African Journal of Biotechnology Vol. 6 (17), pp. 2028-2032, 5 September 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Comparing mannose binding lectin genetic diversity in intracellular and extracellular pathogens

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Accepted 12 June, 2007

One of the important immunological factors in diseases is mannose binding lectin (MBL). The aim of present study is to determine the distribution of the alleles of mannose-binding lectin gene codon 52, 54, 57 and promoter variants H/L, X/Y, P and Q in confirmed VL patients as an intracellular pathogen while compares with extracellular pathogens (in renal infection) and seek correlation between these variants and intracellular and extracellular infections. Fifty eight confirmed VL patients' blood samples were compared with fifty eight blood samples of patients received renal in results of renal infections. MBL genotypes were investigated by polymerase chain reaction and restriction fragment length polymorphism. Frequency of defective allele B in extracellular pathogens was more than intracellular pathogens (P = 0.0001), and in contrary prevalence of wild type allele A in intracellular pathogens was more than extracellular pathogens (P = 0.0001), and in other alleles and variants there was not any significant difference. In conclusion, there was more prevalence of alleles with low mannose binding lectin serum level in extrallelular pathogens which can be consider as a risk factor for these infections. In other hand prevalence of high concentration alleles in intracellular pathogens indicate the role of mannose binding lectin level for susceptibility to intracellular pathogens.

Key words: Extracellular, genotype, infection, intracellular, mannose binding lectin, pathogen.

INTRODUCTION

Different factors have interaction in pathogenesis of intracellular and extracellular pathogens. Genetic factors are most important of them. Studies of twins have confirmed the importance of genetic factors in determining host susceptibility to infection with different pathogens (Comstock., 1978; Lin et al., 1989). Recently several genes have been shown to affect susceptibility to intera and extracellular pathogens such as MHC alleles and different diseases. One of the important immunological factors in diseases is mannose binding lectin (MBL). Mannose-binding lectin is a member of the collectin family of proteins found in serum (Presanis et al., 2003). It binds to mannose and N-acetylglucosamine and activates the complement system independently of antibodies via two associated serin protease, mannose binding lectin-associated serine protease 1 and 2 (Jack et al., 2001; Mass et al., 1998; Thiel et al., 1997). C1q and mannose binding lectin, as well as lung surfactant protein A, share the same phagocytic receptor, which is present on a variety of cells, including phagocytes, platelets, and endothelial cells (Nepomuceno et al., 1999; Nepomuceno et al., 1998).

Human mannose binding lectin is derived from a single gene on chromosome 10 (Sastry et al., 1989); the normal structural mannose binding lectin allele is named A, while the common designation for the 3 variant structural alleles B (mutation in codon 54, Gly to ASP), C (mutation in codon 57, Gly to Glu), and D (mutation in codon 52, Arg to Cys) are O (Hegele et al., 1999; Neth et al., 2001).

In general, individuals with a normal genotype (A/A)

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Table 1. Oligonucleotides used in human MBL genotyping.

