Mannose-binding lectin gene polymorphism and its impact on human immunodeficiency virus 1 infection

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Abstract
Mannose-binding lectin (MBL) is a serum protein whose low concentration is associated with the occurrence of allele variants named MBL*A, MBL*B and MBL*C, whose frequencies were 69%, 22% and 9% among patients and 71%, 13% and 16% among healthy controls, respectively. The presence of the variant MBL*B was associated with higher plasma viral load levels, suggesting the importance of the MBL gene polymorphism in the clinical evolution of HIV-1-infected patients.

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1. Introduction
Mannose-binding lectin (MBL) is a liver-derived pluripotent serum lectin that has a role in the host’s innate immune system (Turner, 2003) by binding with high affinity to mannose or other carbohydrate components existent in viruses, bacteria and yeast (Kuipers et al., 2003). However, MBL function is directly associated with its serum concentrations which are determined by the interplay between promoter and structural gene mutations (Madsen et al., 1995; Juliger et al., 2000).

Three mutations have been described in the structural region of the molecule (codons 52, 54 and 57) from which are derived three allelic variants named MBL*D, MBL*B and MBL*C, respectively. On the other hand, the wild allele is called MBL*A (Madsen et al., 1994). The occurrence of these variants have been associated with MBL serum deficiency, and consequently, to susceptibility/resistance to infection by various pathogens, including HIV-1 (Drogari-Apiranthitou et al., 1997; Garred et al., 1997; Prohászka et al., 1997; Luty et al., 1998; Hoberd et al., 1999; Peterslund et al., 2001; Klabunde et al., 2000; Roy et al., 2002; Song et al., 2003).

The pathogenesis of human immunodeficiency virus 1 infection is very complex and of course influenced by both viral and host factors (Cohen et al., 1997). Recently, studies have focused the attention about the role of MBL gene variants and its serum concentration on the progression of AIDS in HIV-1-infected subjects (Garred et al., 1997; Prohászka et al., 1997).

The present study compares the genotype frequencies in two populational groups composed of HIV-1-infected patients and HIV-1-seronegative controls, and investigates if the occurrence of allele variants could be a factor in the susceptibility to HIV infection or if they have some influence on values of the known predictive markers for disease progression in HIV-1 infection.
2. Materials and methods

2.1. HIV-1-infected patients and seronegative control

A total of 145 blood samples from HIV-1-infected patients obtained in the Unidade de Referência de Doenças Infecciosas e Parasitárias, state of Pará, Brazil (UDEPIE) and Laboratório Central, state of Amapá, Brazil (LACEN-AP) was carried to the Laboratório de Virologia (Centro de Ciências Biológicas da Universidade Federal do Pará), where the samples were submitted to measurements of known predictive markers for disease progression in HIV-1 infection. All patients were under antiretroviral therapy at the moment of the sample collection, according to the Brazilian consensus of the National Program of STD and AIDS of the Ministry of Health (Ministério da Saúde, 2004). Formal inclusion to the study group was preceded by the signature of a thoroughly explained consent form at the first visit.

As a control group, we investigated 99 blood samples from individuals residing in Belém (capital of the state of Pará, Brazil), which were stored at −20 °C at the Laboratório de Virologia. All specimens were previously screened for HIV-1 infection using an enzyme-linked immunosorbent assay (OrthoDiagnostic, US).

2.2. Sample collection

Whole blood samples, from both HIV-1-infected patients and the seronegative control group, were collected in Vacutainer tubes containing K3 EDTA as anticoagulant in order to obtain plasma and peripheral blood mononuclear cells (PBMC). Whole blood was used to achieve quantification of CD4+ T-cells and plasma and PBMC samples were subjected to genomic DNA extraction according to the manufacturer’s instructions (Puregene, Gentra Systems Inc., US).

2.3. Detection of MBL gene polymorphism

In the present study, PCR was performed to amplify 349 bp from exon 1 of the MBL gene, using genomic DNA extracted from HIV-1-infected patients and seronegative subjects. The PCR was accomplished using the Mastercycler Personal Eppendorf thermocycler.

The reactions were carried out in a final volume of 50 μl containing 100 ng genomic DNA, 200 μM dNTPs, 5 pmol primers, KCl 50 mM, MgCl2 2.5 mM, Tris–HCl, pH 8.3, 10 mM and 0.5 U of Taq polymerase (Invitrogen, US). The following pair of primers were used in the genotyping analysis: (mblE01) 5′-AGTCGACCCAGATTTAGGACAGAG-3′ and (mblE02) 5′-AGATCCAGCGATTCTCTGGAAAGG-3′ (Madsen et al., 1995). PCRs were initiated by denaturation step at 94 °C for 2 min, followed by 35 cycles of: 30 s at 94 °C, 60 s at 58 °C and 120 s at 72 °C.

