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ORIGINAL ARTICLE

Mannose-binding lectin gene (MBL-2) polymorphism in oral lichen planus

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Abstract TNF-α may be associated with the etiopathogenesis of oral lichen planus (OLP), and it has been suggested that polymorphism of mannose-binding lectin (MBL) increases the in vitro production of TNF- α. The aim of the present study was to assess the relevance of genetic diversity of MBL in OLP. The study sample comprised 90 individuals, 45 OLP patients and 45 healthy volunteers. MBL-2 gene was amplified using real-time PCR. Frequency of A/A genotype was 55.6% in OLP and 53.3% in healthy volunteers. Likewise, A/0 heterozygote genotype was found in 42.2% and 35.6%; 2.2% and 11.1%, had the recessive 0/0 genotype respectively. Frequencies of the "A" and "0" alleles were 77% and 23% in the OLP group and 71.2% in control group. There were no statistically significant differences regarding genotype frequency (p=0.546) or allele frequency (p=0.497). In conclusion, no significant association was found between polymorphism of MBL-2 gene and OLP.

Keywords Oral lichen planus · Polymorphism · Mannose-binding lectin

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Introduction

Oral lichen planus (OLP) is a common mucocutaneous disorder that typically arises in females in middle to late life [1–3]. The precise etiology of OLP is unknown, but current evidences suggest that it is characterized by a T cell-mediated attack of epithelial surfaces [4–7]. It is plausible that TNF-α plays a central role in these T cell responses and if so may be a therapeutic target. Genetic polymorphisms of TNF-α can influence the production of the pro-inflammatory cytokines involved in T cell reactions and thus might significantly contribute to susceptibility to OLP and other chronic inflammatory disorders [8]. TNF-α stimulates endothelial cells and macrophages to secrete chemokines and induces the chemotaxis of the leukocytes [9].

The mannose-binding lectin gene (MBL-2) is localized in 10q21.1 and codifies for the mannose-binding protein belonging to the family of collectins [10]. This protein plays an important role in innate immunological reactions through molecular recognition mechanisms of a wide

variety of pathogens, including viruses, bacteria, protozoa, and fungi [11], and the activation of the lectin pathway of the complement system, regardless of the presence of specific antibodies [12]. The polymorphism of the MBL-2 gene related to the secretion of pro- and anti-inflammatory cytokines in patients with immunologically mediated diseases has been studied recently, but with controversial results [13] (Table 1). Knowing that OLP is an immunologically mediated disease and that TNF-α may be related to its appearance [6, 22], it is possible that MBL-2 could play a role in the etiopathogenesis of OLP, since polymorphisms of this gene are strongly associated to a reduction of MBL serum levels [12, 23, 24] and, consequently, an increase in the production of interleukins, particularly IL-6 and TNF-α [8]. Although recent studies using real-time polymerase chain reaction (PCR) have enabled the detection of polymorphisms in the codifying region of the MBL-2 gene in order to associate them with the etiopathogenesis of different autoimmune diseases, the possible association with OLP has not yet been established. Based on the information presented above, the aim of the present study was to assess whether MBL-2 gene polymorphisms were associated with the pathogenesis of OLP.

Materials and methods

The present study received approval from the Research Ethics Committee of Center for Health Sciences from Universidade Federal de Pernambuco under process number 251/07. All participating patients gave their informed consent. The study group comprised 45 patients (mean age of 43 years, ranging from 18 to 67) with clinical and histopathological features of OLP. The control group comprised 45 healthy volunteers without history or clinical features of mucocutaneous lichen planus. All patients and control subjects had attended the Oral Medicine unit of Universidade Federal de Minas Gerais, Brazil and Oral Medicine unit of Universidade Federal de Pernambuco, Brazil. Table 2 summarizes clinical characteristics of both patients and control group. Indeed, the control group comprised blood donors paired by age and gender used as healthy controls to define a normal population.

Samples collection

Epithelial smear samples were obtained from the buccal mucosa by using a Cytobrush gynecological brush (Kolplast® Comercial Industrial do Brasil Ltda) [25]. The samples were placed in Eppendorf tubes containing 500 µl of 0.9% saline solution (Laboratório Tayuyna Ltda.) and frozen at −20°C for the subsequent DNA extraction.

DNA extraction

DNA was extracted using the Geneclean® (DNA extraction and purification kit; GENECLEAN® Kit, BIO 101, La Jolla, CA, USA) based on the protocol suggested by Leao et al. [26].

