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Microbial Links to Inflammatory Bowel Disease Development: Potential Interventional Strategies in Treatment

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Commentary

The two major forms of Inflammatory Bowel Disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC), affect an estimated 3.6 million people globally [1]. The main mechanisms responsible for the induction and pathogenesis of IBD remain unknown, but there is a general agreement, those intestinal microbiotas that mediate dysregulation of the immune system resulting in progression and development of IBD, are involved [2]. More recent studies have demonstrated that luminal antigens play an active role, to mediate mucosal immune responses that induce IBD progression. In humans, inflammation is most severe in the part of the gut that contains the highest bacterial concentration [3,4]. It is well known that mice fail to develop colitis or have a reduced severity under germ-free conditions, suggesting a pathologic connection between immune cells and commensal enteric bacteria to develop IBD [5-8]. Due to prolonged mucosal contact in parts of the ileum, rectum and caecum regions, the pathogenic germ(s) may decrease the protective bacteria that induce mucosal permeability and lead to enhanced exposure of bacterial products to Toll-like receptors (TLR), and antigens that directly activate the pathogenic T cell immune responses to induce IBD. This induction also mediates regulatory T cell dysfunction or antigen-presenting cells (APC) that might lead to further decreased tolerance to microbial antigens [9].

The essential features for normal gut function are that the host immune system should be tolerant towards the antigens of commensal gut microbiota. The intestinal bacterial flora contributes in IBD pathogenesis, is supported by several experimental models of colitis as well as clinical studies. In the nineteenth century, Koch's postulation was confirmed later on, that *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD) in cattle [10,11]. After this, a series of studies by many investigators in the field indicate that MAP is a contributing pathogen for CD [12-17]. The granulomas of CD patients also show the presence of *E. coli*, suggesting a secondary role for non-specific pathogen involvement in this disease [18]. Furthermore, rectal mucosa-associated bacterial floras in IBD have demonstrated a reduction in *Bifidobacteria* and an increase in *E. coli* and *Clostridia* [19]. Several reports indicate that childhood environmental factors are involved in pediatric IBD development. The high prevalence of adherent-invasive *E. coli* is associated with CD [20]. Further support at molecular level, suggests a mutation of caspase recruitment domain-15 (CARD-15)/ nucleotide-binding oligomerization domain-2 (NOD-2), in CD patients leads to a loss of antibacterial function in intestinal epithelial cells [21,22]. In summary, *Bacteroides spp, Enterococcus faecalis, Enterobactor cloacae*, intestinal *Helocibacter* spp., *Fusobacterium* Spp., adherent/invasive *E. coli* strains, *Eubacterium* and *Peptostreptococcus* were reported to be harmful bacteria [23] that mediate or contribute to IBD development

and pathology. Based on this information, it is safe to speculate that colonic bacteria manipulation with antibiotic drugs or by probiotic agents will be proven to be more effective than the currently available and utilized immunsuppressive agents in IBD treatment, in the future.

We are excited by several recent reports describing an increase in *Lactobacillus* and *Bifidobacterum* after resveratrol treatment, 20 days prior to DSS induction of colitis [24]. In another report, red wine polyphenols (dimethylhydrazine) were also shown to increase *Lactobacillus*, after 15 weeks of treatment [25]. The increase in lactic acid bacteria after resveratrol treatment demonstrated protection against colitis, by affecting several inflammatory parameters including pro-inflammatory cytokines and oxidative markers of damage [26]. Moreover, there appears to be direct effects of *Lactobacillus* and *Bifidobacterum* administration in preventing proinflammatory responses, in intestinal epithelial cells exposed to pathogenic *Enterobacteria* [27]. Similarly, red wine polyphenols have also been shown to reduce the levels of *Clostridium spp.* and increase *Bifidobacteria* spp. to assist in the protection from IBD [28]. To date, the available treatments for IBD can only reduce the periods of active disease and help to maintain remission. Unfortunately, these treatments often bring marginal results and the disease becomes refractory with a number of side effects. Due to poor responses of this treatment by patients, their clinical use is quite limited [29]. This might be the reason that many IBD sufferers turn to unconventional natural treatments, in the hope of abating symptoms of active disease. It has been estimated that roughly 35-40% of IBD patients use some form of megavitamin therapy, herbal or natural dietary supplement [30]. Our laboratory is working on dietary and natural plant product effects on autoimmune diseases, and we noticed that a plant product, resveratrol, has strong anti-inflammatory and antioxidant properties. Based on the studies described above, there is little doubt that resveratrol influences the microbiota make-up in the gut to protect a host from active IBD. We have recently reported that resveratrol treatment reduces the severity of IBD, using multiple anti-inflammatory pathways as described below.

