndrome in humans. Kim et al. (2001) showed that some substance in the culture supernatant of *Bifidobacterium longum* HY8001 could neutralize the virulence of vero-cytotoxin successfully. In this study, control mice were inoculated intragastrically with *E. coli* O157:H7, and *B. longum* HY8001 culture supernatant were inoculated intragastrically to mice simultaneously. The TNFα and IL-1 levels in sera were decreased in mice treated with *B. longum* culture supernatant compared with those control mice. The concomitant experiment 

Flynn et al. (2002). Inhibition of *Salmonella typhimurium* for cell association and cell invasion was investigated using Caco-2 cells in a study 

Flynn et al., 2002). The induction of hBD-2 may contribute to an increased mucosal barrier to the luminal bacteria, or affect adhesion of pathogens to intestinal epithelial cells (Schierack et al., 2011). Nevertheless, polarized administration of bacteria is critical to control the ensuing immune response as it mimics the physiological entrance of bacteria. Further well designed studies based on intention-to-treat analyses by several independent research groups are still warranted to support the promising results for *E. coli* Nissle in inactive UC and the multispecies product VSL#3 in active UC and inactive pouch patients. So far, no evidence is available to support the use of probiotics in CD (Jonkers et al., 2012).

**PROBIOTICS IN MANAGING IBDs**

The gut microflora is a community that has co-evolved with the host and confers beneficial effects, including nutrient metabolism, immunomodulation and defenses against invading pathogens. However, dysregulation of normal co-evolved homeostatic relationships between gut bacteria and host immune responses can lead to intestinal inflammation, suggesting that luminal flora is a requisite, or perhaps a central factor, in the development of IBDs (Khan et al., 2012). There is abundant evidence that commensal bacteria are involved in the pathogenesis of human IBDs and in experimental colitis (Sartor 2004). The most convincing mechanistic studies have been done in gnotobiotic animal models. An altered balance of beneficial versus spoilage and pathogenic microbial species could lead to a pro-inflammatory luminal milieu that drives chronic intestinal inflammation. Numerous studies have implicated several commensal organisms, such as *E. coli*, *Bacteroides*, *Enterococcus* and *Klebsiella* species, in the pathogenesis of experimental intestinal inflammation and human IBDs (Sartor 2004). By contrast, various *Lactobacillus* and *Bifidobacterium* species have predominantly protective effects and have been used therapeutically as probiotics (Sartor 2004; Kumar et al., 2009; 2010; 2011; 2012a, b, c; Nagpal et al., 2007; 2010; 2012a, b, c; Nagpal and Kaur, 2011; Foye et al., 2012) (Table 3). Several groups have documented alterations in luminal or adherent microbial commensal flora in patients with CD, UC and pouchitis (Swidsinski et al., 2002; Sartor 2004).

An alternative mean of changing the microenvironment in such a way as to stimulate aggressive immune responses is the acquisition of virulence factors by commensal bacteria. As discussed above, enteroadherent and invasive *E. coli* have been found in the neoterminal ileum of patients with post-operative recurrence of CD (Darfeuille-Michaux, 2002). Flagellin from *Clostridium* (sub-phylum cluster XIVa) has been shown to be a dominant antigen in experimental colitis, and to elicit serologic responses in approximately 50% of CD patients (Lodes et al., 2004). The presence of superoxide dismutase activity alters the pathogenicity of *E. faecalis*. Dietary components can alter the composition of microbiota and virulence of enteric commensal bacteria, providing one potential explanation for the marked increase in the incidence of IBDs in Western countries during the second half of the twentieth century, and more recently in Eastern countries, as they adopt Western dietary practices.

It is evident that short-chain fatty acids (SCFAs), predominantly acetate, propionate, and butyrate, are the principal metabolites generated during the catabolism of dietary carbohydrates and proteins. In contrast, protein digestion yields a greater diversity of end products, including SCFAs, amines, phenols, indoles, thiols, *CO*₂, *H*₂, and *H*₂*S*, many of which have toxic properties. The majority of SCFAs are absorbed from the gut and metabolized in various
body tissues, making a relatively small but significant contribution to the body's daily energy requirements (MacFerlane and MacFerlane, 2012). Prebiotics, such as non-absorbed carbohydrates promote the growth of probiotic bifidobacteria and lactobacilli, and provide a substrate for the production of SCFA during gut fermentation. Probiotic *L. acidophilus*, prebiotic inulin or symbiotic may promote host protective immunity and attenuate Cr-induced intestinal inflammation through mechanisms affecting NF-κB and Smad 7 signaling (Foye et al., 2012).

