Abbreviations:

Polysaccharide A (PSA)
Inflammatory bowel disease (IBD)
Lactobacillus rhamnosus GG (LGG)
Crohn's disease (CD)
Antigen presenting cells (APCs)
Lipoteichoic acid (LTA)
Ulcerative colitis (UC)

Introduction:

Despite the substantial progress made in research, the cause of Inflammatory Bowel Disease (IBD) remains unknown. Genetic, environmental, and host immune factors all seem to play a role in the pathophysiology of the disease. In the last two decades, molecular tools have advanced to accurately identify and quantify different intestinal bacteria [1]. With highly sensitive tools now available, many researchers have focused on the environmental effects on the intestinal microflora in promoting inflammation and disease. A disruption in the delicate balance of beneficial and pathologic bacteria in the intestines can induce an inflammatory response, playing a major role in pathogenesis. In light of this bacterial dysbiosis theory came the rise in popularity of using probiotics in conjunction with standard IBD treatment. Probiotics like *Lactobacilus rhamnosus GG* are thought to modulate immune responses to suppress inflammation, compete with pathogenic bacteria for space to flourish, and maintain a healthy mucosa [2]. Although the mechanism by which these beneficial bacteria exert its anti-inflammatory actions is not well understood, probiotics are commonly used in the treatment of IBD. The efficacy of probiotics in maintaining and prolonging remission, however, is still highly debated. Some studies show remarkable improvement in patients' health when they take probiotics while others show no beneficial effect [2]. The moderate and sometimes inconclusive results of probiotics reflect just how little concrete knowledge we possess on its immunomodulatory mechanisms.

In a recent article published in *Nature* of May 2008, a single bacterium previously unknown to have anti-inflammatory actions was found to decrease inflammation in mice through the production of a polysaccharide [3]. Investigation into *Bacteriodes fragilis* and polysaccharide A (PSA) revealed that PSA exerts its effects through the activation of T-cells that corrects the imbalance of leukocytes and cytokines of the intestinal immune system. The study of PSA has started off with a terrific bound with a great potential for further research. The ultimate effect of probiotics and PSA seem similar, but stronger and more concrete knowledge on how each interact and modulate the immune system will be crucial in advancing IBD therapy.

Research on the probiotic *Lactobacillus rhamnosus* and its obstacles:

Lactobacillus rhamnosus GG (LGG) is a gram positive bacteria that is widely used in probiotic therapy. Various studies propose different mechanisms by which LGG promotes a healthy gut. Some propose that LGG dampens the body's adaptive immune response by decreasing the production of pro-inflammatory cytokines. For example, some studies have found that LGG affects T-cell activity through the production of chemical messages that modulate dendritic cell function, ultimately leading to T-cell hyporesponsiveness [4]. Other groups have found specific growth factors produced by LGG, such as granulocyte colony stimulating factor (G-CSF), to induce signal

transducers and activators of transcription (STAT3) activation, reduce c-Jun-N-terminal kinases (JNK) activation, and ultimately inhibit TNF α transcription by macrophages [5]. Other studies suggest that LGG stimulates the host's innate immune response. Inflammatory cytokines such as TNF α and IL-6 are increased with the introduction of LGG, which is thought to protect the intestines from pathologic bacteria [6,7]. Clinical trials have also revealed that LGG stimulates a nonspecific humoral response resulting in an increase of antibody producing lymphocytes [8]. Specific LGG DNA sequences also have been found to stimulate pro-inflammatory cytokines by antigen presenting cells (APCs) and B-cells [9]. LGG modulates the immune system in various ways, making it difficult to identify and distinguish more beneficial actions from less advantageous or even harmful actions. The beneficial effects of LGG most likely arise from a combination of different mechanism that alters the immune system.

The human immune system interacts with various LGG wall components and secretions. Lipoteichoic acid (LTA) rather than capsular proteins and peptidoglycans seem to promote a more significant immune response, although no concrete evidence has been shown [6,7]. Soluble protein factors that are produced and secreted by LGG also seem to act as antigens[10]. The beneficial immune response of LGG is most likely induced by the presentation of different antigens to APCs.

