Interleukin 10 genotypes in irritable bowel syndrome: evidence for an inflammatory component?

W M Gonsalkorale, C Perrey, V Pravica, P J Whorwell, I V Hutchinson

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Background and aims: Inflammation may play a role in the pathogenesis of irritable bowel syndrome in some individuals, such as in those who develop symptoms following a dysenteric illness. Persisting inflammation, resulting from an imbalance of cytokines regulating the inflammatory response, is one possible mechanism. As the elaboration of cytokines is under genetic control, this study was designed to establish whether there might be a genetic predisposition to an altered pattern of anti-inflammatory cytokine production in patients with irritable bowel syndrome.

Subjects: A total of 230 unselected patients with irritable bowel syndrome and 450 healthy, ethnically matched controls were studied.

Methods: DNA was extracted from peripheral blood leucocytes of subjects. Allele and genotype frequencies were determined for the anti-inflammatory cytokine interleukin 10 at the site (–1082) concerned with production in lymphocytes. Transforming growth factor β (codons 10 and 25) genotypes were also examined in a smaller group of subjects.

Results: Patients with irritable bowel syndrome had significantly reduced frequencies of the high producer genotype for interleukin 10 than controls (21% v 32%; p=0.003). There was no apparent relationship with any particular bowel habit subtype. Genotypes for transforming growth factor β, were not altered.

Conclusions: These preliminary results suggest that at least some patients with irritable bowel syndrome may be genetically predisposed to produce lower amounts of the anti-inflammatory cytokine interleukin 10. This lends some support to the hypothesis that there may be an inflammatory or genetic component in some cases of this condition and that further studies in specific irritable bowel syndrome subgroups are justified.
Genotyping methods
Genomic DNA was extracted from peripheral blood leukocytes and genotyping carried out by amplification refractory mutational system-polymerase chain reaction methods, as previously described, using allele specific primers to identify the high and low producer alleles of each biallelic polymorphic site of the IL-10 gene (−1082*G and −1082*A, respectively) and the TGF-β gene (−869*T and +869*C, respectively, codon 10; +915*G and +915*C, respectively, codon 25). Amplified DNA products were then analysed using electrophoresis on 2% agarose gel and viewed under UV light.

Statistical analysis
Allele frequencies for patients and controls were compared by calculation of the odds ratio (OR) and 95% confidence intervals (95% CI). Genotypes were calculated for each individual, with those homozygous for the high producer allele classed as “high producer”, heterozygotes as “intermediate”, and those homozygous for the low producer allele as “low producer” genotype, respectively.

Table 1

<table>
<thead>
<tr>
<th>Allele Frequency§</th>
<th>IBS (n=230)</th>
<th>Controls (n=450)</th>
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<tbody>
<tr>
<td>−1082*G (High)</td>
<td>219 48%</td>
<td>438 52%</td>
</tr>
<tr>
<td>−1082*A (Low)</td>
<td>241 52%</td>
<td>412 48%</td>
</tr>
<tr>
<td>Genotype¶</td>
<td></td>
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<tr>
<td>G/G (High producer)</td>
<td>49 21%</td>
<td>135 32%</td>
</tr>
<tr>
<td>G/A (Intermediate)</td>
<td>121 53%</td>
<td>168 39%</td>
</tr>
<tr>
<td>A/A (Low producer)</td>
<td>60 26%</td>
<td>122 29%</td>
</tr>
<tr>
<td>G/− (G/A)</td>
<td>121 53%</td>
<td>168 39%</td>
</tr>
<tr>
<td>A+/A (A/A or A/G)</td>
<td>181 79%</td>
<td>290 68%</td>
</tr>
</tbody>
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*Odds ratio (OR) 1.17 (95% confidence interval 0.93–1.49), p=0.097.
†Fewer irritable bowel syndrome (IBS) patients with high producer (G/G) genotype (p=1.197, df=2, p=0.003), with more patients carrying A allele (A+ G/A or A/A) (χ²=8.08, OR 1.72 (1.18–2.50), p=0.004)‡.
§n=number of individuals with specific genotype.
¶n=number of individuals with specific genotype.

RESULTS
Table 1 shows allele and genotype frequencies for IL-10. The frequency of the high producer −1082*G allele was slightly lower in IBS patients but this did not reach statistical significance (IBS v controls: 48% v 52%; p=0.097). However, genotype frequencies were found to differ in that there were significantly fewer IBS patients with the high producer (−1082*G/G) genotype (IBS v controls: 21% v 32%; p=0.003), and with an increased proportion of patients being positive for the A allele, being either the low producer genotype −1082*A/A or the heterozygous −1082*G/A (IBS v controls: 79% v 68%; p=0.004). No comparison between bowel habit subtypes was possible because of insufficient numbers in each subgroup. However, there was no obvious relationship between a reduced high producer genotype and a particular pattern of bowel habit.

