# Are Blockers of gp120/CD4 Interaction Effective Inhibitors of HIV-1 Immunopathogenesis?

*Jean-Philippe Herbeuval and Gene M. Shearer*

*Experimental Immunology Branch, Centers for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*

## Abstract

**Objective:** ***The immunopathogenic mechanisms that result in the depletion of CD4+ T-cells after HIV-1 infection remain controversial. We consider here mechanisms that have been suggested, and propose a data-supported model in which CD4+ T-cells undergo apoptosis that is signaled by the binding of viral gp120 to cellular CD4.***

**Procedures:** ***Blood leucocytes from HIV-1-uninfected donors, including CD4+ and CD8+ T-cells, monocytes, myeloid and plasmacytoid dendritic cells (pDC) were cultured with either infectious or noninfectious HIV-1. The cultures were tested for expression of interferon-α, TRAIL, DR5 and apoptosis. Inhibitors of IFNα, TRAIL, DR5 and gp120/CD4 binding were added to the cultures.*** **Ex vivo** ***studies were performed using peripheral blood mononuclear cells (PBMC) from HIV-1-infected patients to test the validity of our*** **in vitro** ***findings.***

**Findings:** ***Both infectious and noninfectious HIV-1 induced pDC to produce IFNα, which induced expression of TRAIL by CD4+ but not CD8+ T-cells. CD4+ T-cells expressed the TRAIL death receptor 5 (DR5), upon HIV-1 binding to CD4. Antibodies against TRAIL and DR5 partly inhibited apoptosis. However, soluble CD4 (sCD4-IgG) efficiently blocked IFNα production, TRAIL and DR5 expression and apoptosis of T helper cells. Studies of HIV-1-infected patients' PBMC indicated increased plasma TRAIL production and CD4+ T-cell DR5 expression, which correlated directly with viral load and inversely with CD4 count.***

**Conclusion:** ***Noninfectious interactions between HIV-1 and CD4 are major contributors to CD4+ T-cell death via IFNα-induced TRAIL expression and HIV-1-induced DR5 expression on CD4+ T-cells. Since noninfectious as well as infectious HIV-1 induces the death cascade resulting in selective apoptosis of CD4+ T-cells, these HIV-1/CD4-dependent binding events would not necessarily be reflected in HIV-1 RNA and DNA expression by the CD4+ target T-cells. Because each step of this model leading to apoptosis requires the binding of gp120 to CD4, we suggest that molecules which block this very early event in virus/target cell interaction will be effective in preventing or reducing the depletion of CD4+ T-cells during progression to AIDS. The above mechanisms and the effect of sCD4-IgG are summarized in our proposed model.*** **(AIDS Reviews 2006;8:3-8)**

## Key words

***HIV-1 immunopathogenesis. interferon-α. Plasmacytoid dendritic cells. CD4+ T-cell apoptosis. TRAIL/DR5. sCD4-IgG therapy.***

**Correspondence to:**
Gene Shearer
Experimental Immunology Branch
Centers for Cancer Research
NCI, NIH, Bethesda, MD, USA
E-mail: shearerg@mail.nih.gov

## Introduction

The immunologic and virologic surrogate markers of AIDS progression are characterized by decreased CD4 count and increased viral load, respectively. An understanding of the pathogenic mechanisms responsible for the depletion of CD4+ T-cells is essential for developing effective therapeutic protocols. Yet, despite more than 20 years of research, depletion of T helper cells is not fully understood. Mechanisms suggested for the loss of CD4+ T-cells include the direct cytopathic effects of HIV-1 infection that involve syncytia formation by infected CD4+ T-cells, accumulation of unintegrated viral DNA or toxic viral proteins, virus-induced changes in target cell membrane permeability, and apoptosis of HIV-infected CD4+ T-cells[1]. Other proposed mechanisms include lysis of infected CD4+ T-cells by CD8+ cytolytic T-cells[2] and tryptophan depletion by indolamine 2,3-dioxygenase[3]. Because the frequency of circulating cells infected with HIV-1 is too low to account for the extensive death of CD4+ T-cells[4], mechanisms involving uninfected CD4+ T-cells have also been suggested[5]. Activation-induced apoptosis was proposed to contribute to this phenomenon[5,6]. However, current models do not account for the preferential loss of CD4+ T-cells during progression to AIDS because both CD4+ and CD8+ T-cells are activated by HIV-1 infection[6].

