International Journal of Infectious Diseases 15 (2011) e551–e557

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Mannose-binding lectin serum levels in patients with leprosy are influenced by age and *MBL2* genotypes

Luydson Richardson Silva Vasconcelos [a,b,*], Juliana Pereira Lopes Fonseca [a], Rodrigo Feliciano do Carmo [a], Taciana Furtado de Mendonça [a], Valeria Rêgo Alves Pereira [c], Norma Lucena-Silva [c,d], Leila Maria Moreira Beltrão Pereira [a,b], Patrícia Moura [a], Maria do Socorro de Mendonça Cavalcanti [a]

[a] *Faculdade de Ciências Medicas, Instituto de Ciências Biológicas, Universidade de Pernambuco, Rua Arnobio Marques 282, CEP 50100-130, Santo Amaro, Recife PE, Brazil*
[b] *Intituto do Fígado de Pernambuco, Rua Arnobio Marques 282, CEP 50100-130, Santo Amaro, Recife PE, Brazil*
[c] *Centro de Pesquisas Aggeu Magalhães, Fiocruz, Av. Prof Moraes Rêgo, CEP 50670-420, Cidade Universitária, Recife PE, Brazil*
[d] *Instituto de Medicina Integral Professor Fernando Figueira, Rua dos Coelhos 300, CEP 50070-550, Boa Vista, Recife PE, Brazil*

ARTICLE INFO

Article history:
Received 8 September 2010
Received in revised form 19 April 2011
Accepted 20 April 2011

Corresponding Editor: William Cameron, Ottawa, Canada.

Keywords:
Mannose-binding lectin
MBL2
Leprosy
Innate immunity
Age
Polymorphism

SUMMARY

Background: Mannose-binding lectin (MBL) activates the complement system promoting opsonophagocytosis, which could represent an advantage for *Mycobacterium leprae*, an intracellular pathogen. Therefore, a single nucleotide polymorphism (SNP) in the *MBL2* gene associated with low levels of MBL could confer protection against the development of leprosy disease.
Methods: In this study, we investigated SNPs of the *MBL2* gene and MBL levels in 228 Brazilian leprosy patients and 232 controls.
Results: There were no differences in the frequencies of variant genotypes and haplotypes of *MBL2* between patients and controls, or between the different clinical forms of leprosy. In the group of patients with a genotype for high expression of *MBL2*, those aged > 40 years had decreased MBL levels compared to patients aged ≤40 years ($p = 0.037$).
Conclusion: Our results demonstrate that age could influence the phenotype of *MBL2*, but no evidence was found for an association of *MBL2* polymorphism with susceptibility to leprosy or its clinical forms.

1. Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, an intracellular pathogen that infects mainly macrophages and Schwann cells.[1] Most individuals do not develop clinical manifestations, even after prolonged exposure to *M. leprae*. Those who do develop clinical manifestations present four major forms: tuberculoid, lepromatous, borderline, and indeterminate. The high degree of clonality in the genome of *M. leprae* isolates from various parts of the world,[2] suggests that the variability in susceptibility to infection with the Mycobacterium is largely due to host genetic factors.[3] The single nucleotide polymorphism (SNP) of several genes that could influence the variability in susceptibility to leprosy has been extensively investigated, showing different results according to the population studied.[4–6]

Positive or negative associations between leprosy and polymorphisms of the immune system molecules have been described, such as the human leukocyte antigen (HLA),[4,7] interleukin 10 (IL-10),[8–10] Toll-like receptor 2 (TLR 2),[8] lymphotoxin alpha (LTA), tumor necrosis factor alpha (TNF-α), receptor for vitamin D (VDR), receptor for component of the complement system (CR1),[8] and mannose-binding lectin (MBL).[8,11,12] These studies have revealed a great genetic complexity involved in controlling susceptibility to leprosy and modulation of the clinical spectrum of leprosy in humans.