The identification of the MBL*A, MBL*B and MBL*C alleles was performed by RFLP analysis of the 349 bp product using BanI and MboI restriction enzymes, followed by a 2% agarose gel electrophoresis, as previously described (Madsen et al., 1995) and a PCR-SSP method was used for discrimination of the MBL*D allele as reported by Steffensen et al. (2000).

2.4. Quantification of plasma HIV-1 viral load and CD4+ T-cells

The plasma viral load was determined by the Nasha method using the NucliSens Reader equipment and kit NucliSensTM produced by Nabsa Diagnostics (Organon Teknika, Botei, The Netherlands).

Blood samples were processed within 4 h of collection and the Tcell subsets count was determined by flow cytometry (FacsCount, Becton & Dickinson, US) using the FacsCountTM Reagents immunomonitoring kit, according to standard protocol recommended by the manufacturer (Becton Dickinson, US).

All patients enrolled in the present study were tested for plasma HIV-1 viral load and CD4+ T-cells at the time of acceptance to join the study and had a second test approximately 4 months later. The study was conducted within a period of 12 months.

2.5. Statistical methods

Allele and genotype frequencies, as well as the Hardy–Weinberg equilibrium, were analyzed, in both populations, using the software “Tools for Population Genetic Analysis – TFPGA 1.3y” (Miller, 1997).

The association analysis between frequencies of variant alleles and the arithmetic mean values of viral load and TCD4+ cells counting were performed by χ2 and ANOVA test using the software BIOSAT (Ayers et al., 2003).

3. Results

3.1. Allele and genotype frequency of MBL

Allele frequency distributions are demonstrated in Table 1. The alleles MBL*A, MBL*B and MBL*D were identified in the present study among both HIV-1-infected patients and the seronegative control group. The occurrence of the mutation MBL*C was not observed. The differences of the allele frequencies between the two groups was significant (χ2 = 8.767; p = 0.0125).

The genotype frequency distributions are listed in Table 2. A higher prevalence of genotypes with the allele MBL*B was observed in the HIV-1-seropositive patients as compared to the control group (χ2 = 2.739; p = 0.098). Furthermore, the genotype B/B was six times more frequent in the HIV-1-infected patient group (χ2 = 4.042; p = 0.044). On the other hand, the...
genotype A/D was the most frequent in the seronegative control ($p^2 = 6.372; p = 0.0116$).

3.2. Association of predictive markers of the progression of HIV-1 infection and the MBL gene polymorphism

Seeking to evaluate the possible association between the predictive markers for disease progression in HIV-1 infection and the MBL gene polymorphism, HIV-1-infected patients were arbitrarily divided in three groups according to the genotype: A (A/A), B (A/B, B/B and B/D) and C (A/D and D/D). These criteria were employed, acknowledging that the presence of the mutation MBL*B, in homo or heterozygosis, is associated with low levels of MBL in the serum. In the comparative analysis group, D was excluded due the small sample size.

The first quantification of the CD4+ T-cells showed lower levels among HIV-1-seropositive patients who carry genotypes with mutation MBL*B, in homo or heterozygosis, is associated with low levels of MBL in the serum. In the comparative analysis group, D was excluded due the small sample size.

The first quantification of the CD4+ T-cells showed lower levels among HIV-1-seropositive patients who carry genotypes with mutation MBL*B, in homo or heterozygosis, is associated with low levels of MBL in the serum. In the comparative analysis group, D was excluded due the small sample size.

The comparative analysis between the first and second measurements of the CD4+ T-cells showed that patients carrying the MBL*A allele presented an improvement of the CD4+ T-cells ($p < 0.001$), a characteristic which was not evident among HIV-1-infected patients carrying the MBL*B allele ($p = 1.000$).

Table 3

<table>
<thead>
<tr>
<th>Evaluation</th>
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<th>Mean (T CD4+ cell/ml)</th>
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<tr>
<td>First evaluation</td>
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<td>370</td>
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<td>36</td>
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<tr>
<td>B</td>
<td>35</td>
<td>341</td>
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<td></td>
</tr>
<tr>
<td>D</td>
<td>04</td>
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<td>Group A vs. Group B</td>
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<td>Second evaluation</td>
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<td></td>
</tr>
<tr>
<td>A</td>
<td>36</td>
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<tr>
<td>B</td>
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<tr>
<td>D</td>
<td>04</td>
<td>597</td>
<td>Group A vs. Group B</td>
<td>0.069</td>
</tr>
</tbody>
</table>

The first quantification of the viral load showed that the HIV-1-infected patients carrying the MBL*A allele presented values lower than that of patients with the MBL*B allele (Table 4). In the second evaluation, this value was 9.8 times higher among the carriers of the MBL*B allele (Table 4). Additionally, a significant reduction of the viral load was observed in the group of patients carrying the MBL*A allele ($p < 0.001$). HIV-1-infected patients carrying the MBL*B allele did not show this reduction of the viral load ($p = 0.999$).