Resveratrol, a polyphenolic stilbene found in grape skins, berries,

and nuts was originally described as a plant anti-fungal resistance factor, that exerts several biological activities in humans and rodents [31,32]. Interestingly, resveratrol consumption in animals increases life span [33]. More recent studies have demonstrated that resveratrol treatment also reduces inflammation [34], prevents cancer [35], protects against neurodegeneration [36] and reduces severity in autoimmune diseases like EAE [37]. Resveratrol also modulates early inflammation in colitis [38]. While the anti-inflammatory mechanism(s) of resveratrol administration is currently unknown, reductions in the expression of COX-1 and COX-2 after resveratrol treatment, have been reported [39]. Treatment with polyphenol has also been shown to prevent or delay the progression of IBD [40]. We have reported that orally administered resveratrol ameliorates both dextran sodium sulphate (DSS)- induced and IL-10$^{-/-}$ chronic colitis in mice [41,42]. Our studies suggest that resveratrol targets multiple signaling pathways, including silent mating type information regulation-1 (SIRT1) gene expression, which has not been previously investigated with regard to its association with colitis. To this end, colitis induction may down-regulate SIRT1 levels and promote both NF-κB activation and cytokine expression in the colon, and resveratrol reverses these effects by up-regulating SIRT1 [41]. Furthermore, resveratrol treatment suppresses all indicators of inflammation including cytokine production and Th1 cell development as well as COX-2 expression and activation, thereby diminishing colitis. We have also shown that resveratrol reduces tumor incidence and lesion numbers, in DSS-induced colon cancer models [43]. In additional studies, our data has also demonstrated that resveratrol induces the generation of immunosuppressive CD11b$^+$ Gr-1+ myeloid cells that express arginase-1 (ARG-1), which correlates with colitis amelioration [42]. The correlation between the induction of the immunosuppressive CD11b$^+$ Gr-1$^+$ cells by resveratrol and the reversal of colitis, suggest that these cells may also contribute towards the reversal of chronic colitis in IL-10$^{-/-}$ mice. Resveratrol also reduced the number of CXCR3$^+$ T cells in the spleen, mesenteric lymph nodes (MLN), and lamina propria (LP). It is well known that IL-10$^{-/-}$ mice display large amounts of CXCL10 in the colon, by the recruitment of more CXCR3$^+$ leukocytes to propagate disease, while resveratrol treatment reduces the systemic CXCL10 levels, as well as frequency of CXCR3$^+$ T cells.

In summary, these results demonstrate that resveratrol protects against colitis and colitis-associated colon cancer development through multiple pathways, primarily via the up-regulation of SIRT1, the down-regulation of NF-κB activation in immune cells, the induction of immunosuppressive myeloid-derived suppressor cells (MDSCs), and the down-regulation of CXCR3+-expressing CD4+ T cells in the LP. Based on these studies, an overall concept emerges that resveratrol might be a useful, non-toxic complementary and alternative strategy to abate colitis and colon cancer associated with colitis. It is also possible that resveratrol treatment may influence the microbiota in colitis, further contributing to the therapeutic effects of polyphenolics in IBD and colitis. Most likely, there are a number of variables that may influence IBD development including age, nutritional status, environmental exposures and genetics. Changes in diet as well as drug or microbial supplementation, can influence a host's microbiota, thus promoting a more-friendly gut environment, by preventing and/or balancing the stressors that influence inflammation, microbial leakage and tissue damage.

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References

1. Loftus EV Jr (2004) Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. Gastroenterology 126: 1504-1517.

2. Scaldaferri F, Fiocchi C (2007) Inflammatory bowel disease: progress and current concepts of etiopathogenesis. J Dig Dis 8: 171-178.

