Virus Research 147 (2010) 284–287

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Short communication

Telomerase activity in peripheral blood mononuclear cells from HIV and HIV–HCV coinfected patients

Rita Reynoso[a,b], Natalia Laufer[a], Federico Bolcic[a,b], Jorge Quarleri[a,b,*]

[a] Centro Nacional de Referencia para el SIDA, Dto. Microbiología, Facultad de Medicina, Universidad de Buenos Aires, Argentina
[b] CONICET, Argentina

ARTICLE INFO

Article history:
Received 9 October 2009
Accepted 13 November 2009
Available online 20 November 2009

Keywords:
HIV
HCV
Coinfection
Telomerase activity

ABSTRACT

Replicative senescence of peripheral blood mononuclear cells (PBMC) plays an important role in the pathophysiology of chronic viral infections. Although there are controversial reports concerning telomerase activity in HIV monoinfected subjects, no data on HIV–HCV coinfected individuals is available. In this cross-sectional study telomerase activity was quantified in non-stimulated and mitogen-stimulated PBMC lysates from HIV-1 monoinfected and HIV–HCV coinfected individuals using real-time PCR.

Up-regulation of telomerase activity after mitogen stimulation was impaired in PBMC of HIV monoinfected and HIV–HCV coinfected patients. The lack of an appropriate induction of this enzymatic activity after stimulus could partly account for immunosuppressive conditions observed in such patients.

Telomeres are nucleoprotein structures at chromosome termini crucial for genomic stability. The progressive shortening of telomeres after each cell division is suggested to act as a mitotic clock that can trigger cellular senescence in normal somatic cells. To prevent replicative senescence, telomere length can be maintained by activation of telomerase, an RNA-dependent DNA polymerase (Holt and Shay, 1999). In normal human peripheral blood mononuclear cells (PBMC) telomerase activity is detected at low but measurable levels and is upregulated after cell activation (Weng et al., 1997). This mechanism is essential for the extensive cloning expansion involved in the effective immune response.

Decreased telomerase activity in PBMC has been linked to immunosenescence and has been observed in patients affected by advanced HIV infection, as well as in patients chronically infected with the hepatitis B (HBV) or C virus (HCV) (Satra et al., 2005).

Chronic HCV infection is currently one of the leading causes of morbidity and mortality in HIV infected individuals in developed countries. The bidirectional interferences between HCV and HIV hinder the management of coinfected individuals. Several studies have verified the unfavorable impact of HIV coinfection at HCV RNA levels, liver disease progression, and response rates to HCV treatment (Sulkowski and Thomas, 2003). Both viruses may directly interact when they coexist in a given cellular scenario such as PBMC (Blackard et al., 2006).

The aim of this cross-sectional study is to evaluate telomerase activity in stimulated PBMC from HIV infected individuals as a sole infection and with HCV coinfection in order to elucidate a possible involvement of telomerase dysfunction in the impairment of cellular immune responses in HIV–HCV coinfection.

Twenty-eight consecutive HIV infected patients followed up at the National Reference Center for AIDS and 15 healthy individuals were included in the present study. HIV infected patients were classified into two groups: HIV monoinfected patients ($n = 13$) and, patients coinfected with HIV–HCV ($n = 15$). Descriptive statistics (N, mean, standard deviation) were used to summarize continuous variables. Fisher's Exact Test was used to analyze quantitative variables. All reported p values are two-sided; $p < 0.05$ was considered statistically significant.

HIV monoinfected patients were younger (11 males; 35 ± 8.8 years old) than HIV–HCV coinfected ones (14 males; 43.64 ± 6.6, $p = 0.01$), and had a shorter time of known HIV infection (2.11 ± 3.79 years vs. 16.7 ± 4.7 years, $p = 0.000$).

The diagnosis of chronic HCV infection was based on clinical, laboratory and histological evaluation. Only HIV–HCV coinfected patients were under ARV therapy but none was under pegylated-interferon-based treatment. All subjects consented to participate in the study. The Local Ethics Committee of the School of Medicine, University Buenos Aires approved the study protocol.

