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The Role of Mannan-Binding Lectin (MBL) Gene Polymorphism in Ulcerative Colitis

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Summary **Series studies suggest that enteropathogenic microorganisms play a substantial role in the clinical initiation and relapses of ulcerative colitis (UC). Mannan-binding lectin (MBL) is an important constituent of the innate immune system, and deficiency of MBL has been reported to increase the overall susceptibility of an individual to infectious disease. This study was aimed to investigate the associations between polymorphisms of the MBL gene and UC. Recruited in this study were 108 Japanese patients with UC and 144 healthy control subjects. Polymorphism at codon 54 of exon 1 of the MBL gene was investigated by polymerase chain reaction based restriction fragment length polymorphism. In general, no significant difference in MBL polymorphism was found between UC patients and health controls. However, the frequency of A carriers was significantly higher in the relapsing cases than controls (Odds ration = 2.19, 95%CI, 1.10–4.34; $p = 0.023$), and similar tendency was also found in A/A genotype. In conclusion, the polymorphism at codon 54 of exon 1 of the MBL gene associated with the susceptibility to the relapsing phenotype of ulcerative colitis. It suggests that codon 54 A variants of MBL gene may have an increased risk for the flare-ups of UC.**

Key Words: **genetic polymorphism, mannan-binding lectin, ulcerative colitis**

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD), the primary constituents of inflammatory bowel disease (IBD), are precipitated by a complex interaction of environmental, genetic, and immunoregulatory factors [*1*, *2*]. However, the pathogenesis of IBD is only partially understood. Some genes are associated with IBD itself, while others increase the risk of UC or CD are associated with disease phenotypes [*3*, *4*]. Both UC and CD are characterized by intestinal inflammation mainly caused by a disturbance in the balance between cytokines and increased complement (C) activation [*5*, *6*].

Mannan-binding lectin (MBL) is an important constituent of the innate immune system and one of the proteins of the complement system [*7*]. Research over the past decade indicates that MBL provides a distinct third pathway of complement activation, and phygiologenetic studies suggest that it may have been the first such pathway to have evolved [*8–10*]. The biological importance of this pathway is also indicated by the clinical phenotypes of the deficiency state. In many human populations, MBL deficiency is relatively common and there have been a large number of studies attempting to link the deficiency state with particular clinical presentations [*11–13*]. This study was designed to investi-

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gate the association between polymorphisms of the MBL gene and the pathogenesis of chronic UC in Japanese population.

Materials and Methods

Patients and controls

A total of 108 chronic UC patients (57 male and 51 female, mean age, 39.3 ± 11.6 years) and 144 health control subjects (85 male and 59 female, mean age, 36.1 ± 13.5 years) were recruited in the study. Blood or colonic mucosal biopsy samples were obtained from all the subjects in Fujita Health University Hospital from April to August 2006. The diagnosis of UC was made on the basis of clinical manifestations, endoscopic findings as well as histopathological examinations according to the conventional criteria. Informed consent was obtained from all subjects after a full explanation of the project, and the specimen collecting procedures were approved by the Ethics Committee in Fujita Health University.

Classification

According to their clinical courses, chronic UC cases were classified into one episode, relapsing and continuous phenotypes [*14*]. UC were also classified as pancolitis or distal colitis according to the location and extension of the inflammatory lesions judged by endoscopic findings. Moreover, cases that need continual intravenous or oral steroid therapy were identified as steroid dependent, and those had one onset over 6 months or 2 onsets within one year were defined as refractory cases.

Genotyping for MBL point mutation

Point mutations at codon 54 of exon 1 of the *MBL* gene were detected by polymerase chain reaction (PCR) based restriction fragment length polymorphism [*15*]. In brief, PCR was performed using a total of 50 μl of the following mixture: 200 ng of genomic DNA, 1 unit Taq DNA polymerase, 50 pmol of each primer, 200 ng of each dNTP and 1.5 mM Mg^{2+}. The codon 54 of exon 1 of the *MBL* was amplified by the primers (5'-ccttccctgagttttcacac-3' and 5'-atcagtctcctcatatcccc-3') under the following conditions: an initial denaturation at 95°C for 5 min, followed by 38 cycles of 95°C for 40 sec, 55°C for 30 sec, and 72°C for 40 sec. The final extension step was prolonged to 5 min.