Dynamic community of intestinal microflora plays a critical role in maintaining intestinal health serving metabolic, digestive, trophic as well as protective functions (Guarner et al., 2003). Increased numbers of adherent invasive *Escherichia coli* (AIEC) have been found in CD patients. Probiotics are viable microorganisms with beneficial physiologic and therapeutic properties that when ingested in specific numbers exert health benefits beyond those of basic nutrition (Huebner et al., 2011; Kumar et al., 2009; 2010; 2011; 2012a, b, c; Nagpal et al., 2007; 2010; 2012a, b, c; Nagpal and Kaur, 2011). Examples of bacteria demonstrated to have beneficial effects include *Lactic acid bacteria, Lactobacillus, Bifidobacterium, E. coli Nissle 1917, Streptococcus salivarius* subsp. *therophilus* and a non-pathogenic yeast *Saccharomyces boulardii* (Fioramonti et al., 2003; Nagpal et al, 2007; 2012). Levels of commensal bacteria appear to be altered in IBDs patients with increased numbers of bacteroides, adherent/invasive *E. coli, enterococci* and decreased *Bifidobacterium* and *Lactobacillus* species (Neut et al., 2002).

**MODULATING IMMUNE RESPONSE OF INTESTINAL EPITHELIA AND MUCOSAL IMMUNE CELLS**

Intestinal macrophages and dendritic cells act in a synergistic fashion with intestinal epithelial cells and microbiota to initiate the triad that governs the intestinal immune responses (whether inflammatory or regulatory) (Khan et al., 2012). The adaptive immune system allows individual organisms to mount defensive reactions against unanticipated pathogens by developmentally creating a diverse repertoire of clonally distributed receptors capable of recognizing a multitude of antigens and then expanding as effector cell populations comparable those that can recognize signals from the invading or endogenous pathogens (Schwartz, 2005). Probiotic treatment can down-regulate the expression of proinflammatory cytokines such as TNFα, IL1β, and IFN-γ, inducible nitric oxide synthase, and matrix metalloproteinase activity in inflamed mucosa of active IBDs or experimental colitis (Tables 1 and 4) (Dieleman et al., 2003; Furrie et al., 2005; Mileti et al., 2009). Lavasani et al., (2010) have shown that therapeutic effect of probiotic lactobacilli is associated with induction of transferable tolerogenic Tregs in MLNs, but in the periphery and the central nervous system, mediated through an IL-10-dependent mechanism. These findings indicate a therapeutic potential of orally administered probiotics, and provide a platform to understand host-commensal interactions that contribute to beneficial effects in autoimmune diseases. In inflammatory conditions of IBDs, immune cells secrete excessive inflammatory products such as cytokines chemokines.

**FIGURE 2.** Possible mechanisms involved in beneficial effects of probiotics, and inhibition of gut inflammation. The probiotics inhibit invading pathogenic microbes through various mechanisms including competitive inhibition, production of antagonistic compounds including antimicrobial peptides, bacteriocins, organic acids, hydrogen peroxide, diacetyl etc. The disturbance in population of probiotics can lead to enhance number of pathogenic microorganisms which leads to inflammatory responded and pathological conditions. The dotted arrows represent multistep metabolic pathways.
and active oxides. Over-production of cytokines affect the biological action of epithelial cells, for instance, TNF-α induces epithelial cells secreting IL8, expressing membrane toll-like receptors (TLR)-4 excessively (Hausmann et al., 2002).

