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Mannose-binding lectin gene polymorphisms in relation to periodontitis

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Abstract

Aim: To investigate the correlation of six functional polymorphisms in the MBL gene with MBL plasma levels in relation to periodontitis.

Material and Methods: A total of 92 periodontitis patients and 70 controls, all of Caucasian origin, were included. Patients and controls were genotyped for the L/H, X/Y, P/Q, A/D, A/B and A/C polymorphisms. Distributions of genotypes, rate of allele carriage and allele frequencies were compared between patients and controls. Patients and controls were subdivided in groups of genotypes. Plasma MBL levels were compared between different genotype groups.

Results: On the basis of genotyping, three phenotypes with regard to mannose-binding lectin (MBL) production were distinguished: high-producers, low-producers and deficient subjects. No differences in the genotype frequencies were observed between patients and controls. Within patients and controls, subjects with the high-producing genotypes had significantly higher MBL plasma levels than low-producers and deficient subjects ($p < 0.001$). Plasma MBL was higher in low-producer patients compared with low-producer controls ($p_{adjusted} = 0.021$).

Conclusion: No association could be observed between MBL gene polymorphisms and susceptibility to periodontitis in Caucasians. However, now that genotyping could distinguish the low producing and deficient subjects from the high-producers, it was observed, for the first time, that MBL acts as a weak acute-phase protein in periodontitis.

Key words: acute phase reactant; genotyping; MBL; periodontitis

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Periodontitis is considered to be a complex disease. Complex diseases are associated with variations in multiple genes, each having a small overall contribution and relative risk for the disease process; complex diseases are typically polygenic (Tabor et al. 2002). The disease genes in complex diseases are therefore considered disease modifying genes (Hart et al. 2000). Currently, very little is known about which genes and which variations within these genes may be involved in periodontitis as disease modifying genes. A number of candidate gene polymorphisms have been investigated in relation to periodontitis (Loos et al. 2005). As the immune system plays a crucial role in the pathogenesis of periodontitis, researchers have concentrated on the identification of genetic polymorphisms in several aspects of host immunity (e.g. Kornman et al. 1997, Loos et al. 2003, D'Aiuto et al. 2004, Brett et al. 2005, Huynh-Ba et al. 2007, Nibali et al. 2008, Stein et al. 2008).

Conflict of interest and source of funding statement

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Mannose-binding lectin (MBL) is a protein belonging to the family of collectins and is considered to be an important component of the innate immune system (Turner 1996). MBL is primarily produced in the liver and circulates in the blood. It primarily recognizes and binds to specific sugar groups that are displayed on the surface of microorganisms. Interestingly, the periodontal pathogens *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* also appear to have mannan-containing polysaccharides on the cell surface, which can be a potential target for MBL binding (Bramanti et al. 1989, Califano et al.

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1991). Upon binding to microorganisms, MBL activates the lectin pathway of the complement system by the action of MBL-associated serine proteinases (MASPs) (Thiel et al. 1997). MBL may also act as an opsonin, enhancing phagocytosis by binding to cell-surface receptors present on phagocytic cells. It is also recognized to play a role in the modulation of inflammation probably through an effect on cytokine release from monocytes (Dommett et al. 2006).

There are two human MBL genes (MBL-1 and MBL-2) that are closely positioned on the long arm of chromosome 10 (q11.2–q21). MBL-1 is a pseudogene and only MBL-2 encodes a protein product. The nucleotide sequence of the human MBL-2 gene was resolved in 1989 (Sastry et al. 1989, Taylor et al. 1989). The protein-encoding region of the gene consists of four exons interrupted by three introns. The promoter region of the gene contains a number of regulatory elements, which affect the transcription of the protein (Garred et al. 2006).

