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

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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 mannan or other carbohydrate components of viruses, bacteria and yeasts¹. The performance of MBL is directly related to its serum concentration, which is determined by the interplay between the promoter and structural gene mutations²,³.

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, respectively⁴. 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 pathogens⁵-⁸.

Low levels of serum MBL have been primarily associated with a significant increase⁹ or reduction¹⁰ 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 death¹¹.

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™ kit, following the manufacturer’s instructions (NucliSens 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™ 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 protocols2,12. The reactions were performed in a final volume of 50µl containing 100ng genomic DNA, 200µM dNTPs, 5pmol primers, 50mM KCl, 2.5mM MgCl₂, 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’-AGGATCCAGGCAGTTTCCTGGAAGG-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 software13. 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, 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).

| TABLE 1 - Distribution of the allele and genotype frequencies among HIV-1 infected individuals. |
|-----------------------------------------------|---------------|---------------|-------|-------|
| Genetic profile | HIV-1 | n | % | Control | n | % | χ² | 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.
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. 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. The frequency of the mutation in exon 1 of the MBL gene has been recorded in several populations from Europe, Africa, Asia, and Melanesia. 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.

Garred et al. 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., 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. 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. 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.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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