Pivotal role of HIV and EBV replication in the long-term persistence of monoclonal gammopathy in patients on antiretroviral therapy

David Eric Ouedraogo1,2,3, Alain Makinson4, Jean-Pierre Vendrell1,2,3, Marie-Laure Casanova4, Nicolas Nagot1, Renaud Cezar2, Karine Bollore1,2,3, Yassine Al Taaba1,3, Vincent Foulongne1,3, Stéphanie Badiou5, Johannes Viljoen1,6, Jacques Reynes4, Philippe Van de Perre1,2,3, and Edouard Tuaillon1,2,3

1INSERM U1058, Université Montpellier 1, France
2CHRU de Montpellier, Institut de Recherche en Biothérapies, France
3CHRU de Montpellier, Département de Bactériologie-Virologie, France
4CHRU de Montpellier, UMI 233, Département des Maladies Infectieuses Tropicales, France
5CHRU de Montpellier, Département de Biochimie, France
6Africa Centre for Health and Population Studies, University of KwaZulu-Natal, South Africa

Running head: HIV-associated monoclonal gammopathy persistence

Correspondence:

David Eric Ouedraogo (corresponding author)
Institute of Research in Biotherapy
CHU Montpellier Hôpital Saint-Eloi
80, av. Augustin Fliche
34295 Montpellier Cedex 5, France
Phone: 33 467 338 972
Fax: 33 467 330 113
E-mail: daverid32@yahoo.fr
Key points: Immunological and virological factors associated with monoclonal gammopathy persistence in HIV-infected patients; B Lymphocytes activation and Epstein-Barr virus replication are key features of monoclonal gammopathy.

Abstract

High prevalence of monoclonal gammopathy (MG) is observed in HIV-infected patients. We explored the conditions associated with long term persistence of serum monoclonal protein (M protein) in HIV-infected patients on antiretroviral therapy (ART). Of 21 patients with monoclonal gammopathy, M Protein disappeared in 12 patients (58%) over 5 years of ART. Higher level of serum gamma-globulin and higher percentages of circulating plasmablasts and plasma cells were observed in patients with persistent MG in comparison to patients with transient MG. MG persistence was associated with the cumulative time of detectable plasma HIV RNA after ART initiation, detection of EBV DNA in plasma and high level of EBV DNA in B cells. Poor control of HIV replication and detectable EBV replication in plasma were both associated with long-term MG persistence in patients on ART. In case of viral control, MG associated with HIV infection is usually transient.

Key words: Monoclonal gammopathy, Epstein-Barr virus, ART, B-cells, HIV, Lymphocytes activation.
Introduction

HIV induces numerous impairments in B cell function \(^1\). Polyclonal hypergammaglobulinemia is one of the major manifestations of B cell deficiencies associated with HIV replication \(^2\). Monoclonal and oligoclonal proteins detected in serum are also frequently observed in HIV-infected patients \(^3\). With the advent of widespread use of combination antiretroviral treatment (ART), it has become clear that long-term effective ART dramatically reverses many B cell abnormalities including polyclonal activation, autoimmune response, plasmacytosis and hypergammaglobulinemia \(^4-6\).

In HIV-infected individuals, the prevalence of MG is estimated to be between 3% and 26% \(^3\) and occurs at a younger age when compared to the general population, in which the prevalence is estimated to be 3.2% in persons over 50 years old and 5.3% in those over 70 \(^7\). The significance and evolution of monoclonal gammopathy (MG) after ART initiation remains unclear.

Previous studies reported that the impairment of specific T-cell function in HIV-infected individuals is associated with an increase of EBV DNA load in PBMC \(^8\). B-cell hyperactivation and terminal differentiation into plasmablasts and plasma cells induce EBV reactivation and contribute to the increase of its reservoir \(^9-10\). The close relationship between EBV and lymphoproliferative disorders may include MG in HIV-infected patients. We hypothesized that the persistence of MG in HIV-infected individuals requires a continuous B cell stimulation fueled by both HIV and EBV replications.

In the present study, we investigated whether immune activation and HIV and EBV replication were associated with the long term persistence of MG in HIV-infected patients on ART exhibiting a monoclonal band at the time of HIV diagnosis.