Three single-base substitutions positioned in exon-1 of the MBL-2 gene at codon 52 (allele D), codon 54 (allele B) and codon 57 (allele C) have been reported (Sumiya et al. 1991, Lipscombe et al. 1992, Madsen et al. 1994). The effect of these exon-1 mutations on the protein product is impaired oligomerization leading to functional deficiency (Sumiya et al. 1991, Wallis & Cheng 1999). In addition, polymorphisms in the downstream promoter and the 5′-untranslated region of the gene have been reported to further modulate the amount of protein produced: $-619G>C$ (L/H), $-290G>C$ (Y/X) and $-66C>T$ (P/Q) (Madsen et al. 1995, 1998, den Dunnen & Antonarakis 2000). Strong linkage disequilibrium exists between the known genetic MBL variants resulting in seven widespread haplotypes namely: HYPA and LYQA giving high serum MBL levels, LYPA intermediate levels, LXPA low levels and LYPB, HYPD and LYQC, resulting in the absence of MBL (defective haplotypes) (Madsen et al. 1995, 1998, Bouwman et al. 2006). There is a difference in the distribution of the above-mentioned haplotypes among different ethnic groups.

The combination of structural gene and promoter polymorphisms may to a large extent account for the variations in MBL serum concentrations in apparently healthy individuals (Caucasians: range <20–10,000 ng/ml). MBL deficiency, defined as an MBL serum concentration below 100 ng/ml has been estimated to occur in about 5–10% of the general white population (Turner 1991). In addition, it has been shown that MBL is an acute phase reactant (Ezekowitz et al. 1988). It has been shown that MBL levels can increase between 1.5 and threefold during the acute phase, but this response is variable between individuals (Thiel et al. 1992). Epidemiological studies have suggested that MBL insufficiency is associated with increased risk for infections especially when a coexisting immune deficiency is present. Further, variations in MBL serum levels are thought to influence the susceptibility to and the course of different types of autoimmune, cardiovascular and other diseases (Turner & Hamvas 2000, Kilpatrick 2002). Thus, it is conceivable that variations in the MBL levels due to genetic polymorphisms may be part of the susceptibility to periodontitis. In a previous study (Maffei et al. 2005), an attempt was made to correlate periodontal disease with MBL deficiency, the latest being defined on the basis of plasma levels. Because MBL plasma levels have been reported to increase during infectious and inflammatory processes, low MBL plasma levels were regarded as a non-reliable indicator of true state of deficiency and therefore it was not possible to draw with certainty conclusions regarding a possible role of MBL deficiency in the pathogenesis of periodontitis.

The aim of this study was to investigate whether genetically determined MBL deficiency was associated with periodontitis. The occurrence of six known, functional polymorphisms in the MBL gene in periodontitis patients and controls was investigated and the resultant genotypes were correlated with the plasma MBL levels.

Material and Methods

Study population

The study population included 92 consecutive Caucasian patients who were referred to the Department of Periodontology of the Academic Centre of Dentistry Amsterdam (ACTA) for diagnosis and treatment of periodontitis. All patients were initially screened in our ''new patient intake clinic'' and had not been treated for periodontal disease before. For each patient, a set of full-mouth radiographs was available and the individual number of teeth present was determined. Subsequently, all teeth were radiographically examined on the mesial and distal aspects; the site with the most bone loss was used to characterize the amount of periodontal destruction per tooth. Patients were selected according to the criteria for a ''periodontitis case'' as defined in the Fifth European Workshop in Periodontology, i.e. presence of proximal attachment loss $\geqslant 3$ mm in $\geqslant 2$ non-adjacent teeth (Tonetti & Claffey 2005).

Further, 70 control Caucasian subjects, who were registered for restorative dental procedures or who visited the dental school for regular dental check-ups, were included in the study. Control subjects were included if they were not missing more than one tooth per quadrant (the third molar excluded), and if they had at all interproximal sites a distance between the cemento-enamel junction and the alveolar bone crest of $\leqslant 3$ mm on bite-wing radiographs $\leqslant 1$ year old. Control subjects may have had gingivitis to some extent.

