Article/Artigo

Characterization of mannose-binding lectin plasma levels and genetic polymorphisms in HIV-1-infected individuals

Caracterização dos polimorfismos genéticos e dos níveis plasmáticos da lectina ligante de manose em indivíduos infectados pelo HIV-1

Antonio Carlos Rosário Vallinoto[1], Felipe Bonfim Freitas[1], Isabella Guirelli[1], Luiz Fernando Almeida Machado[1], Vânia Nakauth Azevedo[1], Izaura Cayres-Vallinoto[1], Marluísa Oliveira Guimarães Ishak[1] and Ricardo Ishak[1]

ABSTRACT

Introduction: The present study investigated the association between mannose-binding lectin (MBL) gene polymorphism and serum levels with infection by HIV-1. **Methods:** Blood samples (5mL) were collected from 97 HIV-1-infected individuals resident in Belém, State of Pará, Brazil, who attended the Special Outpatient Unit for Infections and Parasitic Diseases (URE-DIPE). $CD4^+$ T-lymphocyte count and plasma viral load were quantified. A 349bp fragment of exon 1 of the MBL was amplified via PCR, using genomic DNA extracted from controls and HIV-1-infected individuals, following established protocols. MBL plasma levels of the patients were quantified using an enzyme immunoassay kit. **Results:** Two alleles were observed: MBL*O, with a frequency of 26.3% in HIV-1-infected individuals; and the wild allele MBL*A (73.7%). Similar frequencies were observed in the control group ($p > 0.05$). Genotype frequencies were distributed according to the Hardy-Weinberg equilibrium in both groups. Mean MBL plasma levels varied by genotype, with statistically significant differences between the AA and AO ($p < 0.0001$), and AA and OO ($p < 0.001$) genotypes, but not AO and OO ($p = 0.17$). Additionally, CD4+ T-lymphocytes and plasma viral load levels did not differ significantly by genotype ($p > 0.05$). **Conclusions:** The results of this study do not support the hypothesis that MBL gene polymorphism or low plasma MBL concentrations might have a direct influence on HIV-1 infection, although a broader study involving a large number of patients is needed.

Keywords: HIV-1. MBL gene polymorphism. Amazon region.

RESUMO

Introdução: O presente estudo investigou a associação entre o polimorfismo no gene da lectina ligante de manose (MBL) e os níveis séricos da proteína com a infecção pelo HIV-1. **Métodos:** As amostras de sangue (5mL) foram coletadas de 97 indivíduos infectados pelo HIV-1 residentes em Belém, Estado do Pará, Brasil, que frequentavam a Unidade de Referência Especial para Doenças Infecciosas e Parasitárias Especiais (URE-DIPE). Os níveis de linfócitos T CD4+ e da carga viral plasmática foram quantificados. Um fragmento de 349pb do exon 1 da MBL foi amplificado via PCR, utilizando DNA genômico extraído das amostras controles e dos indivíduos portadores do HIV-1, seguindo protocolos previamente estabelecidos. O nível plasmático de MBL nos pacientes foi quantificado usando kit de ensaio imunoenzimático. **Resultados:** Dois alelos foram observados - MBL*O, com uma frequência de 26,3% em indivíduos infectados e o alelo selvagem MBL*A (73,7%). Frequências similares foram observadas no grupo controle ($p > 0,05$). As frequências genotípicas estavam em equilíbrio de Hardy-Weinberg em ambos os grupos. A média dos níveis plasmáticos MBL variou por genótipo, com diferenças significativas entre os genótipos AA e AO ($p < 0,0001$), e AA e OO ($p < 0,001$), mas não entre AO e OO ($p=0,17$). Além disso, os linfócitos T CD4+ e os níveis plasmáticos de carga viral não diferiram significativamente de acordo com o genótipo ($p>0,05$). **Conclusões:** Os resultados deste estudo não apoiam a hipótese de que o polimorfismo no gene MBL ou baixa concentração plasmática de MBL poderia ter uma influência direta sobre a infecção pelo HIV-1, embora um estudo com número maior de pacientes seja necessário.