3. Linskens RK, Huijsdens XW, Savelkoul PH, Vandenbroucke-Grauls CM, Meuwissen SG (2001) The bacterial flora in inflammatory bowel disease: current insights in pathogenesis and the influence of antibiotics and probiotics. Scand J Gastroenterol Suppl 29-40.

4. Russel MG, Stockbrügger RW (1996) Epidemiology of inflammatory bowel disease: an update. Scand J Gastroenterol 31: 417-427.

5. Eckburg PB, Relman DA (2007) The role of microbes in Crohn's disease. Clin Infect Dis 44: 256-262.

6. Strober W, Fuss I, Mannon P (2007) The fundamental basis of inflammatory bowel disease. J Clin Invest 117: 514-521.

7. Sartor RB (2006) Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol 3: 390-407.

8. Schultz M, Tonkonogy SL, Sellon RK, Veltkamp C, Godfrey VL, et al. (1999) IL-2-deficient mice raised under germfree conditions develop delayed mild focal intestinal inflammation. Am J Physiol 276: G1461- G1472.

9. Sartor RB (2008) Microbial influences in inflammatory bowel diseases. Gastroenterology 134: 577-594.

10. Collins MT (2003) Paratuberculosis: review of present knowledge. Acta Vet Scand 44: 217-221.

11. Harris NB, Barletta RG (2001) Mycobacterium avium subsp. paratuberculosis in Veterinary Medicine. Clin Microbiol Rev 14: 489-512.

12. Autschbach F, Eisold S, Hinz U, Zinser S, Linnebacher M, et al. (2005) High prevalence of Mycobacterium avium subspecies paratuberculosis IS900 DNA in gut tissues from individuals with Crohn's disease. Gut 54: 944-949.

13. Naser SA, Schwartz D, Shafran I (2000) Isolation of Mycobacterium avium subsp paratuberculosis from breast milk of Crohn's disease patients. Am J Gastroenterol 95: 1094-1095.

14. Naser SA, Ghobrial G, Romero C, Valentine JF (2004) Culture of Mycobacterium avium subspecies paratuberculosis from the blood of patients with Crohn's disease. Lancet 364: 1039-1044.

15. Shafran I, Piromalli C, Decker JW, Sandoval J, Naser SA, et al. (2002) Seroreactivities against Saccharomyces cerevisiae and Mycobacterium avium subsp. paratuberculosis p35 and p36 antigens in Crohn's disease patients. Dig Dis Sci 47: 2079-2081.

16. Chamberlin W, Graham DY, Hulten K, El-Zimaity HM, Schwartz MR, et al. (2001) Review article: Mycobacterium avium subsp. paratuberculosis as one cause of Crohn's disease. Aliment Pharmacol Ther 15: 337-346.

17. Feller M, Huwiler K, Stephan R, Altpeter E, Shang A, et al. (2007) Mycobacterium avium subspecies paratuberculosis and Crohn's disease: a systematic review and meta-analysis. Lancet Infect Dis 7: 607-613.

18. Ryan P, Kelly RG, Lee G, Collins JK, O'Sullivan GC, et al. (2004) Bacterial DNA within granulomas of patients with Crohn's disease--detection by laser capture microdissection and PCR. Am J Gastroenterol 99: 1539-1543.

19. Mylonaki M, Rayment NB, Rampton DS, Hudspith BN, Brostoff J (2005) Molecular characterization of rectal mucosa-associated bacterial flora in inflammatory bowel disease. Inflamm Bowel Dis 11: 481-487.

20. Darfeuille-Michaud A, Boudeau J, Bulois P, Neut C, Glasser AL, et al. (2004) High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease. Gastroenterology 127: 412-421.

21. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, et al. (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 411: 603-606.

22. Hisamatsu T, Suzuki M, Reinecker HC, Nadeau WJ, McCormick BA, et al. (2003) CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. Gastroenterology 124: 993-1000.

23. Ewaschuk JB, Dieleman LA (2006) Probiotics and prebiotics in chronic inflammatory bowel diseases. World J Gastroenterol 12: 5941-5950.