All subjects were both verbally and written informed about the purposes of the investigation and gave written informed consent. The study was approved by the Medical Ethical Committee of our institution. An extensive medical history was taken both by a written questionnaire and by interview. All subjects that were included in the study were medically healthy. For all participants smoking habits were recorded and subjects were classified as smokers or non-smokers. Smokers were defined as those subjects who were current smokers or had stopped smoking in the previous 6 months. Subjects who had never smoked or had stopped smoking >6 months previously were defined as non-smokers.

Furthermore height and weight were determined for all subjects and the body mass index (BMI) was calculated. MBL plasma levels for all subjects were available from a previous study performed in our department (Maffei et al. 2005).

DNA isolation

From each patient 10 ml of venous blood was obtained by venepuncture. Blood was collected in EDTA-containing vacuum tubes and genomic DNA was isolated with a commercially available kit (GENTRA Systems, Minneapolis,

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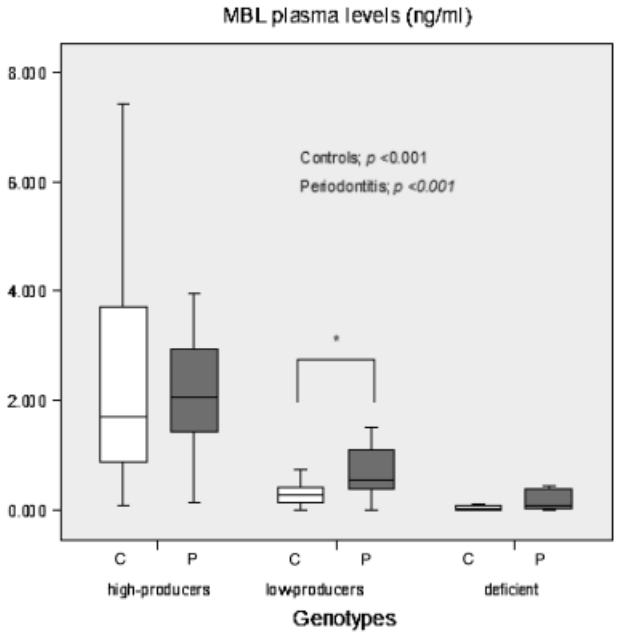

Fig. 1. Box plot for MBL plasma levels in periodontitis patients and controls subdivided, based on genotypes, in high-producers, low-producers and deficient subjects. The horizontal line inside each box (interquartile range) indicates the median (50 percentile). The *p*-values for the Kruskal–Wallis tests used to test for differences in MBL plasma levels between different genotype subgroups within patients and controls are indicated inside the graph. $^{*}p = 0.001$ (Mann–Whitney test). P, periodontitis patients; C, controls.

MN, USA) according to the manufacturer's instructions. The aliquots of DNA used for the analysis of single-nucleotide polymorphisms (SNPs) were diluted 10 times with sterile distilled water and stored at -20°C until use.

Analysis of SNPs in the MBL-2 gene

SNP analysis was performed with six different real time PCR allelic discrimination TaqMan assays. The assays included non-labeled forward and reverse primers together with two fluorescent TaqMan oligonucleotide probes. Two different primer pairs and six probe pairs were used to detect the six different polymorphisms (H/L, Y/X, P/Q, A/D, A/B and A/C) (Fig. 1). TaqMan probes corresponding to the alleles L, Y, P and A were labelled with VIC™ and probes corresponding to alleles H, X, Q, D, B and C were labelled with FAM™ (PE Applied Biosystems, Foster City, CA, USA). The design of primers, probes and positive controls is described elsewhere (Catsburg et al. 2007).

