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Short communication

Macrophage HIV-1 infection in duodenal tissue of patients on long term HAART

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ABSTRACT

Mucosal surfaces play a major role in human immunodeficiency virus type 1 (HIV-1) transmission and pathogenesis. Since the role of intestinal macrophages as viral reservoirs during chronic HIV-1 infection has not been elucidated, we investigated the effects of successful therapy on intestinal HIV-1 persistence. Intestinal macrophage infection was demonstrated by the expression of p24 antigen by flow cytometry and by the presence of proviral DNA, assessed by PCR. Proviral DNA was detected in duodenal mucosa of HIV-infected patients under treatment with undetectable plasma viral load. These findings confirm that intestinal macrophages can act as viral reservoirs and permit HIV-1 production even after viral suppression following antiretroviral therapy.

Current antiretroviral therapy inhibits viral replication but does not eradicate long-lived cellular reservoirs of HIV-1. Eradication efforts need to address the mucosal tissue sites directly as such tissues may continue to promote low-level replication due to, among other factors, the intrinsic difference in susceptibility to infection of GALT T cells compared to PBMC and the presumed lower concentrations of antiviral drugs in tissue than in plasma. The gastrointestinal mucosa is potentially the largest reservoir of HIV-1-infected macrophages in the body and constitutes a productive source of HIV-1 (Smith et al., 1997). However, effects of successful HAART on the persistence of the intestinal reservoir remain to be elucidated.

We have previously reported that the duodenal mucosa can act as an HIV reservoir, sustaining HIV-1 persistence even during successful HAART (Belmonte et al., 2007). Therefore, we aimed at further investigating the duodenal cells that harbor HIV in aviremic patients.

For this purpose, fresh duodenal biopsies were collected from 30 HIV-1-infected and 13 HIV-uninfected subjects. All the HIV-1-infected patients were under HAART for at least 5 years: 21 males, 9 females, 20 with successful treatment, plasma viral load (pVL) < 50 copies/ml, median CD4 cell count 341 cell/mm^3 (7–879); 10 who failed treatment, with pVL >100,000 copies/ml, median CD4 cell count 8 cell/mm^3 (2–198). The reported pVL corresponds to the time of sample collection. The 13 HIV-uninfected subjects were recruited from a population undergoing routine screening endoscopy. Endoscopic biopsies were obtained from the second or third part of the duodenum and a total of 6 tissue samples were taken. This protocol was reviewed and approved by the Ethics Committee of Academia Nacional de Medicina (Buenos Aires, Argentina) and each individual gave a written informed consent.

After acquisition, two of the 6 biopsies were immediately frozen at −80 °C for subsequent genomic DNA extraction and HIV-1 DNA detection. Genomic DNA was isolated from duodenal tissue (GenElute™ Mammalian Genomic DNA Miniprep Kit, Sigma–Aldrich Co.) according to the Manufacturer. Polymerase chain reaction (PCR) was performed with primers *SK145* (5′-AGTGGGGGGACATCAAGCAGCCATGCAAAT-3′) and *SKCC1B* (5′-TACTAGTAGTTCCTGCTATGTCACTTCC-3′) to amplify a 155-nucleotide sequence of the HIV-1 gag gene (Michael et al., 1999). Samples were thermal-cycled on a PerkinElmer 2400 as follows: 5 μl of DNA in a total volume of 50 μl containing each primer at 0.4 μM, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, each deoxynucleoside triphosphate at a concentration of 200 μM and 1.25 U of Taq DNA polymerase (Promega, Madison, WI). After an initial denaturation step at 95 °C for 5 min, 40 cycles of PCR at 95 °C for 30 s, 53 °C for 30 s and 72 °C for 30 s were carried out with a final extension of 10 min. The PCR products were analyzed on a 1.5% agarose gel stained with ethidium bromide. The biopsies were labeled as *"HIV +"* if HIV-DNA was detected by PCR.