In refining probiotic research, it also may be necessary to rethink who really benefits from probiotic organisms. Based on clinical trials, LGG seems to benefit patients with ulcerative colitis (UC) much more than patients with Crohn's disease (CD). LGG alone successfully maintains remission in patients with UC, while having no benefit in maintaining remission in patients with CD [11,12]. Whether or not the same probiotics would benefit both patients with UC or CD is questionable, yet many physicians advise their use. As in the case of LGG, certain bacteria may be more beneficial to one type of IBD more then the other, raising the idea of specially formulated probiotics unique for UC or CD (see Table 1) [11,13]. The difference in response to LGG may be due to the fact that the inflammation of UC is predominantly mediated by Th2 T-cells while CD is a T-cell Th1/Th17 inflammation [2]. Certain Lactobacillus strains have been noted to induce regulatory T-cells to decrease inflammation [14]. Inflammation in UC may benefit more from the cytokines produced by regulatory T-cells. Such discoveries reinforce the importance of understanding the mechanism of probiotics to better restrict its use only in conditions that would truly benefit from its actions. Patients with IBD take many pills ranging from vitamin supplements to steroids, and taking ten to twenty pills a day may not be uncommon. Therefore proper identification of beneficial organisms is crucial in relieving patients from unnecessary pills and medication.

Polysaccharide A and its Overall effects:

The recent discovery of the immunoregulatory actions of PSA has opened a door to a completely new area of study, specifically on how polysaccharide bacterial antigens are processed by the human immune system. *B. fragilis* produces and encases itself within a capsular polysaccharide complex. There are two main polysaccharides, PSA and PSB, that comprise this complex but it has been noted that this complex can contain up to eight different types of polysaccharides [15,16]. PSA has a high molecular weight of

roughly 110kD and is made up of several hundred repeating units of a tetrasaccharide [17]. The unique immunomodulatory effects that PSA exhibits seem to come from the zwitterionic nature of the sugar [3].

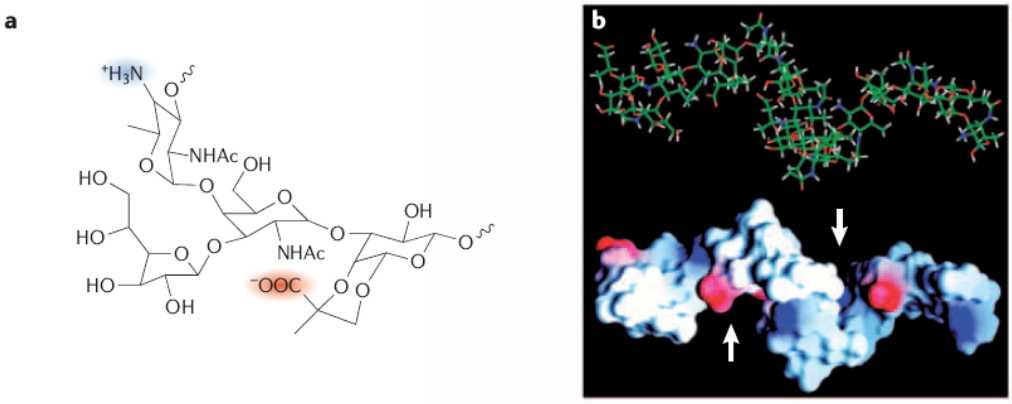

Fig. 1. a) Single unit of PSA tetrasaccharide. b) 3-D model of four tetrasaccharide units from PSA2 (a molecule similar to PSA that is produced by *B. fragilis*). In the lower image, blue areas represent positive charges and red represents negative charges. Adapted by permission from Macmillan Publishers Ltd: *Nature Reviews Immunology* [18], (2006).

Intestinal epithelia and leukocytes interact with bacteria to produce a variety of different chemical messages that modulate inflammation. When disease animal models were colonized with B. fragilis producing PSA, the expression of pro-inflammatory cytokines TNF α, IL-23, and IL-17 were suppressed while the immunomodulatory cytokine IL-10 expression increased. Animals were also protected from body wasting due to diarrhea and colonic hyperplasia secondary to inflammation [3]. Perhaps the most exciting discovery in the studies of PSA is its active role in IL-10 production. IL-10 produced by activated T-cells allows for control of APC activity by inhibiting the production of inflammatory cytokines [19,20]. This results in a large anti-inflammatory effect since multiple cell types are affected and various inflammatory cytokines are reduced. LGG, on the other hand, does not exert such strong control over APCs, thus the anti-inflammatory effects it may have may be minute when compared to PSA. In short, IL-10 may be the key player in modulating the immune system.

