ORIGINAL ARTICLE

Possible hidden cause of human immunodeficiency virus type 1 latency and role of interferon

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Abstract

Latent human immunodeficiency virus type 1 (HIV-1) infected cells under antiretroviral therapy are reported to be resting memory CD4+ T cells; however, the mechanisms of HIV-1 latency is unclear. We demonstrate that long-term culture of interleukin-2-dependent CD4+ T cells with a memory phenotype mimicked latently HIV-1-infected cells in the presence of interferon-α. These cells are mostly resting and contained HIV-1 proviruses that could be re-activated by stimulation. Our findings suggest a potential role of type-1 interferon in HIV-1 latency.

Key words: HIV-1 latency, interferon, CD4+ T cells.

Introduction

Highly active antiretroviral therapy (HAART) efficiently suppresses human immunodeficiency virus type 1 (HIV-1) replication and improves the prognosis of HIV-1 infection.[1-3] Interruption of HAART leads to rebound in viremia, because antiviral drugs can only limit the active viral replication but not eliminate latently infected cells.[4-7] As a result, lifelong antiretroviral therapy is required for infected individuals. This is accompanied by various side effects including a heavy financial burden, because of the cost of treatment. It would be advantageous to determine how latently HIV-1-infected cells can be eliminated in vivo.

In HIV-1-infected individuals after long-term HAART, latent HIV-1-infected cells remaining in peripheral blood were reported to be resting memory type CD4+ T cells. They exhibit a CD4+ CD45RA- CD45RO+ HLA-DR- phenotype and possess integrated HIV-1 provi-ruses that do not replicate unless activated.[4,6,8] However, it is not well understood how HIV-1 persists in cells under latent conditions.

In the present study, we attempted to reproduce HIV-1 latency in memory CD4+ T cells that had been cultured for a long period and whose growth is dependent on antigen stimulation. We also examined the effects of type-1 interferon (IFN-α) on HIV-1 latency. The αIFNs are produced by many kinds of cells in vivo even in physical conditions as well as in HIV-1-infected individuals and known to restrict viral replication and induce cell cycle arrest in peripheral blood mononuclear cells (PBMCs).[9-13] The aim of the present study was to identify possible hidden cause of HIV-1 latency and role of IFN.

Materials and Methods

We isolated CD4+ CD25- CD45RO- T cells from PBMCs of a healthy individual using

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immunomagnetic beads. The cells were then stimulated with beads coated with anti-CD3 and -CD28 antibodies (CD3/CD28 beads, Invitrogen) for 5 days in the presence of 10 nM all trans retinoic acid (ATRA), 50 U/ml interleukin (IL)-2 and 5 ng/ml transforming growth factor (TGF)-β, and further cultured in the same medium for three weeks, then maintained in medium containing IL-2 and TGF-β for more than 5 months, periodically stimulated with CD3/CD28 beads. The resulting CD4+ T cell line was designated the LTiT4 (long term inducible T cell positive for CD4) line.

Results and discussion

LTiT4 cells exhibit a memory T cell phenotype (CD45RA-CD45RO+ CD27+ CD127+) (Fig.1A). Stimulation with CD3/CD28 beads strongly induced CD25 expression (Fig. 1B) and tumor necrosis factor-alpha (TNF-α) production in LTiT4 cells (Fig. 1C). Thus, LTiT4 cells are non-tumor memory-type CD4+ T cells that can be maintained in the presence of IL-2 and proliferate in response to antigen stimulation.

