Increased expression of SAMHD1 in a subset of HIV-1 elite controllers

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Received 21 May 2014; returned 14 June 2014; revised 18 June 2014; accepted 23 June 2014

Objectives: SAMHD1 and the CDKN1A (p21) cyclin-dependent kinase inhibitor have been postulated to mediate HIV-1 restriction in CD4+ cells. We have shown that p21 affects HIV replication through its effect on SAMHD1. Thus, we aimed at evaluating the expression of SAMHD1 and p21 in different HIV+ phenotypic groups.

Patients and methods: We evaluated SAMHD1 and CDKN1A mRNA expression in CD4+ T cells from HIV+ individuals including elite controllers (n=12), individuals who control HIV without the need for antiretroviral treatment, viraemic progressors (n=10) and HIV-1 seronegative healthy donors (n=14). Immunological variables were measured by flow cytometry.

Results: We show that a subset of HIV+ elite controllers with lower T cell proliferation levels (Ki67+ cells) expressed higher SAMHD1 compared with healthy donors or viraemic progressors. Conversely, there was no difference in p21 expression before or after T cell activation with a bispecific CD3/CD8 antibody.

Conclusions: Our results suggest that SAMHD1 may play a role in controlling virus replication in HIV+ individuals and slow the rate of disease progression.

Keywords: restriction factors, p21, viral control

Introduction

HIV susceptibility and disease progression show a substantial degree of individual heterogeneity, ranging from fast progressors to long-term non-progressors or elite controllers (EC).1,2,4,5 EC are a small subgroup of HIV-1-positive individuals who control infection without the need for antiretroviral therapy. CD4+ T cells from different donors show differential susceptibility to infection3 and cells from EC have been shown to be relatively resistant to HIV-1 infection when compared with CD4+ cells from healthy donors (HD).2,3,5 However, the molecular basis for this restriction remains poorly defined.6 Elite control of infection may be considered as a model for a functional cure or serve to identify therapeutic or vaccine strategies against HIV.7

SAMHD1 is a deoxynucleotide triphosphate (dNTP) triphosphohydrolase that regulates the size of the intracellular dNTP pool, reducing the availability of dNTPs for the HIV-1 reverse transcriptase and limiting infection.8–12 SAMHD1 restricts HIV-1 infection in myeloid and lymphoid cells, but its role in controlling HIV-1 infection in vivo is undefined.

We and others have shown that the cyclin-dependent kinase (CDK) inhibitor CDKN1A (p21) affects HIV-1 replication through the control of the dNTP pool required for reverse transcription, by regulating dNTP availability either through ribonucleotide reductase13 or SAMHD1 functions.14 Increased p21 levels have been associated with elite control of HIV-1 and might be important in maintaining HIV-1 latency.15 Therefore, we aimed at evaluating the expression of SAMHD1 and p21 in different HIV+ phenotypic groups.

Patients and methods

All patients participating in the study gave informed consent. The work was approved by the Ethics Committee of Hospital Germans Trias i Pujol. Criteria for selection of EC (n=12) were confirmed HIV-1 infection with sustained plasma viral load (VL) below the limit of detection in the absence of antiretroviral treatment (non-consecutive blips of <2000 copies/mL) were allowed if present only in <20% of VL determinations). The mean VL and CD4+ T cell count of EC were <40 RNA copies/mL and 706 cells/µm3 (range: 498–880), respectively. Cells from viraemic progressors (VP) corresponded to a set point previous to antiretroviral treatment (n=10, mean VL=491 531 copies/mL, CD4+ T cell count=398 cells/µm3, range: 370–544). Six EC were identified as expressing protective major histocompatibility complex (MHC) class I alleles: two HLA-B*5701 and one each of *5703, *2702, *3501 and *3503 (Table 1). For relative mRNA quantification, RNA was extracted using the Qiagen RNeasy Kit (Qiagen), including the DNase I treatment step. Reverse transcription was performed using the High
Capacity cDNA Kit (Life Technologies). Relative levels of mRNA were measured by two-step quantitative RT–PCR (qPCR) and normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA expression using the ΔCt method. For mRNA quantification, all measurements were normalized to the mean ΔCt value of the HD. Primers and DNA probes were purchased from Life Technologies (TaqMan gene expression assays). Data were analysed with the PRISM statistical package.

**Results**

The degree of cellular proliferation in all subjects was quantified by intracellular Ki67 staining (Figure 1a) as previously reported. EC patients showed similar levels of cell proliferation to HD. Conversely, the mean of Ki67+ cells was significantly different between VP and HD (\(P = 0.0002\)) or VP and EC (\(P = 0.0003\)), Table 1.