Methods

Patients and study design

Twenty one patients in whom monoclonal protein (M protein) was identified by combined PEP and immunofixation were included between 1998 and 2005. All patients were treated and regularly followed up for HIV therapeutic monitoring at the Infectious Disease Department of the Montpellier University Teaching Hospital. MG was detected before treatment initiation. Concentrations of the monoclonal protein ranged from unquantifiable (15/21 cases) to 19.3
g/L with a median of 3.1 g/L when quantifiable. Fifteen HIV-infected subjects without monoclonal band were included as controls. The long-term persistence of MG was controlled in 2011 after at least 5 years of ART by PEP (Capillars 2 SEBIA, Evry, France) and immunofixation (Hydrasys 2 (SEBIA, Evry, France). We defined MG as “persistent” when the monoclonal peak remained detectable and confirmed and as “transient” when the monoclonal peak disappeared. Patients' characteristics and treatments are presented in Table 1. EBV DNA in B cell, plasma EBV DNA, and ex vivo EBV associated B cells were analyzed as previously described 10-11 and detailed in supplemental data. This study was conducted in accordance with the Declaration of Helsinki. Study protocol and sample collection were registered at the French Health Ministry (number DC-2008-417).

**Statistical analysis**
The Wilcoxon rank-sum test was performed using the R software to compare median values of non-parametric variables. Fisher’s exact test was used in the case of proportion comparisons. The exact logistic regression and multivariate analysis were performed to search independent associations between the persistence of the monoclonal peak and HIV RNA viremia, EBV DNA load in plasma, gain of CD4+ T-cells, hepatitis B and C co-infection, and CMV reactivation.

**Results and discussion**
Out of the 21 patients with detectable MG by PEP and confirmed by immunofixation before ART initiation, 9 (42%) patients still harbored MG after a prolonged period on ART (≥ 5 years) while the monoclonal peak disappeared for the other 12 (58%) patients. This observation reveals a labile characteristic of MG associated with HIV infection and ART in contrast to the MG detected in MGUS, myeloma or lymphoma where MG is maintained lifelong 12.

In line with previous reports 7, we observed that MG occurs at a younger age among HIV-infected individuals when compared to the general population (Table 1). No differences were found at baseline between the transient and persistent MG group for plasma HIV RNA load and high expression of CD38 on CD8+ T lymphocytes. Most of the patients studied had low CD4+ T cell nadir but there was no significant difference in nadir of CD4+ T cell count or in
CD4+ T cell replenishment over the ART period between patients with persistent MG versus transient MG. The exact logistic regression showed that the persistence of MG was associated with the number of years of detectable HIV RNA load during therapy (OR = 2.2, CI95%: 1.1-5.9; \(P\) = .01, supplemental Table 1). After a prolonged period on ART, a 2 fold higher percentage of CD8+ T-cell activation (% of CD38bright CD8+ T-cells) was observed in the persistent MG group as compared to the transient MG group although the difference was not significant (\(P\) = .06). Polyclonal CD8+ T-cell hyperactivation is known to be a robust marker of the immune activation driven by HIV and an independent predictive marker of HIV disease progression.\(^{13-14}\)

The persistence of the MG was accompanied by a higher serum immunoglobulin level (\(P\) = .003) (Fig.1B), mainly restricted to IgG isotype. Hypergammaglobulinemia was observed in 11/21 patients (52%), namely 8/9 (89%) in the persistent MG group and 3/12 (25%) in the transient MG group (\(P\) < .001). High frequencies of circulating plasmablasts and plasma cells were observed in the persistent MG group (\(P\) = .01 and .02, respectively), (Fig. 1A and C). These results provide evidence that mature B-cells are prompted to undergo terminal differentiation into antibody-producing cells and may be indicative of persistent B-cell activation through systemic antigenic stimulation.\(^{15}\)