Palavras-chaves: HIV-1. Polimorfísmos no gene MBL. Região amazônica.

1. Virus Laboratory, Institute of Biological Science, Federal University of Para, Belem, PA, Brazil.
Address to: Dr. Antonio Carlos Rosário Vallinoto. Lab. Virologia/ICB/UFPA. Cidade Universitária Prof. José da Silveira Netto, Guamá, 66075-910 Belém, PA.
Tel: 55 91 3201-8410
e-mail: vallinoto@ufpa.br
Received in 22/04/2010
Accepted in 19/10/2010

INTRODUCTION

Mannose-Binding Lectin (MBL) is a liver-derived pluripotent serum lectin that plays an important role in the host's innate immune system. It binds with high affinity to mannose or other carbohydrate components of viruses, bacteria and yeasts[1]. The performance of MBL is directly related to its serum concentration, which is determined by the interplay between the promoter and structural gene mutations[2,3].

Three mutations of the wild allele MBL*A have been identified in the structural region of the molecule (codons 52, 54 and 57) with three allelic variants named MBL*D, MBL*B and MBL*C, respectively[4]. These variants have been associated with MBL serum deficiency and, consequently, variations in the susceptibility or resistance to infection in carriers by a number of pathogens[5-8].

Low levels of serum MBL have been primarily associated with a significant increase[9] or reduction[10] in survival time following a diagnosis of AIDS, as well as with an increase in the time between seroconversion and the development of AIDS and death[11].

The present study investigated a possible association between MBL gene polymorphism and serum levels and infection by HIV-1.

METHODS

Population group

Blood samples (5mL) were collected from 97 HIV-1-infected individuals resident in Belém, State of Pará, Brazil, who attended the Reference Unit for Special Infections and Parasitic Diseases (URE-DIPE), in order to determine $CD4^+$ T-lymphocyte counts and plasma viral load. None of the patients were receiving antiretroviral therapy at the time of sample collection. The study only included patients over 18 years old and seropositive for HIV-1 infection.

Control group

As a control group, 99 blood samples from seronegative individuals residing in Belém (capital of the State of Pará, Brazil) were investigated, which were stored at -20°C at the virus laboratory. All specimens were previously screened for HIV-1 infection using an enzyme-linked immunosorbent assay (OrthoDiagnostic, US). The study only included individuals over 18 years old and seronegative to HIV-1 infection.

Quantification of plasma viral load and CD4$^+$ T-lymphocyte counts

Plasma viral load was determined by the Nasba method, using a NucliSens Reader and the NucliSens^{TM} kit, following the manufacturer's instructions (Nasba Diagnostics, Organon Teknika, Boxtel, Netherlands).

Whole blood samples were processed within 4 hours of collection to determine T-lymphocyte subset counts by flow cytometry (FacsCount, Becton & Dickinson, USA) using the FacsCount^{TM} Reagents immunomonitoring kit, following the protocol recommended by the manufacturer (Becton Dickinson, USA).

Detection of the MBL gene polymorphism

In the present study, PCR was performed to amplify 349bps of exon 1 of the MBL gene, using genomic DNA extracted from the HIV-1-infected patients, according to standard protocols[2,12]. The reactions were performed in a final volume of 50µl containing 100ng genomic DNA, 200µM dNTPs, 5pmol primers, 50mM KCl, 2.5mM $MgCl_2$, 10mM Tris-HCl pH 8.3 and 0.5U of Taq polymerase (Invitrogen, US). The following pair of primers was used for genotyping: (mblE01) 5'-AGTCGACCCAGATTGTAGGACAGAG-3' and (mblE02) 5'-AGGATCCAGGCAGTTTCCTCTGGAAGG-3'. The PCRs were initiated by a denaturation step at 94°C for 2min, followed by 35 cycles of: 30sec at 94°C, 60sec at 58°C and 120sec at 72°C.

