

# 193 HIV-1 Induces Apoptosis in primary Osteoblasts: an Alternative Mechanism in the Osteopenia/Osteoporosis Development

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## Abstract

**Background:** Several HIV-1 infected patients show bone loss and osteopenia/osteoporosis. The mechanisms underlying this degenerative process are unsettled and the interaction between HIV-1 and osteoblasts/osteoclasts cross-talk regulation is still unknown. We analysed whether the primary osteoblasts are permissive to different HIV-1 strains infection and HIV-1 interaction with cell membrane by gp120 to study the mechanisms involved in the bone loss in HIV-1 infection.

**Methods:** Human hipbone osteoblasts, obtained from commercial sources or isolated from HIV-1 negative subjects (enrolled after giving their informed consent), were challenged by HIV-1 X4 and R5 classical strains. HIV-1 proviral DNA and viral RNA load were determined by PCR and RT-PCR respectively. Flow cytometry procedures were used for apoptosis and membrane markers analysis whereas TNF- $\alpha$  supernatant analysis was performed by commercial kit.

**Results:** Human osteoblasts, challenged by HIV-1, did not show any positive signal of active (RNA load) or latent (DNA viral load) infection. On the other hand, HIV-1, heat-inactivated HIV-1 and HIV-1 gp120 treatment induced a significant apoptotic activation at 72-96 hours ( $p < 0.01$ ), that is tackled by soluble CD4 treatment, suggesting an interaction between gp120 and cell membrane proteins. CD4, CXCR4 and CCR5 mRNAs were detectable even though CD4 and CXCR4 proteins were expressed at very low density and in few cells, whereas CCR5 fig 2B protein was significantly more expressed, suggesting a binding between CD4 and HIV-1 gp120 in absence of HIV entry. As observed in CD34+ hematopoietic cells this phenomenon might be due to a possible lack of functional association between receptor and co-receptor. Further experiments demonstrated that HIV-1, heat-inactivated HIV-1 and HIV-1 gp120 treatment were able to induce TNF- $\alpha$  (a well-known apoptosis-inducer in the osteoblasts) mRNA and a protein increase at 24-96 hours. Moreover, anti-TNF- $\alpha$  pre-treatment tackled the apoptosis induction suggesting a direct role of TNF- $\alpha$  in the HIV-1 activation of apoptotic process.

**Conclusions:** These results indicate that HIV-1 triggers apoptosis in osteoblasts without infection but through gp120 interaction with cell membrane suggesting a novel mechanism in the HIV-1 related impairment of the bone mass structure homeostasis.

## Introduction

Several HIV-1 infected patients show bone loss and osteopenia/osteoporosis. Bone remodelling is a continuous physiological process due to dynamic balanced action by osteoblasts and osteoclasts. The mechanisms underlying this degenerative process are unsettled and the interaction between HIV-1 and osteoblasts/osteoclasts cross-talk regulation is still unknown.

In this study we investigated the interaction between HIV-1 and primary human osteoblasts on cell membrane by gp120 and its effects on cell survival to disclose the mechanism(s) involved in HIV-1 related bone damage.

## Methods

- Human hipbone osteoblasts of three HIV-1 negative subjects were obtained from Promocell (Promocell, Heidelberg, Germany) or isolated from biopsies of trabecular bone derived from healthy patients undergone to surgery following acute trauma enrolled after giving their informed consent following the Helsinki declaration.
- The osteoblasts were challenged by HIV-1 X4 and R5 classical strains. HIV-1 proviral DNA and viral RNA load were determined by PCR and RT-PCR respectively.
- HIV-1<sub>IB</sub> (lymphotropic strain) and HIV-1<sub>ADA</sub> (monotropic strain) stocks were titrated by ELISA HIV-1 p24 antigen (Biomerieux, Marcy L'Etoile, France). All HIV-1<sub>IB</sub> and HIV-1<sub>ADA</sub> stocks were determined at 400 pg/ml of gag p24.
- We investigated whether HIV-1 affects the survival/proliferation of osteoblasts. To do so 5x10<sup>5</sup> primary osteoblasts inoculated by 400pg/ml of purified infectious HIV-1 (IB or Ada) or with the same concentration of heat-inactivated HIV-1.
- Flow cytometry procedures were used for apoptosis and membrane markers analysis whereas TNF- $\alpha$  supernatant analysis was performed by commercial kit.
- The apoptosis cell percentage was analyzed at 24, 48, 72, 96 hours after HIV-1 strains and/or recombinant proteins treatment.
- To establish whether TNF- $\alpha$  is involved in the HIV-1-related apoptosis, we analyzed TNF $\alpha$  mRNA and protein expression in HOBIT and primary osteoblasts. The results from osteoblasts were compared with these of a previous study (Gibellini et al.2008)

