Markers of HIV-1 infection and its pathogenesis
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Chapter 5

Presence of the variant mannose-binding lectin alleles associated with slower progression to AIDS. Amsterdam Cohort Study


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Presence of the variant mannose-binding lectin alleles associated with slower progression to AIDS

Jaap Maas, Ana-Maria de Roda Husman*, Margreet Brouwer*, Anneke Krol, Roel Coutinho, Ireneus Keet, Remko van Leeuwen and Hanneke Schuitemaker* from the Amsterdam Cohort Study

Objective: To examine the association between mannose-binding lectin (MBL) polymorphism and progression to AIDS and death in HIV-1 infection.

Design and methods: In 131 HIV-1-infected homosexual seroconverters, survival analyses were performed to determine both the association between MBL genotype and time from HIV-1 seroconversion to AIDS and death, and time from AIDS to death.

Results: Of the 131 seroconverters, of whom 61 developed AIDS, 76 were typed as homozygous wild-type and 55 as carriers of variant alleles (52 heterozygous and three homozygous variant alleles). A survival analyses suggested that HIV-1-infected men with the variant alleles progressed somewhat slower to AIDS [relative hazard (RH), 0.62; 95% confidence interval (CI), 0.36–1.10] and death [RH, 0.73; 95% CI, 0.42–1.25]. Interestingly, CD4+ T-cell count determined at the moment of AIDS was found to be significantly lower among persons with the mutation (97 x 10^9/l versus 204 x 10^9/l; P = 0.03). Furthermore, when AIDS-free times before the diagnosis of an opportunistic infection were compared with those preceding a diagnosis of Kaposi's sarcoma, Kaposi's sarcoma diagnosis was more postponed than that of an opportunistic infection (RH, 0.21; 95% CI, 0.05–0.95; versus RH, 0.67; 95% CI, 0.35–1.27).

Conclusion: Indications for a weak pre-AIDS protective effect of variant MBL alleles were demonstrated.

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Keywords: HIV-1, AIDS, genetic markers, coreceptors, mannose-binding lectin, mannose-binding protein, progression, genotype, survival, HIV-1 seroconverters, Kaposi's sarcoma, opportunistic infections, human herpesvirus type 8

Introduction

Insight into the role of host genetic factors in the natural history of HIV-1 infection is rapidly increasing. Recently, mutations in the gene encoding mannose-binding lectin (MBL), also known as mannose-binding protein, were found to be associated with a significantly shorter survival time after AIDS [1]. Interestingly, no association between the occurrence of these variant MBL alleles and progression to AIDS could be found. However, the moment of HIV-1 seroconversion was unknown in this study and therefore an early effect could have been missed.

Serum MBL is a liver-synthesized protein with binding specificity towards mannose and N-acetylglycosamines, which can be found on the surface of several bacteria, yeast and the envelope of HIV-1 (gp120) [2], and which plays a role in first-line defence immunity. Low serum levels are closely associated with opsonization...
defects and impaired phagocytosis [2-5]. After opsonization of the organisms, both the alternative and the classical complement pathway are activated [6]. Lower MBL serum concentrations are explained by the dominant actions of point mutations in codons B (Asp-54), C (Cla-57) and D (Cys-52) [7]. According to the studies of Ezekowitz et al. [8], serum MBL can inhibit HIV-1 infection in vitro, whereas Nielsen et al. [9] was unable to find any relationship between MBL serum levels and progression of disease.

The aim of this study was to analyse whether the variant MBL alleles are associated with progression to AIDS and death as well as with the survival time after AIDS diagnosis. In addition, the correlation between MBL genotype and the occurrence of specific AIDS-defining diseases was studied.

Materials and methods

Subjects
Since October 1984, HIV-seronegative and seropositive homosexual men have been enrolled in the Amsterdam Cohort Study on the natural history of HIV-1 infection and AIDS [10]. As of October 1996, 131 men with a mean age of 36 years (range, 21-58 years) seroconverted for HIV-1 during follow-up, of whom 61 developed AIDS according to the 1987 Centers of Disease Control and Prevention case definition [11]. The median time between the last HIV-1-negative and the first HIV-1-positive blood sample was 3.1 months (range, 1.6-11.1 months). All participants were seen at 3-monthly visits at which a medical history was taken, a physical examination was performed, and blood was drawn for immunological and virological laboratory evaluations.