Memory B lymphocytes are the main reservoir of EBV and their differentiation into plasma cells can reactivate this reservoir. As shown in Figure 1D, EBV DNA was detectable in the plasma of 1/12 patients in transient MG and in 0/15 of HIV-infected controls, whereas 6 out of 9 patients with persistent MG on ART (66%) exhibited quantifiable EBV DNA in plasma (\(P\) < .001). Multivariate analysis showed that EBV DNA level in plasma was associated with persistence of MG independent of detectable HIV RNA load and CD4+ T-cell replenishment (OR = 18.6; CI95%: 2.02-infinity, \(P\) = .007, supplemental Table 1). EBV DNA B-cell reservoir was 5 fold higher in persistent MG (\(P\) = .006) and 4 fold higher than controls (\(P\) = .007), (Figure 1E). EBV DNA spontaneously produced by infected B-cells maintained in culture for 48h was more than 10 fold higher in persistent MG as compared to transient MG (\(P\) = .002) and to controls (\(P\) = .001), (Figure 1F). Altogether these observations indicate that: i) markers of EBV replication, ii) number EBV latently infected B cells, and iii) EBV infected cells primed to enter and complete the EBV cycle, were associated with the persistence of MG.
Besides the young age at time of MG diagnosis, virus stimulation of B cells and reversion to a transient state are two important characteristics of HIV-associated MG. In the general population MG occurrence is related to molecular pathogenic events such as IgH translocations, aneuploidy, chromosome 13 deletion, and dysregulation of a CYCLIN D gene. Chronic bacterial antigen stimulation has also been associated with an increased risk of monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma. Reduction of the level of M protein after initiation of combined ART has been previously reported. In HIV infected individuals ongoing EBV replication may fuel persistent lymphocyte activation and consequently be part of an amplification loop that adversely impact the host immune response and favors MG persistence. MG associated with HIV infection may share common features with post-transplant MG since EBV reactivation and increased frequency of EBV latently infected B cells are observed in these cases as well. These features make the physiopathological mechanisms behind HIV-associated MG different from the MGUS observed in the general population and closer to the MG observed in post transplant patients.

Acknowledgments
This study was supported by student grant from Infectiopole Sud. We thank Sophie Bendriss for the technical assistance and Audrey De Jong for statistical analysis.

Contribution to authorship
D.E.O. designed the study, performed research, analyzed data, and wrote the paper; A.M., J-P.V., P. V. and E.T. designed the study and wrote the paper; J.V., M-L. C., N.N., R.C., Y.A, S.B., K.B., V.F. and J.R. analyzed data and wrote the paper.

Conflict of interest: the authors declare to have no financial conflict of interest.
References


Table 1. Patient characteristics at baseline, ie at time of detection of M protein before ART initiation, and after a long term period on ART ranging from 5 to 12 years.

<table>
<thead>
<tr>
<th>Characteristic c (median, IQR)</th>
<th>Transient MG</th>
<th>Persistent MG</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.5 (35.5-58)</td>
<td>43 (20-55.5)</td>
<td>.49</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>10/2</td>
<td>6/3</td>
<td>.61</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>14.4 (12.6-15)</td>
<td>13.7 (12.8-14.3)</td>
<td>.77</td>
</tr>
<tr>
<td>Calcium (µM)</td>
<td>2.5 (2.4-2.5)</td>
<td>2.48 (2.37-2.55)</td>
<td>.81</td>
</tr>
<tr>
<td>Total urine protein (g/L)</td>
<td>0.15 (0.13-0.31)</td>
<td>0.14 (0.05-0.54)</td>
<td>.78</td>
</tr>
<tr>
<td>Chronic Viral Hepatitis</td>
<td>4/12 (3 HCV, 1 HBV)</td>
<td>4/9 (3 HCV, 1 HBV)</td>
<td>.67</td>
</tr>
<tr>
<td>CMV reactivation*</td>
<td>4/12</td>
<td>1/9</td>
<td>.34</td>
</tr>
<tr>
<td>CD4+ count (cells/µL)</td>
<td>221 (87-285)</td>
<td>145 (72-227)</td>
<td>.65</td>
</tr>
<tr>
<td>Plasma HIV RNA (copies/mL)**</td>
<td>23339 (10805-319000)</td>
<td>29540 (9305-688000)</td>
<td>.58</td>
</tr>
<tr>
<td>Type of MG</td>
<td>6 IgGκ, 6 IgGλ</td>
<td>6 IgGκ, 3 IgGλ</td>
<td>.66</td>
</tr>
<tr>
<td><strong>After prolonged ART (≥5 years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+ count (cells/µL)</td>
<td>326 (228-554)</td>
<td>304 (172-558)</td>
<td>.93</td>
</tr>
<tr>
<td>Nadir CD4+ count (cells/µL)</td>
<td>116 (31-195)</td>
<td>61 (31-93)</td>
<td>.5</td>
</tr>
<tr>
<td>Cumulative detectable HIV**</td>
<td>0.5 (0-1)</td>
<td>1.5 (0-4)</td>
<td>.04</td>
</tr>
<tr>
<td>CDB8 CD38bright (%)</td>
<td>6.2 (3.7-11.4)</td>
<td>12.2 (7.9-38.9)</td>
<td>.06</td>
</tr>
<tr>
<td>ART regiment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRTI+NNRTI</td>
<td>5/12</td>
<td>3/9</td>
<td>1</td>
</tr>
<tr>
<td>NRTI+PI</td>
<td>6/12</td>
<td>6/9</td>
<td>.66</td>
</tr>
<tr>
<td>Other</td>
<td>1/12</td>
<td>0/9</td>
<td>1</td>
</tr>
<tr>
<td>Duration of ART</td>
<td>9 (7-10)</td>
<td>9 (7-12)</td>
<td>.95</td>
</tr>
</tbody>
</table>