A total of 20–50 ml peripheral blood were drawn and PBMC were isolated by means of Ficoll-Hypaque density gradient centrifugation. PBMC were cultured at densities of 0.5×10^6/ml. Culture medium RPMI 1640 (GIBCO, Paisley, UK) was supplemented with 100 U penicillin/ml, 100 μg streptomycin/ml, 10% fetal bovine serum and 2 mM glutamine. PBMC were expanded using phyto-

* Corresponding author at: Centro Nacional de Referencia para el SIDA, Paraguay 2155, Piso 11, 1121 Buenos Aires, Argentina. Tel.: +54 11 4508 3689; fax: +54 11 4508 3705.
E-mail address: quarleri@fmed.uba.ar (J. Quarleri).

0168-1702/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2009.11.006

haemagglutinin (PHA) stimulation 4 μg/ml (Sigma, St Louis, MO). Viable cell counts were determined by trypan blue exclusion.

To study the effect of PHA stimulation, the expression of CD69 and CD25 was measured, using flow cytometry. PBMC were cultured for 48 h in either plain culture medium or in culture medium supplemented with PHA 4 μg/ml. Prior to expansion, few PBMC expressed CD25 and CD69 activation antigens and no significant differences were observed among HIV, HIV–HCV and, healthy individuals (CD25: 0.93 ± 0.95; 0.43 ± 0.49; 1.35 ± 2.24, $p > 0.05$; CD69: 2.63 ± 3.54; 0.87 ± 1.00; 2.53 ± 1.66, $p > 0.05$). As expected, post-PHA both CD25 (37.47 ± 13.99; 29.05 ± 9.40; 23.58 ± 2.51) and CD69 (47.6 ± 16.86; 35.65 ± 12.23; 27.83 ± 4.34) were dramatically increased without significant differences between healthy donors and both groups of HIV infected patients, indicating a comparable level of cell activation in the three groups (Fig. 1a and b).

The HCV genotype was determined by restriction fragment-length polymorphism (RFLP) analysis at the 5′-untranslated region (5′-UTR). The substrates for RFLP were nested-PCR products obtained from both serum and PBMC for each patient following our previously reported protocol (Bolcic et al., 2008).

HIV viral load (VERSANT HIV RNA 3.0 Assay [bDNA]) was $6.3 \times 10^5 \pm 4.2 \times 10^5$ copies/ml (median ± SD) among monoinfected patients; in contrast, it was undetectable (<50 copies/ml) in those coinfected with HCV. The median (±SD) HCV viral load (ROCHE Cobas Amplicor Monitor HCV test version 2.0) was 5.8×10^6 copies/ml ($\pm 5.2 \times 10^6$). No statistically significant differences were found in CD4+ T cell counts (HIV group: 213.9 ± 87.33 vs. HIV–HCV group: 455 ± 156.8, $p = 0.35$). All but one HIV/HCV coinfected patients exhibited HCV genotype 1, demonstrating coincidence in both PBMC and serum compartments for each patient sample. Liver biopsies were available in 6/15 (66.6%) of the patients exhibiting METAVIR F3–F4.

Telomerase activity was quantified in duplicate in both non-stimulated and PHA-stimulated PBMC lysates using real-time PCR as described elsewhere (Hou et al., 2001). Briefly, PBMC (1.0×10^6) were lysed in 200 μl of CHAPS buffer and incubated for 30 min on ice. After incubation, lysates were centrifuged at 14,000 rpm for 20 min at 4 °C. The protein concentration was measured using the Bradford assay; and the whole cell extracts were diluted to a final concentration of 0.01 μg/μl. The total volume of the reaction mixture was 25 μl per well, containing 12.5 μl 1× SYBR Green Master Mix (Biosystems), 0.1 μg of TS primer (5′-AATCCGTCGAGCAGAGTT-3′), 0.05 μg of ACX primer (5′-GCGCGGCTTACCCTTACCCTTACCCTAACC-3′), 1 μl of RNase-free water, and 10 μl of sample.

The reaction mixture was incubated for 20 min at 25 °C. The PCR was then started at 95 °C for 15 min, followed by a 40-cycle amplification (95 °C for 15 s and 60 °C for 60 s). RNase-free water and heat-inactivated eluates (85 °C for 10 min) were used as negative controls. The threshold cycle values (Ct) were determined from semilog amplification plots (log increase in fluorescence versus cycle number) and compared with standard curves generated from serial dilutions of protein extracts from Jurkat E6 cells (0.025, 0.05, 0.1, 0.15 μg), where the assay was linear. Relative telomerase activity (RTA) was expressed as a percentage of enzymatic Jurkat cells activity.