We assessed the effects of IFN-α on LTiT4 cells (Fig 2A). The cell growth rate of LTiT4 cells was diminished by IFN-α in a dose-dependent manner at concentrations of 300 and 1000 U/ml. IFN-α -treatment markedly reduced expression of CD25, while the CD45RA- CD45RO+ HLA-DR- phenotype was not largely altered irrespective of IFN-α treatment (Fig. 2B). Through cell cycle analysis, we observed that the majority of LTiT4 cells at 2 and 4 weeks after stimulation were
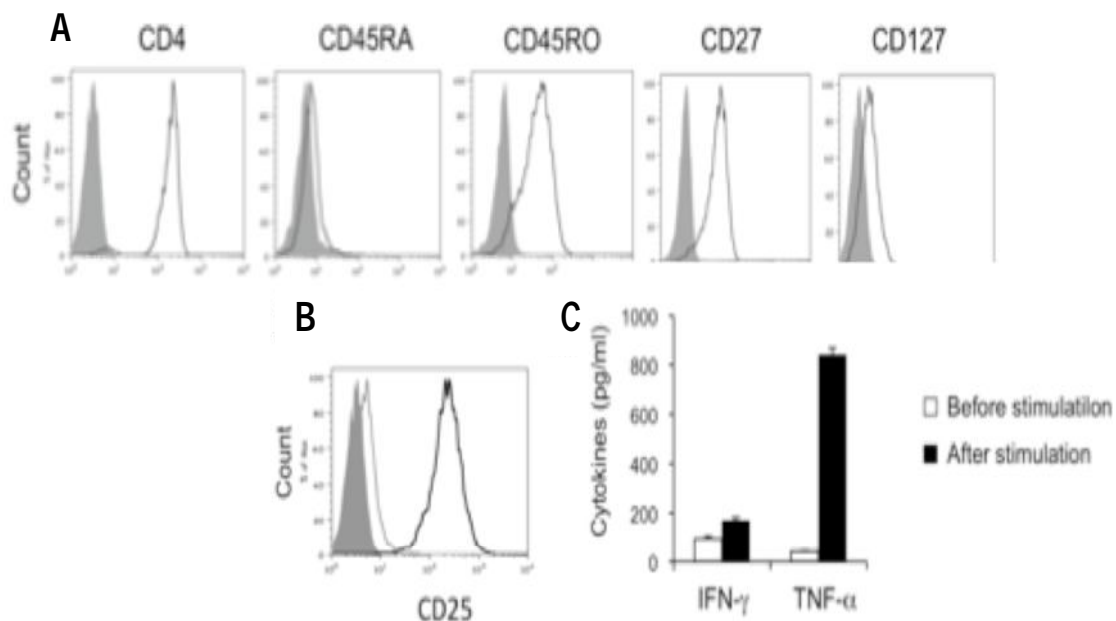