Origin of gene	Sequence of primers								
Codon 57 (wild type)	Forward	GAG	GCT	TAG	ACC	TAT	GGG	GCT	AG
	Reverse	TAC	CTG	GTT	CCC	CCT	TTT	CTC	
Codon 57 (mutant)	Forward	GAG	GCT	TAG	ACC	TAT	GGG	GCT	AG
	Reverse	TAC	CTG	GTT	CCC	CCT	TTT	CTT	
Codon 54 (wild type)	Forward	GAG	GCT	TAG	ACC	TAT	GGG	GCT	AG
	Reverse	CCC	CTT	TTC	TCC	CTT	GGT	GC	
Codon 54 (mutant)	Forward	GAG	GCT	TAG	ACC	TAT	GGG	GCT	AG
	Reverse	CCC	CTT	TTC	TCC	CTT	GGT	GT	
Codon 52 (wild type)	Forward	CTT	CCC	AGG	CAA	AGA	TGG	GC	
	Reverse	CAG	GCA	GTT	TCC	TCT	GGA	AGG	
Codon 52 (mutant)	Forward	CTT	CCC	AGG	CAA	AGA	TGG	GT	
	Reverse	CAG	GCA	GTT	TCC	TCT	GGA	AGG	
MBL allele H	Forward	GCT	TAC	CCA	GGC	AAG	CCT	GTG	
	reverse	CAG	GCA	GTT	TCC	TCT	GGA	AGG	
MBL allele L	forward	GCT	TAC	CCA	GGC	AAG	CCT	GTC	
	reverse	CAG	GCA	GTT	TCC	TCT	GGA	AGG	
MBL allele P	forward	GTA	GGA	CAG	AGG	GCA	TGC	TC	
	reverse	CAG	GCA	GTT	TCC	TCT	GGA	AGG	
MBL allele Q	forward	GTA	GGA	CAG	AGG	GCA	TGC	TT	
	reverse	CAG	GCA	GTT	TCC	TCT	GGA	AGG	
haplotypes Hy	forward	GCT	TAC	CCA	GGC	AAG	CCT	GTG	
	reverse	GGA	AGA	CTA	TAA	ACA	TGC	TTT	CC
haplotypes Ly	forward	GCT	TAC	CCA	GGC	AAG	CCT	GTC	
	reverse	GGA	AGA	CTA	TAA	ACA	TGC	TTT	CC
haplotypes Lx	forward	GCT	TAC	CCA	GGC	AAG	CCT	GTC	
	reverse	GGA	AGA	CTA	TAA	ACA	TGC	TTT	CG
haplotypes Hx	forward	GCT	TAC	CCA	GGC	AAG	CTT	GTG	
	reverse	GGA	AGA	CTA	TAA	ACA	TGC	TTT	CG

have mannose binding lectin concentration in serum that are 6 - 8 times higher that those in individuals heterozygous for one of the variant alleles (A/O: A/B, A/C or A/D), while individuals with a defective genotype (2 variant alleles B/B, C/C, D/D, B/C, B/D or C/D) have almost undetectable mannose binding lectin serum levels (Garred et al., 1999; Gradual et al., 2000; Dornelles et al., 2006).

Moreover, mannose binding lectin expression is influenced by polymorphic sites in the upstream part of the mannose binding lectin gene (Crosdale et al., 2001; Ip et al., 1998). Nucleotides substitutions at position -550, -221 and +4 give rise to H/L, Y/X and P/Q, respectively and cause to different haplotypes, while LX haplotype is associated with low mannose binding lectin plasma levels (Santos et al., 2001; Soborg et al., 2003).

In this study we investigated role of mannose binding lectin in leishmania infantum as an intracellular pathogen and renal infections as an extracellular pathogens to find relation of mannose binding lectin genetic diversity and pathogenesis of these infections.

MATERIALS AND METHODS

Samples

Blood samples were obtained from 58 confirmed visceral leishmaniasis patients detected by clinical signs and direct smear and direct agglutination test and 58 patients with severe renal infection which had been caused to renal dysfunction. DNA was isolated from both granulocytes and mononuclear cells by the modified proteinase K, SDS and CTAB (Asgharzadeh et al., 2007).

Genomic PCR

PCR was performed in 20 to 100 µl volumes that contained 50 to 500 ng of genomic DNA, 0.5 µM of specific primers (Table 1) in the presence of 1.5 mM $MgCl_2$, 100 µM of each dNTP, 50 mM KCl, 20 mM Tris-HCl, pH 8.4, and 1 to 2.5 unit recombinant Taq DNA polymerase (Cinnagen, Iran). DNA was amplified by general PCR and SSP-PCR. All PCRs were initiated by a 4 min denaturation step at 94ºC and completed by a 7 min extension step at 72ºC. The temperature cycles for different types of PCRs were as follows. 32 cycles of 40 s at 94ºC, annealing temperatures for 40 s and 72ºC for 55 s. Annealing temperatures which were used as follow: 60, 63,

Table 2. Genotype frequency of mannose-binding lectin structural alleles in intracellular pathogens and extracellular pathogens.