Non-stimulated PBMC in the three experimental groups showed low and indistinguishable levels of RTA activity (HIV group: 18.75 ± 9.98, HIV–HCV group: 9.50 ± 8.89; healthy controls: 13.5 ± 7.85; $p > 0.05$). After mitogenic stimulus, RTA was up regulated in all groups (HIV group: 26.00 ± 22.07, HIV–HCV group: 15.25 ± 6.23; healthy control: 26.25 ± 6.55). When comparing the enzymatic activity pre- and post-PHA stimulus, only a significant increase was observed in the control group ($p = 0.02$). Similarly but not significantly HIV and HIV–HCV individuals also increased their RTA ($p = 0.223$ and, $p = 0.165$, respectively). Moreover, after antigen stimulation, we found that RTA of activated PBMC from coinfected patients but no from HIV monoinfected ones, was significantly lower ($p = 0.05$) than that observed in PBMC from healthy

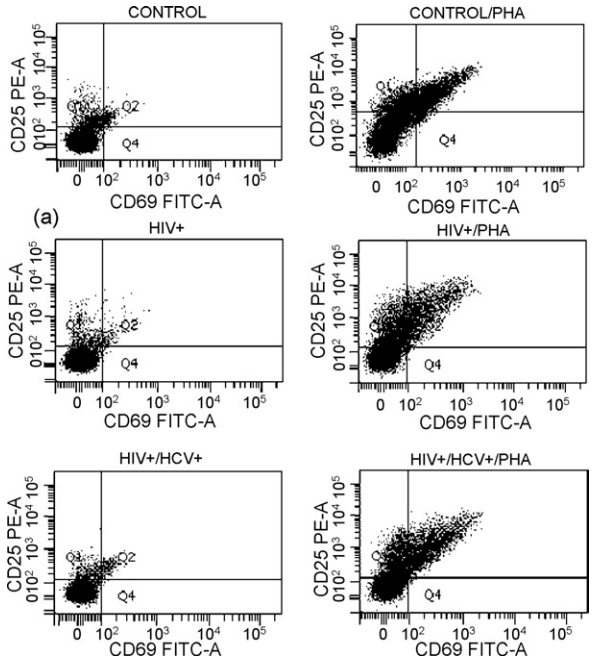

Fig. 1. (a) CD69 and CD25 expression on PBMC derived from healthy control, HIV, and HIV–HCV coinfected patients in the presence or absence of phytohaemagglutinin (PHA). Representative flow cytometric analysis using two-colour immunofluorescence staining is shown. PBMC were stained with PE-labelled anti-CD25 and FITC-labelled anti-CD69. One representative experiment is shown for each group. (b) Mean percentages of CD69 (left panel) and CD25 (right panel) cells within each group of patients' PBMC in the presence or absence of PHA. (c) Mean of relative telomerase activity (RTA) expressed as percentage of Jurkat cells activity in PBMC prior and post-PHA stimulation. The horizontal bars within boxes represent the mean values, and the upper and lower boundaries of boxes represent the 75th and 25th percentiles, respectively. Whiskers (error bars) represent the standard deviation. The p values for comparing group mean were calculated by the Student t test.

