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## Probiotics in Inflammatory Bowel Disease: Yet Another Mechanism of Action?

**See “*Saccharomyces boulardii* inhibits inflammatory bowel disease by trapping T cells in mesenteric lymph nodes” by Dalmasso G, Cottrez F, Imbert V, Lagadec P, Peyron J-F, Rampal P, Czerucka D, Groux H, on page 1812.**

Since the discovery of the beneficial effect of sulfasalazine in ulcerative colitis, the number of agents used for treatment of

inflammatory bowel disease (IBD) has steadily grown to include corticosteroids, 5-aminosalicylic acid, immunosuppressives, elemental diets, antibiotics, and biologicals. One of the latest additions to this vast therapeutic armamentarium are probiotics, defined as live microbial feed supplements, which beneficially affect the host by improving intestinal microbial balance.<sup>1</sup> The indication of probiotics in IBD is grounded on a number of human and animal studies indicating that the enteric flora is centrally involved in the pathogenesis of Crohn’s disease and ulcerative colitis.<sup>2</sup> Commensal bacteria–host interactions are

essential to health and immune homeostasis,<sup>3,4</sup> and thus it is not too surprising that disruption of the physiologic bacteria-immune balance may lead to gut inflammation. Manipulation of the effector arm of the immune system to suppress its uncontrolled tissue-damaging response seems logical and successful, as demonstrated by the effectiveness of corticosteroids, immunosuppressives, and biologicals in resolving inflammation in most IBD patients. Alternatively, one could aim at the target of the unrestrained immune response by manipulating the enteric flora, which can be accomplished with antibiotics or probiotics.<sup>5</sup> The use of antibiotics has limited value in IBD, with the exception of special situations like fistulas, abscesses, bacterial overgrowth, or pouchitis. In addition, prolonged use of antibiotics can cause serious side effects and drastically alter the flora with further undesirable consequences. However, by definition, probiotics “beneficially affect the host by improving intestinal microbial balance,”<sup>1</sup> a highly desirable behavior with implied safety. So, what is the scientific rationale behind the use of probiotics in IBD?<sup>6</sup> Is the enteric flora abnormal in IBD? Is there objective evidence that probiotics benefit IBD patients? What is (are) the mechanism(s) behind the therapeutic effect of probiotics in IBD? Unfortunately, the answers to each of these critical questions are not straightforward, at least for now.

Various abnormalities of the gut flora have been described in Crohn’s disease and ulcerative colitis patients, but available data are inconsistent and inconclusive.<sup>7</sup> Compared with the healthy gut, an increased number of mucosa-associated bacteria, quantitative and qualitative differences, and instability of flora composition have all been reported.<sup>8</sup> How these findings relate to IBD pathogenesis is still unclear. Evidence that probiotics provide therapeutic benefits in IBD is growing but needs solidifying. On the positive side, the probiotic mixture VSL3# is effective in both maintenance and prophylactic treatment of pouchitis,<sup>9,10</sup> and helps to induce remission in ulcerative colitis patients<sup>11</sup>; the nonpathogenic *Escherichia coli* Nissle 1917 is as effective as mesalazine in preventing relapse of ulcerative colitis,<sup>12</sup> and *Saccharomyces boulardii* appears useful as maintenance treatment for Crohn’s disease.<sup>13</sup> On the negative side, *L. rhamnosus* GG (LGG) and *Lactobacillus johnsonii* LA1 are ineffective in preventing postoperative recurrence of Crohn’s disease,<sup>14,15</sup> and LGG fails to prolong time to relapse in pediatric Crohn’s disease.<sup>16</sup> Support for a favorable action of probiotics in gut inflammation also comes from animal models, including interleukin (IL)-10- and IL-2-deficient mice, dextran sodium sulfate- and hapten-induced colitis, and HLA-B27 transgenic rats.<sup>17–20</sup> A miscellanea of disparate biological mechanisms brings about the good deeds of probiotics, including modulation of host immune response (changes of dendritic cell phenotype and function, modulation of nuclear factor [NF]- $\kappa$ B and AP-1 pathways, modulation of innate immunity through toll-like receptor engagement by CpG-DNA motifs, altered cytokine release, induction of regulatory T cells, modulation of apoptosis, and induction of PPR- $\gamma$ ), enhanced epithelial barrier function (enhanced tight junction protein phosphorylation, mucus production, epithelial cell glycosylation, and sIgA production), and antimicrobial activity (decreased luminal pH, secretion of antimicrobial peptides, inhibition of pathogen invasion, and blockade of bacterial adhesion to epithelial cells).<sup>21,22</sup>

