

Interleukin 7 Reduces the Levels of Spontaneous Apoptosis in CD4⁺ and CD8⁺ T Cells from HIV-1-Infected Individuals

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Abstract

Background. Apoptosis is considered one of the primary mechanisms of CD4⁺ T-cell depletion during the course of human immunodeficiency virus type I (HIV-1) infection. Thus, therapeutic strategies that reduce apoptosis should prolong T-cell survival and facilitate immune reconstitution in HIV-1-infected subjects. **Methods.** Apoptosis levels were studied in PBMC and purified CD4⁺ and CD8⁺ T-cell subsets obtained from 29 HIV-1-infected subjects at different stages of disease and 14 HIV-1-seronegative controls. Apoptosis levels were measured daily for 7 days of *ex vivo* culture by evaluating Annexin-V binding, propidium-iodide staining and caspase-3 activation. Cellular proliferation was assessed by Ki67 staining and absolute cell counting. HIV-1 replication was determined by p24 antigen release. Recombinant human interleukin-7 (IL-7) was added at the onset of culture. **Results.** Levels of spontaneous apoptosis over 7 days of *ex vivo* culture were significantly higher in HIV-1-infected subjects than in HIV-1-seronegative controls (p<0.0001). Treatment with IL-7 at 0.5-5.0 ng/mL exerted significant anti-apoptotic effects on PBMC from HIV-1-infected subjects (p<0.0001), but not from seronegative controls. The degree of apoptosis reduction was inversely correlated with the number of circulating CD4⁺ T cells, indicating a higher sensitivity to IL-7 effects in patients with more advanced disease. Analysis of purified CD4⁺ and CD8⁺ T cells demonstrated that both subsets were sensitive to the protective effects of IL-7. The anti-apoptotic effect of IL-7 was uncoupled from the induction of cellular proliferation. Moreover, at doses that reduced apoptosis, IL-7 did not trigger or increase the levels of endogenous HIV-1 replication. **Conclusions.** The anti-apoptotic activity of IL-7 documented in HIV-1-infected subjects provides a further rationale for consideration of this cytokine as an agent of immune reconstitution in HIV-1 infection.

Background

CD4⁺ T-cell-depletion is one of the hallmarks of HIV-1 infection, that severely compromises the immune system and ultimately leads to AIDS (Lane, H. C., Fauci, A. S., *Annu. Rev. Immunol.*, 1985)

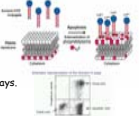
Among the mechanisms that contribute to the CD4⁺ T cell-loss, spontaneous apoptosis is believed to play a very important role (McCune, J. M. *et al.*, *Nature*, 2001). High levels of apoptosis have been documented both *in vivo* (Maynard *et al.*, *Science*, 1992; Lewis *et al.*, *J. Immunol.*, 1994; Muro-Cacho *et al.*, *J. Immunol.*, 1995) and *ex vivo* (Lewis *et al.*, *J. Immunol.*, 1994; Muro-Cacho *et al.*, *J. Immunol.*, 1995; Gougeon *et al.*, *J. Immunol.*, 1996) in T-lymphocytes from HIV-1-infected individuals; of note, the large majority of the CD4⁺ T cells that undergo spontaneous apoptosis in lymphoid tissues are uninfected, bystander cells (Finkel *et al.*, *Nat. Med.*, 1995)

The advent of HAART has greatly improved the treatment of HIV-1 infection, but the current protocols often fail to fully restore the immune function (Lange and Lederman, *J. Antimicrob. Chemother.*, 2003). Thus, innovative approaches based on the use of immunomodulatory agents, such as IL-2 and IL-7, are currently under evaluation

IL-7 is a non-redundant cytokine that plays essential roles in the development and homeostasis of the T-cell compartment of the immune system (Fry and McKall, *J. Immunol.*, 2005)