The remaining 4 biopsies were disrupted by mechanical methods. Immediately after separation, the mucosal cells obtained were

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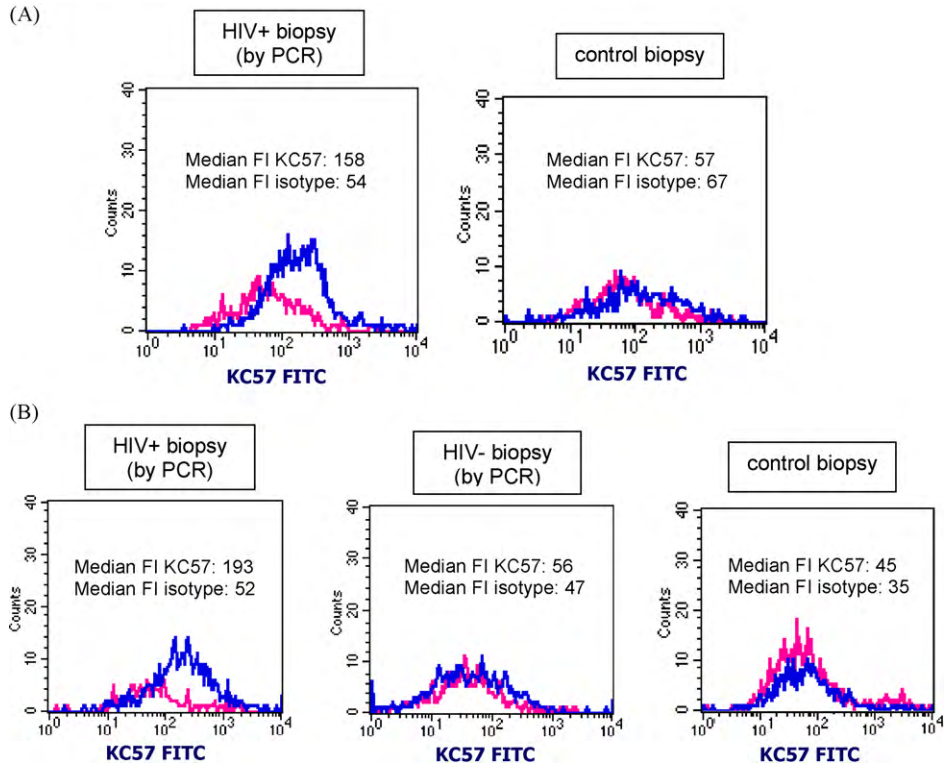

Fig. 1. HIV-infected mucosal CD68+ and CD64+ cells. (A) Representative histograms of p24 expression in gated CD68+ macrophages from HIV+ biopsies and control biopsies (HIV-uninfected subjects). % CD68+ cells in HIV+ patients: 14.4 ± 3.9 and controls: 12.6 ± 1.4 (mean ± SEM). (B) Representative histograms showing p24 expression in gated CD64+ cells from HIV+ and HIV− biopsies and control biopsies. % CD64% cells: 21.2 ± 2.7 in HIV+ patients and 21.5 ± 2.6 in controls. Histogram plots corresponding to control isotope are shown in pink and intracellular p24 in blue. FI: fluorescence intensity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

washed with PBS/1% fetal calf serum (PBS/FCS) and resuspended in PBS/FCS containing antibodies for flow cytometry.

Monoclonal anti-human CD3-PerCP (BD Pharmingen), anti-human CD4-FITC and -PE (BD Pharmingen), anti-human CD64-PE (BD Pharmingen) were used to characterize the mucosal cells. Irrelevant mAbs of the same isotype were included in each experiment. After staining, cells were washed with PBS/FCS, fixed with 1% paraformaldehyde and processed for flow cytometry. To detect HIV-infected cells, intracellular staining of the core protein viral p24 Ag was performed. Cells were fixed and permeabilized using the Fix and Perm kit (Caltag) following the instructions of the suppliers and stained with anti-p24mAb (FITC, clone Kc57, Beckman Coulter/Immunotech, Hialeah, FL) used at a 1/160 dilution or control isotype antibody. Anti-human CD68-PE (BD Pharmingen) was also stained intracellularly. Cells were acquired on FACScan (Becton Dickinson, San Jose, CA) and the analyses were done using CellQuest software (Becton Dickinson, San Jose, CA). Twenty thousands events were gated on a living cell population identified by forward- and side-scatter parameters. Macrophages were identified by gating on SSC/CD68. Gated macrophages were then examined for p24 expression relative to background staining of uninfected cells. We also examined CD64+ cells (monocytes/macrophages) by gating on SSC/CD64 for p24 expression.

The significance of the observed differences was calculated using Mann–Whitney test.

The results were considered to be statistically significant when $p < 0.05$.

Duodenal HIV-DNA was detected in 6 out of 10 (60%) patients with pVL >100,000 copies/ml and in 9 out of 20 (45%) of those with undetectable pVL. No significant correlation was observed between pVL and HIV-DNA in the duodenum. These results confirm that HIV-1 remains present in duodenal mucosa in HIV-infected patients even after successful HAART.