The Fas/FasL apoptotic pathway has been studied extensively in AIDS patients[7], and was suggested to contribute to apoptosis resulting from HIV-1 infection[8-11]. However, other studies demonstrated that CD4+ T-cell depletion was not due to the Fas/FasL mechanism[12,13]. Another TNF family member, TNF-related apoptosis-inducing ligand (TRAIL), was suggested to contribute to HIV-induced CD4+ T-cell apoptosis[14,15]. TRAIL has five receptors: two death receptors (DR4 and DR5) that induce apoptosis and three others that lack the death domain[16-18]. The two biologically active forms of TRAIL, membrane-bound (mTRAIL) and soluble TRAIL (sTRAIL), are regulated by type I interferon (IFNα and IFNβ)[19-21]. TRAIL is secreted by leukocytes, including T lymphocytes[22], natural killer cells[23], dendritic cells[24,25], monocytes and macrophages[26]. TRAIL induces apoptosis of tumor and virus-infected cells[26,27], but not of normal cells[28]. Several studies now indicate that TRAIL is involved in HIV-1 immunopathogenesis. Soluble TRAIL was found in the serum of HIV-1-infected patients[29,30]. CD4+ and CD8+ T-cells from HIV-1-infected patients are more susceptible to TRAIL-induced apoptosis *in vitro* than T-cells from healthy, uninfected donors[31-33]. TRAIL induced apoptosis of CD4+ T-cells in the SCID-Hu-PBL model of AIDS[34]. Furthermore, antigen-presenting cells such as monocytes and dendritic cells[35] have been implicated in TRAIL-induced pathogenesis[14,36]. TRAIL is also involved in HIV-1-induced dementia, since macrophages found in brain tissue of AIDS patients expressed TRAIL[37-39]. We recently found that plasma TRAIL levels in HIV-1-infected patients directly correlate with viral load[40]. We also demonstrated that CD4+ T-cells exposed to HIV-1 underwent apoptosis by a TRAIL/DR5-dependent mechanism, which was inhibited by anti-type I IFN antibodies[41]. Finally, we demonstrated that IFNα produced by HIV-1-exposed plasmacytoid dendritic cells (pDC) induced TRAIL expression on primary CD4+ but not on CD8+ T-cells[41]. We review here the effect of antiretroviral therapy on TRAIL/DR5 expression *in vitro* and *in vivo,* and propose blocking the gp120/CD4 interaction as a way to reduce immunopathogenesis, defined as HIV-1-induced CD4+ T-cell depletion.

## Effect of HAART on immunologic markers

Current therapy for HIV-1-infected patients involves the combination of multiple antiretroviral drugs (highly active antiretroviral therapy, HAART) and has effectively reduced HIV-1-induced morbidity and mortality[42,43]. HAART decreases HIV-1 load[44], improves circulating CD4 cell counts, and reduces the level of immune activation in blood and lymphoid tissue (LT)[45]. HAART also reduces apoptosis of CD4+ and CD8+ T-cells in LT[46-48], and is suggested to reduce the sensitivity of CD4+ T-cells to FAS-mediated apoptosis[46].

Because TRAIL-mediated apoptosis may contribute to HIV-1-induced CD4+ T-cell depletion, we tested whether HAART would affect the TRAIL/DR5 apoptotic pathway. We found that when HAART decreased viral load, a concomitant decrease in plasma TRAIL was observed[40]. We also investigated CD4+ T-cell death, TRAIL, and DR5 expressions in a longitudinal study of HIV-1-infected patients who received HAART. The HAART-induced decline in plasma viral load was paralleled by a decrease in the level of plasma TRAIL[40]. Furthermore, HAART-induced increases in CD4 count were associated with decreasing DR5 mRNA expression in CD4+ T-cells[40]. The fact that these levels of HAART-induced TRAIL and DR5 were reflected in the FDA-approved surrogate markers of viral load and CD4 count strongly suggests that TRAIL and DR5 are involved in HIV-1 disease progression.