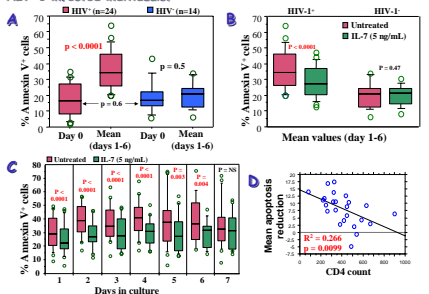
Given the increased propensity of lymphocytes derived from HIV-1-infected individuals to undergo spontaneous apoptosis *ex vivo* and the relevance of this phenomenon *in vivo*, we investigated the effect of IL-7 on spontaneous apoptosis in a series of HIV-1-infected subjects.

Apoptosis was assessed by testing cell surface binding of Annexin V (on the right), which detects even the earliest apoptotic changes, as well as the activation of caspase-3, a common marker of the intrinsic and extrinsic apoptosis pathways.



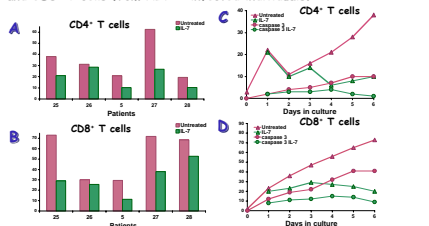
Results

1) IL-7 reduces the levels of spontaneous apoptosis in PBMC from HIV-1 infected individuals.



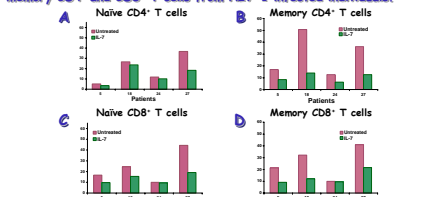
A Mean levels of Annexin-V binding on PBMC from 24 HIV-1-infected individuals and 14 healthy subjects cultured *ex vivo* for 6 days in the absence of cytokines. **B** Mean levels of Annexin V-binding on PBMC cultured *ex vivo* for 6 days in the absence or presence of IL-7 (5 ng/ml). **C** Day-by-day mean levels of Annexin V-binding on PBMC from the 24 HIV-1-infected individuals cultured *ex vivo* in the absence or presence of IL-7 (5 ng/ml). **D** Correlation between the anti-apoptotic effect of IL-7 *ex vivo* and the CD4 counts *in vivo*.

2) IL-7 reduces the levels of spontaneous apoptosis in purified CD4⁺ and CD8⁺ T cells from HIV-1 infected individuals.



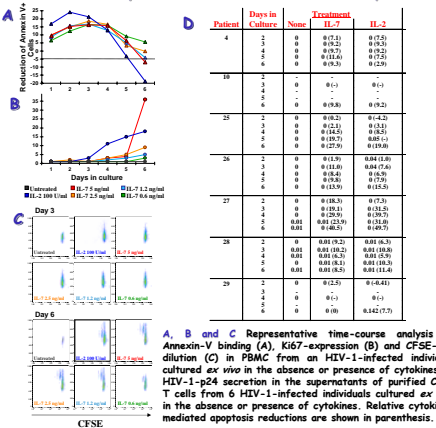
A and **B** Mean levels of Annexin-V binding on purified CD4⁺ and CD8⁺ T cells from 5 HIV-1-infected individuals cultured *ex vivo* for 6 days in the absence or presence of IL-7 (5 ng/ml). **C** and **D** Time-course analysis of Annexin-V binding and Caspase-3 activation in purified CD4⁺ and CD8⁺ T cells from a representative HIV-1-infected individual cultured *ex vivo* in the absence or presence of IL-7 (5 ng/ml).

3) IL-7 reduces the levels of spontaneous apoptosis in naive and memory CD4⁺ and CD8⁺ T cells from HIV-1 infected individuals.