![Image](https://academic.oup.com/jac/article-abstract/69/11/3057/2911282)

**Figure 1.** SAMHD1 mRNA is increased in unstimulated EC cells. The percentage of Ki67+ cells of unstimulated (a) or stimulated (d) peripheral blood mononuclear cells from seronegative HD, VP or EC, measured by flow cytometry. Relative mRNA expression of SAMHD1 (b and e) and CDKN1A (p21) (c and f) genes in unstimulated (b and c) and stimulated (e and f) cells from HD, VP and EC. Horizontal bars indicate mean values. All data were normally distributed. \(P\) values were calculated using an unpaired, two-tailed, Student’s t-test.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Number</th>
<th>Median (IQR) VL at set point, copies/mL</th>
<th>CD4 cell count at set point, cells/mm(^3)</th>
<th>Protective HLA-B alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD</td>
<td>14</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>EC</td>
<td>12</td>
<td>&lt;40</td>
<td>706 (498–880)</td>
<td>5701 (n = 2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5703 (n = 1)</td>
<td>2702 (n = 1)</td>
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<td></td>
<td></td>
<td></td>
<td>3501 (n = 1)</td>
<td>3503 (n = 1)</td>
</tr>
<tr>
<td>VP</td>
<td>10</td>
<td>491531 (384261–730000)</td>
<td>398 (370–544)</td>
<td>ND</td>
</tr>
</tbody>
</table>
| ND, not determined.
confirming increased T cell activation and proliferation as a marker of disease progression in HIV+ individuals, a feature not present in the EC, who show cell proliferation values undistinguishable from uninfected subjects.

When the expression of SAMHD1 mRNA was analysed by qPCR in peripheral blood mononuclear cells, SAMHD1 levels were found significantly increased in EC subjects (Figure 1b), suggesting that increased levels of SAMHD1 expression in EC might play a role in the outcome of infection. Of note, when the three outliers corresponding to the EC group were removed, statistical difference was still found when comparing with the HD group ($P=0.0004$). In contrast, we could not identify significant differences in the mRNA levels of the CDKN1A gene when comparing EC with HD and only a slight but significant ($P=0.0298$) decrease in p21 expression was observed in VP compared with HD or EC (Figure 1c).

After stimulation with an anti-CD3/CD8 bispecific antibody as described in Chen et al., cells from both HIV+ groups (VP and EC) reached similar T cell proliferation capacity, slightly higher than HD (Figure 1d). No differences were observed in the percentage of naive, central memory and effector memory T cells or in the surface expression markers CD69, CD25, CXCR4 or CCR5 (data not shown). SAMHD1 levels in EC remained significantly higher when compared with VP, but not with HD (Figure 1e). However, CDKN1A levels were decreased in EC ($P=0.0186$) (Figure 1f).

**Discussion**

We have shown that knockdown of p21 induced an increase in viral DNA formation and HIV-1 replication in monocyte-derived macrophages. This effect disappeared when SAMHD1 was degraded by expression of HIV-2 Vpx. CDK-dependent phosphorylation of SAMHD1 is strongly associated with the SAMHD1 capacity to control the intracellular dNTP pool and restrict HIV-1 replication. Importantly, CDK2, the target of p21, appears to block SAMHD1 function in primary cells. Thus, it would be expected that p21 over-expression would increase HIV-1 replication. Although we could not demonstrate the relationship between high p21 expression and elite control of HIV-1 infection, we cannot discard a role of p21 in SAMHD1-mediated restriction of HIV-1 replication.

Significant correlations between CD4+ T cell activation and expression of several viral restriction factors, including SAMHD1, have been observed in HLA-B*57-positive individuals, suggesting distinct associations between host restriction factors and protective HLA class I genotypes. Only half of the EC in our study carry protective HLA-B alleles (evaluated as described in Ballana et al.), indicating that increased SAMHD1 mRNA expression may not be an exclusive condition associated with cell-mediated immunity. Moreover, differences in SAMHD1 mRNA levels were significant when comparing EC individuals harbouring ($n=6; P=0.0010$) or not ($n=6; P=0.0003$) the protective HLA alleles with the HD group (data not shown). Thus, increased SAMHD1, a mediator of the interferon response and tightly linked to cell cycle progression, may not be a cause but the consequence of the protective condition against HIV-1 infection.

It is possible that differences in the CD4 T cell count, VL or both could be the cause (and not the consequence) of the observed differences in SAMHD1 levels. However, when comparing extreme phenotypes, EC appear to resemble healthy HIV-negative individuals in the degree of T cell proliferation and SAMHD1 expression, despite infection. Further studies comparing EC with individuals on highly active antiretroviral chemotherapy (HAART) with a similar immunological condition and longitudinal evaluation of EC are warranted.

Together, our results suggest that innate immune protection from HIV-1 infection may be associated with the inability of the virus to surpass cell restriction without negatively affecting the cells’ proliferative capacity. SAMHD1 has been recognized as an innate antiviral factor affecting the replication of several viruses. Our findings may be of relevance for a wide range of viral infections.

**Funding**

This work was supported by the Spanish MINECO projects BFU2012-31569 and P113/01083, by HIVACAT and grants from the Bill and Melinda Gates Foundation and Gala Contra la SIDA. EP, BM and AR are research fellows from MINECO.

**Transparency declarations**

None to declare.

**References**