INFLAMMATORY BOWEL DISEASE

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

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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_1 (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_1 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.

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I rritable bowel syndrome (IBS) is a multifactorial disorder and a number of pathophysiological mechanisms have been proposed, including abnormalities of motility,[1 2] visceral sensation,[3] and even alterations of gut fermentation.[4] In addition, psychological factors are important in at least some patients.[5] There is also a familial tendency to IBS and, although this could be psychosocial in origin, a genetic influence is a possibility.

More recently, it has been proposed that inflammation may play a role in IBS, based on several lines of evidence.[6] For instance, increased numbers of mast cells have been found in the terminal ileum of IBS patients[7 8] and some patients with ulcerative colitis have IBS-like symptoms when in remission.[9] In addition, a substantial proportion of cases are apparently precipitated by gastrointestinal infection[10–12] where a continuing inflammatory response has been demonstrated.[13]

The production of cytokines is under genetic control, and these molecules are involved in the regulation of immune and inflammatory responses. Some, including tumour necrosis factor α and interferon γ, are proinflammatory while others, such as interleukin 10 (IL-10) and transforming growth factor β_1 (TGF-β_1), have anti-inflammatory properties. By studying the polymorphisms in the genes coding for these cytokines, alleles associated with high or low production ("high" or "low" producer alleles) have been identified.[14–17] Thus individuals can be divided into high, intermediate, and low producers of a particular cytokine, those who are homozygous for the high producer allele making the highest amounts, those homozygous for the low producer allele the least, while heterozygotes have intermediate levels.

The genetic predisposition to produce higher or lower amounts of a particular cytokine may alter susceptibility to certain diseases or affect their clinical expression. For example, possession of the high producer allele at position −308 in the gene coding for tumour necrosis factor α is associated with worse outcome in cerebral malaria.[18] Mice lacking the IL-10 gene spontaneously develop a form of enterocolitis.[19] We have previously shown that patients with ulcerative colitis are more likely to have genotypes associated with lower IL-10 production.[20]

The aim of this study therefore was to test the hypothesis that if there is an inflammatory component in some cases of IBS, there may be a genetic predisposition for an altered pattern of production of the anti-inflammatory cytokines IL-10 and TGF-β_1 in such individuals.

METHODS

Study subjects

Blood samples were obtained, after informed consent, from 230 consecutive patients fulfilling the Rome I criteria for IBS[21] attending the Gastroenterology Out-Patients Clinic. Patients were of all bowel habit subtypes, 24% being constipation predominant, 38% being diarrhoea predominant, and a further 38% having an alternating pattern. A total of 450 ethnically matched controls were used for comparison. Initially, only the IL-10 genotype was assessed but subsequently preliminary screening of TGF-β_1 genotypes was also carried out on 134 patients and 127 controls. The study was approved by the local ethics committee.

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Abbreviations: IL-10, interleukin-10; IBS, irritable bowel syndrome; TGF-β_1, transforming growth factor β_1; OR, odds ratio; PI-IBS, post infective irritable bowel syndrome.

Table 1 Interleukin 10 (IL-10) (−1082) polymorphism allele and genotype distribution

	IBS (n=230)		Controls (n=450)	
	n	%	n	%
IL-10				
Allele frequency§				
−1082*G (high)	219	48*	438	52
−1082*A (low)	241	52	412	48
Genotype¶				
G/G (high producer)	49	21†	135	32
G/A (intermediate)	121	53	168	39
A/A (low producer)	60	26	122	29
Genotype¶				
A− (G/G)	49	21‡	135	32
A+ (A/A or G/A)	181	79	290	68

*Odds ratio (OR) 1.17 (95% confidence interval 0.93–1.49); p=0.097.
†Fewer irritable bowel syndrome (IBS) patients of high producer (G/G) genotype (χ^2=11.97, df=2, p=0.003), with more patients carrying A allele (A+: G/A or A/A) (χ^2=8.08, OR 1.72 (1.18–2.50), p=0.004)‡.
§n= number of alleles in each group, being twice the number of individuals as two alleles are inherited.
¶n=number of individuals with specific genotype.

Genotyping methods

Genomic DNA was extracted from peripheral blood leucocytes and genotyping carried out by amplification refractory mutational system-polymerase chain reaction methods, as previously described,[22 23] 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_1 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 producer", and those homozygous for the low producer allele as "low producer" genotype, respectively.[15 19] Genotype frequencies were then compared by χ^2 analysis.

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_1 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%; χ^2=1.07, p=0.584) or at codon 25 (IBS v controls: allele

frequency: +915G (high producer allele): 91% v 91%, OR 1.05 (0.57–1.97), p=1.00; genotype frequencies: +915G/G: 84% v 82%; G/C: 13% v 17%; C/C: 3% v 1%; χ^2=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_1, 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.[15 20 24] Lymphocytes have been found in excessive numbers in the intestinal mucosa in cases of post infective irritable bowel syndrome (PI-IBS)[13] 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 forme fruste 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 gene 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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