Probiotic strains interact with intestinal epithelia, and attenuate synthesis of inflammatory effector molecules elicited by diverse proinflammatory stimuli (Otte et al., 2004; Bai et al., 2004; Lammers et al., 2002). This immunosuppressive effect involves inhibition of the inhibitor kB/nuclear factor kB (IkB/NF-kB) pathway by block of IkB-α degradation, which prevents nuclear translocation of active NF-kB dimmer and subsequent relevant gene expression (Neish et al., 2000; Neish et al., 2004). Hence, the probiotics can be responsible for the unique tolerance of the GI mucosa to proinflammatory stimuli. The intestinal apical di-/tripeptide transporter PepT1, responsible for the uptake of a broad array of small peptides transports bacterial proteoglycan derived muramyl dipeptide (MDP). PepT1 expression in chronic colonic inflammation is increased substantially (Vavricka et al., 2004). In colonic epithelial cells, MDP taken up by hPepT1 activates NF-kB and chemokine production. In this way, PepT1 may play an important part in promoting colonocyte participation in host defense and pathogen clearance through increased uptake of MDP. Probiotic LAB such as L. casei can change the function of intestinal PepT1 (Neudeck et al., 2004). In view of scarce data on this topic, more studies should be carried out to explore probiotic-intestinal transporter interactions, and to find out how probiotics participate in host defense and pathogen clearance from the gut.

Some probiotic strains (Table 4) may modify immune response of the intestinal immune cells (Borruel et al., 2002). Mucosal specimens from CD patients and controls were cultured for 24 hours alone or with probiotic strains as non-pathogenic E. coli, L. casei DN-114001, L. bulgaricus LB10, or L. crispatus. Release of TNFα by inflamed CD mucosa was significantly reduced by co-culture with L. casei or L. bulgaricus, whereas changes induced by L. crispatus or E. coli were not significant. The co-culture with L. casei and L. bulgaricus reduced the number of CD4 cells as well as TNFα expression among intraepithelial lymphocytes from CD mucosa. None of the bacteria induced changes in non-inflamed mucosa. Probiotics interact with immunocompetent cells using the mucosal interface and modulate locally the production of proinflammatory cytokines. Toll-like receptors (TLRs) are mediators of pathogen recognition in innate immune system (Andreakos et al., 2004). Preliminary data from experimental colitis show that TLR9 signaling is essential in mediating the anti-inflammatory effect of probiotics (Rachmilewitz et al., 2004). Such recognition results in the initiation of an inflammatory immune response and subsequent instruction of the adaptive immune system, both of which are designed to provide protection against invading pathogens.

Probiotics can also modify mucosal immune function via TLR signaling (Rachmilewitz et al., 2004). The protective effect of probiotics is mediated by their own genome via TLR9 signaling pathway, rather than by their metabolites or ability to colonize the colon (Rachmilewitz et al., 2004). Sturm et al. (2005) have demonstrated that one probiotic strain, E. coli Nissle 1917, could affect cell cycling and apoptosis of peripheral blood T-cells (PBT). When anti-CD3 stimulated PBT are treated with E. coli Nissle 1917 conditioned medium, E. coli Nissle 1917 medium inhibit cell cycling and

### Table 4. Some potential probiotics proposed for their efficacy against IBDs.

<table>
<thead>
<tr>
<th>Probiotic strains</th>
<th>References</th>
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<tbody>
<tr>
<td>Lactobacillus reuteri (R2LC)</td>
<td>Madsen et al., (1999)</td>
</tr>
<tr>
<td>L. salivarius UCC118</td>
<td>O’Mahony et al., (2001)</td>
</tr>
<tr>
<td>L. casei GG (L. GG)</td>
<td>Malin et al., (1996)</td>
</tr>
<tr>
<td>VSL#3 (Vivomixx in Europe and Visbiome in USA)</td>
<td>Madsen (2001)</td>
</tr>
<tr>
<td>Bifidobacterium Infantis; Bifidobacterium Bb-12</td>
<td>Tanabe et al., (2008)</td>
</tr>
<tr>
<td>S. bouardii</td>
<td>Guslandi et al., (2000)</td>
</tr>
<tr>
<td>L. brevis HY7401</td>
<td>Lee et al., (2008)</td>
</tr>
<tr>
<td>L. johnsonii NCC 533</td>
<td>Pridmore et al., (2008)</td>
</tr>
<tr>
<td>Lactococcus Lactis (engineered)</td>
<td>Steidler et al., (2000)</td>
</tr>
<tr>
<td>Bifidobacterium infantis 1222; Bifidobacteria sp. 3B1; B. infantis DSM 15158</td>
<td>Shiha et al., (2003); Osman et al., (2004)</td>
</tr>
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expansion of peripheral blood T-cells, decrease cyclin D2, B1 and retinoblastoma protein expression contributing to the reduction of T cell proliferation, and regulate the decreased expression of IL2, TNFα and interferon gamma but increased IL-10 production in PBT. The inhibition of PBT proliferation by *E. coli* Nissle 1917 conditioned medium is TLR2-dependent, as shown by using TLR-2 knockout mice.