Fig. 1. A. Cell surface phenotype of LTiT4 cells in the resting stage was evaluated by flow cytometry following staining with FITC conjugated CD4, CD27 (Biolegend), CD127, CD45RO, and CD45RA (BD Bioscience) (lined histogram). Closed histrogram indicates control stained with isotype antibody. B. Cell surface CD25 expression in LTiT4 cells with (dark line) or without (light line) CD3/CD28 beads stimulation for 24 h. C. IFN-γ and tumor necrosis factor-alpha (TNF-α) in the supernatants of LTiT4 cells without (blank bar) and with (closed bar) CD3/CD28 beads stimulation for 24 h were analyzed by using Th1/Th2 11 plex Kit (eBioscience).
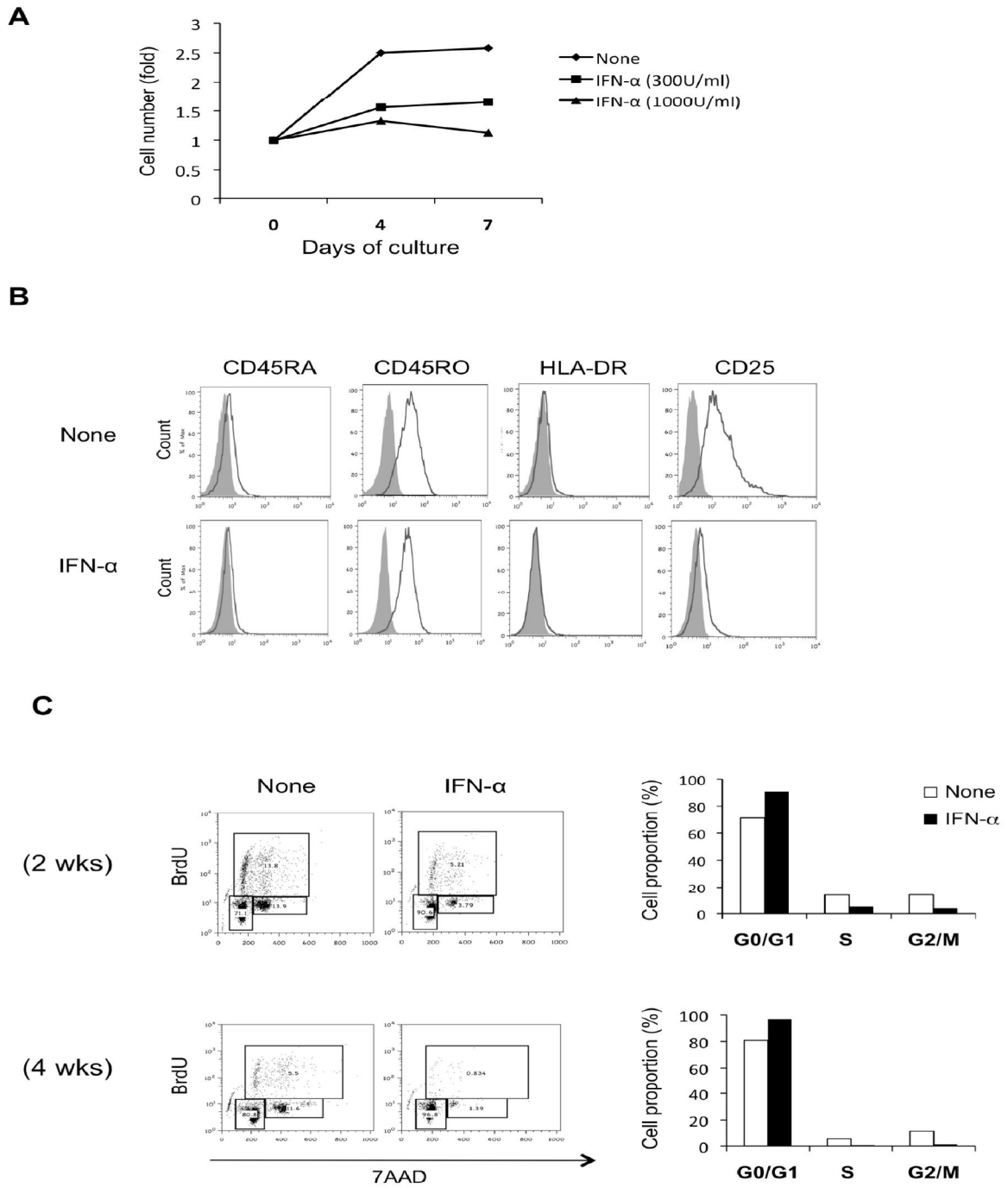
Fig. 2. A. LTiT4 cells were pre-stimulated with CD3/CD28 beads for 3 days, then cultured in the presence of 0 (◆), 300 (■) and 1000 (▲) U/ml of IFN-α, and the growth of the cells was evaluated. B. LTiT4 cells were cultured in the presence or absence of 1000 U/ml IFN-α and the cell surface phenotype on day 14 was analyzed by flow cytometry. Closed histogram indicates isotype control. C. LTiT4 cells in (B) were cultured for a longer period with medium replenished once a week. Cells were subjected to cell cycle analysis after 2 and 4 weeks in culture. The results of flow cytometry (left panels) were indicated also in the bar graphs (right panels), with (closed bar) or without (open bar) IFN-α.
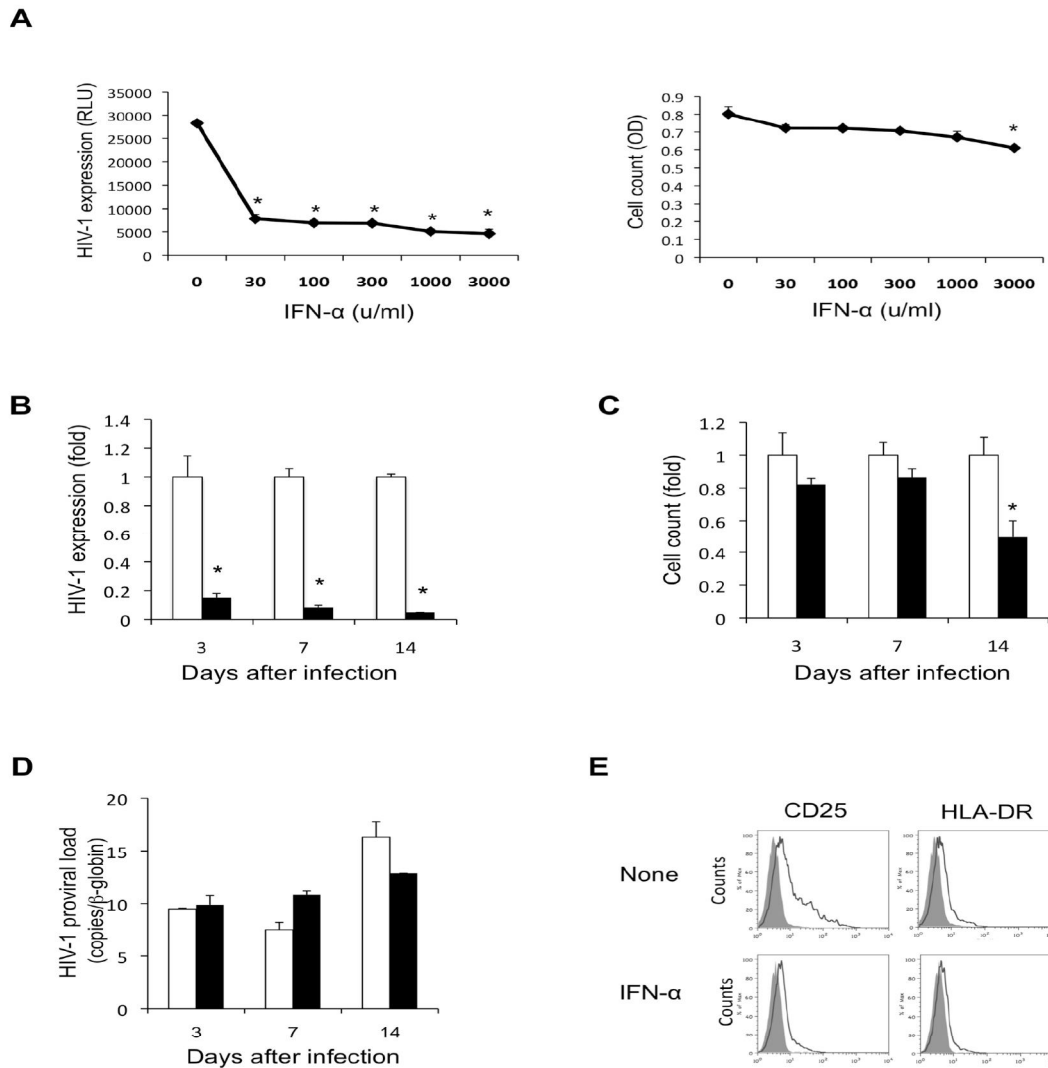