In a nested matched case-control study, 16 seroprevalent non-progressors (AIDS-free after at least 9 years of seroconverter follow-up with a mean CD4 T-cell count above 400 x 10^3/l in years 8 and 9 of follow-up) were individually matched with two seroprevalent progressors (AIDS within 2-7 years) for age (±10 years) and CD4+ T-cell counts (±250 x 10^3/l) in year 2 of HIV-1-seroconverter follow-up. Due to the small number of carriers of homozygous (O/O) variant MBL alleles, heterozygous (A/O) and homozygous (O/O) variant allele carriers were analysed together. All 179 participants analysed were Caucasian.

Statistical methods
In survival analyses, Kaplan-Meier and Cox proportional hazards analyses were performed to estimate the time between the moment of HIV-1 seroconversion and the following endpoints: AIDS according to the Centers for Disease Control and Prevention case definitions of 1987 [11], Kaposi's sarcoma (KS), AIDS-related opportunistic infections and death. Those who had not reached an endpoint were censored at 1 January 1996. We used the mid-point between the last HIV-1-negative and the first HIV-1-positive blood sample as the estimated time of HIV-1 seroconversion. The Cox proportional hazards analyses were repeated with AIDS, KS and opportunistic infection as starting point and death as endpoint. Furthermore, we examined the association between the MBL genotype and AIDS-free follow-up after adjusting for the following markers of progression: HIV-1 RNA load, determined 1 year after HIV-1 seroconversion and dichotomized at above and below 10 000 copies/ml [12] and CD4+ T-cell count, dichotomized at above and below 200 x 10^3/l. All covariables, except MBL genotype and HIV-1 RNA, were analysed as time-dependent variables.

In all seroconverters, differences between MBL genotype and continuous variables with a normal distribution, such as T-cell subsets at moment of seroconversion (range, 6 months) and mean CD4+ T-cell decline per year, were tested using the Student's t-test. The individual slope of CD4+ T-cell decline was determined using linear least squares regression for each HIV-1-positive subject with at least three CD4+ T-cell counts available for analysis. Variables with a skewed distribution, such as HIV-1 RNA levels and CD4+ T-cell count less than 6 months before AIDS, were used after log transformation and square-root transformation, respectively. If normality was not reached, a non-parametric test was applied.

Conditional logistic regression analysis was used to study the genotypic frequency in a matched case-control study, in which the non-progressors were the reference group. Statistical significance was determined by log-likelihood ratio statistics (P < 0.05).

Laboratory methods
For MBL genotyping, genomic DNA was extracted from PBMNC of 131 seroconverters and 48 seroprevalent individuals using the QIAamp blood kit (Qiagen, Dusseldorf, Germany). As previously described, amplification using a general PCR and a site-directed mutagenesis PCR was followed by detection of MBL variant alleles by restriction fragment-length polymorphism analyses [7]. Briefly, general PCR products were subjected to BanI digestion, which only cleaves the A allele, and to MbolI digestion cleaving only the C allele. Subsequently, site-directed mutagenesis PCR was performed, which introduced an HflI or Mrnl restriction site into the A and D alleles, respectively. Digestion with HflI only leaves the D allele uncleaved, whereas Mrnl specifically cleaves the D allele. Restriction fragments were analysed by agarose gel electrophoresis.
**Results**

Of the 131 seroconverters, 76 (58%) were typed as homozygous wild-type (A/A; MBL-WT). The remaining 55 were typed as carriers of the MBL variant alleles [MBL-VA; 32 heterozygous A/B (24%), two A/C (2%), 18 A/D (14%), and three homozygous B/B (2%)]. At the moment of HIV-1 seroconversion there was no difference in CD3+, CD4+ and CD8+ T-cell counts or HIV-1 RNA levels between MBL-WT and MBL-VA carriers (Table 1).

Compared with HIV-1 MBL-WT seroconverters, carriers of MBL-VA progressed somewhat slower to AIDS [relative hazard (RH), 0.62; 95% confidence interval (CI), 0.36–1.1; Fig. 1a, Table 2] and death (RH, 0.73; 95% CI, 0.42–1.25; Fig. 1b). We did not observe an effect of the MBL genotype on survival after AIDS (Fig. 2, Table 2).

Despite the indications of somewhat slower progression in the group of MBL-VA carriers, there was no difference in the rate of CD4+ T-cell decline (101 x 10^{3/4}/l and 94 x 10^{3/4}/l per year for MBL-WT and MBL-VA carriers, respectively). However, interestingly, the group with MBL-VA appeared to have lower absolute CD4+ T-cell counts at time of AIDS diagnosis (204 x 10^{3/4}/l and 97 x 10^{3/4}/l for MBL-WT and MBL-VA carriers, respectively; \( P = 0.03 \)).