Quantification of MBL serum levels

MBL plasma concentrations of the patients were quantified using an enzyme immunoassay kit (EASIA Sanquin Reagents - Amsterdam, Netherlands), according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed by the BioEstat 5.0 software[13]. The genotypic and allelic frequencies of the patient group were calculated by direct counting. The Hardy-Weinberg equilibrium and the comparison of genotype and allelic frequencies between groups were performed using the Chi square test.

The nonparametric Mann-Whitney test was used to assess the possible correlation between MBL plasma concentrations and genetic background. A p value of < 0.05 was considered to be statistically significant.

Ethical considerations

All participating individuals, including HIV-1 infected patients and seronegative controls, were briefed on the study and signed a free, informed consent form before the collection of samples. The study was approved by the Human Ethics Committee of the Medical Sciences Institute of the Federal University of Para.

RESULTS

In HIV-1-infected individuals, a frequency of 26.3% was determined for the allelic variants MBL*B, MBL*C and MBL*D, represented by the MBL*O allele, whereas a frequency of 73.7% was determined for the wild allele MBL*A. Similar frequencies were observed in the control group ($p > 0.05$).

The AA genotype was the most frequent in healthy controls in comparison with the HIV-1 infected group, whereas the heterozygous AO genotype was slightly more frequent in HIV-1 patients, although once again, the difference was not significant **(Table 1)**. Furthermore,

TABLE 1 - Distribution of the allele and genotype frequencies among HIV-1 infected individuals.

	HIV-1		Control			
Genetic profile	n	%	n	%	χ^2	*p*
Genotype						
AA	54	55.7	65	65.7		
AO	35	36.1	29	29.3		
OO	8	8.2	5	5.0		
total	97	100.0	99	100.0	2.251	0.3244
Allele						
A	143	73.7	159	80.3		
O	51	26.3	39	19.7		
total	194	100.0	198	100.0	2.049	0.1523

n: number of individuals or chromosomes analyzed, AA: wild genotype, AO: heterozygous and OO: mutant homozygous.

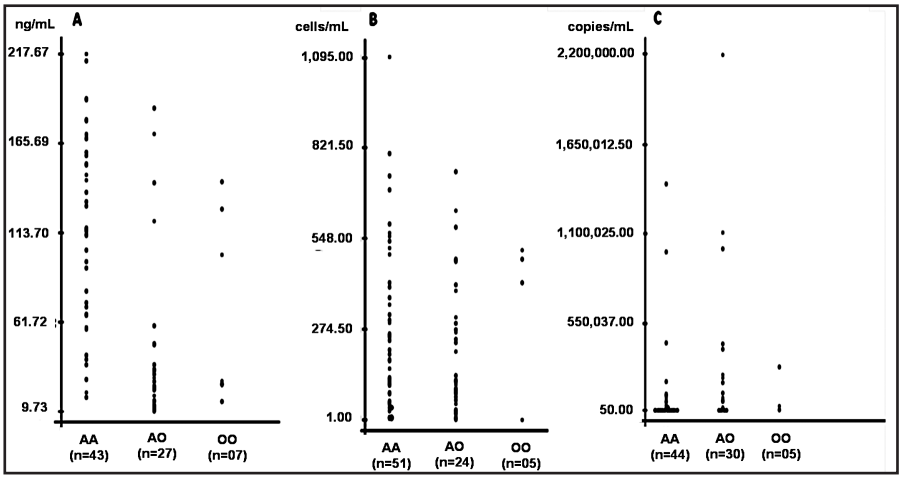

FIGURE 1 - A: Plasma concentrations of mannose-binding lectin in HIV-1 patients according to genotype. B: T cell CD4$^+$ levels (cells/mm^3) according to genotype. C: Viral load (copies/mm^3) according to genetic background. AA: wild genotype, AO: heterozygous and OO: mutant homozygous.

the distribution of genotype frequencies in both groups was in agreement with the Hardy-Weinberg equilibrium ($p > 0.05$).