MBL is a lectin of the innate immune system that plays an important role as a pattern recognition molecule in the identification of pathogens; it is able to activate the complement system, promoting phagocytosis and modulation of inflammation.[13] SNPs in the MBL gene (*MBL2*) are responsible for reduced serum levels of the protein. Three SNPs in the promoter region modulate the expression of the molecule, of which the alleles are H/L (position – 550), X/Y (position – 221), and P/Q (position +4).[14] Exon-1 of *MBL2* presents allelic variants B (Gly54Asp), C (Gly57Glu), and D (Arg52Cys), collectively termed allele 'O', while the wild-type allele is called 'A'. The amino acid changes affect the oligomerization of variant MBL, which has a lower molecular weight and is dysfunctional compared to normal MBL; this influences the biological activity of the lectin and also reduces its serum concentration.[15]

* Corresponding author. Tel.: +55 81 3183 3512; fax: +55 81 3183 3510.
E-mail address: luydson@yahoo.com.br (L.R.S. Vasconcelos).

1201-9712/$36.00 – see front matter © 2011 International Society for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijid.2011.04.008

The exon-1 allelic variants that are associated with low serum levels of MBL predispose to infection by various pathogens,[14,16] as well as to the development of autoimmunity.[17] However, genotypes of low expression of MBL have been associated with protection against the development of visceral leishmaniasis,[18] tuberculosis,[19] and leprosy.[11] It is suggested that *MBL2* may have undergone natural selection pressures, which induce genetic polymorphisms, resulting in biological advantages. However, a selective neutrality test with respect to the complete sequencing of the gene showed significant results only for the population of individuals of European origin, but not for African-Americans or Hispanics.[20] Indeed, a larger study focusing on evolutionary insights into the high prevalence of *MBL2* allele deficiency worldwide, supports the idea that the role of MBL is redundant in human host defense.[21]

Studies of the association of *MBL2* polymorphisms with leprosy present conflicting results. Fitness et al.[8] found no association in a study of the first exon variant alleles in leprosy in a population of paucibacillary patients from the northeast of Africa. In contrast, de Messias-Reason et al.,[11] studying Brazilian patients with leprosy in the southern region, which has a large European colonization influence, showed a positive association between high-expression haplotypes of *MBL2* with susceptibility to leprosy per se, whereas those with low expression were associated with the tuberculoid form. In addition, in an Indian population from Nepal, the B variant allele was present in 4.3% of patients with the tuberculoid vs. 1.5% of patients with the lepromatous form, however the authors stated that the statistical significance was modest and that more studies were needed for conclusive validation.[12]

Due to the complex genetic regulation of susceptibility to leprosy, the influence of polymorphism in *MBL2* could vary according to the genetic background of the population studied. Boldt et al.[22] demonstrated that the frequencies of polymorphisms of exon-1 and the promoter of *MBL2* are different among Brazilians of different genetic backgrounds. Therefore, this study aimed to investigate the association of *MBL2* polymorphism and serum levels of MBL in a group of patients with different forms of leprosy from the northeast of Brazil.

2. Patients and methods

2.1. Patients and controls

We recruited 232 healthy blood donors as the control group and 228 patients with leprosy from the city of Recife, northeast of Brazil, matched by place of origin. The control group had a mean age of 33 ± 8.3 years, and 58% of subjects were male. In the leprosy group, the clinical forms were distributed as follows: 26% lepromatous, 31% borderline, 16% tuberculoid, 7% indeterminate, and 20% were not classified. The average age of patients was 44.8 ± 16 years, with 59% male subjects. The classification of disease with regard to the operational class – paucibacillary (PB) and multibacillary (MB) – was based on the information provided by SINAN (Technical Information System for Notifiable Diseases/Ministry of Health, Brazil, 2008), which uses the Ridley and Jopling classification (1966).[23]

This study was approved by the Research Ethics Committee of Aggeu Magalhães (CEP/CPqAM/Fiocruz, record 45/06). All subjects in the study signed a consent form.

2.2. DNA extraction and genotyping

DNA samples were extracted from whole blood in anticoagulant solution (EDTA) using the QIAamp Mini Spin Columns Kit (Qiagen, Basel, Switzerland) following the manufacturer's instructions.