Fig. 3. A. LTiT4 cells at 3 days after stimulation were infected with VSV-G/pNL4-3Luc pseudotype virus (100 ng of HIV-1 p24/10^6 cells)[14] overnight in the presence of 100 U/ml IL-2. The cells (10^5/well) were incubated for another day after wash, then treated with various concentrations of IFN-α for 3 days. HIV-1 expression (left panel) and viable cell number (right panel) in each well was evaluated by a luciferase assay and a colorimetric cell counting method, respectively. **B-D.** LTiT4 cells infected with VSV-G/pNL4-3Luc in the presence (closed bar) or absence (open bar) of 1000 U/ml IFN-α were subjected to analysis of luciferase activity (B), viable cell number (C) and the amount of HIV-1 proviral DNA (D) at 3, 7, and 14 days post-infection. For B and C, the relative values to the sample without IFN-α for each time points are indicated as the mean and standard deviation. To quantify proviral DNA, chromosomal DNA were subjected to quantitative PCR with R/Gag primer set. **E.** LTiT4 cells were infected with VSV-G/pNL4-3Luc and cell surface CD25 and HLA-DR expression was evaluated after 14 days of culture in the presence or absence of IFN-α. *: $p < 0.05$ (Student's *t*-test).
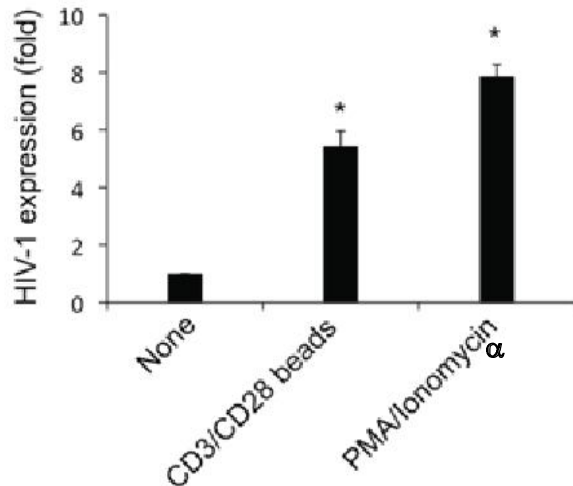
Fig. 4. LTiT4 cells were infected with VSV-G/pNL4-3Luc and maintained with 1000 U/ml IFN-α for 13 days, and 10^5 cells/well were stimulated without or with CD3/CD28 beads (8×10^4/well), or PMA (50 ng/ml) and Ionomycin (500 ng/ml) for 24 hrs, and then subjected to luciferase assays. Relative values against the sample without stimulation were indicated as the mean and SD of duplicate samples. *: $p < 0.05$.