The 298 bp PCR product (10 μl) was cleaved by *Ban I* (New England Biolabs. Inc., Bevely, MA) in an appropriate buffer at 37°C for 2 hours, resulting in two fragments of 195 bp and 103 bp for 54G/G, three bands (298 bp, 195 bp and 103 bp) for 54G/A (heterozygote), and a single band of 298 bp in the case of 54A/A (homozygote).

Statistic evaluation

The data of age was expressed as mean ± SD. Allele and genotype frequencies were calculated by direct counting. The allele counts were compared between UC and its subgroups and health controls by a 2×2 table using Chi-squared test. Furthermore, the strength of association between allele frequencies and the disease was assessed by calculating the odds ration (OR) and 95% confidence intervals (CI). Mean ages between 2 groups were compared with Student's *t* test. A probability value of less than 0.05 was considered statistically significant.

Results

The results of electrophoresis using clinical samples are shown in Fig. 1. All of the genotypes were clearly identified. A total of 108 UC patients and 144 health control subjects were recruited for the study. The characteristics and the allele frequency of the MBL gene were shown in Table 1. There were no significant differences in age and sex ratio between the 2 groups. Polymorphism at codon 54 of exon 1 of the MBL gene was typed in all 252 subjects. The frequency of MBL genotypes in the control group did not deviate significantly from the results expected under the Hardy-Weinberg equilibrium ($p = 0.39$).

Statistic comparison of genotype frequencies was carried

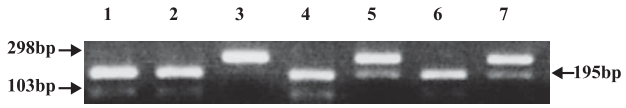

Fig. 1. *Detection of polymorphism at codon 54 of exon 1 of the MBL gene by the PCR-RFLP assay.* The 298 bp PCR product was cleaved by *Ban I* at 37°C for 2 hours, resulting in two fragments of 195 bp and 103 bp for 54G/G (lane 1,2,4 and 6), three bands (298 bp, 195 bp and 103 bp) for 54G/A (heterozygote, lane 5 and 7), and a single band of 298 bp in the case of 54A/A (homozygote, lane 3).

Table 1. Characteristics of the subjects and allelic frequency

	HC group	UC group
number of sample	144	108
mean age ± SD	36.1 ± 13.5	39.3 ± 11.4
(age of onset)		(31.2 ± 12.8)
male:female	85:59	57:51
MBL genotype		
G/G	96	63
G/A	45	41
A/A	3	4
A allele frequency	17.7%	22.7%

Table 2. The association between MBL gene polymorphism and ulcerative colitis

	Genotype (*n*)			A/A vs G/G	A carrier vs G/G
	G/G	G/A	A/A	OR (95%CI)	OR (95%CI)
Overall					
HC group (144)	96	45	3	reference	reference
UC group (108)	63	41	4	2.30 (0.44–9.39)	1.43 (0.85–2.39)
Male					
HC group (85)	54	29	2	reference	reference
UC group (57)	35	20	2	1.54 (0.21–11.5)	1.09 (0.55–2.19)
Female					
HC group (59)	42	16	1	reference	reference
UC group (51)	28	21	2	3.00 (0.26–34.7)	2.03 (0.92–4.46)

Table 3. The association between MBL polymorphism and phenotype of ulcerative colitis

Variables (n)	A/A vs G/G	A carrier vs G/G
	OR (95% CI)	OR (95% CI)
HC group (144)	reference	reference
Age of Onset		
20≥(19)	ND	1.80 (0.69–4.72)
>20 (89)	2.42 (0.52–12.2)	1.36 (0.79–2.35)
Clinical type		
One episode (9)	ND	0.89 (0.26–3.13)
Relapsing (44)	4.57 (0.86–24.2)	**2.19 (1.10–4.34)***
Continuous (51)	0.97 (0.10–9.65)	1.09 (0.56–2.13)
Extension		
Pancolitis (58)	0.91 (0.09–9.08)	1.31 (0.70–2.97)
Distal colitis (50)	3.43 (0.66–17.9)	1.64 (0.86–3.15)
Response to treatment		
steroid-dependent (28)	ND	1.78 (0.59–3.19)
refractory (38)	2.78 (0.44–17.6)	1.22 (0.58–2.58)