Real time PCR was performed on the ABI PRISM 7000 (Applied Biosystems, Foster City, CA, USA). The reactions were performed with 5 μl DNA in a total volume of 15 μl containing 1 × TaqMan Universal PCR Master Mix (Applied Biosystems), 400 nM of each primer and 100 nM of each probe with the exception of the probe used for allele D, which was 50 nM. After an initial activation step of the AmpliTaq Gold DNA polymerase for 10 min. at 94°C, 40 cycles were run of a two step PCR consisting of a denaturation step at 95°C for 30 s and an annealing and extension step at 60°C for 1 min. Each run included standard known genomic DNA controls for each different genotype and a negative control with distilled water.

After PCR amplification, all products were subjected to an allelic discrimination plate read and analysis. Based on the fluorescence measurements made during the plate read we determined which SNPs were present in each DNA sample. Identification of the genotypes was done manually by two of the authors (AL, TS).

Statistical Analysis

Differences among subject groups in background characteristics were analysed with *t*-tests or Fisher's two-tailed exact tests. Genotype frequencies were tabulated by direct counting and allele frequencies were calculated from the observed number of genotypes. Combined genotypes were constructed based on the seven common haplotypes reported in the literature (Madsen et al. 1995, 1998). As reported in the literature, MBL genotypes determine MBL production (high, low or deficient). Therefore, on the basis of genotyping, both patients and controls were subdivided into three groups, regarding MBL production: high-producers, low-producers and deficient subjects (Bouwman et al. 2006). Chi-square analyses were used for each individual SNP to test for deviation from the Hardy–Weinberg equilibrium. Genotype, allele and carrier frequencies were compared between the control and periodontitis groups by Chi-square or Fisher's two-tailed exact tests. Odds ratios (ORs) and confidence intervals were also determined. Logistic regression analyses were used to further explore the data and to adjust for potential confounders (age, gender, smoking and BMI). MBL plasma concentrations were compared *between* patients and controls with similar genotypes using both the *t*-test and the Mann–Whitney test. Also exploratory general linear models (GLM) were created to control for the above-mentioned potential confounders. MBL plasma levels *within* groups (patients and controls) were compared between subjects carrying different genotypes using the Kruskal–Wallis test. *P*-values < 0.05 were considered to be statistically significant. Only in case of significant difference for MBL levels between the three above-mentioned groups (high-producers, low-producers and deficient) within patients or controls, post-hoc correction for multiple testing was performed. All data analyses were performed using a statistical package (SPSS Inc., version 14.0, Chicago, IL, USA).

Results

A total of 162 subjects of Caucasian origin were included in the study. From those, 92 were periodontitis patients and 70 were healthy controls. The background characteristics of patients and controls are presented in Table 1. The mean ages for patients and controls, the distribution of individuals for gender and the BMI did not differ among the two groups. Almost half of the periodontitis patients were smokers. The con-

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Table 1. Characteristics of the study population [mean ± SD or number (%) of subjects]

	Patients ($n = 92$)	Controls ($n = 70$)
Mean age (years)	43.6 ± 10.1	40.7 ± 12.6
Range	21–75	21–75
Gender		
Male	40 (43.5%)	28 (40.0%)
Female	52 (56.5%)	42 (60.0%)
Smoking status*		
Smokers	43 (46.7%)	20 (28.6%)
Non-smokers	49 (53.3%)	50 (71.4%)
BMI (kg/m^2)	24.2 ± 3.5	23.6 ± 3.1
Number of teeth		
Total†	26.5 ± 3.1	27.7 ± 1.8
Without bone loss	7.3 ± 7.0	27.7 ± 1.8
With ⩾30% bone loss	9.3 ± 4.8	0
With ⩾50% bone loss	7.0 ± 6.8	0
MBL plasma levels (ng/ml)	1544 ± 1262	1617 ± 1926
Range	0–6794	0–8684

n, number of subjects; BMI, body mass index; MBL, mannose-binding protein.
*$p = 0.023$.
†$p = 0.003$.

trol subjects had on average more teeth than the periodontitis patients ($p = 0.018$). Periodontitis patients had on average 10 teeth with bone loss beyond the coronal 1/3 of the root length and seven teeth with ⩾50% bone loss. MBL plasma levels ranged in both patients and controls from non-detectable to more than 6000 ng/ml in patients and more than 8000 ng/ml in controls, respectively (Table 1).