The promoter region of *MBL2* was genotyped by real-time PCR, using specific probes, performed by the TaqMan system. The probes and validated protocols for the regions − 550 and − 221 are available at http://snp500cancer.nci.nih.gov. For determination of alleles H/L, the following probes and primers were used: FAM-MGB-AGCCTGTGTAAAAC, VIC-MGB-CCTGTCTAAAACACC, CCAACGTAGTAAGAAATTTCCAGAGA-forward, and reverse-CAACC-CAGCCCAGAATTAACTG. For alleles X/Y, the following probes and primers were used: FAM-CATGCTTTCCGTGGCAG-MGB, VIC-MGB-CATGCTTTCGGTGGCAG, GCACGGTCCCATTTGTTCTCA-forward, and reverse-GCGTTGCTGCTGGAAGACTATAAA.

Genotyping of the structural region (exon-1) of *MBL2* was performed using the technique of real-time PCR with melting temperature assay (MTA), as described by Hladnik et al.,[24] using the following primers: forward primer 5′-AGGCAT-CAACGGCTTCCCA-3′, reverse primer 5′-CAGAACAGCCCAACACG-TACCT-3′. The three allelic variants of the *MBL2* gene in codon positions 52, 54, and 57 in exon-1 were designated 'O', and the wild-type allele designated 'A'.

2.3. MBL serum concentration

The serum concentrations of MBL were determined using a commercial capture enzyme-linked immunosorbent assay (ELISA; Antibody Shop, Copenhagen, Denmark). Sera were diluted 1:100 and added to ELISA plates coated with monoclonal antibody against the binding carbohydrate domain. The bound MBL was detected by a second biotin-labeled antibody and the streptavidin–peroxidase system. Tetramethylbenzidine was used as substrate. Reading of the reaction was performed at 450 nm using an ELISA plate reader (BioRad, CA, USA). According to the manufacturer, the concentrations of MBL in normal serum are classified as low at levels of < 100 ng/ml, intermediate at 100–1000 ng/ml, and high at >1000 ng/ml.

2.4. Grouping of genotypes according to serum MBL and haplotypes

Genotypes of the promoter region (−550 H/L, −221 X/Y) were grouped with genotypes of the exon-1 (A/O) and correlated to MBL serum concentrations in the leprosy patients to categorize groups of low, intermediate, and high expression.[14] The following genotypes were considered as high expression (total n = 87, median = 3190 ng/ml): HYA/HYA (n = 22, median = 3063 ng/ml), HYA/LYA (n = 32, median = 2658 ng/ml), LYA/LYA (n = 33, median = 3667 ng/ml); intermediate expression (total n = 105, median = 848 ng/ml): LYA/LXA (n = 19, median = 2121 ng/ml), HYA/LXA (n = 27, median = 2694 ng/ml), HYA/HYO (n = 6, median = 1805 ng/ml), HYA/LYO (n = 18, median = 564 ng/ml), LYA/LYO (n = 29, median = 338 ng/ml), LXA/LXA (n = 6, median = 946 ng/ml); and low expression (total n = 36, median = 30 ng/ml): HYO/LXA (n = 7, median = 190 ng/ml), HYO/LYO (n = 6, median = 16 ng/ml), LYO/LYO (n = 8, median = 20 ng/ml), LXA/LYO (n = 15, median = 59 ng/ml). The haplotypes were then divided into groups of low (LXA, HYO, LYO) and high (HYA, LYA) expression of the *MBL2* gene.[14]

2.5. Determination of serum C-reactive protein

C-reactive protein (CRP) levels were used as a marker of inflammation. The exam was performed using the Turbidimetric Ultra-sensitive CRP Kit (Biotech, Minas Gerais, Brazil), with sample collection and the test protocol as per the manufacturer's instructions, and the results were read on a TARGA 3000 (Rome, Italy). The reference concentration for CRP indicated by the kit manufacturer was <3 mg/l.

2.6. Statistical analysis

The Mann–Whitney or Kruskal–Wallis test was employed when appropriate for comparison of the variation in MBL serum