Table 1 Studies involving autoimmune diseases and the polymorphism of the MBL gene

References	Autoimmune diseases	Patients	Results
[14]	SLE	91 patients (83 women and 8 men) and 250 controls	Mutant allele frequency was significantly increased in SLE patients (p=0.01)
[15]	SLE	125 patients and 138 controls	Mutant allele frequency "0" was higher than in controls (p=0.007)
[16]	Rheumatoid arthritis	68 patients and 250 controls	Mutant allele of MBL increased the risk of development of RA (p=0.02)
[17]	Rheumatoid arthritis	211 patients and 196 controls	RA patients showed higher MBL mutation rates when compared with controls (p=0.027)
[18]	Sjögren's syndrome and SLE	266 patients and 129 controls	MBL mutant homozygous genotypes O/O had higher risk of developing SLE (p=0.0431) and SS (p=0.0479)
[19]	Celiac disease	117 patients and 130 controls	The frequency of mutant homozygous 0/0 was significantly higher in CD patients (p=0.0405)
[20]	Celiac disease	120 patients (92 women, 28 men) and 147 controls	The frequency of mutant homozygous 0/0 was significantly higher in CD patients when compared with controls (p=0.014)
[21]	SLE	96 Europeans (90 women, 6 men)	Mutant allele "O" was a risk factor for developing SLE (p=0.001)
[13]	Pemphigus foliaceous	114 patients and 100 controls	Low levels of MBL were not associated with PF (p=0.32)

Table 2 Summary of patients and control group characteristics

Characteristics		Values	
		Control	OLP
Age	Median age years	45	45
	Years range	19–65	18-–7
	Standard deviation	8.9	9.5
Patient gender	Male, *n* (%)	17 (37.8%)	17 (37.8%)
	Female, *n* (%)	28 (62.2%)	28 (62.22%)
Clinical OLP	Reticular		36 (80.0%)
	Erythematosus		3 (6.7%)
	Erosive		6 (13.3%)
Sites committed	Unique site		13 (28.9%)
	Multiple sites		32 (71.1%)
Dermatological manifestations	Present		6 (13.3%)
	Absent		39 (86.7%)
Symptoms	Present		8 (17.8%)
	Absent		37 (82.2%)

Real-time PCR and genotyping

The detection of the polymorphisms of the Exon 1 of the MBL-2 gene was based on the curve of the "melting temperature assay", using the Rotor Gene™ RG 3000 (Uniscience-Cobert Research) as the platform as well as specific primers (forward initiator 5'-AGGCATCAACGGCTTCCCA-3' and reverse initiator 5'-AGAACAGCCCAACACGTACCT-3') [27–30]. The three SNPs (at positions −52, −54, and −57 of exon 1 of the MBL gene) were analyzed in the same real-time PCR product through the dissociation curve and grouped in the "0" allele when mutation occurred in the codons, while the wild-type allele was called "A" [31].

The amplification of exon 1 of the MBL-2 was carried out for a final volume of 25 μl with SyBR Green PCR Master Mix 1× (Applied Biosystems), 0.5 μl of each primer (as describe above) and approximately 2 μl of the genomic DNA mold. The thermal condition of the reaction began with denaturation at 95°C for two minutes, followed by 35 cycles of denaturation at 95°C for 15 s, annealing, and extension at 60°C for 1 min. Following the amplification of exon 1, the resulting product was submitted to the Melting Curve (Rotor Gene™ RG 3000—Uniscience-Cobert Research).

The protocol began with a slow heating from 60°C to 95°C, and the calculations referring to the variation of the fluorophore emission were performed automatically by the program (Rotor Gene™ RG 3000 version 6.0 (build 41); Uniscience-Cobert Research), generating dissociation curves and allowing the detection of the SNPs.

Data analysis

In the data analysis, statistical measures were obtained (descriptive statistical methods) using tables for the qualitative variables. The central tendency measure (mean) and variability (standard deviation) were calculated for the quantitative variables. For the inferential statistical analysis regarding age and gender, the Mann–Whitney test for non-parametric variables was employed. The likelihood ratio was determined from the calculation of the MBL-2 genotype frequency. Fisher's exact test was used for the analysis of allele frequency. The level of significance for all statistical tests was set at 5%. The data were entered on an Excel spreadsheet, and the Statistical Package for the Social Sciences (version 13, Chicago, IL, USA) was employed for the statistical analysis.

Results

Twenty five of the 45 (55.6%) patients with OLP were homozygous, i.e., had the A/A genotype (wild type). Nineteen of the 45 were heterozygous, having A/0 genotype, and one individual had a recessive homozygotous MBL-2 genotype (0/0 genotype). Likewise, 24/45 (53.3%) of the control subjects had the A/A genotype (normal wild), 16/45 (35.6%) had the A/0 genotype (heterozygote), and 5/45 (11.1%) had the 0/0 genotype (recessive homozygote). The likelihood ratio indicated that mutant genotypes did not constitute a risk factor for the development of oral lichen planus (p=0.546) (Table 3).

In the analysis of allele frequency, both groups exhibited similar frequencies for both alleles. The frequency of the dominant A allele in the group of patients with OLP was 69/90 (77%), whereas the frequency of this allele in the group of healthy volunteers was 64/90 (71.2%). Likewise, the frequency of the recessive 0 allele was 21/90 (23%) in the experimental group and 26/90 (28.8%) in the control

Table 3 Distribution of genotypic frequency of MBL-2 in patients with oral lichen planus (OLP) and control subjects

Genotypic frequency	Group			OR	IC (95%)	*p* value
	OLP	Control				
A/A	25 (55.6%)	24 (53.3%)	49	1.254	0.72–1.85	0.546[a]
A/0	19 (42.2%)	16 (35.5%)	35			
0/0	1 (2.2%)	5 (11.2%)	6			
Total	45	45	90			

OR odds ratio, *A/A* homozygous, *A/0* heterozygote, *0/0* homozygous recessive

[a] Using likelihood ratio test

group. Mutant alleles were not contributing factors for the development of OLP (p=0.497) (Table 4).