T-cell recognition of PSA:

PSA recognition by leukocytes seems to differ from the typical way in which polysaccharides are detected. It does not act as a T-cell independent antigen or a superantigen. PSA activates CD4 T-cells through presentation on APCs expressing MHC-II. PSA is recognized by APCs (dendritic cells, macrophages, and B-cells) and is engulfed into endosomes. It has been proposed that dendritic cells of the gut-associated lymphoid tissue (GALT) detect PSA crossing the epithelia or actively sample PSA in the gut by extending their arms through the epithelia [18]. Once internalized, PSA is broken down into smaller fragments through the action of nitric oxide synthase 2 (NOS2) and the nitric oxide that it produces. Fragments are then presented on HLA-DR molecules on MHC-II [3]. The zwitterionic nature of PSA is thought to play a critical role in its docking to the MHC. The "groove –binding model" suggests that the repetitive positive and

negative units on each sugar unit twist the polysaccharide chain into a helical structure with the charges facing the outside [21]. It is proposed that PSA fragments fit within the alpha-helices of the peptide-binding groove on the HLA-DR molecule of the MHC and becomes displayed for T-cell activation. Thus presentation of PSA on APCs mimic protein antigen presentation almost entirely, except for the difference in fragmentation, as protein antigens are cleaved by proteolytic enzymes.

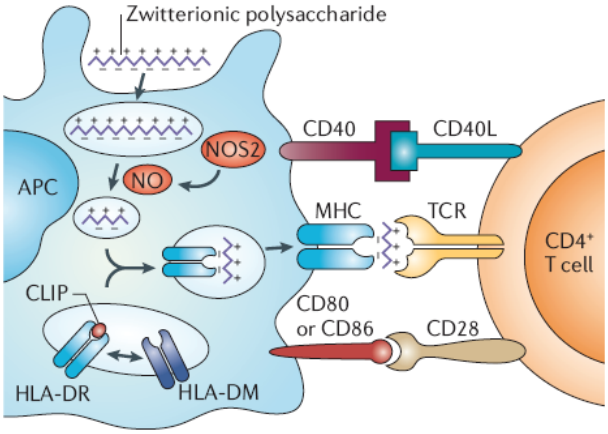

Fig 2. PSA presentation by APCs. Adapted by permission from Macmillan Publishers Ltd: Nature Reviews Immunology [18], (2006).

Once PSA is internalized, MHC-II molecules as well as co-stimulatory molecules CD40, CD80, and/or CD86 are up-regulated to the dendritic cell surface [18]. The APC travels through the lymph to the mesenteric lymph nodes where it meets naïve T-cells. A CD4 T-cell receptor physically interacts with the MHC molecule presenting the PSA fragment, and along with the proper co-stimulatory signals, becomes activated. In animal models, these mature CD4 T-cells protect the intestines from inflammation by regulating inflammatory cytokine production [18].

Up until recently, the beneficial effects of *B. fragilis* have been unknown. However, with the clear identification of PSA as an antigen, researchers will be able to focus their studies on its interaction with the immune system. Further study of *B. fragilis* and PSA may lead to future use of it in IBD therapy. Once the safety of purified PSA is established, it may be evaluated in clinical trials for the treatment of IBD. Investigation of other PSA induced responses such as unwanted side effects may be an interesting area of study, along with how PSA is absorbed, distributed, metabolized, and excreted by the body. LGG and *B. fragilis* are two different organisms, one widely used in IBD treatment but with recent faltering popularity, and the other just entering the spotlight but with immense potential. How each will prove its worth in the following years will largely depend on what further research reveals.

Table 1 Major outcomes from three clinical trials with the probiotic *Lactobacillus rhamnosus* (LGG).

Ref. #	Type of trial	Population size (n)	Treatment groups	Duration	Clinical monitoring	Outcome

UC: Maintenance of remission						
11	Randomized, open label	187	LGG vs LGG + Mesalamine vs Mesalamine	12 mo	Physical examination every 3 mo, colonoscopy at 0, 6, & 12 mo.	No difference in % of pts in remission among treatment groups.
CD: Inducing remission						
22	Randomized, double blind	11	LGG vs placebo	6 mo	Follow up visits at 2, 4, 8, 12, 18, & 24 wks.	No benefit in inducing remission.
CD: Maintenance of remission						
12	Randomized, double blind	75	LGG vs placebo (with concomitant maintenance drugs, mesalamine, 6-MP, corticosteroids)	2 yrs or until relapse	Follow up visits with physical exam and lab tests every 3 mo.	No change in time to relapse or likelihood of relapse.