* CMV reactivation detected by pp65 antigenemia

**Median years (IQR) with detectable viremia during the follow-up

Cytomegalovirus (CMV), Hepatitis B virus (HBV), Hepatitis C virus (HCV)
Figure legends

Figure 1. Analysis of circulating plasmablast and plasma cells, polyclonal immunoglobulin levels and EBV DNA levels in HIV infected patients with transient MG or persistent MG. (A) Gating strategy and phenotypes of circulating plasmablast and plasma cells. Circulating cells were stained with CD19, CD20, CD27 and CD138 monoclonal antibodies. Plasmablasts were identified as CD19+, CD20-, CD27+, CD138- cells. Plasma cells were identified as CD19+, CD20-, CD27+, CD138+ cells. Plasma cells are large cells expressing higher level of CD27 than memory B cells. Circulating plasma cells represented 0.1 to 5% of B cells in HIV infected patients. (B) Plasmablasts and plasma cells in the blood of transient MG, persistent MG and control patients. Percentage of plasmablasts and plasma cells among CD19+ B cells were determined. (C) Polyclonal immunoglobulin levels in patients with persistent and transient MG. Serum levels of gammaglobulins were determined by protein electrophoresis at least after 5 years of follow-up under ART. (D) EBV DNA load in plasma (Log copies/mL). (E) EBV DNA load in B cells (Log copies/10^6 cells), numbers near plots mean corresponded HIV RNA load. (F) EBV DNA in B-cell culture supernatant after 48h of culture. The ends of the boxes indicate the 25th and 75th percentiles, with a line at the median; error bars represent the 10th and 90th percentiles. (***) define a $P < .05$ and (*) define a $P >.05$. 
Figure 1. Analysis of circulating plasmablast and plasma cells, polyclonal immunoglobulin levels and EBV DNA levels in HIV infected patients with transient MG or persistent MG.
Serum gammaglobulins (g/L)

(B)

persistent MG

transient MG

Serum gammaglobulins (g/L)

**
EBV DNA in B-cells (log copies/10^6 B-cells)

controls  persistent MG  transient MG

(E)
EBV DNA in supernatant (log copies/mL)

controls  persistent MG  transient MG

(F)
Pivotal role of HIV and EBV replication in the long-term persistence of monoclonal gammopathy in patients on antiretroviral therapy

David Eric Ouedraogo, Alain Makinson, Jean-Pierre Vendrell, Marie-Laure Casanova, Nicolas Nagot, Renaud Cezar, Karine Bollorre, Yassine Al Tabaa, Vincent Foulongne, Stéphanie Badiou, Johannes Viljoen, Jacques Reynes, Philippe Van de Perre and Edouard Tuaillon