Discussion

OLP is a chronic disorder that probably represents a T cell-mediated attack of the oral mucosal epithelium [4–7]. Although the exact trigger for such events is unknown, it is likely to be local as disease rarely affects other sites [32]. An underlying bacterial cause has never been found, although as the oral cavity has an abundant microflora [33], such a notion cannot be excluded. Even if bacteria are important in initiating or indeed perpetuating this chronic disorder, it is unlikely that OLP solely reflects infection by distinct oral microbes as it would then be expected that there would be some clustering of affected individuals—which does not arise. Instead, a defective immune response to oral microbes would seem more likely. One possibility is that there is a defective innate immunity that thereby allows a strong, perhaps uncontrolled acquired immune response that ultimately gives rise to clinical disease. The present study has evaluated the potential role of MBL, a soluble mediator of innate immunity [34] that has been found in some previous studies to have a modulating role in a number of other autoimmune diseases [16, 17, 35, 36]. Garred et al. [14] and Turner [37] have suggested a protocol for the detection of polymorphisms in this gene, in which the wild (normal) allele for the MBL is designated A and the usual designation for the variant alleles is 0. Individuals who are homozygous (0/0) for a mutant allele produce MBL in amounts that are undetectable by ELISA, whereas individuals who are heterozygotous (A/0) for the mutation have significantly lower serum concentrations in comparison to individuals who are homozygotes (A/A) for the wild allele [16]. In addition, 0/0 and A/0 genotypes are associated with a low antigenic capacity and low functional levels of MBL (compared with the A/A genotype) [38, 39].

Table 4 Distribution of allele frequency in patients with oral lichen planus (OLP) and control subjects

Allele frequency	Group		Total	OR	IC (95%)	*p* value
	OLP	Control				
A	69 (77.0%)	64 (71.2%)	133	1.33	0.81–1.66	0.497[a]
0	21 (23.0%)	26 (28.8%)	47			
Total	90	90	180			

OR odds ratio, *CI* confidence interval, *A* allele dominant, *0* allele recessive

[a] Using Fisher's exact test

In the present study, the frequency of the 0 allele in the control group was slightly higher than that of the experimental group. However, this difference was not statistically significant. However, in studies by Boniotto et al. [19] with individuals affected by celiac disease, the frequency of the mutant 0 allele was greater than that of the control group, thereby corroborating results described by Villarreal et al. [15], Lee et al. [21], and Garred et al. [14], who found that the frequency of the mutant 0 allele was also significantly greater in patients with systemic lupus erythematosus than in healthy individuals.

Regarding the genotype study for OLP in the present study, there was a greater prevalence of the wild A/A genotype, followed by frequencies of the A/0 heterozygote and 0/0 recessive homozygote genotypes, thereby corroborating results described in other conditions such as celiac disease [20] and systemic lupus erythematosus [18]. In the present study, however, there was no statistically significant association between the polymorphism of the MBL-2 gene and the occurrence of OLP. Similar studies involving other autoimmune disease—such as the investigation by Mullighan et al. [30] involving individuals with Sjögren's syndrome and a recent study carried out by Messias-Reason et al. [13] involving patients diagnosed with Pemphigus Brasiliensis—also found no association with the polymorphism of the MBL gene. A possible association was expected, as the polymorphism of the MBL-2 gene appears to be involved in other autoimmune processes [13–21]. Jack et al. [8] suggest that MBL-2 polymorphism induces a reduction in serum concentration levels of the protein and that reduced concentrations of MBL profoundly enhance the production of the interleukins IL-6 and IL-1b as well as TNF-α. This enhanced generation of TNF-α is relevant to OLP as significantly high levels of TNF-α are found in the oral mucosa [40], serum [41], and oral fluids [42] of patients with OLP. Furthermore, recent studies have found a significant association between the polymorphism of TNF-

α and the etiology of OLP [2, 22, 43]. It is possible that the lack of a causal relationship in the results of the present study reflects innate immunity having no etiological significance in the development of OLP and indeed points away from any local, potentially microbial etiology. Instead, OLP seems to be driven by mechanisms derived from acquired immunity.

The present evidence thus indicates that variation in the MBL-2 gene is unlikely to be of relevance to the etiology of OLP. Further studies, possibly with a larger number of individuals, together with detailed studies of other aspects of the innate immune system may better clarify the role of this and other relevant components of the role of innate immunity in the modulation of the inflammatory response of OLP.

Conclusion

The preliminary results obtained in the present study, with the limitation of the low number of patients and controls, demonstrate that it was not possible to associate the polymorphism of the MBL-2 gene with the etiopathogenesis of oral lichen planus.

Conflict of interest The authors declare that they have no conflict of interest.

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