References:

1. Vaughan EE, de Vries MC, Zoetendal EG, Ben-Amor K, Akkermans AD, de Vos WM. The Intestinal LABs. *Antonie Van Leeuwenhoek.* 2002;82:341-52.
2. Boirivant M, Strober W. The mechanism of action of probiotics. *Current Opinions in Gastroenterology*. 2007;23:679-692.
3. Mazmanian S, Round J, Kasper D. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*. 2008;453:620-625.
4. Braat H, Brande J, Tol E, et al. Lactobacillus rhamnosis induces peripheral hyporesponsiveness in stimulated CD4+ T cells via modulation of dendritic cell function. *Am J Cli Nutr*. 2004;80:1618-1625.
5. Kim SO, Sheikh HI, Ha SD, Martins A, Reid G. G-CSF-mediated inhibition of JNK is a key mechanism for Lactobacillus rhamnosus-induced suppression of TNF production in macrophages. *Cell Microbiol*. 2006;8:1958-71.
6. Korhonen R, Korpela R, Moilanen E. Signalling Mechanisms Involved in the Induction of Inducible Nitric Oxide Synthase by Lactobacillus Rhamnosus GG, Endotoxin and Lipoteichoic Acid. *Inflammation*. 2002;26:207-214.
7. Miettinen M, Vuopio J, Varkila K. Production of Human tumor Necrosis Factor Alpha, Interleukin-6, and Interleukin-10 Is Induced by Lactic Acid Bacteria. *Infection and Immunity*. 1996;64:5403-5405.
8. Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, Arvilommi H. Enhancement of the circulating antibody secreting cell response in human diarrhea by a human Lactobacillus strain. *Pediatr Res.* 1992;32:141-4.

9. Iliev ID, Kitazawa H, Shimosato T, Katoh S, Morita H, He F, Hosoda M, Saito T. Strong immunostimulation in murine immune cells by Lactobacillus rhamnosus GG DNA containing novel oligodeoxynucleotide pattern. *Cell Microbiol*. 2005;7:403-14.
10. Peña JA, Versalovic J. Lactobacillus rhamnosus GG decreases TNF-alpha production in lipopolysaccharide-activated murine macrophages by a contact-independent mechanism. *Cell Microbiol*. 2003;4:277-85.
11. Sartor RB. Probiotic therapy of intestinal inflammation and infections. *Curr Opin Gastroenterol*. 2005;1:44-50.
12. Bousvaros A, Guandalini S, Baldassano RN, Botelho C, Evans J, Ferry GD, Goldin B, Hartigan L, Kugathasan S, Levy J, Murray KF, Oliva-Hemker M, Rosh JR, Tolia V, Zholudev A, Vanderhoof JA, Hibberd PL. A randomized, double-blind trial of Lactobacillus GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease. *Inflamm Bowel Dis*. 2005;9:833-9.
13. Fedorak RN, Madsen KL. Probiotics and prebiotics in gastrointestinal disorders. *Curr Opin Gastroenterol*. 2004;2:146-55.
14. Smits HH, Engering A, van der Kleij D, de Jong EC, Schipper K, van Capel TM, Zaat BA, Yazdanbakhsh M, Wierenga EA, van Kooyk Y, Kapsenberg ML. Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. *J Allergy Clin Immunol*. 2005;115:1260-7.
15. Krinos C.M. et al. Extensive surface diversity of a commensal microorganism by multiple DNA inversions. *Nature*. 2001;414:555-558.
16. CerdenoTarraga A.M. et al. Extensive DNA inversion in the B. fragilis genome control variable gene expression. *Science*. 2005;307:1463-1465.
17. Baumann H, Tzianabos A. O., Brisson J. R. Kasper D L., Jennings H. J. Structural elucidation of two capsular polysaccharides from one strain of B. fragilis using high-resolution NMR spectroscopy. *Biochemistry*. 1992;31:4081-4089.
18. Mazmanian S, Kasper D. The love-hate relationship between bacterial polysaccharides and the host immune system. *Nat Rev Immunol*. 2006;11:849-58.
19. Williams I, Rich B, Kupper T. "Cytokines" from Dermatology, http://www.accessmedicine.com/content.aspx?aID=2975389&searchStr=interleukin-10 (2008) Accessed Nov. 21, 2008.
20. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol.* 2001;19:683-765.
21. Wang Y, Kalka-Moll WM, Roehrl MH, Kasper DL. Structural basis of the abscess-modulating polysaccharide A2 from B. fragilis. *Proc. Natl. Acad. Sci. USA*. 2000;97:13478-13483.
22. Schultz M, Timmer A, Herfarth HH, Sartor RB, Vanderhoof JA, Rath HC. Lactobacillus GG in inducing and maintaining remission of Crohn's disease. *BMC Gastroenterol.* 2004;4:5.