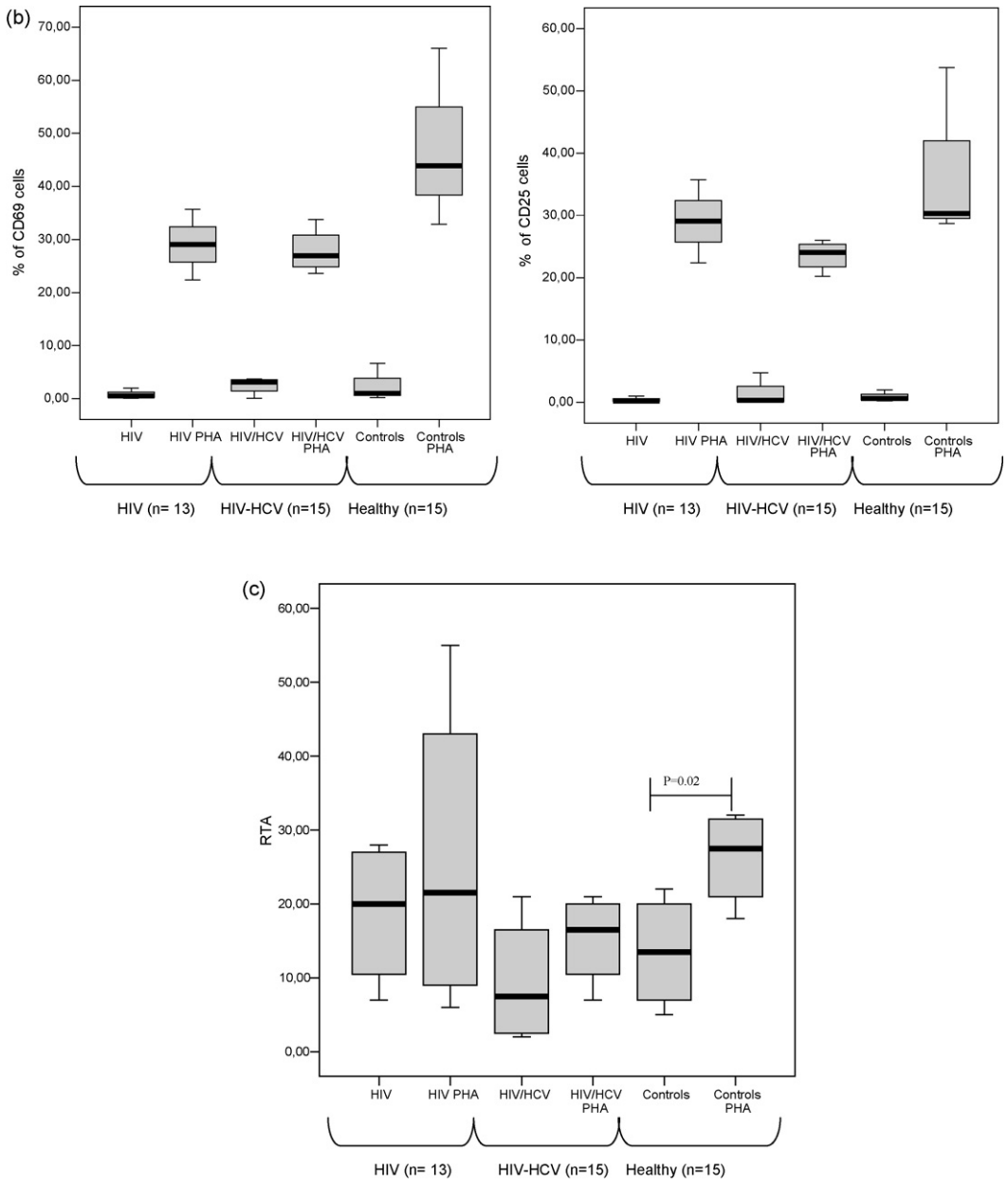

Fig. 1. (*Continued*).

donors (Fig. 1c). It has been reported that reverse transcriptase inhibitors show anti-telomerase activity (Beltz et al., 1999; Tendian and Parker, 2000; Olivero, 2007). Because of this, it is mandatory to consider that HIV–HCV coinfected patients were exposed to ARV therapy. In this group none of them received AZT but 7 out of 15 were treated with abacavir. Nevertheless, no significant difference in RTA was detected between those exposed (15.9 ± 9.7) and non-exposed (15.7 ± 3.9) to this drug.

Telomere length and telomerase activity are closely associated with PBMC development, differentiation and replicative capacity. Since telomerase contributes to the protection from telomere shortening in PBMC, it plays a critical role in immune function. A decrease of telomerase in PBMC has been linked to several chronic pathologies and to immunosenescence.

In this study, we have found that PBMC from HIV monoinfected patients showed impaired telomerase activity in response to mitogen exposure. This result is in agreement with previous reports (Franzese et al., 2007). In other studies, however, enzymatic activity was normal in T lymphocyte subsets (Wolthers et al., 1996). It should be noted that most of these studies were cross-sectional. Thus, discrepant results may be due to different patient characteristics (i.e., at early or late disease stages) as well as to specific cell lineages, considering the heterogeneity of the cell population analyzed.