Since KS is an AIDS-defining event that generally occurs at a higher CD4+ T-cell count, we compared the progression rates of KS and opportunistic infection cases for the two genotypic groups. Indeed, carriers of MBL-VA showed a significantly lower risk for developing KS (RH, 0.21; 95% CI, 0.05–0.96). The percentage of KS cases (n = 15) compared with all AIDS cases (n = 61) with MBL-WT was 31% (13 out of 42), versus 11% (two out of 19) for MBL-VA carriers (\( P = 0.12 \)). Three persons were diagnosed with AIDS diagnoses other than opportunistic infections or KS. This significantly lower progression rate to develop KS and the lower percentage of KS cases together with a lower CD4+ T-cell count in the KS-diagnosed individuals (347 x 10^{3/4}/l and 49 x 10^{3/4}/l for MBL-WT and MBL-VA carriers, respectively; \( P = 0.11 \)), although not significant, were suggestive for a protective association of the MBL-VA carriers towards developing KS, when compared with MBL-WT carriers.

A borderline significant difference in CD4+ T-cell count at time of AIDS diagnosis between genotypic groups was, however, also observed when an opportunistic infection was the AIDS-defining event (154 x 10^{3/4}/l and 83 x 10^{3/4}/l for MBL-WT and MBL-VA carriers, respectively; \( P = 0.06 \)). The percentage of opportunistic infection cases (n = 43) compared with all AIDS cases (n = 61) in MBL-WT was 60% (29 out of 42) versus 74% (14 out of 19) for MBL-VA carriers.

**Table 1.** Immunological and virological baseline characteristics found in all 131 HIV-1 seroconverters participating in the Amsterdam Cohort Study at moment of seroconversion.

<table>
<thead>
<tr>
<th>Markers of progression</th>
<th>MBL variant alleles (n = 55)</th>
<th>Wild-type alleles (n = 76)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunological markers * (x10^3/4)</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CD3+ T cells</td>
<td>1588 (678)</td>
<td>1561 (668)</td>
<td>0.82</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>775 (319)</td>
<td>733 (279)</td>
<td>0.43</td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>684 (308)</td>
<td>732 (501)</td>
<td>0.38</td>
</tr>
<tr>
<td>Virological markers</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>HIV-1 RNA (log_{10} copies/ml)</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*Student's t-test. \*Obtained 1 year after seroconversion. MBL, Mannose-binding lectin.

**Fig. 1.** Kaplan–Meier analysis of (a) AIDS-free survival time and (b) overall survival in 131 HIV-infected seroconverters of the Amsterdam Cohort Study, of whom 55 were carriers of the variant mannose-binding lectin (MBL) alleles. Starting point was HIV seroconversion, and endpoint was (a) AIDS diagnosis, according to the Centers for Disease Control and Prevention 1987 case definition and (b) death.
Likewise, as seen in individuals who developed KS, we also found in those who developed opportunistic infections indications for a trend of slower progression among MBL-VA carriers (RH, 0.67; 95% CI, 0.35–1.27). No effect of the MBL genotype on survival after KS and opportunistic infection diagnoses could be found (Table 2).

In the multivariate Cox proportional hazards model, after adjustment for CD4+ T cell and plasma HIV-1 RNA, the RH for MBL-VA remained similar at 0.59 (95% CI, 0.33–1.06; \( P = 0.08 \)). This was suggestive for the fact that the protective effect of the MBL mutation was working by another mechanism than that of the established markers.

Finally, the matched case–control study confirmed the direction of the protective association with an odds ratio of 0.61 (95% CI, 0.15–2.42), with seven (44%) out of 16 non-progressors compared with 11 (34%) out of 32 progressors carrying MBL-VA.

**Table 2. Median AIDS-free follow-up and survival time observed in all 131 HIV-1 seroconverters.**

<table>
<thead>
<tr>
<th>Endpoints and markers</th>
<th>Median years of survival (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MBL variant alleles (n = 55)</td>
</tr>
<tr>
<td>HIV-1 seroconversion to AIDS*</td>
<td>7.9 (6.0–9.8)</td>
</tr>
<tr>
<td>HIV-1 seroconversion to Kaposi's sarcoma</td>
<td>11.3</td>
</tr>
<tr>
<td>HIV-1 seroconversion to AIDS-defining opportunistic infection</td>
<td>10.0 (7.3–12.6)</td>
</tr>
<tr>
<td>AIDS to death</td>
<td>1.7 (0.9–1.7)</td>
</tr>
<tr>
<td>Kaposi's sarcoma to death</td>
<td>1.9</td>
</tr>
<tr>
<td>AIDS-defining opportunistic infection to death</td>
<td>1.1 (0.8–1.5)</td>
</tr>
</tbody>
</table>

*AIDS diagnosis according the 1987 Centers for Disease Control and Prevention case definition. \(^*\)Six patients died of reasons other than AIDS or the cause of death remained unclear. \(^*\)Log-likelihood ratio. \(^*\)Estimated median survival time exceeded the maximal length of follow-up. \(^*\)Calculation of confidence interval (CI) was not possible due to small numbers.