Mean MBL plasma concentrations according to genotype **(Figure 1)** were 113.41 ± 55.95ng/mL (AA), 53.30 ± 51.49ng/mL (AO) and 66.58 ± 55.06ng/mL (OO). The differences between the AA and AO genotypes ($p < 0.0001$) and AA and OO ($p < 0.001$) were statistically significant, although between the AO and OO genotypes the difference was not significant ($p = 0.17$). Additionally, CD4+ T-lymphocyte counts and plasma viral load levels did not vary significantly according to genotype ($p > 0.05$).

DISCUSSION

In the present study, possible associations between HIV-1 infection and MBL plasma concentrations and polymorphism of the MBL gene were investigated. MBL is an important serum protein that is involved in the innate immune response by triggering complement activation[14]. Several studies have shown an association between the presence of single nucleotide polymorphisms (SNPs) in exon 1 of the MBL gene and the occurrence of immunodeficiency and chronic infectious diseases[6,12,15].

The frequency of the mutation in exon 1 of the MBL gene has been recorded in several populations from Europe, Africa, Asia and Melanesia[3,10,16,17]. In the present study, MBL*O presented frequencies of 19% and 26% in HIV-infected patient and control groups, respectively. These relatively high values can be attributed to the European and African genetic background of the population of Belém, estimated at about 49% and 16%, respectively[18].

Garred et al[10] observed a significantly higher prevalence of mutant OO homozygotes (8%) in HIV-1-infected patients in comparison with healthy controls (0.8%). In the present study, the frequency of this genotype was repeated in HIV-1 infected individuals, although a very similar frequency was also recorded in the control group. This difference may reflect the tri-hybrid genetic background of the Belém population, in comparison with that studied by Garred et al[10], which was composed exclusively of Caucasians.

Several studies have verified that MBL deficiency increases susceptibility to HIV-1 infection or affects the process of infection[9,19-21]. In the present study, the comparative analysis of genotype frequencies between the two groups revealed only a slightly higher prevalence of the MBL*O variant in HIV-1-seropositive patients compared to the healthy controls, while the allele and genotype differences were not significant. Thus, the present study did not confirm any association between this genotype and any increased susceptibility to HIV-1 infection, at least in the population of the Brazilian City of Belém.

MBL gene polymorphism and low plasma concentrations of MBL may affect HIV-1 infection by reducing activation of the complement system, resulting in an increase in the plasma viral load[7,10]. However, the results presented here do not support this conclusion, given the absence of any relation between the presence of the MBL*O allele and viral load, despite significant differences in MBL plasma concentrations according to genotype. This result could be related to the relatively small number of individuals carrying the *OO* genotype. Given the implications of the results of the present study, it would be necessary to conduct a much broader study, involving a large number of patients in order to more systematically assess the impact of this mutation on the HIV-1 viral load of carriers.

ACKNOWLEDGMENTS

The authors would like to thank all the individuals who participated in the present study.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

FINANCIAL SUPPORT

PN-DST & AIDS and the CNPq.