Notably, the decrease in telomerase activity observed in cells from coinfected individuals was more important. Interestingly, previous observations showed that HCV down-modulates telomerase in PBMC by diminishing the level of its catalytic subunit, hTERT, mRNA. Considering that HIV infection can affect telomerase, in patients with advanced HIV infection (Lichterfeld et al., 2008), a possible synergic action of both viruses could affect the enzymatic activity in a more pronounced way.

Although further experiments have to be conducted, the present study constitutes the first description of the concomitant effect of both HIV and HCV on telomerase activity in mononuclear cells. The observed reduction in mono- and coinfected patients suggests an

involvement of the enzyme in the immunopathogenesis of coinfection.

Disclosure

The authors declare that they have no conflict of interest.

Acknowledgements

The authors thank Sergio Mazzini for his assistance during the manuscript preparation.

This work was supported by a grant from the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Argentina, no. PIP6116

References

Beltz, L., Moran, R., Elsawy, O., Sadler, J., Jurgenson, J., 1999. The effects of telomerase inhibitors on lymphocyte function. Anticancer Res. 19 (4B), 3205–3211.

Blackard, J.T., Kemmer, N., Sherman, K.E., 2006. Extrahepatic replication of HCV: insights into clinical manifestations and biological consequences. Hepatology 44, 15–22.

Bolcic, F., Bull, L., Martinez, L., Reynoso, R., Salomon, H., Arduino, R., Barnett, B., Quarleri, J., 2008. Analysis of sequence configurations of the PKR-interacting HCV proteins from plasma and PBMC as predictors of response to interferon-alpha and ribavirin therapy in HIV-coinfected patients. Intervirology 51 (4), 261–264.

Franzese, O., Adamo, R., Pollicita, M., Comandini, A., Laudisi, A., Perno, C.F., Aquaro, S., Bonmassar, E., 2007. Telomerase activity, hTERT expression, and phosphorylation are downregulated in CD4(+) T lymphocytes infected with human immunodeficiency virus type 1 (HIV-1). J. Med. Virol. 79 (5), 639–646.

Holt, S.E., Shay, J.W., 1999. Role of telomerase in cell proliferation and cancer. J. Cell. Physiol. 180, 10–11.

Hou, M., Xu, D., Björkholm, M., Gruber, A., 2001. Real-time quantitative telomeric repeat amplification protocol assay for the detection of telomerase activity. Clin. Chem. 47 (3), 519–524.

Lichterfeld, M., Mou, D., Cung, T.D., Williams, K.L., Waring, M.T., Huang, J., Pereyra, F., Trocha, A., Freeman, G.J., Rosenberg, E.S., Walker, B.D., Yu, X.G., 2008. Telomerase activity of HIV-1-specific CD8+ T cells: constitutive up-regulation in controllers and selective increase by blockade of PD ligand 1 in progressors. Blood 112 (9), 3679–3687.

Olivero, O.A., 2007. Mechanisms of genotoxicity of nucleoside reverse transcriptase inhibitors. Environ. Mol. Mutagen. 48 (3–4), 215–223.

Satra, M., Dalekos, G.N., Kollia, P., Vamvakopoulos, N., Tsezou, A., 2005. Telomerase reverse transcriptase mRNA expression in peripheral lymphocytes of patients with chronic HBV and HCV infections. J. Viral Hepat. 12 (5), 488–493.

Sulkowski, M., Thomas, D., 2003. Hepatitis C in the HIV-infected person. Ann. Intern. Med. 138, 197–207.

Tendian, S.W., Parker, W.B., 2000. Interaction of deoxyguanosine nucleotide analogs with human telomerase. Mol. Pharmacol. 57 (4), 695–699.

Weng, N.P., Palmer, I., Levine, B.L., Lane, C.H., June, C., Hodes, R.J., 1997. Tales of tails: regulation of telomere length and telomerase activity during lymphocyte development, differentiation, activation and aging. Immunol. Rev. 160, 43–54.

Wolthers, K.C., Wisman, G.B.A., Otto, S.A., et al., 1996. T cell telomere length in HIV-1 infection: no evidence for increased CD4+ T cell turnover. Science 274, 1543–1547.