Allele and genotype frequencies for TGF-β, were no different in IBS patients and controls either at codon 10 (IBS v controls: allele frequency: +869T (high producer allele): 61% v 63%, OR 0.91 (95% CI 0.63–1.30), p=0.593; genotype frequencies: +869T/T: 39% v 39%; T/C: 44% v 48%; C/C: 17% v 13%; χ²=1.07, p=0.584) or at codon 25 (IBS v controls: allele frequency: +915G (high producer allele): 91% v 91%, OR 1.05 (0.37–1.97), p=1.00; genotype frequencies: +915G/G: 84% v 82%; G/C: 13% v 17%; C/C: 3% v 1%; χ²=2.22, p=0.330).

DISCUSSION
The results of this study demonstrate a significant association between IL-10 genotypes and IBS, with fewer patients having the high producer genotype compared with healthy controls. The lower prevalence of the high producer genotype in IBS suggests that high production of IL-10 may have some protective role or, conversely, that individuals predisposed to produce lower amounts of this cytokine might be more likely to develop the condition. A genetic predisposition to lower anti-inflammatory cytokine production could mean that control of the inflammatory response may be compromised in some individuals and may help to explain why gastrointestinal infections, for example, can sometimes lead to continuing problems. It is possible that an inflammatory process is perpetuated by failure of downregulation secondary to an inadequate anti-inflammatory cytokine response.

There are a number of points however that need to be considered in the interpretation of these findings. IL-10 is only one of the anti-inflammatory cytokines involved in regulation of immune and inflammatory responses, and the possible involvement of other cytokines in the inflammatory process cannot be ruled out. However, examination of allelic and genotypic frequencies for the biallelic polymorphisms of TGF-β, albeit on a smaller number of patients, did not reveal any significant differences from healthy controls. The IL-10 gene does have a number of different polymorphisms but the site selected in this study (−1082) is known to influence IL-10 production in lymphocytes, and differences in IL-10 levels have been measured in serum and from activated peripheral blood cells. Lymphocytes have been found in excessive numbers in the intestinal mucosa in cases of post infective irritable bowel syndrome (PI-IBS) but the question of whether in vivo IL-10 production within the intestine is similarly influenced by genotype remains to be addressed.

IBS is almost certainly a multifactorial condition with different combinations of factors operating in any one individual. Thus it is likely that a certain facet of the condition under genetic control will only account for a relatively small proportion of patients. Furthermore, if that facet has no biological marker, identifying appropriate subgroups for more indepth evaluation presents difficulties. At first sight, PI-IBS might seem a relatively straightforward group to study but this will inevitably contain patients with pre-existing forms of IBS as well as de novo cases, either of which might have the genetic trait under scrutiny. Similar pitfalls could accompany another potentially fruitful line of enquiry—that is, those patients with a family history. It is highly likely that this group could contain patients in whom learning from parents is just as important as inheritance from the same. This study was not powered to assess an association between our findings and any particular characteristic of the condition, such as diarrhoea, constipation, bloating, pain, or even apparent precipitation by dysentery. However, the fact that in such an unselected group of patients a significant trend emerged strongly suggests that it might contain a subgroup in whom genes controlling inflammation might be important and this warrants further investigation.

Future studies on PI-IBS will have to be very carefully designed to ensure homogeneity of the groups under study as far as possible. This should include documenting the infecting organism, not forgetting viruses, considering measurement of markers of inflammation, both serological and mucosal, and possibly assessing gastrointestinal physiology or mucosal permeability. In addition, the role of dietary antigens may also have to be taken into account.
In conclusion, the association found between IL-10 genotype frequencies in an unselected group of IBS patients suggests there may be a subgroup of patients in whom this genotype is critically important to the development of their disease. In addition, these results provide some support for the concept that genetic factors may also contribute to the pathogenesis of this condition.

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Authors’ affiliations
W M Gonsalkorale, P J Whorwell, Department of Medicine, University Hospital of South Manchester, Nell Lane, Manchester M20 2LR, UK
C Perrey, V Pravica, I V Hutchinson, Immunology Research Group, School of Biological Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, UK

REFERENCES