Genotype and allele frequencies of the six tested polymorphisms in the MBL-2 gene

The distribution of genotypes, rate of allele carriage and allele frequencies for the six tested polymorphisms in the MBL-2 gene are presented in Table 2. The frequencies of all genotypes in the control group were found to be in accordance with those expected by the Hardy–Weinberg equilibrium ($p > 0.05$). 53% of the patients were carrying the L/L genotype, 31% the L/H and 16% the H/H genotype *versus* 36%, 53% and 16% of the controls, respectively ($p = 0.020$). A trend towards a lower frequency of the H allele carriage in patients compared with controls was observed ($p = 0.050$).

There was no significant difference among patients and controls in the distribution of the Y/X, P/Q, A/D, A/B and A/C genotypes (Table 2). Among the three variant alleles in exon-1 (B, C and D) the frequency of allele B was higher than the frequency of D or C allele both in patients and controls (14% *versus* 6% and 0.5%, respectively). Almost 25% of patients and controls were carrying the variant allele B, while none of the patients or the controls was homozygous for the rare allele C.

Genotypes and MBL plasma concentrations

The polymorphisms in exon-1 of the MBL-2 gene are also known as structural polymorphisms (A/D, A/B and A/C). The variant alleles B, C and D are commonly referred to as O alleles, with the A allele being the normal allele (Bouwman et al. 2006). Combination of these alleles result in three structural genotypes: AA (common allele homozygosity), AO (heterozygosity) and OO (variant allele homozygosity or compound heterozygosity). The structural genotypes associate with polymorphisms in the promoter region of the gene leading to the formation of combined genotypes (Table 3).

The distribution of the combined genotypes and the MBL plasma concentrations in patients and controls are presented in Table 3. The HYA/LYA genotype, which is a high-producing genotype, was more frequent in controls than in periodontitis patients ($p = 0.007$). For the remainder of the combined genotypes, no significant difference in the frequency was observed between patients and controls. The genotypes leading to low MBL plasma levels (LXA/LXA, A/O, O/O) were almost equally prevalent in periodontitis patients and controls (frequencies 2–14%).

Both in periodontitis patients and controls, the MBL plasma concentrations corresponded with the observed genotypes. Subjects homozygous for the frequent allele A (AA) had higher MBL plasma levels compared with heterozygous individuals (AO) or individuals homozygous for the rare allele O. This difference in MBL plasma levels was statistically significant both in periodontitis patients and controls ($p < 0.001$). When MBL plasma concentrations were compared between patients and controls, it was observed that periodontitis patients with low-producing genotypes had significantly higher MBL plasma levels compared with control subjects with the same genotypes ($p = 0.021$, adjusted for age, gender, smoking and BMI). Higher plasma MBL levels were also observed for patients when low-producing and deficient genotypes were combined ($p = 0.029$, adjusted for age, gender, smoking and BMI) (Table 3).

A graphic presentation showing the MBL concentrations in plasma for patients and controls is given in Fig. 1; genotypes were clustered to the high-producing, low-producing and deficient genotypes. The MBL concentrations both in patients and controls were significantly higher in subjects carrying the high-producing genotype than in subjects with the low-producing and deficient genotype (in both cases $p < 0.001$). This difference was also highly significant when low-producing and deficient subjects were grouped ($p < 0.001$). Among subjects with the low producing genotype, patients had significantly higher MBL serum levels than controls (Table 3; Fig. 1).