**Fig. 2. Kaplan–Meier analysis of 61 HIV-infected seroconverters of the Amsterdam Cohort Study.** Starting point was the moment of AIDS diagnosis, according to the Centers for Disease Control and Prevention 1987 case definition, and endpoint was death.

**Discussion**

In this study, we found indications for a protective association of variant MBL alleles with AIDS-free survival amongst a group of HIV-1-infected individuals with a well-documented moment of seroconversion. This association was confirmed in a matched case–control study with seroprevalent HIV-1-infected cases. The mean CD4+ T-cell count found at the moment of AIDS diagnosis was lower in the group of individuals that carried MBL-VA than the group of MBL-WT carriers, although CD4+ T-cell decline was the same in both genotypic groups. It thus seems that the delayed onset of AIDS in MBL-VA carriers is simply due to the fact that AIDS develops at a lower level of CD4+ T-cell count in MBL-VA carriers.

However, if we look at the median survival times after AIDS diagnosis, in both the overall analysis and in persons diagnosed with only opportunistic infections, survival was somewhat shorter in MBL-VA carriers. This is in accordance with the results presented in the study of Garred et al. [1]. It may well be that the discrepancy between our study and the study by Garred et al. [1] is due to the fact that we studied well-defined seroconverters, thus disregarding possible biases that may be introduced by studying seroprevalent cases.

In this study we did not find any person with a B/D subtype [13,14] as would be expected, which is possible due to the relatively small sample size of the study. Furthermore, we also observed a significantly lower progression rate of KS in the group of MBL-VA carriers, an AIDS-defining event that in general occurs at a higher CD4+ T-cell count. Indeed, a weak protective effect of MBL-VA on the time to KS was observed.
Another explanation may be that carriers of MBL-VA are more resistant to developing AIDS per se, even in the presence of only low CD4+ T-cell counts.

Soluble serum MBL [3,15] bear close resemblance to the first complement factor of the classical complement pathway (C1q) [16], both of which belong to the family of collectives. After binding to the terminal mannos and N-acetylgalactosamine moieties of some bacteria, yeast, Pneumocystis carinii [15] and the HIV-1 envelope [2], both the alternative and classical [6,7] complement pathways are activated, leading to the killing of the microorganism.

Recently, Prohaszka et al. [17] showed that fixation of C1q to intact virions results in an enhanced productive HIV-1 infection in MT-4 cell cultures. Haurum et al. [2] found that binding of the HIV envelope gp120 and gp110 by MBL was capable of initiating the classical complement pathway. Furthermore, in another recent study by Prohaszka et al. [18], significantly lower MBL serum levels were found in asymptomatic HIV-1-infected persons when compared with healthy HIV-1-seronegative individuals and persons with AIDS. In addition, they also found a negative correlation between MBL serum levels and CD4+ T-cell counts. It can therefore be hypothesized that, due to the close molecular resemblance between MBL and C1q, the mechanism of infection enhancement also applies to MBL. In other words, due to the association between high serum levels of the complement factor C1q and infection enhancement, low serum levels of MBL as seen in carriers of MBL-VA [2,19,20] may be beneficial. In particular, it seems that the MBL-VA genotype is associated with a protective effect against KS. Furthermore, we found indications that the MBL-VA genotype postponed the moment of KS diagnosis. However, the numbers were small and the results far from conclusive. Nevertheless, these results remain intriguing, especially in light of a study of Uccini et al. [21], who found a nearly 100% MBL receptor expression in KS lesions. Human herpesvirus (HHV)-8 is known to be associated with Kaposi’s sarcoma [22,23]. We therefore also examined the association between the MBL genotype and the presence of HHV-8 antibodies. We did not find an association between the presence of HHV-8 antibodies and the MBL genotype in homosexual men (N. Renwick and J. Goudsmit, personal communication, 1998).

In this study, indications for a weak pre-AIDS protective effect of MBL-VA was demonstrated. In contrast to the findings reported by Garred et al. [1], we did not observe an association between MBL variant alleles and a decreased survival time after AIDS diagnosis. However, such a post-AIDS effect cannot be ruled out, because both studies comprised relative small numbers of individuals. Therefore, additional analyses with larger numbers as well as detailed analyses on the possible mechanism by which MBL genotype may influence AIDS pathogenesis should be subject of future studies.

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