Alleles	Frequency (%)*		P-value
	Internal pathogens (Leishmania infantum) (n = 58)	External pathogens (renal infection) (n = 58)	
Codon 54 mutation (Allele B)	6.9	35.34	0.0001
Codon 57 mutation (Allele C)	1.7	6.9	0.3219
Codon 52 mutation (Allele D)	3.45	9.48	0.1648
Wild type (Allele A)	87.93	48.27	0.0001

*Mannose-binding lectin variants frequency in patients and controls.
†Each patient has two alleles on its genotype.

Table 3. Frequency of promoter variants and positions -550 and + 4 in intracellular pathogens and extracellular pathogens.

Variant	Frequency (%)*		P-value
	Internal pathogens (Leishmania infantum) (n = 58)	External pathogens (renal infection) (n = 58)	
H	45.68	29.31	0.0652
L	54.31	70.69	0.0652
P	74	74	1
Q	26	26	1

*Mannose-binding lectin Variants frequency in patients and controls.
†Each patient has two variants on its genotype.

63, 62, 66, 63, 66, 67, 64, 67, 67, 65, 65, and 66ºC for codon 57 (wild type), 57 (mutant), codon 54 (wild type), 54 (mutant), codon 52 (wild type), 52 (mutant), allele H, L, P, Q, haplotype Hy, Ly, Lx and Hx amplification, respectively (Crosdale et al., 2000; Madsen et al., 1995; Sullivan et al., 1996).

PCR-RFLP

In addition to SSP-PCR, B and C alleles were detected by BanI and MboII restriction enzyme digestion of the 320 bp product amplified by the alleles P and Q primers, respectively (Table 1), followed by a 2.5% agarose gel electrophoresis. BanI cleaves the A allele into two fragments (245 and 83 bp) and leaves the B allele undigested, while MboII specifically cleaves the C allele into two fragments (266 and 62 bp) (Madsen et al., 1995).

Statistical analysis

The distribution of alleles and genotypes between groups were compared using chi-square ($\chi 2$ test) and $P < 0.05$ was considered significant.

RESULTS

The association between mannose binding lectin and pathogenesis of intracellular or extracellular pathogens cannot be explain by confounding factors such as difference in age and disease duration (Garred et al., 1999). These overall frequencies differ significantly between intracellular and extracellular pathogens. Table 2 shows the frequency of defective allele B in extracellular pathogens was more than intracellular pathogens (P = 0.0001), and in contrary prevalence of wild type allele A in intracellular pathogens was more than extracellular pathogens (P = 0.0001).

Frequency of promoter variants and position +4 (Table 3) indicate that there is a prevalence of H variant in intracellular pathogens (P = 0.0652) and more prevalence of L variants in extracellular pathogens (P = 0.0652). Promoter haplotypes frequency in intracellular pathogens and extracellular pathogens were not significantly different and only a very rare haplotype of Hx in patients with leishmaniasis was found (Table 4).

DISCUSSION

In this study we investigated the association of exon 1 and promoter haplotypes and pathogenesis upon intracellular infection (leishmania infantum) and extracellular pathogen (renal infection). The results showed that geno-

Table 4. Promoter haplotypes frequency in intracellular pathogens and extracellular pathogens.