4. Discussion

In the present study, we evaluated the association between the predictive markers for disease progression in HIV-1 infection and the MBL gene polymorphism. MBL is an important acute-phase serum protein involved in the innate immune response that can trigger complement activation (Wong et al., 1999). Additionally, several studies have shown an association between the presence of allele mutation in the exon 1 of the MBL gene and the occurrence of immunodeficiency and chronic infectious diseases (Turner, 1998).

The frequency distribution of the mutation in the exon 1 of the MBL gene has been described in several populations in Europe, Africa, Asia and Melanesia (Garred et al., 1997; Madsen et al., 1998; Jülicher et al., 2002; Malik et al., 2003). The MBL*B variant occurs varying from 13% to 28% in European, Chinese and Eskimos populations. The MBL*D mutation is present in European and African populations with frequencies of about 14%. On the other hand, MBL*C is present in Sub-Saharan African populations with frequencies ranging from 50% to 60% (Kilpatrick, 2002; Turner, 2003). In the present study, the MBL*B and MBL*D were present with frequencies of 13–22% and 9–16%, respectively. This high prevalence can be attributed to the European and African genetic background of the general population of Belém where the White and Black contributions were 49% and 16%, respectively (Santos and Guerreiro, 1995). On the other hand, the absence of MBL*C, which has high frequencies in the Sub-Saharan Africa, could be attributed to the small sample size or to the origin of African slaves brought to northern region of Brazil, who were brought from Angola, Mozambique, Kenya and Tanzania.
and Figueiredo, 1990). Further studies involving a large number of Amazonian populations, including semi-isolated African descendant communities, will be necessary to determine the real frequency of the MBL*C allele in this region.

Garred et al. (1997) observed a significantly higher prevalence of homozygotes in the MBL gene mutations (8%) in HIV-1-infected patients than in healthy controls (0.8%). The genotype distribution found herein does not support this previous report. Perhaps this difference reflects the genetic background of the Belem population, which has a tri-hybrid model, distinguished from the population studied by Garred et al. composed exclusively of Caucasians.

Several studies highlight that MBL deficiency increases the susceptibility to HIV-1 infection or affects the progression of infection (Nielsen et al., 1995; Prohászka et al., 1997; Pastinen et al., 1998). In the present study, the genotype frequency comparative analysis between the two groups showed a high prevalence of the MBL*B variant among HIV-1-seropositive patients as compared to the healthy control subjects. Furthermore, the genotype B/B was six times more frequent among HIV-1-infected patients, but the allele and genotype differences were not significant. Thus, it is premature to affirm that the presence of this genotype can be associated with the increased susceptibility to HIV-1 infection in Belem.

The MBL gene polymorphism can have a direct role in HIV-1 infection. The low serum concentration of MBL, and subsequent reduction of complement system activation, can contribute to the increasing of the plasma viral load (Garred et al., 1997). The results found in the current study support this hypothesis, where a higher plasma viral load was detected among HIV-1-seropositive patients that carried MBL*B variant, as previously described in a group of HBV-seropositive patients (Song et al., 2003). The current results suggest that the presence of the MBL*B variant, which correlates with low serum levels of MBL, could be associated with an inefficient elimination of the virus from the blood of HIV-1-infected patients, and consequently, an increasing of plasma viral load. We have ruled out the possibility of the high viral load to be due to the occurrence of drug resistance mutations in the HIV-1 strains, because our previous study has showed the absence of antiretroviral resistance mutations in patients carrying MBL*B variant (data not shown).

On the other hand, the decrease of viral load observed among patients that carried MBL*A allele could be explained by the direct elimination of the virus from the blood as a result of the usual MBL activity that has been associated with this allele. Furthermore, it is not possible to rule out the possibility that the allele MBL*A is also a marker associated to a better response to an adequate antiretroviral therapy.

It has been suggested that the MBL serum concentration derived from the MBL*D variant can be partially incorporated in the functional oligomeric structure of the MBL molecule when in association with the MBL*A wild allele. This characteristic is not observed in the protein chains coded by MBL*B and MBL*C mutations (Garred et al., 2003), which could explain the normal serum levels of MBL in subject carriers of the genotype A/D. This observation explains why, in the present study, a high viral load was not found among patients carrying the allele MBL*D.

On the other hand, it is necessary to conduct a broad study involving a large number of patient carriers of this variant to verify the real impact of this mutation on the HIV-1 viral load.

Taking into account the results obtained herein, we can conclude that the MBL gene polymorphism characterization in HIV-1-infected patients can be an important auxiliary biological marker in the evaluation of the progression of the HIV disease, in its association with the measurements of CD4+ T-cells and plasma viral load.

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