HIV-1 reservoirs could be maintained by CD4+ T cell proliferation but several studies both in macaques infected with simian immunodeficiency virus and in humans infected with HIV-1 have shown that the infection rapidly kills most CD4+ T cells at mucosal surfaces during primary infection (Veazey et al., 1998; Guadalupe et al., 2003). Also, incomplete restoration of these intestinal CD4+ T cells has been reported in chronically HIV-1-infected patients following HAART (Guadalupe et al., 2003; Mehandru et al., 2004). The intestinal macrophage population was described to be refractory to HIV during early infection (Meng et al., 2000), but the role of these cells as viral reservoirs is not new; macrophages contribute to support viral population during the chronic phase of HIV-1 infection, especially when CD4 T cells are limiting (Smith et al., 2003).

Here we also report that duodenal CD4+ T lymphocytes were depleted in our cohort of chronically HIV-infected patients relative to controls (HIV+: 4.5 ± 1.1% vs C: 15.1 ± 3.7%, $p = 0.0013$). The percentage of duodenal CD4+ T lymphocytes did not differ significantly ($p = 0.56$) between patients with successful HAART and those with HAART failure (patients with undetectable pVL: 5.2 ± 1.6% vs patients with high pVL: 3.8 ± 1.5%). These results agree with previous data demonstrating that duodenal CD4+ T lymphocytes counts were not restored, even after effective HAART. Even if these results are not innovative, we wanted to corroborate intestinal CD4 depletion in patients during chronic infection.

Then we attempted to reveal the cells mediating viral persistence, as we detected HIV-DNA even after viral suppression following antiretroviral therapy. We investigated the persistence of HIV-1 in tissue CD68+ macrophages, the second major target cell type in which HIV can replicate productively. HIV-1 infection of macrophages in gastrointestinal mucosa had been demonstrated

previously (Smith et al., 2000). Although the prevalence of HIV-1-infected mucosal macrophages is low (0.06% of lamina propria mononuclear cells), the large size of the gastrointestinal mucosa confers to intestinal macrophages a principal role as HIV-1 reservoir (Smith et al., 2003). However, direct effects of HAART on this reservoir remains to be demonstrated. Likewise, it is not known if HIV can establish latent infection in this cell type.

Intestinal CD68+ macrophages were examined by flow cytometry to determine the occurrence of intracellular p24. We demonstrated a detectable p24 expression in intestinal CD68+ macrophages, indicating that these cells can support HIV-1 infection. Interestingly, infected CD68+ macrophages were identified both, in aviremic patients (on successful HAART) and in patients who failed HAART only when HIV-DNA was detected by PCR (Fig. 1A).

It is known that monocytes and macrophages also express the high-affinity receptor for IgG, FcγRI (CD64) on their surfaces (Rogovyi et al., 2007); therefore, we next aimed to confirm our results, examining p24 levels in intestinal CD64+ cells. As expected, HIV-1 replication has been recovered from CD64+ cells, demonstrating that monocytes/macrophages who carry integrated HIV-1 DNA, can serve as a long-term reservoir of HIV-1 (Fig. 1B). Even if other phagocytic cells like neutrophils and dendritic cells, which principally mediate viral dissemination, and also express CD64, no evidence of productive HIV infection was demonstrated for these cells.

In conclusion, this study demonstrates that HIV-DNA could be detected in duodenal mucosa in patients who failed treatment as well as in those with undetectable pVL, suggesting that HAART was less efficient at the intestinal level than in peripheral blood.

Concerning the nature of cells that harbour HIV infection during the chronic phase of disease, we have shown that macrophages may host persistent HIV-1 infection and could be considered as a reservoir of HIV-1 even after successful HAART. Moreover, p24 was identified in mucosal macrophages only when HIV-DNA was detected by standard PCR, confirming the lack of correlation between pVL and duodenal HIV-1 infection. This important aspect must be taken into account when considering the dynamics of HIV-1 re-emergence after HAART interruption. Because latently infected cells are almost impossible to control with most current therapeutic protocols and because HIV-1 replication continues at a low level during prolonged antiretroviral treatment, complete clearance of HIV-1 is very unlikely in the short run.

Our results reinforce the studies indicating that macrophages require higher concentrations of antivirals to inhibit HIV-1 replication compared with T-cells (Aquaro et al., 1998). Therefore, macrophages may represent not only an important target for HIV-1 infection but also a persistently infected cellular reservoir where virus production is difficult to control by currently available antiviral therapy.

Competing interests

The authors declare that they have no competing interests.

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