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 across cell-cell interaction is still unknown. We analysed whether the primary osteoblasts are permissive to different HIV-1 strains infection and HIV-1 interaction with cell membranes by gp120 to study the mechanisms involved in the bone loss in HIV-1 infection.

Methods: Human osteoblasts, obtained from commercial sources or isolated from biopsies of trabecular bone derived from healthy human donors were infected with HIV-1 strains or cross-linked gp120 stocks were titrated by ELISA and viral RNA load were determined by PCR and RT-qPCR, respectively. Flow cytometry analyses were used for apoptosis and membrane markers analysis whereas TNF-α 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 (viral RNA 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). In this case, it is tackled by soluble CD4 treatment, suggesting an interaction between gp120 and cell membrane proteins. CD4, CXCR4 and CCR5 mRNA were detected even though CD4 and CXCR4 proteins were expressed at very low density and in few cells, whereas CCR5 gp120 protein was significantly more expressed, suggesting a binding between CD4 and HIV-1 gp120 in absence of HIV entry. As observed in CD4+ lymphoblastoid 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-α (a well-known apoptosis inducer in the osteoblasts) mRNA and protein increase at 24-96 hours. Moreover, anti-TNF-α pre-treatment tackled the apoptosis induction suggesting a direct role of TNF-α in the HIV-1 activation of apoptotic process.

Conclusion: 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 actions by osteoblasts and osteoclasts. The mechanisms underlying this degenerative process are unsettled and the interaction between HIV-1 and osteoblasts/osteoclasts across cell-cell interaction is still unknown.

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

Materials and Methods

Human osteoblasts of three HIV-1 negative subjects were obtained from Promocell (PromoCell, Heidelberg, Germany) or isolated from biopsies of trabecular bone derived from healthy primary osteoblasts to surgery following same means infected after giving their informed consent according to the Helsinki declaration.

The osteoblasts were challenged by HIV-1 IIIb and HIV-1 ADA clinical strains. HIV-1 proviral DNA and viral RNA load were determined by qPCR and RT-qPCR, respectively. Human osteoblasts were permissive to different HIV-1 strains infection and HIV-1 interaction with cell membranes by gp120 to study the mechanisms involved in the bone loss in HIV-1 infection.

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Results

HIV-1 IIIb, HIV-1 ADA, heat-inactivated HIV-1 and cross-linked recombinant gp120-challenged osteoblasts at 48-96 hours post-treatment in comparison with sample treated by gp24, gp120 polymeric antibody or sCD4 pre-treated HIV-1 strain. HIV-1IIIb, HIV-1ADA, a significant increase of apoptotic cell number apoptosis at 48-96 hr (p<0.01) in all tested cell models. Data are expressed as means SD of three separate experiments performed in duplicate. (Fig. 2)

Discussion

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

Conclusion

This report demonstrated that:

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

References