REFERENCES

1. Turner MW. The role of mannose-binding lectin in health and disease. Immunobiology 2003; 199:327-339.
2. Madsen HO, Garred P, Thiel S, Kurtzhals JAL, Lamm LU, Ryder LP, et al. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. J Immunol 1995; 155:3013-3020.
3. Jüliger S, Luckner D, Mordmüller B, May J, Weierich A, Lell B, et al. Promoter Variants of the Mannose-Binding Lectin Gene Show Different Biding. Biochem Biophys Res Comm 2000; 275:617-622.
4. Madsen HO, Garred P, Kurtzhals JA, Lamm LU, Ryder LP, Thiel S, et al. A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. Immunogenetics 1994; 40:37-44.
5. Kuipers S, Aerts PC, van Dijk H. Differential microorganism-induced mannose-binding lectin activation. Medical Microbiol Immunol 2003; 36:33-39.
6. Pontes GS, Tamegão-Lopes B, Machado LFA, Azevedo VN, Ishak MOG, Ishak R, et al. Characterization of Mannose-Binding Lectin Gene Polymorphism among Human T-Cell Lymphotropic Virus 1 and 2-Infected Asymptomatic Subjects. Hum Immunol 2005; 66:892-896.
7. Vallinoto ACR, Menezes-Costa MR, Alves AEM, Machado LFA, Azevedo VN, Souza LLB, et al. Mannose-binding lectin gene polymorphism and its impact on human immunodeficiency virus 1 infection. Mol Immunol 2006; 43:1358-1362.
8. Alves AEM, Hermes RR, Lopes BPT, Azevedo VN, Machado LFA, Ishak MOG, et al. Polymorphism in the promoter region of the mannose-binding lectin gene among human T-cell lymphotropic virus infected subjects. Mem Instituto Oswaldo Cruz 2007; 102:991-994.
9. Prohászka Z, Thiel S, Ujhelyi E, Szlávik J, Banhegyi D, Füst G. Mannan-binding lectin serum concentrations in HIV-infected patients are influenced by the stage of disease. Immunol Lett 1997; 58:171-175.
10. Garred P, Madsen HO, Balslev U, Hofmann BO, Pedersen C, Gerstoft J, et al. Susceptibility to HIV infection and progression of AIDS in relation to variant alleles of mannose-binding lectin. Lancet 1997; 349:236-240.
11. Maas J, Husman AMR, Brouwer M, Krol A, Coutinho R, Keet I, et al. Presence of the variant mannose-binding lectin alleles associated with slower progression to AIDS. AIDS 1998; 12:2275-2280.
12. Vallinoto ACR, Pinheiro da Silva RF, Hermes RH, Amaral ISA, Miranda ECB, Barbosa MSB, et al. Mannose-binding lectin gene polymorphisms are not associated with the susceptibility to HCV infection in the Brazilian Amazon region. Hum Immunol 2009; 70:754-756.
13. Ayres M, Ayres JR, Ayres DL, Santos AS. BioEstat: Aplicação estatística nas áreas das ciências biológicas e médicas [CD-ROM]. Versão 5.0. Belém: Sociedade Civil Mamirauá; 2009.
14. Wong NK, Kojima M, Dobo J, Ambrus G, Sim RB. Activities of the MBL-associated serine proteases (MASPs) and their regulation by natural inhibitors. Mol Immunol 1999; 36:853-861.
15. Turner MW. Mannose-binding lectin (MBL) in health and disease. Immunobiology 1998; 199:327-339.
16. Madsen HO, Satz ML, Hogh B, Svejgaard A, Garred P. Different molecular events result in low protein levels of mannan-binding lectin in populations from Southeast Africa and South America. J Immunol 1998; 161:3169-3175.
17. Malik S, Arias M, Di Flumeri C, Garcia LF, Schurr E. Absence of association between mannose-binding lectin gene polymorphisms and HIV-1 infection in a Colombian population. Immunogenetics 2003; 55:49-52.
18. Santos SEB, Guerreiro JF. The indigenous contribution to the formation of the population of the Brazilian Amazon Region. Rev Bras Genetica 1995; 18:311-315.
19. Nielsen SL, Andersen PL, Koch C, Jensenius JC, Thiel S. The level of the serum opsonin, mannan-binding protein in HIV-1 antibody positive patients. Clin Exp Immunol 1995; 100:219-222.
20. Pastinen T, Liitsola K, Niini P, Alminen M, Syvänen AC. Contribution of the CCR5 and MBL genes susceptibility to HIV type I infection in the Finnish population. AIDS Res Hum Retroviruses 1998; 14:171-175.
21. Catano G, Agan BK, Kulkarni H, Telles V, Marconi VC, Dolan MJ, et al. Independent effects of genetic variations in mannose-binding lectin influence the course of HIV disease: the advantage of heterozygosity for coding mutations. J Infect Dis 2008; 198:72-80.