The peroxisome proliferator-activated receptors (PPARs, namely PPARα, γ and δ) are transcription factors that translate nutritional signals into specific gene-expression patterns that control cellular bioenergetics. The receptors act as nutritional sensors, regulating metabolism across organs to modify systemic metabolism (Dubuquoy et al., 2006; Ament et al., 2012). Probiotics can modulate the inflammatory response in inflamed mucosa of IBDs patients through PPAR pathway that has been proposed as a key inhibitor of colitis through attenuation of NF-kb activity (Nencioni et al., 2003). However, PPAR expression is impaired in patients with ulcerative colitis, compared with normal controls (Dubuquoy et al., 2003).

**FUTURE DIRECTIONS AND CONCLUSION**

There is a rapid increase in the understanding of how microbes can be employed to deliver health benefits. Gut bacteria play an important role in the pathogenesis of IBDs, its complications and symptoms. While the exact causes are unclear, IBDs, including the most common manifestations i.e. CD and UC, are known to be the result of an overactive immune response that is linked to an imbalance of the normal types of bacteria inhabiting the gut. With recent evidences implicating disruption in the balance of the gut microbiome and gut immunity as a potential trigger for IBDs and related pathologies, there has been growing interest in developing and utilizing gut microbes as probiotics as an adjunct to anti-inflammatory and immune suppressing therapies. Probiotics can have inflammatory activities in both healthy and IBDs tissue, and are regarded as safer alternative for the treatment of patients with IBDs in acute inflammatory phase. Recent advances deciphering the complex gut microbiome will highlight novel insights into the cellular and molecular bases of IBDs, and will provide opportunities to manipulate gut microbiome through intake of potential probiotics to manage pathologies associated with IBDs and related diseases. Future studies should focus on specific disease subtypes and disease location. New knowledge about the role of microbial communities in the microbiota of the GI tract and their microbiome, in chronic diseases opens new opportunities for therapeutic interventions. Insight into the etiology of IBDs and the cellular and molecular mechanisms of probiotic actions will aid in selecting probiotic strains for specific disease entities and disease locations. Valid preclinical data on proper model systems should, therefore, be generated before specific probiotic strains are recommended for clinics, especially if administered during acute inflammatory responses. As for the potential of a gut-flora analysis to drive a more tentatively specific probiotics intervention in IBDs, it can be noted the recent availability of the next-generation sequencing (NGS) methodology that by combining multiple samples in a single sequencing run, allows the analyte of the whole microbial community within a minute sample. This powerful technology uses clonal amplification and sequencing with simultaneously targeting of DNA bases emitting a unique fluorescent signal.

Although the massive amount of data still needs to be fully unfolded and hierarchically prioritized, it is worth mentioning that a multi-team approach to these data may already start be amenable to clinical practice. In particular, this has been quite recently achieved by a spin off of dedicated genetists and biologist (Next Genomics, Prato, Italy) which avail itself of a kit allowing small sampling and remaining stable for 14 days at room temperature and with a detection accuracy of 0.0001% of the original sample. The report gathers all the above mentioned expertise so to make it also a tool of potential clinical use, with all due limitation of exploring this new field, to possibly unveil subtle disease risk-prone conditions, early signs of IBDs flare ups and to tentatively shape up a tailored intervention and an objective follow up monitoring.

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