
doi:10.1053/j.gastro.2004.05.050

**Crohn’s Disease Is Associated With a Toll-like Receptor-9 Polymorphism**

Dear Sir:

In their stimulating article Rachmilewitz et al. convincingly demonstrate that the protective effect of probiotic bacteria in experimental colitis is mediated via toll-like receptor (TLR)-9 following recognition of immunostimulatory DNA. This work extends previous findings of the authors who described a preventive role of bacterial DNA in experimental and spontaneous murine colitis. Immunostimulatory DNA sequences denote unmethylated CpG motifs which are common in bacterial and viral DNA but are underrepresented in mammalian genomes. TLR9 is of paramount importance for the maturation of dendritic cells and the release of proinflammatory cytokines such as tumor necrosis factor, interleukin-6, and interleukin-12 from macrophages after exposure to unmethylated CpG-rich bacterial DNA. Although TLR9 is highly conserved across species, there are distinct species-specific sequence differences with respect to the optimal stimulatory CpG motifs, which imply that genetic variation in TLR9 could play a role in diseases like asthma. The gene encoding for TLR9 is mapped to chromosome 3p21.3. In view of the aforementioned observations and the findings of the authors who described a preventive role of bacterial DNA in experimental and spontaneous murine colitis, we hypothesized that genetic variation in TLR9 might be associated with inflammatory bowel disease.

We assessed the frequency of 2 recently described polymorphisms of the TLR9 gene (−1237 C/T and 2848 A/G) in 174 German patients with Crohn’s disease, 138 patients with ulcerative colitis, and 265 healthy blood donors who were matched with respect to gender and age. In previous studies, it had been shown that both polymorphisms unambiguously distinguish between the 4 most common TLR9 haplotypes in Caucasians. Genotyping was performed by polymerase chain reaction and restriction fragment length polymorphism analysis using the restriction enzymes BstN I for the −1237 T/C polymorphism and Nco I for the 2848 A/G polymorphism and confirmed by sequencing. Patients with Crohn’s disease were stratified according to the NOD2/CARD15 genotype. Statistical analysis was performed using the χ² test and Fisher exact test, respectively.

The frequency of the −1237 C allele and the C carrier status were significantly increased in patients with Crohn’s disease when compared to the controls (19.3% vs. 11.9%, \( P = 0.0036 \) and 34.5% vs. 22.6%, \( P = 0.0093 \), respectively) (Table 1). For the 2848 A/G polymorphism, no significant differences in the allele frequencies were noted between Crohn’s disease, ulcerative colitis, and controls. The frequency of the 2848 A/G genotype was slightly decreased in Crohn’s disease when compared to the controls (43.1% vs. 54%, \( P = 0.0328 \)). Among the study groups the genotype distribution for both polymorphisms corresponded to Hardy–Weinberg equilibrium. Associations between TLR9 polymorphisms and the presence of mutations in NOD2/CARD15 were not observed.

In summary, the association of Crohn’s disease with a promotor polymorphism in the TLR9 further sustains a pivotal role of bacterial DNA sensing in the pathophysiology of inflammatory bowel disease.

**HELGA–PAULA TORÖK**

Chirurgische Klinik und Poliklinik
Medizinische Poliklinik
Standort Innenstadt
Ludwig-Maximilians University
Munich, Germany

**JÜRGEN GLAS**

Medizinische Poliklinik
Standort Innenstadt
und Poliklinik für Zahnkrankheiten und Parodontologie
Ludwig-Maximilians University
Munich, Germany

**LAURIAN TONENCHI**

Medizinische Poliklinik
Standort Innenstadt
Ludwig-Maximilians University
Munich, Germany

**GUENTER BRUENNLER**

Labor für Immunogenetik
Kinderklinik und Kinderpoliklinik
Ludwig-Maximilians University
Munich, Germany

---

**Table 1.** Allele and Genotype Frequencies for the −1237 T/C and the 2848 G/A Polymorphisms of the TLR9 Gene

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Allele/genotype frequencies</th>
<th>Crohn’s disease (N = 174)</th>
<th>Ulcerative colitis (N = 138)</th>
<th>Controls (N = 265)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−1237 T/C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td>C (67/19.3%)</td>
<td>39 (14.1%)</td>
<td>63 (11.9%)</td>
<td></td>
</tr>
<tr>
<td>frequencies</td>
<td>T (281/80.7%)</td>
<td>237 (85.9%)</td>
<td>467 (88.1%)</td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>7 (4%)</td>
<td>3 (2.2%)</td>
<td>3 (1.1%)</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>53 (30.5%)</td>
<td>33 (23.9%)</td>
<td>57 (21.5%)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>114 (65.5%)</td>
<td>102 (73.9%)</td>
<td>205 (77.4%)</td>
<td></td>
</tr>
<tr>
<td>2848 G/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td>G (153/44%)</td>
<td>120 (43.5%)</td>
<td>227 (42.8%)</td>
<td></td>
</tr>
<tr>
<td>frequencies</td>
<td>A (195/56%)</td>
<td>156 (56.5%)</td>
<td>303 (57.2%)</td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>39 (22.4%)</td>
<td>22 (15.9%)</td>
<td>41 (15.5%)</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>75 (43.1%)</td>
<td>76 (55.1%)</td>
<td>144 (54.3%)</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>60 (34.5%)</td>
<td>40 (29%)</td>
<td>80 (30.2%)</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** The offsets for the polymorphisms were calculated taking the A of the TLR9 ATG start codon as position 1 based on GenBank Accession No. NM_017442, as described by Lazarus et al.

\(^{a}P \leq 0.01.\)

\(^{b}P \leq 0.05.\)
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.1 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 dextrose 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 the severity of the histological scores. The authors scoring system clinically relevant colitis? The low MPO levels are inconsistent with the severity of the histological scores. The authors scoring system characterized by several degrees of crypt damage.

The study has merit in describing a potential mechanism of action of probiotic strain extracts, but the findings cannot rule out other attributes of probiotics, including by-products not found in VSL#3 strains, and immunomodulatory activity induced via other pathways, such as IL-10 synthesis and secretion. The primary author previously failed to show a remediation of immune-mediated dinitrobenzene sulfonic acid-induced colitis,2 again illustrating that clinical studies are needed before over-interpreting these animal data in relation to a disease in humans.

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.

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. By defining a product or active strain properly, and proving efficacy along with mechanism of action, we will collectively advance this important emerging field, and determine how best to apply the knowledge to retention and restoration of health in our patients.

Numerous publications and trade journals have picked up the press release that followed this paper, including Nature online,10 who refused a rebuttal, and taken literally the concluding message that viable probiotics are “not required.” Sadly, this is to the detriment of probiotic research generally and at a time when it is turning the corner from a somewhat murky folklore to a scientific and clinically sound field offering new light in the many dark tunnels of intestinal and other diseases.

MATTHIAS FOLWACZNY
Poliklinik für Zahnheilkunde und Parodontologie
Ludwig-Maximilians University
Munich, Germany

CHRISTIAN FOLWACZNY
Medizinische Poliklinik
Chirurgische Klinik und Poliklinik
Standort Innenstadt
Ludwig-Maximilians University
Munich, Germany


6. Rudensky B, Akira S, Takeda K, Raz E, immumomodulatory activity induced via other pathways, such as IL-10 synthesis and secretion. The primary author previously failed to show a remediation of immune-mediated dinitrobenzene sulfonic acid-induced colitis, again illustrating that clinical studies are needed before over-interpreting these animal data in relation to a disease in humans.