Discussion

In the present case–control study limited to Caucasian subjects, we investigated the possible association between periodontitis and genetically determined MBL levels. We investigated six polymorphisms in the promoter region and in exon-1 of the MBL gene, which have been shown to have an effect on the circulating MBL levels. In general, we could not detect any differences in the genotype frequencies between patients and controls. One significant difference was observed for the frequency of the high-producing HYA/LYA genotype,

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Table 2. Genotype, allele carriage rate and allele frequencies for the 6 tested SNPs in the MBL gene

MBL gene polymorphism (position/substitution/rs number)	Genotype/carriage rate/allele frequency	Patients ($n = 92$)	Controls ($n = 70$)	p-value*	OR (95%CI) [OR_{adj} (95%CI)]
L/H	Non-typable	4	4		
(− 619G > C)	L/L	47 (53%)	24 (36%)		
(rs11003125)	H/L	27 (31%)	35 (53%)		
	H/H	14 (16%)	7 (11%)	0.020	
	Carriage allele H	41 (47%)	42 (64%)	0.050	0.50 (0.26–0.96) [0.49 (0.25–0.98)]
	H	55 (31%)	49 (37%)		
	L	121 (69%)	83 (63%)	0.330	0.77 (0.48–1.24)
Y/X	Non-typable	–	1		
(− 290G > C)	Y/Y	56 (61%)	49 (71%)		
(rs7096206)	Y/X	29 (31%)	18 (26%)		
	X/X	7 (8%)	2 (3%)	0.274	
	Carriage allele X	36 (39%)	20 (29%)	0.242	1.58 (0.81–3.07) [1.63 (0.81–3.31)]
	X	43 (23%)	22 (16%)		
	Y	141 (77%)	116 (84%)	0.123	1.61 (0.91–2.84)
P/Q	Non-typable	–	1		
(− 66C > T)	P/P	54 (59%)	37 (54%)		
(rs7095891)	P/Q	34 (37%)	29 (42%)		
	Q/Q	4 (4%)	3 (4%)	0.803	
	Carriage allele Q	38 (41%)	32 (46%)	0.526	0.81 (0.43–1.53) [0.84 (0.43–1.62)]
	Q	42 (22%)	35 (25%)		
	P	142 (78%)	103 (75%)	0.600	0.87 (0.52–1.46)
A/D	Non-typable	–	–		
(154C > T)	A/A	81 (88%)	63 (90%)		
(rs5030737)	A/D	11 (12%)	6 (9%)		
	D/D	0	1(1%)	0.414	
	Carriage allele D	11 (12%)	7 (10%)	0.803	1.22 (0.45–3.33) [1.25 (0.44–3.54)]
	D	11 (6%)	8 (6%)		
	A	173 (94%)	132 (94%)	1.000	1.05 (0.41–2.68)
A/B	Non-typable	–	3		
(161G > A)	A/A	69 (75%)	51 (76%)		
(rs1800450)	A/B	20 (22%)	14 (21%)		
	B/B	3 (3%)	2 (3%)	0.986	
	Carriage allele B	23 (25%)	16 (24%)	1.00	1.06 (0.51–2.21) [1.33 (0.61–2.89)]
	B	26 (14%)	18 (13%)		
	A	158 (86%)	116 (87%)	1.000	1.06 (0.55–2.03)
A/C	Non-typable	–	1		
(170G > A)	A/A	91 (99%)	67 (97%)		
(rs1800451)	A/C	1 (1%)	2 (3%)		
	C/C	0	0	0.577	
	Carriage allele C	1 (1%)	2 (3%)	0.577	0.37 (0.03–4.15) [0.50 (0.04–5.93)]
	C	1 (0.5%)	2 (1%)		
	A	183 (99.5%)	136 (99%)	0.58	0.37 (0.03–4.14)

*χ^2 test or Fisher's two-tailed exact test, when applicable. Values represent number of subjects and percentages (%).

n, number of subjects; OR, odds ratio; CI, confidence interval; OR_{adj}, odds ratio adjusted for age, gender, smoking and BMI; BMI, body mass index; SNP, single-nucleotide polymorphism; MBL, mannose-binding lectin.

with more controls (25%) than patients (9%) displaying the HYA/LYA genotype. However, in light of the multitude of the investigated genotypes, we conclude that MBL gene polymorphisms are not associated with susceptibility to periodontitis.