24. Larrosa M, Yañéz-Gascón MJ, Selma MV, González-Sarrías A, Toti S, et al. (2009) Effect of a low dose of dietary resveratrol on colon microbiota, inflammation and tissue damage in a DSS-induced colitis rat model. J Agric Food Chem 57: 2211-2220.

25. Dolara P, Luceri C, De Filippo C, Femia AP, Giovannelli L, et al. (2005) Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. Mutat Res 591: 237-246.

26. Fooks LJ, Gibson GR (2002) Probiotics as modulators of the gut flora. Br J Nutr 88: S39- S49.

27. Candela M, Perna F, Carnevali P, Vitali R, Ciati P, et al. (2008) Interaction of probiotic Lactobacillus and Bifidobacterium strains with human intestinal epithelial cells: adhesion properties, competition against enteropathogens and modulation of IL-8 production. Int J Food Microbiol 125: 286-292.

28. Queipo-Ortuño MI, Boto-Ordóñez M, Murri M, Gomez-Zumaquero JM, Clemente-Postigo M, et al. (2012) Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. Am J Clin Nutr 95: 1323-1334.

29. Mouser JF, Hyams JS (1999) Infliximab: a novel chimeric monoclonal antibody for the treatment of Crohn's disease. Clin Ther 21: 932-942.

30. Head K, Jurenka JS (2004) Inflammatory bowel disease. Part II: Crohn's disease--pathophysiology and conventional and alternative treatment options. Altern Med Rev 9: 360-401.

31. Gholam PM, Flancbaum L, Machan JT, Charney DA, Kotler DP (2007) Nonalcoholic fatty liver disease in severely obese subjects. Am J Gastroenterol 102: 399-408.

32. Chan MM (2002) Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin. Biochem Pharmacol 63: 99-104.

33. Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, et al. (2006) Resveratrol improves health and survival of mice on a high-calorie diet. Nature 444: 337-342.

34. Rahman I, Biswas SK, Kirkham PA (2006) Regulation of inflammation and redox signaling by dietary polyphenols. Biochem Pharmacol 72: 1439-1452.

35. Lee KW, Lee HJ (2006) The roles of polyphenols in cancer chemoprevention. Biofactors 26: 105-121.

36. Tredici G, Miloso M, Nicolini G, Galbiati S, Cavaletti G, et al. (1999) Resveratrol, map kinases and neuronal cells: might wine be a neuroprotectant? Drugs Exp Clin Res 25: 99-103.

37. Petro TM (2011) Regulatory role of resveratrol on Th17 in autoimmune disease. Int Immunopharmacol 11: 310-318.

38. Martín AR, Villegas I, La Casa C, de la Lastra CA (2004) Resveratrol, a polyphenol found in grapes, suppresses oxidative damage and stimulates apoptosis during early colonic inflammation in rats. Biochem Pharmacol 67: 1399-1410.

39. Martín AR, Villegas I, Sánchez-Hidalgo M, de la Lastra CA (2006) The effects of resveratrol, a phytoalexin derived from red wines, on chronic inflammation induced in an experimentally induced colitis model. Br J Pharmacol 147: 873-885.

40. Biasi F, Astegiano M, Maina M, Leonarduzzi G, Poli G (2011) Polyphenol supplementation as a complementary medicinal approach to treating inflammatory bowel disease. Curr Med Chem 18: 4851-4865.

41. Singh UP, Singh NP, Singh B, Hofseth LJ, Price RL, et al. (2010) Resveratrol (trans-3,5,4'-trihydroxystilbene) induces silent mating type information regulation-1 and down-regulates nuclear transcription factor-kappaB activation to abrogate dextran sulfate sodium-induced colitis. J Pharmacol Exp Ther 332: 829-839.

42. Singh UP, Singh NP, Singh B, Hofseth LJ, Taub DD, et al. (2012) Role of resveratrol-induced CD11b(+) Gr-1(+) myeloid derived suppressor cells (MDSCs) in the reduction of CXCR3(+) T cells and amelioration of chronic colitis in IL-10(-/-) mice. Brain Behav Immun 26: 72-82.

43. Cui X, Jin Y, Hofseth AB, Pena E, Habiger J, et al. (2010) Resveratrol suppresses colitis and colon cancer associated with colitis. Cancer Prev Res (Phila) 3: 549-559.