Frequency %*			
Haplotypes	**Internal pathogens (Leishmania infantum) (n = 58)**	**External pathogens (renal infection) (n = 58)**	**P-value**
Hy	44.8	38.33	0.4775
Ly	33.6	42.9	0.3040
Lx	20.7	18.75	0.7920
Hx	0.9	0	0.4785

* Mannose-binding lectin Variants frequency in patients and controls.

types with high mannose binding lectin serum level were more frequent among patients with extracellular pathogens (renal infection) (P = 0.0001) and there was more prevalence of wild type genotype A in intracellular pathogen (P = 0.0001). Mannose binding lectin in gram negative organisms act as a complement activator to kill these organisms directly via the member attack complex or to enhance complement mediated phagocytosis, through the increased deposition of C3 fragments (Jack et al., 2001a). Previous studies about the role of mannose binding lectin in aspergillosis showed that codon 52 mutation was particularly common demonstrating that mannose binding lectin low serum level as a risk factor for chronic necrotizing pulmonary aspergillosis (Crosdale et al., 2001). Previous studies in gram negative pathogens in our study and other extracellular pathogens studies confirmed that mannose binding lectin deficiency confer a long-life risk of these infections (Summerfield et al., 1995; Jack et al., 2001a).

Also recent studies about relation of mannose binding lectin and *Neisseria* showed that this blood factor can bound to the surface of *Neisseria* and activate complement, which leads to an increase in bacterial killing (Jack et al., 1998; Jack et al., 2001a). Similar experiments have shown cidal activity of mannose binding lectin of *Escherichia coli* (Kawasaki et al., 1989) and *Salmonella* (Ihara et al., 1991). However some reports could not find any effect of mannose binding lectin on complement activation upon extracellular infection of *Staphylococcus aureus* (Cunion et al., 2001).

In intracellular infections, there is conversion and deficiency confers protection against these infections. A new study suggests a protective role for mannose binding lectin deficiency against development of the most severe and multibacillary form of the leprosy (but not tuberculoid form) (Dornells et al., 2006). In tuberculosis, it has been demonstrated that heterozygosity for mannose binding lectin variant allele (XA/O), which encodes low serum mannose binding lectin level is associated with protection against clinical tuberculosis (Bellamy and Hill, 1998; Soborg et al., 2003). It has also been shown that lack of mannose binding lectin enhances survival in a mouse model of acute septic peritonitis (Takahashi et al., 2002).

In some specific intracellular pathogens like *Leishmania*, increasing concentration of mannose binding lectin cause increasing internalization of pathogens. Mannose binding lectin modulate the function of *Leishmania chagasi*-infected cells and high levels of circulating mannose binding lectin are directly correlated to the development of visceral leishmaniasis upon infection with *Leishmania chagasi* (Santos et al., 2001). Mannose binding lectin can promote the attachment of microorganisms to phagocytic cells (Jack and Turner, 2003) and increase the release of TNF-α and interleukin 6 from monocytes contaminated with parasite (Jack and Turner, 2003). Also mannose binding lectin binds to the parasite surface and is most intense at base of the flagella in many parasites, on area of the plasma membrane that is the major site for exocytosis for these cells and contains a high concentration of surface antigen (Pimento et al., 1991). Regarding mannose binding lectin role in the internalization of intracellular microorganism by phagocytic cells, it has been considered a candidate molecule for modifying the disease progression of intracellular pathogens (Ambrosio and Messias-Reason, 2005; Dornelles et al., 2006). This observation and our study suggest that mannose binding lectin deficiency could be advantageous to the host in intracellular pathogens but is completely dis-advantageous in the case of extracellular pathogens. It seems plausible that under certain circumstance, mannose binding lectin binding to pathogens would lead to excessive activation of complement being useful for the host defense against extracellular pathogens.

In conclusion, our study confirmed that difference of mannose binding lectin gene variants can be a risk factor or protective factor in extracellular and intracellular pathogens. There is more prevalence of alleles with low mannose binding lectin serum level in extrallelular pathogens. In other hand, prevalence of high concentration alleles in intracellular pathogens indicates the role of mannose binding lectin level during susceptibility to intra-

cellular pathogens.

ACKNOWLEDGMENTS

We thank the Drug applied Research center of Tabriz University of Medical Sciences and the renal transplant-tation section of Imam Khomeini Hospital, Tabriz-Iran. Naghmeh Badroghli provided entire background and information of patients, and Parvaneh Salahsh ia appreciated for assistance in obtaining visceral leishnaniasis patients' information.

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