A, B, C and **D** Mean levels of Annexin-V binding to naive and memory CD4⁺ and CD8⁺ T cells from 4 HIV-1-infected individuals. CD4⁺ and CD8⁺ T cells were purified from PBMC by negative selection and cultured *ex vivo* in the absence or presence of IL-7 (5 ng/ml). Naive and memory phenotypes were identified by multi-color-flow cytometry.

4) The anti-apoptotic effect of IL-7 can be dissociated from the induction of cellular proliferation and activation of HIV-1-replication.



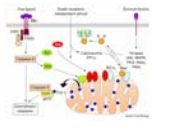
A, B and **C** Representative time-course analysis of Annexin-V binding (**A**), Ki67-expression (**B**) and CFSE-dye dilution (**C**) in PBMC from an HIV-1-infected individual cultured *ex vivo* in the absence or presence of cytokines. **D** HIV-1-p24 secretion in the supernatants of purified CD4⁺ T cells from 6 HIV-1-infected individuals cultured *ex vivo* in the absence or presence of cytokines. Relative cytokine-mediated apoptosis reductions are shown in parenthesis.

Conclusions

- In line with previous observations, we found that PBMC from HIV-1-infected individuals showed a higher propensity to undergo spontaneous apoptosis compared to PBMC from healthy subjects
- IL-7 significantly reduced the levels of spontaneous apoptosis in both unfractionated PBMC and purified CD4⁺ and CD8⁺ T cells from HIV-1-infected individuals, whereas PBMC from uninfected healthy subjects were less sensitive to the antiapoptotic effects of IL-7
- IL-7 exerted anti-apoptotic effects over a wide dose range
- The anti-apoptotic effect of IL-7 *ex vivo* was inversely correlated with the CD4 counts *in vivo*. IL-7 was more effective on cells derived from patients with more advanced disease
- The anti-apoptotic and proliferative effects of IL-7 could be uncoupled: in our experiments IL-7 protected the cells from apoptosis since the very early time points, whereas cell cycling and proliferation became detectable only at later time points; moreover, at the lowest doses tested, IL-7 was still protective without inducing any cellular proliferation
- At concentrations that showed anti-apoptotic effects, IL-7 did not induce HIV-1 replication in both unfractionated PBMC and purified CD4⁺ T cells

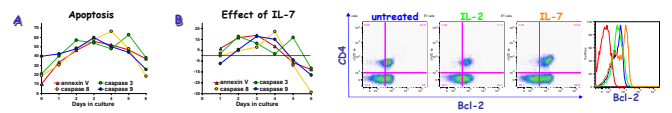
Ongoing and future studies

- Investigation of the mechanisms that mediate spontaneous apoptosis in HIV-1-infected individuals
- Investigation of the mechanisms of impairment of the IL-7/IL-7R system during the course of HIV-1 infection
- Investigation of the mechanisms of IL-7-mediated apoptosis reduction in HIV-1 infection:
 - evaluation of the intrinsic and extrinsic pathways of apoptosis
 - evaluation of anti-apoptotic and pro-apoptotic proteins

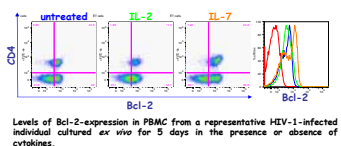


Preliminary Data

- IL-7 reduces the levels of activation of both caspase 8 and 9 in PBMC from HIV-1 infected individuals.
- IL-7-treated cells show an increased level of expression of the anti-apoptotic protein Bcl-2.



A Mean levels of Annexin V-binding and caspase 3, 8 and 9-activation in PBMC from a representative HIV-1-infected individual cultured *ex vivo* for 6 days in the absence of cytokines. **B** Reduction of Annexin V-binding and caspase 3, 8 and 9-activation mediated by IL-7 (5 ng/ml).



Levels of Bcl-2-expression in PBMC from a representative HIV-1-infected individual cultured *ex vivo* for 5 days in the presence or absence of cytokines.

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