In this issue of GASTROENTEROLOGY, Dalmasso et al<sup>23</sup> add a new and surprising effect to the already intricate picture of probiotics’ bioactivities. These authors fed *S. boulardii* to animals

with experimental colitis induced by the transfer of CD45R<sup>high</sup> T cells into severe combined immunodeficient (SCID) mice and measured clinical, histological, and immune parameters. Daily feeds of *S. boulardii* both prevented and improved colitis and the associated wasting disease, and decreased mucosal NF- $\kappa$ B activity and pro-inflammatory cytokine (tumor necrosis factor- $\alpha$ , IL-1 $\beta$ , interferon [IFN]- $\gamma$  and IL-6) expression. These are positive results but certainly not unanticipated. However, when they measured IFN- $\gamma$  production by CD4<sup>+</sup> T cells in *S. boulardii*-fed mice, it was reduced in the colon but increased in the mesenteric lymph nodes, a surprising finding suggesting a redistribution of IFN- $\gamma$ -producing T cells. These results displayed some specificity; CD4<sup>+</sup> T cells did not excessively accumulate in Peyer’s patches. In addition, mice given *S. boulardii* did not overexpress IL-10 or TGF- $\beta$ , suggesting that improved colonic inflammation was not due to induction of T-regulatory cells. To explore the mechanisms underlying these novel observations, Dalmasso et al fed *S. boulardii* to healthy mice and reproduced the increased accumulation of T cells in the mesenteric lymph nodes but not the spleen, an effect unrelated to the cell’s state of activation. As further experiments failed to show that the *S. boulardii*-mediated redistribution of T cells was due to enhanced expression of adhesion molecules, like integrins, the authors looked for an increased expression of homing receptors in T cells and/or the mesenteric lymph nodes. In transfer experiments, enhanced accumulation of CD4<sup>+</sup> T cells occurred when the receiver, but not the donor, mice were given the probiotic, indicating that T-cell retention was due to an action at the level of the mesenteric lymphatic tissue and not the circulating cells. Because increased chemokine production was not responsible for this retaining action, the authors finally discovered that a factor contained in supernatants of *S. boulardii* cultures enhanced the ability of lymph node murine endothelial cells to mediate T-cell rolling (and presumably adhesion and translocation), apparently through amplification of selectin-mediated interactions between T cells and endothelial cells.

The bottom line, at the end of this long series of imaginative experiments, is the discovery of yet another biological property of probiotics, namely, the capacity to affect immune cell redistribution by improving the competence of lymphatic endothelial cells to trap T lymphocytes. At first glance, this seems almost an “out of character” action of probiotics, unrelated to and unlike any of the more direct biological effects, such as modulation of host immune response, enhanced epithelial barrier function, or antimicrobial activity. This puzzling “endothelial cell-oriented” activity of *S. boulardii* raises a series of interesting issues. The first obvious one is whether the lymphocyte trapping activity is limited to *S. boulardii* or is shared with other probiotics. To address this important question culture supernatants of *Saccharomyces cerevisiae* were used as a control for the treatment of lymph node endothelial cells, but they failed to enhance T-cell rolling. This result does not fully answer the question of a possibly unique action of *S. boulardii* because *S. cerevisiae*, although a related yeast, can hardly be considered a probiotic, and one could even argue that *S. cerevisiae* may have detrimental effects, considering that an immune response against this baker’s yeast is common in IBD patients.<sup>24</sup> Culture supernatants of more traditional probiotics, like lactobacilli or bifidobacteria, might have been a more suitable choice. An equally important issue is whether the lymphocyte trapping-dependent anti-inflammatory activity of *S. boulardii* occurs in

other experimental colitis models. Unlike other models where inflammation is induced by lack of anti-inflammatory cytokines, like in IL-10-deficient mice, or local instillation of irritants, like dextran sodium sulfate or trinitrobenzene sulfonic acid enemas, the CD45R<sup>high</sup> T-cell transfer model is based on repopulation of T cells throughout the body. Here, lymphocyte trafficking obviously plays a key role and it may be particularly susceptible to the endothelial cell-modulatory activity of *S. boulardii*, whereas this may not occur in other models where colonic infiltration requires far less complex and extensive lymphocyte movements. Thus, studies comparing *S. boulardii* to other probiotics with different mechanisms of action in various IBD models would be extremely desirable. An additional tantalizing question is this: What product(s) and mechanism(s) does *S. boulardii* utilize to enhance T-cell rolling by endothelial cells? Based on culture experiments, viable *S. boulardii* colonies were easily recovered from Peyer's patches but hardly from lymph nodes and spleen of mice given the probiotic, suggesting that intact yeast may not be needed to increase endothelial cells' T-cell trapping capacity. Endothelial cells express toll-like receptors and yeast components, such as cell wall-derived zymosan, could be tested for their ability to activate endothelial cells and alter their phenotype and function.

Several other fascinating studies should follow the novel findings of Dalmasso et al.<sup>23</sup> such as whether the animals were colonized or not, whether dendritic cell function was altered, and whether epithelial barrier function or antimicrobial effects were concomitantly induced by *S. boulardii*. From a clinical perspective, instead, one should investigate whether the lymphocyte trapping capacity of *S. boulardii* does occur in humans and, if so, whether this makes this probiotic more desirable for IBD patients than others tested so far. We already know that all probiotics are not created equal and some of them, like *S. boulardii* itself, can even cause serious systemic infections,<sup>25</sup> and only a head-to-head comparison with other probiotics can answer that question. Regrettably, this would require patients, resources, and time that are simply not available. Thus, from a practical standpoint, it makes more sense to take advantage of "all" beneficial properties of probiotics by giving IBD patients combinations of multiple bacteria and yeasts with anti-inflammatory and protective effects, rather than hoping that a single probiotic will fight alone and win the battle against an overwhelming and hostile army of gut flora.