## Results

HIV-1<sub>IB</sub>, HIV-1<sub>ADA</sub>, heat-inactivated HIV-1 strains and cross-linked recombinant gp120 elicited apoptosis at 48-96 hours post-treatment in comparison with samples treated by p24, anti-gp120 polyclonal antibody or sCD4 pre-treated HIV-1 strains. HIV-1<sub>IB</sub>, HIV-1<sub>ADA</sub>, a significant increase of apoptotic cell number apoptosis at 48-96 hr ( $P < 0.05$ ) in all tested cell models. Data are expressed as means SD of three separate experiments performed in duplicate. [Fig.1]

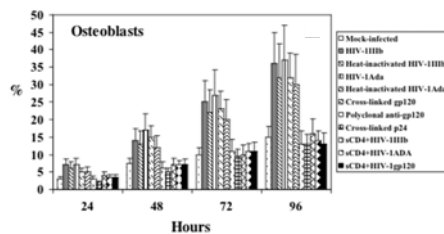


Fig.1

We also investigated whether HIV-1 productively infected primary osteoblasts. Real time PCR and b-DNA assays did not detect HIV-1 proviral DNA and HIV-1 genomic RNA respectively, suggesting that apoptosis was induced by interaction between HIV-1 and cell membrane [Fig.2]

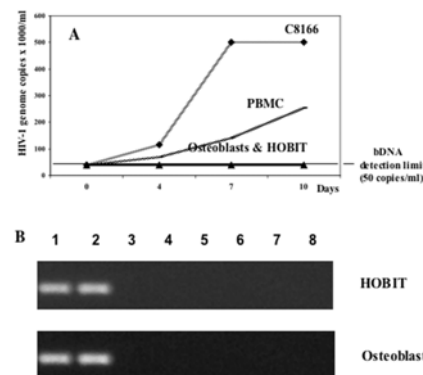


Fig 2

Quantitative real time RT-PCR showed an increase of TNF $\alpha$  mRNA by HIV-1 strains or cross-linked gp120 treatment at 24 hours with an expression peak achieved at 48-72 hours. On the other hand, HOBIT and primary osteoblasts yielded a significant amount of TNF $\alpha$  protein in the cellular supernatant at 48 hours ( $p < 0.05$ ) with detection peaking at 72-96 hours after virus or cross-linked gp120 challenges. [Fig.3 A and B] Polyclonal antibody the apoptosis induction is clearly tackled ( $p < 0.05$ ) at 72-96 hours. [Fig. 4]

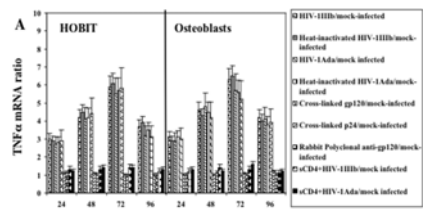


Fig. 3 A

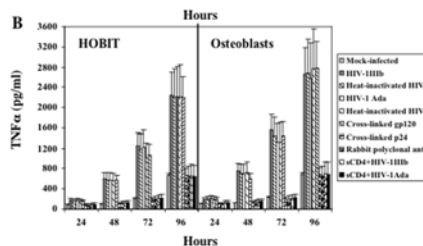


Fig. 3 B

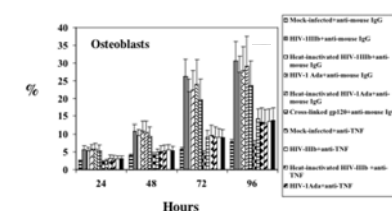


Fig. 4

## Discussion

Our data showed that HIV-1 significantly induced osteoblasts or HOBIT cells towards apoptosis at 48-96 hours after challenge. Interestingly, in our experimental conditions, programmed cell death was linked to engagement between gp120 and cell membrane. Heat-inactivated HIV-1 or recombinant gp120 elicited apoptosis activation of primary osteoblasts and HOBIT cells as when infectious HIV-1 was employed.

Both HOBIT and primary osteoblasts showed the presence of all three mRNAs, but when membrane protein expressions were analyzed, we observed a weak detection of CD4 and CXCR4 in a small percentage of HOBIT and primary osteoblasts, whereas CCR5 was consistently expressed in the 15% of these cells.

## Conclusions

This report demonstrated that:

- HIV-1, heat-inactivated HIV-1 and recombinant gp120 triggered apoptosis in primary osteoblasts;
- HIV-1 does not infect osteoblasts and HOBIT cells and then the apoptosis induction is related to gp120/cell membrane interaction;
- TNF $\alpha$  is secreted after HIV-1 challenge and neutralizing anti-TNF $\alpha$  antibody tackled the HIV-1 related apoptosis both in osteoblasts and HOBIT cells suggesting a direct role of TNF $\alpha$  in apoptosis induction

## References

Gibellini D, De Crignis E, Ponti C, Cimatti L, Borderi M, Tschon M, Giardino R, Re MC, HIV-1 triggers apoptosis in primary osteoblasts and HOBIT cells through TNF $\alpha$  activation Journal of Medical Virology 2008 Sep;80(9):1507-14