Original article

Mannose Binding Lectin Gene Polymorphism and the Severity of Chronic Periodontitis

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summary

OBJECTIVE: Mannose binding lectin (MBL) is a key molecule in first line defense against various microorganisms. Polymorphism of the MBL gene greatly affects serum MBL concentration, and is known to be associated with occurrence of infectious diseases. We aimed to determine whether there is a relationship between occurrence or severity of chronic periodontitis (CP) and polymorphism of the MBL gene.

PATIENTS AND METHODS: Ninety-eight CP patients and 63 healthy subjects with no periodontitis were typed for the codon 54 polymorphism of the MBL gene by PCR–restriction fragment length polymorphism method.

RESULTS: Genotype frequencies of the codon 54 polymorphism were similar between patients and control subjects. When patients were categorized to mild, moderate and severe periodontitis groups, possession of the low serum MBL concentration allele was a risk factor for having severe CP, as assessed by logistic regression analysis.

CONCLUSION: Patients with MBL genotypes that cause lower serum MBL concentration may be at risk of having severe periodontitis. MBL gene typing may become useful to predict the prognosis of CP.

Key words——periodontium; genetics; immunology; infectious diseases

Introduction

Chronic periodontitis (CP) is considered as one of the most common infectious diseases. Bacteria consisting mainly of gram-negative anaerobes continuously reside in the affected lesions, causing prolonged inflammation and bone absorption that ultimately leads to premature loss of teeth. Factors that enhance the progression of CP include improper maintenance of oral hygiene, diabetes mellitus and smoking (for review, see ref. 1). Since the host immune system is thought to have a preventive role against progression of periodontitis, its dysfunction may be a risk factor for occurrence or increased severity of CP.

Mannose binding lectin (MBL) is a protein that plays a key role in innate immunity. MBL binds to carbohydrates on the surface of microorganisms and exerts an opsonin effect. In addition, MBL activates

the lectin pathway of the complement cascade²⁾. Thus, MBL is a protein actively involved in first line defense against infection. Many studies have described the association of MBL deficiency with various infectious diseases^{3~5)}. Since CP is caused by bacterial infection, we assumed that MBL may have a defensive role in the occurrence or progression of CP. Serum MBL concentration is greatly influenced by the single nucleotide polymorphisms (SNP) in the MBL gene. The MBL gene MBL2, located on the long arm of chromosome 10 at 10q11.2–q21, contains 4 exons⁶⁾. Codon 52(+223 C/T), 54(+230 G/A) and 57(+239 G/A) polymorphisms are all on exon 1, and presence of any of the minority alleles results in an amino acid substitution and significant reduction of serum MBL concentration^{7~9)}. We have previously confirmed that, in the Japanese population, the presence of codon 54 minority allele (allele B) is significantly associated with low serum MBL concentration, and that individuals homozygous for allele B has almost undetectable serum MBL¹⁰⁾. Codon 52 and 57 polymorphisms, common in some ethnicities, are not present or extremely rare in the Japanese¹⁰⁾. We reasoned that there might be some association between

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the codon 54 polymorphism of the MBL gene and occurrence or severity of CP.

To test this hypothesis, we genotyped the codon 54 polymorphism of the MBL gene, and compared allele frequencies and genotype frequencies between CP patients and healthy individuals. In addition, we aimed to determine whether possession of allele B is related to having more severe CP.

Patients and Methods

Genomic DNA from 98 Japanese CP patients (52 males and 46 females, mean age 48.2 years) was used. Sixty three Japanese individuals (31 males and 32 females, mean age 27.0 years) with no signs of periodontitis served as controls. All subjects were checked for the presence and severity of periodontitis before typing the MBL genotype, at the Periodontal Clinic of Niigata University Medical and Dental Hospital. The severity of periodontitis was determined according to our criteria (Table 1), which is modified from that by Kornman et al¹¹⁾. CP patients and controls were categorized to 2 groups according to their smoking status; current smokers and non-smokers. All clinical parameters were obtained by dentists unaware of the patients' MBL genotype.

Codon 54 polymorphism of the MBL gene was analyzed by PCR–restriction fragment length polymorphism (PCR–RFLP) method as reported

previously^{9,10)}. Here, the wild-type allele is designated allele A, and the codon 54 substitution (glycine to aspartic acid) is designated allele B. Briefly, genomic DNA purified from peripheral blood mononuclear cells was amplified by polymerase chain reaction (PCR) using primers encompassing the site of the polymorphism. Amplified products were digested by restriction enzyme *Ban I*, which digests allele A, but not allele B. Genotypes were determined according to the sizes of the digested products.

