Discussion on Toll-like Receptor 9 Signaling Mediates the Anti-inflammatory Effects of Probiotics in Murine Experimental Colitis

Dear Sir:

We have some concerns with the Rachmilewitz et al. article published in GASTROENTEROLOGY recently, that concluded “live microorganisms are not required to attenuate experimental colitis.”

Animal models per se have limitations, particularly when the sample size is limited (here <10 per group). The dextrone sodium sulfate model can be difficult to interpret. Was mild disease created and if so how much inflammation was ameliorated, in terms of clinically relevant colitis? The low MPO levels are inconsistent with pancreatic secretion, very rich in feature in this model characterized by several degrees of crypt damage.

Probiotics are defined as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host.” As such, strains that do not confer a benefit, for example in treating colitis, would not be termed probiotic for that application. This might seem a petty comment, but in practical terms it is critical that healthcare professionals know exactly what it means to be a probiotic. Hence, interpreting these animal data in relation to a disease in humans.

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The aim of our investigations was to explore the mechanism of actions of probiotics in models of experimental colitis. Regarding these mechanisms, our studies provide genetic, immunologic, and biochemical evidence, rather than opinionated scholarship. Since in this work we demonstrated that immunostimulatory properties of the bacterial DNA are responsible for the anti-inflammatory effects mediated by viable probiotic bacteria, it was unavoidable to reach other conclusions than those that we had raised. The current definition of probiotics as live bacteria was not being challenged by our study. The mechanisms attributed to probiotics were challenged. Below are specific comments in response to several of the points raised in the letter:

Because of the drawbacks of every model of experimental colitis, including DSS induced colitis, our work showed that the probiotic DNA is effective also in TNBS--induced colitis and in spontaneous colitis in IL-10 knockout mice. The fact that oral administration of the probiotic DNA was effective rules out a complete efficacy of pancreatic DNase. In fact, this issue was tested in Figure 3A. The data presented in this Figure indicate the percentage of the oral dose of naked pDNA that is absorbed could be detected at systemic sites. For the delivery of irradiated probiotics, we assumed an additional protective role for the probiotic bacterial cell wall on probiotic DNA from enzymatic digest by pancreatic DNase. Therefore, the probiotic DNA is available to mediate its effect (see Figure 3C). These results are in contrast to an in vitro enzymatic activity of DNase, which was titrated to provide a complete digest of the reaction’s substrate (probiotic DNA).

The activation of NF-kB pathway (Figure 1) was presented in bone marrow--derived macrophages and was used in order to show that probiotic DNA has immunostimulatory properties. We selected the bacterial preparation VSL-3 only because of the solid published clinical data with this preparation. To demonstrate that our findings do not relate only to this preparation, E. coli DNA was also used and shown to mediate similar properties as was shown for VSL-DNA. We agree with Reid that it is worthwhile to elaborate on whether all the strains in the published clinical data with this preparation. To demonstrate that our results provide a simple method to do so, i.e., evaluation of the immunostimulatory properties of each of the probiotic bacteria in this preparation.

Nowhere in the paper did we claim that unreliable probiotic preparations should be used to treat colitis.

We did not rule out possible involvement of other mechanisms including the secretion of IL-10 (see Discussion). However, Reid’s suggestion that induction of IL-10 may be involved in our studies is unlikely as our data indicate anti-inflammatory effects of probiotic DNA in IL-10 KO mice (Table 8).

We agree with Reid et al. that it is now important to demonstrate that viable probiotics, irradiated probiotics or synthetic ISS–ODN are effective in modulation of human disease such as IBD. We do believe that a critical scientific approach leads to new findings, adds knowledge, and provides insight. Only this discipline can turn the probiotic field from murky folklore to a solid and sound science and consequently to the expansion of probiotic uses for the benefit of humankind.


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