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## Genetically Defined Models of Chronic Pancreatitis

**See “A mouse model of hereditary pancreatitis generated by transgenic expression of R122H trypsinogen” by Archer H, Jura N, Keller J, Jacobson M, Bar-Sagi D, on page 1844; and “Primary cilia deletion in pancreatic epithelial cells results in cyst formation and pancreatitis” by Cano DA, Sekine S, Hebrok M, on page 1856.**

The concept that acute pancreatitis was not an infection, but was caused by autodigestion of the pancreas through activation of digestive enzymes, was first proposed by Chiari in 1896.<sup>1</sup> It is now generally accepted that, in most cases, acute pancreatitis begins with trypsinogen activation to trypsin within the pancreas. Anti-trypsin protective mechanisms are overwhelmed with further activation of trypsinogen and other zymogens, thereby resulting in pancreatic injury and initiation of an acute inflammatory response.<sup>2</sup> A major breakthrough that strongly supported Chiari's hypothesis of autodigestion and that linked trypsin activity to both acute and chronic pancreatitis was the discovery in 1996 that mutations in the cationic trypsinogen gene (PRSS1) were associated with hereditary pancreatitis.<sup>3</sup>

Hereditary pancreatitis is an uncommon, autosomal-dominant disorder that was first described by Comfort and Steinberg<sup>4</sup> in 1952 in a kindred spanning 3 generations comprising 4 affected persons and 2 others suspected of being obligate carriers of the disease. The typical family member who carries a major PRSS1 gene mutation develops recurrent acute pancreatitis at around 10 years of age, and a majority go on to develop various degrees of chronic pancreatitis within the next 10–15 years; of these who develop chronic pancreatitis, up to 40% develop pancreatic cancer.<sup>5,6</sup> The fact that each manifestation of pancreatic disease is indistinguishable from the sporadic form, except for the family history and the lack of other etiologic factors, has made hereditary pancreatitis a very important human model for investigating disease mechanisms.<sup>7</sup> Indeed, insights from this disease have revolutionized our conceptualization of sporadic pancreatic diseases.

To date, over 25 mutations have been identified in the cationic trypsinogen gene, with the most common being PRSS1 R122H and N29I.<sup>8,9</sup> The mutations generally cluster around the 2 calcium-binding pockets that are critical in regulating

trypsinogen activation and trypsin inactivation.<sup>10</sup> For example, trypsin has a built-in self-destruction (or autolysis) site at R122 that can only be accessed by another trypsin molecule when the site is not being protected by calcium occupying one of the binding sites. Biochemical studies have proven that eliminating the R122 autolysis site because of a mutation preserves trypsin survival in solutions with low calcium concentrations,<sup>11,12</sup> such as those that exist within acinar cells. Taken together, studies of the cationic trypsinogen mutations in humans with hereditary pancreatitis point to the importance of unregulated trypsin activity in initiating acute pancreatitis. They also indicate the significance of maintaining low calcium concentrations within the acinar cell, which facilitates trypsinogen activation and prevents trypsin inactivation and can therefore lead to trypsin related injury and acute pancreatitis.<sup>13</sup>

The importance of mutated trypsin in hereditary pancreatitis led researchers to investigate other molecules in humans that normally protect the pancreas from inappropriate trypsin activity. Indeed, mutations in the pancreatic secretory trypsin inhibitor (PSTI) gene (or serine protease inhibitor Kazal type 1, SPINK1) were found to be associated with chronic pancreatitis in children,<sup>14</sup> families,<sup>15</sup> tropical pancreatitis,<sup>16</sup> and to a lesser degree, in alcoholics.<sup>17,18</sup> Because SPINK1 is expressed as an acute phase protein, it likely becomes relevant only after inflammation has occurred,<sup>19</sup> and therefore protects against recurrent acute pancreatitis rather than an initial attack. Other factors within the acinar cell that can activate trypsinogen include cathepsin B,<sup>20,21</sup> a lysosomal enzyme that is normally segregated from trypsinogen, and is located in zymogen granules in a cell's cytoplasm. In experimental animals in which acute pancreatitis is being induced, colocalization of these vesicles appears to be associated with trypsin activation and worsens pancreatitis.<sup>22</sup> Recently, this potential mechanism has also been linked to humans because mutations in the cathepsin B gene alter the risk of tropical pancreatitis.<sup>23</sup> Finally, the pancreas must generate a bicarbonate-rich fluid to “flush” trypsinogen and the other zymogens out of the pancreatic duct following secretion from the acinar cell. This action depends on the cystic fibrosis transmembrane membrane conductance regulator (CFTR), a regulated anion channel in the pancreatic duct cells that is permeable to chloride, and to a lesser degree, bicarbonate. Mutations in the CFTR gene lead to cystic fibrosis,<sup>24,25</sup> a multisystem genetic disorder causing chronic pancreatitis beginning in utero. Severe CFTR mutations, as well as some more moderate ones, are predicted to limit CFTR-dependent bicar-