Relationships between MBL allele frequencies or genotype frequencies and occurrence or severity of CP were analyzed by chi-square analysis. P value equal or lower than 0.05 was considered significant. Effects of age gender, smoking status, % sites with plaque and B allele positivity to the severity of CP were analyzed by logistic regression analysis.

All subjects gave written informed consent and the study was approved by the ethical committees of Niigata and Tsukuba Universities.

Results

No significant relationship between MBL genotypes and occurrence of chronic periodontitis

Allele and genotype frequencies of codon 54 polymorphism of the MBL gene were determined in Japanese CP patients and healthy subjects. Frequencies of the MBL low producing allele (allele B) tended to be higher in CP patients than in controls, but without statistical significance (Table 2–1). Frequencies of subjects possessing the MBL low producing allele (genotypes AB or BB) seemed to be higher in the patients group than the control group, but the difference did not achieve statistical significance (Table 2–2).

Possession of MBL low producing allele may be a risk factor for severe chronic periodontitis

Distribution of the MBL genotypes and alleles were compared between patients with severe CP and controls, and between patients with severe CP and patients with mild/moderate CP. MBL genotypes of patients with CP were compared with the severity of the disease. Frequency of the B allele was higher in severe CP patients than in controls, and in severe CP patients than in mild/moderate CP patients. In addition, B allele positivity (percentage of individuals who carry at least 1 B allele) was higher in severe CP patients than in controls, and in severe CP patients than in mild/moderate CP patients (Table 3). These results indicate that possession of allele B, and hence,

Table 1. Demographics of chronic periodontitis (CP) patients and controls

	Severe CP (n=51)	Mild/ Moderate CP (n=47)	Controls (n=63)
Age (years)	46.9±1.5	49.8±1.6	27.0±0.5
Female/male	23/28	23/24	32/31
%smokers	27.5	23.4	20.6
Number of missing teeth	2.9±0.4	1.3±0.2	0
Probing depth (PD) (mm)	4.2±0.1	1.8±0.1	<3.0
Clinical attachment level (CAL) (mm)	5.0±1.4	3.2±0.1	<3.0
% bone loss	45.9±1.4	22.4±0.9	<5.0
% sites with plaque	50.0±1.6	52.0±3.1	ND

PD : probing depth

CAL : clinical attachment level

ND : not determined

Values represent mean + standard error

Definition of mild/moderate/severe periodontitis.

Mild : no sites with probing pocket depth (PPD) >3 mm and mean alveolar bone loss (BL) ≤15%.

Moderate : interproximal sites with BL ≥ 50% within 4, and mean BL 17 to 28%.

Severe : interproximal sites with BL ≥ 50% more than 7, and mean BL ≥ 34%.

Table 2. Mannose binding gene alleles and genotypes in patients with chronic periodontitis (CP) and in controls.

2-1 Allele frequencies				
	CP	Controls	Total	<i>p</i> -value
Allele A	152 (77.6%)	106 (84.1%)	258 (80.1%)	0.1490
Allele B	44 (22.4%)	20 (15.9%)	64 (19.9%)	
Total	196	126	322	

2-2 Genotype frequencies				
	CP	Controls	Total	<i>p</i> -value
AA	58 (59.2%)	44 (69.8%)	102	0.1708
AB	36 40 (40.8%)	18 19 (30.2%)	54	
BB	4	1	5	
Total	98	63	161	

A : codon 54 majority allele (high serum concentration allele)

B : codon 54 minority allele (low serum concentration allele)

P values by chi-square analysis. In table 2-2, *p* value is for AA vs AB/BB.

Table 3. Distribution of mannose binding lectin genotypes and alleles in chronic periodontitis (CP) patients and controls.

	Severe CP		Mild/ Moderate CP		Controls		Controls vs Severe		Mild/Moderate vs Severe	
	%	n=51	%	n=47	%	n=63	<i>p</i> -value	OR (95% CI)	<i>p</i> -value	OR (95% CI)
Genotypes										
AA	49.0	25	70.2	33	69.8	44	0.044		0.035	
AB	43.1	22	29.8	14	28.6	18				
BB	7.8	4	0	0	1.6	1				
Allele frequency*										
A	70.6	72	85.1	80	84.1	106	0.014	2.21	0.015	2.38
B	29.4	30	14.9	14	15.9	20		(1.11–4.40)		(1.11–5.15)
Minority allele positivity†										
B+	51.0	26	29.8	14	30.2	19	0.024	2.41	0.033	2.45
B–	49.0	25	70.2	33	69.8	44		(1.04–5.59)		(1.00–6.14)

A : codon 54 majority allele (high serum concentration allele)

B : codon 54 minority allele (low serum concentration allele)

* Total number of alleles : controls 2n=126, Mild/moderate CP 2n=94, Severe CP 2n=102

† Percentage of individuals who carry at least one copy of polymorphic allele B

For definition of severe, moderate and mild CP, see legend for Table 1.