Note. *n*, number of samples; A carrier, A/A + A/G.; + ND, no data; *: $p = 0.023$

out between healthy control group and each subgroup of UC patients as well as UC overall (Table 2, 3). In general, there were no significant difference in the frequencies of A/A and A/G genotypes at codon 54 of exon 1 of the MBL gene between healthy control group and patients with UC (OR, 2.03; 95%CI, 0.44–9.39; and OR, 1.43; 95%CI, 0.85–2.39, respectively). However, the frequency of A carriers in the relapsing cases was significantly higher than that in controls (OR, 2.19; 95%CI, 1.10–4.34; $p = 0.023$), and similar tendency was also found in A/A carrier ($p = 0.053$). No significant association was found between the frequencies of genotypes of the MBL gene with the rest clinical features such as gender, age, age of the first onset, or extension of colitis.

Discussion

Heterogeneity of disease susceptibility in humans and rodents suggest that multiple mechanisms are responsible for the etiology of IBD. In particular, deficiencies in anti-inflammatory and immune-suppressive mechanisms play an important role in the development of the disease [*16–18*]. Therefore, IBD is thought to result from a dysregulated interaction between the host immune system and its commensal microflora [*19*, *20*]. However, it is unknown how the enteric microflora stimulates the immune system and how this response is regulated.

MBL is a pattern recognition molecule of the innate immune system. It belongs to the collectin family of proteins in which lectin domains are found in association with collagenous structures [*7*, *21*]. This permits the protein to

interact with a wide selection of viruses, bacteria, yeasts, fungi and protozoa decorated with such sugars. Unlike the other collectins, MBL bound to microbial surfaces is able to activate the complement system in an antibody and C1-independent manner [*22*]. This activation is mediated by complexes of MBL with MBL-associated serine protease 2 (MASP-2), which specifically cleaves C4 and C2 to create a C3 convertase. MBL may also interact directly with cell surface receptors and thereby promote opsonophagocytosis by a complement-independent pathway [*23–25*]. Research over the past decade has shown that MBL plays an important role in the first hours/days of any primary immune response to a sugar-decorated pathogen. This provides the host with a first-line of defense before the adaptive immune system becomes operative and in humans may be particularly important between 6 and 18 months of age when the adaptive system is still immature [*26–28*]. MBL deficiency arises primarily from three single nucleotide polymorphisms in codons 52, 54 and 57 of exon 1 of the MBL-2 gene, which result in a failure to assemble fully functional multimeric protein [*29*, *30*]. These polymorphisms are associated with decreased MBL plasma concentrations and increased susceptibility to various infectious diseases [*31*]. If these MBL polymorphisms could lead to susceptibility to putative IBD-etiological microbial agents, or could temper the complement-mediated mucosal damage in IBD, MBL could function as the link between certain microbial, immunological and genetic factors in IBD [*32*, *33*].

Results from our study demonstrated that the frequency of polymorphism at codon 54 of exon 1 of the MBL gene was significantly higher in the relapsing cases than that in controls. It indicates that polymorphism of the MBL gene may be associated with an increased risk for the flare-ups of UC. The reason for the association between MBL gene polymorphism and relapsing of UC is not clear. Recent studies indicate that enteric pathogens could cause initial onset of UC and are associated with reactivation of quiescent disease. Despite their self-limited character, these infections initiate a cascade of inflammatory events leading to chronic, relapsing disease in a genetically susceptible host ("hit-and-run" hypothesis) [*34*, *35*]. Epidemiological and microbiologic studies also suggest that enteropathogenic microorganisms play a substantial role in the clinical initiation and relapses of IBD [*36*]. Based on these results, we presume that MBL gene polymorphism may increase susceptibility of an individual to enteric infection, which induces the relapse of inflammation in patients with chronic UC.

Rector *et al.* first reported that the MBL gene polymorphisms had a protective effect against UC but not CD [*37*]. Another study suggests that genetic variants of the MBL gene are associated with immune reactivity to mannans in CD [*5*]. We could not replicate the described association between the MBL allele and UC: It is possible that the association seen in other studies may be due to population stratification and different constitutions of patients, or to the *MBL* polymorphism being in linkage with the real disease-causing variant(s).

Conclusions

Overall, the polymorphism at codon 54 of exon 1 of the MBL gene was not significantly associated with the susceptibility to UC. However, the frequency of A allele carriers of this polymorphism was significantly higher in the relapsing cases than that in controls. It suggests that codon 54 A variants of MBL gene may have an increased risk for the flare-ups of UC.

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