In a previous study conducted in our department regarding the relation of MBL plasma levels with periodontitis (Maffei et al. 2005), no difference in levels between patients and controls could be detected and the question that was addressed i.e. whether MBL deficiency was associated with periodontitis

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Table 3. Haplotypes and MBL concentrations in periodontitis patients and controls

Structural genotype	Combined genotype	Phenotype* (MBL production)	Genotype†		MBL concentration† (ng/ml)	
			patients	controls	patients	controls
A/A	HYA/HYA	High	9 (10%)	4 (6%)	2681 ± 963	3174 ± 3685
	LYA/LYA	High	11 (12%)	6 (9.5%)	2386 ± 1836	2075 ± 1292
	HYA/LYA	High	8 (9%)‡	16 (25%)	2002 ± 938	2604 ± 1990
	HYA/LXA	High	7 (8%)	9 (14%)	1963 ± 1038	2812 ± 2508
	LYA/LXA	High	14 (16%)	6 (9.5%)	1953 ± 822	1520 ± 1345
	LXA/LXA	Low	7 (8%)	1 (2%)	716 ± 430	282
A/O§	HYA/O	Low	12 (14%)	8 (13%)	999 ± 849¶	337 ± 238
	LYA/O	Low	12 (14%)	9 (14%)	552 ± 404 ‖	223 ± 147
	LXA/O	Deficient	5 (6%)	3 (5%)	251 ± 180	54 ± 51
O/O§		Deficient	3 (3%)	1 (2%)	12 ± 22	0
High-producers			49 (56%)	41 (65%)	2194 ± 1179	2470 ± 2106
Low-producers			31 (35%)	18 (28%)	762 ± 634**	277 ± 192
Deficient genotypes			8 (9%)	4 (7%)	162 ± 184	41 ± 49
Low-producers and deficient			39 (44%)	22 (35%)	639 ± 619††	234 ± 197

Values represent number of subjects (%) or mean ± SD.

*Based on Bouwman et al. (2006).

†The non-typable subjects ($n = 11$, 4 patients and 7 controls) were excluded from the statistical analyses; $n = 88$ for patients, $n = 63$ for controls.

‡$p = 0.007$ (Fisher's two-tailed exact test).

§O denotes B, C, D alleles and O/O includes genotypes B/B, C/C, D/D, B/C, B/D and C/D.

¶$p = 0.047$ (*t*-test), $p = 0.031$ (Mann–Whitney), $p_{GLM} = 0.274$ adjusted for age, gender, smoking and BMI.

‖$p = 0.032$ (*t*-test), $p = 0.041$ (Mann–Whitney), $p_{GLM} = 0.057$ adjusted as above.

**$p = 0.003$ (*t*-test), $p = 0.001$ (Mann–Whitney), $p_{GLM} = 0.021$ adjusted as above.

††$p = 0.004$ (*t*-test), $p = 0.004$ (Mann–Whitney), $p_{GLM} = 0.029$ adjusted as above.

could not be clearly answered. In that study, deficiency was arbitrary defined as MBL plasma levels below 800 ng/ml. In the present study, on the basis of genotyping, both patients and controls could be subdivided in three clearly defined groups, regarding MBL production: high-producers, low-producers and deficient subjects.

In a recent review on MBL (Garred et al. 2006), frequencies of the low-producing and deficient genotypes of around 40% were reported for two Caucasians populations (Danish Caucasians and British Caucasians). Our findings correspond with the reported frequencies. In the present study, the prevalence of genotypes encoding for low and very low MBL plasma levels was 44% for periodontitis patients and 35% for controls respectively. It has been suggested that the persistence of MBL gene polymorphisms at high frequencies may offer a potential advantage to the host (Garred et al. 1994). Currently, there are two main hypotheses suggesting positive pressure for variant MBL alleles. The first suggests that low levels of functional MBL could reduce possible deleterious consequences owing to complement activation and subsequent release of inflammatory mediators that may lead to tissue damage. The other suggests that MBL may contribute to enhanced uptake and virulence of certain intracellular microbes, whereas low MBL levels may be protective (Garred et al. 2006).