P values by chi-square analysis.

OR : odds ratio.

being a low MBL producer, may be a risk factor of developing severe CP.

We further evaluated the possible risk factors for severe CP by logistic regression analysis. Results showed that possession of at least 1 B allele is a independent risk factor for increased severity of CP (Table 4).

Discussion

MBL is an important member of the first line defense cells and molecules that defend the host

Table 4. Logistic regression analysis for increased severity of chronic periodontitis (CP).

Variable	OR	95% CI	<i>p</i> -value
Age	0.96	0.92-1.00	NS*
Gender	0.74	0.29-1.91	NS*
Smoking	1.05	0.35-3.13	NS*
% sites with plaque	1.00	0.97-1.02	NS*
Positivity of the B allele	3.01	1.23-7.35	0.015

Logistic regression with the disease status (0 : mild/moderate CP ; 1 : severe CP) as dependent variable, independent variables : age as continuous, gender (0 : male ; 1 : female), % sites with plaque as continuous, smoking status (0 : subjects who had never smoked ; 1 : smoker), and positivity of the MBL B allele (0 : negative ; 1 : positive).

* NS : Not significant

against microorganisms. MBL, by virtue of its multiple lectin domains, recognizes the carbohydrates on microorganisms, activates the complement cascade, and exerts an opsonin effect. Many microorganisms are readily recognized by MBL, as they harbor on their cell walls and capsules carbohydrates such as mannose, *N*-acetyl-D-glucosamine or fucose in alignments that allow effective binding by MBL. Most carbohydrates on the surface of eukaryotic cells end with sialic acid, which is not recognized by MBL. MBL has been shown to efficiently bind many gram-negative bacteria, including *Salmonella* and *Neisseria*¹²⁾. Binding to these bacteria leads to activation of the lectin complement pathway¹³⁾. Microorganisms responsible for occurrence and progression of CP are gram-negative species such as *Porphyromonas gingivalis*. Although binding of MBL to *Porphyromonas gingivalis* has not been directly proven, it is conceivable that MBL and the lectin complement pathway has a protective role in the pathogenesis of CP, and these organisms are expected to be recognized efficiently by MBL. These bacteria reside in the plaque formed on the surface of the teeth, in the periodontal pockets, and in some cases, in the affected periodontal tissue. Gingival crevicular fluid (GCF), present in the periodontal pockets, are composed of serum and tissue exudates, and thus contain many serum components and inflammatory molecules¹⁴⁾. Therefore, MBL, although primarily a serum protein, may play a defensive role against bacteria in the GCF and periodontal tissues of patients with CP. We speculated that individuals with low serum MBL alleles might be prone to occurrence or enhanced progression of CP. When we compared the genotypes of the MBL gene between CP patients and controls, a tendency towards the patients possessing at least 1 low MBL producer allele (allele B) was observed, but significance was not achieved. In addition, allele frequencies or genotype frequencies did not differ between patients with mild/moderate CP and controls (Table 3). Other confounding factors such as oral hygiene and smoking habits may be more important in defining as to whether an individual would develop periodontitis or not. We assumed that MBL might have a more prominent role after occurrence of CP, when host defense at the periodontal pockets becomes more important. In accord with this assumption, we found that CP patients that possess at least one allele B has a significantly higher probability of having severe CP compared to patients homozygous for the high serum MBL allele (allele A) (Table 3).

Theoretically, presence of one allele B would reduce serum MBL concentration to 1/8 compared to serum MBL concentration in individuals homozygous for allele A, and being homozygous for allele B would lead to near complete MBL deficiency. Unfortunately, serum samples from the patients involved in this study are not available. However, we have previously determined that serum MBL concentration in the Japanese population is heavily influenced by the MBL genotype examined in this study¹⁰⁾.

Our findings imply that MBL has a significant role in the host defense of CP patients against bacteria responsible for the progression of periodontitis, and that patients lacking this protein (genotypes AB or BB) may have a poorer prognosis compared to patients with normal serum MBL concentration (genotype AA). Thus, genotyping the MBL gene may enable us to identify patients at a higher risk of developing severe CP, and to provide those patients with more intensive periodontal care to avoid premature loss of teeth. Serum MBL concentration in patients with CP and its relationship with the severity of CP should also be studied in the future.

In conclusion, we have shown that having the low serum MBL allele may be a risk factor for having severe CP and that typing the MBL gene may be beneficial for these patients. Additional studies are required to confirm this finding.

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