already in the G0/G1 phase. Treatment with IFN-α (1000 U/ml) further increased the numbers of cells in the G0/G1 phase, and decreased those in the S and G2/M phases (Fig. 2C). These findings suggest that IFN-α might accelerate LTiT4 cells entering a resting stage.

We investigated the effects of IFN-α on HIV-1 replication in LTiT4 cells by using a pseudo-typed HIV-1 (VSV-G/NL4-3-luc)14 (Fig. 3). The LTiT4 cells well supported HIV-1 gene expression, as determined by luciferase assays. We added different doses of IFN-α for 3 days post-infection and found that doses between 30-3000 U/ml significantly suppressed HIV-1 gene expression with a mild dose dependency (Fig. 3A left). Cell numbers were also slightly decreased by IFN- but not significantly except when 3000 U/ml IFN-α was applied as it was toxic (Fig. 3A right).

Long-term effects were evaluated by maintaining infected cells in the presence or absence of IFN-α (1000 U/ml). The HIV-1 gene expression was significantly suppressed by IFN-α on day 3 and further decreased on days 7 and 14 (Fig. 3B). Cell

numbers, as determined by a colorimetric cell-counting kit, were not significantly altered by IFN-α treatment.

HIV-1-infected cells were maintained in cultures with IFN-α; we detected HIV-1 proviral DNA in cells on days 3, 7 and 14. Relative copy numbers of HIV-1 proviruses in these cells, standardized with those of β-globin, were comparable with the cultures lacking IFN-α (Fig. 3D). CD25 expression was reduced by IFN-α as similarly observed in uninfected LTiT4 cells (Fig. 3E). These observations confirmed that, in the presence of IFN-α the HIV-1-infected cells entered a resting stage to a greater extent, and the HIV-1 existed as integrated proviral DNA but was hardly replicate.

Finally, we examined whether immune stimulations could reactivate viral expression in latently HIV-1-infected LTiT4 cells (Fig. 4). We added CD3/CD28 beads into the HIV-1-infected LTiT4 cells that were rendered after 2 weeks of culture in the presence of IFN-α. At 24 h post-stimulation, HIV-1 gene expression was significantly enhanced in these cells. Viral expression was also induced by stimulation with phorbol 12-myristate 13-acetate (PMA) and Ionomycin.

Our findings indicated that LTiT4 cells can mimic resting memory T cells in the presence of IFN-α, exhibiting post-integration latency following HIV-1-infection. IFN-α seemed to suppress both cell cycle progression and HIV-1 gene expression. There are many reports of type-1 IFN-mediated suppression of HIV-1 replication at the early and late stages of the HIV-1 replication cycle.[15-18] Additionally, elevation of type-1 IFNs in HIV-1 infected individuals and its undesirable role in acquired immunodeficiency syndrome development by chronic immune activation has been widely debated.[19] However, little interest has focused on the role of IFNs during HIV latency. Here, we hypothesized a significant role of type-1 IFNs in inducing memory CD4+ T cells towards a resting stage, thereby leading to HIV-1 latency.

In conclusion, type-1 IFNs induced cell cycle arrest and post-integration latency of HIV-1 infection in memory-type CD4+ T cells derived from a healthy individuals in vitro, mimicking the latently HIV-1-infected resting memory CD4+ T cells detected in HIV-1-infected individuals undergoing HAART. An antigen-specific CD4+ T cell line such as LTiT4 in the presence of IFN-α should be a useful model in understanding the mechanisms of latent HIV-1 infection, and assist the development of novel therapeutic strategies to eradicate latent HIV-1-infected cells.

Acknowledgments

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