Within the genotype groups, MBL plasma levels were compared between patients and controls. It was a new finding that patients belonging to the low-producing and deficient groups had significantly higher MBL plasma levels compared with the control subjects of the same groups. This can be explained by the fact that MBL has been shown to be an acute-phase reactant. The promoter region of the MBL gene contains several acute-phase regulatory elements (Taylor et al. 1989). Indeed, in clinical trials MBL plasma levels have been reported to increase during infections and inflammatory processes. For example, the circulating levels of MBL were found to increase 1.5- to 3- fold in patients undergoing surgery or suffering from malaria (Thiel et al. 1992, Aittoniemi et al. 1997). This seems also to be the case in the present study and is in accordance with previous observations that several acute phase proteins, like C-reactive protein and fibrinogen, are up-regulated in periodontitis (Loos et al. 2000). Interestingly, this acute-phase response was not observed in the high-producing groups, most likely because of the constitutively high gene expression.

It should be noted that genotype is a good predictor of MBL plasma concentration for groups or populations and not for individuals. There are some subjects whose MBL plasma levels cannot be accounted for by their genotypes (Crosdale et al. 2000). Recent studies have shown that there are additional polymorphisms in the MBL-2 gene that may contribute to the regulation of circulating MBL levels (Bernig et al. 2004, 2005). Alternatively, it is possible that a cofactor essential for MBL transcription may be defective or absent in these individuals (Crosdale et al. 2000).

Periodontitis is considered to be a complex disease. It is generally accepted that the presence of bacteria is necessary but not sufficient for the development of periodontitis. Host immune responses, genetic and environmental factors determine part of the susceptibility and severity of periodontitis (Page et al. 1997). In a review on genetic risk factors associated with periodontitis (Loos et al. 2005), it was concluded that the majority of studies concerning candidate genes for periodontitis investigate single polymorphisms, many of which have no functional effects on the encoding protein. In the present study, we investigated six polymorphisms in the MBL-2 gene and all of them have been reported to affect the function or modulate the

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amount of the circulating protein. We were not able to find any association of these polymorphisms with susceptibility for periodontitis. Furthermore, no association with severity could be established (data not shown). In this context, a disadvantage of the present study is the relative low number of subjects per group of genotype. It has been suggested that candidate gene studies need to be large scale in order to be able to have sufficient power for robust conclusions on associations of genetic polymorphisms and disease (Ioannidis et al. 2003). Another difficulty of genetic studies is the variable influence of genetic variations in different ethnic population. In the present study, only subjects of Caucasian origin have been included. It would have been of interest to investigate if there is any association between MBL gene polymorphisms and periodontitis in subjects of other ethnic groups.

In conclusion, in the present study investigating the MBL gene polymorphisms in periodontitis patients, no association with the susceptibility or severity of periodontitis was found. However, in this study we showed for the first time that MBL behaves as an acute-phase reactant in periodontitis. The potential role of this up-regulation in terms of complement activation and increased phagocytosis of periodontal pathogens needs to be further elucidated.

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Clinical Relevance

Scientific rationale for the study: Periodontitis is a multifactorial disease, involving bacterial, environmental and genetic factors. MBL is a protein that activates the complement and enhances phagocytosis. Genetic polymorphisms influence MBL plasma levels. Because MBL deficiency is associated with diseases, the association of MBL genetic variants and plasma levels in relation to periodontitis was investigated.
Principal findings: No association was observed between MBL gene polymorphisms and susceptibility to periodontitis in Caucasians. Nevertheless it was observed, for the first time, that MBL acts as a weak acute-phase protein in periodontitis.
Practical implications: MBL may represent an immune component that plays a role in the effective immune response against invading microorganisms.

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