**Table 1. Summary of blocking of TRAIL, DR5, Apoptosis and IFNα that results from *in vitro* exposure to HIV-1**

| | TRAIL | | DR5 | Apoptosis | IFNα |
|---|---|---|---|---|---|
| | Monocytes | CD4+ T-cells | CD4+ T-cells | CD4+ T-cells | pDCs |
| HAART | Yes | Yes | Yes | Yes | ? |
| sCD4-IgG | Yes | Yes | Yes | Yes | Yes |
| AMD-3100 | No | No | No | No | No |

## Therapeutic implications

Despite its success by increasing CD4 count and reducing plasma viral load and patient mortality[42,43], HAART is limited by viral resistance, drug toxicity, and complex dosing regimens, and many patients eventually exhaust their therapeutic options[49,50]. Consequently, there is an urgent need for new strategies of AIDS therapy. As noted above, when HAART decreased viral load we observed a concomitant decrease in plasma TRAIL[40]. Furthermore, HAART-induced increases in CD4 count were also associated with decreasing DR5 mRNA expression in CD4+ T-cells[51]. Considered together, these results suggest that the TRAIL/DR5 apoptotic pathway is a major contributor to CD4+ T-cell death during progression to AIDS. Therefore, blocking TRAIL/DR5-mediated cell death, and possibly other apoptotic mechanisms such as Fas/FasL, may provide a new therapeutic strategy.

Three different therapeutic strategies that inhibit TRAIL/DR5-mediated CD4+ T-cell apoptosis *in vitro* will be considered here:

- Antibodies against TRAIL and DR5;
- Antibodies against type I interferon; and
- Molecules that block the interaction between HIV-1 gp120 and cellular CD4. We found that antibodies against TRAIL and DR5 partly blocked the apoptosis induced by AT-2 HIV-1[51], raising the possibility that TRAIL/DR5 is not the only death mechanism induced by HIV-1, and that these antibodies may not be effective *in vivo.* Since we found that type I IFN regulates TRAIL gene expression on CD4+ T-cells, we were able to inhibit HIV-1-initiated TRAIL and apoptosis of CD4+ T-cells using antibodies against IFNα/β[51]. However, type I IFN is pleiotropic and has broad-spectrum antiviral activity including against HIV-1[52]. Therefore, the use of such antibodies might be harmful to patients. Molecules that have the potential to block the interaction between HIV-1 and CD4 such as soluble CD4 (sCD4) have also been studied.

## Effect of sCD4 on the TRAIL/DR5 pathway

The entry of HIV-1 into target cells proceeds *via* a cascade of events that provide opportunities for therapy. Soluble CD4-IgG is an HIV-1 early entry inhibitor that binds the HIV-1 envelope glycoprotein gp120 with high affinity and neutralizes primary HIV-1, regardless of genotype or phenotype[53]. We recently reported that sCD4-IgG completely inhibited sTRAIL expression by monocytes and mTRAIL by CD4+ T-cells[40,51]. Coreceptor inhibitors, such as AMD-3100, which block CXCR4 co-receptors[54], did not inhibit TRAIL expression[40]. Furthermore, HIV-1-induced apoptosis of CD4+ T-cells was blocked by sCD4-IgG, but not by AMD-3100. Finally, sCD4-IgG, but not AMD-3100 blocked HIV-1-induced production of IFNα by pDC[41]. The above results are summarized in table 1 and indicate that the interaction between gp120 and CD4 is required to initiate the signals that activate TRAIL and DR5, leading to CD4+ T-cell death.

## Therapeutic strategies

Based on the above-noted findings, we propose that agents that block the initial interaction between CD4+ leucocytes and HIV-1 gp120 could provide an effective strategy for stopping HIV-1 infection, and also for reducing apoptosis-mediated CD4+ T-cell depletion leading to AIDS pathogenesis. These agents would block CD4+ T-cell apoptosis that results from noninfectious as well as infectious binding of viral gp120 to cellular CD4. An additional potential advantage of gp120/CD4 blockers is that such a therapeutic strategy should not be susceptible to the viral mutations such as those that result in resistance to HAART. Mutation of gp120 that results in failure to bind the CD4 molecule should be self-defeating for the virus. Thus, viral mutations that circumvent CD4-gp120 binding would prevent the interactions of HIV-1 with its primary receptor. The entry inhibitor PRO 542, a tetrameric derivative of sCD4-IgG, was reported to effectively reduce the viral loads of

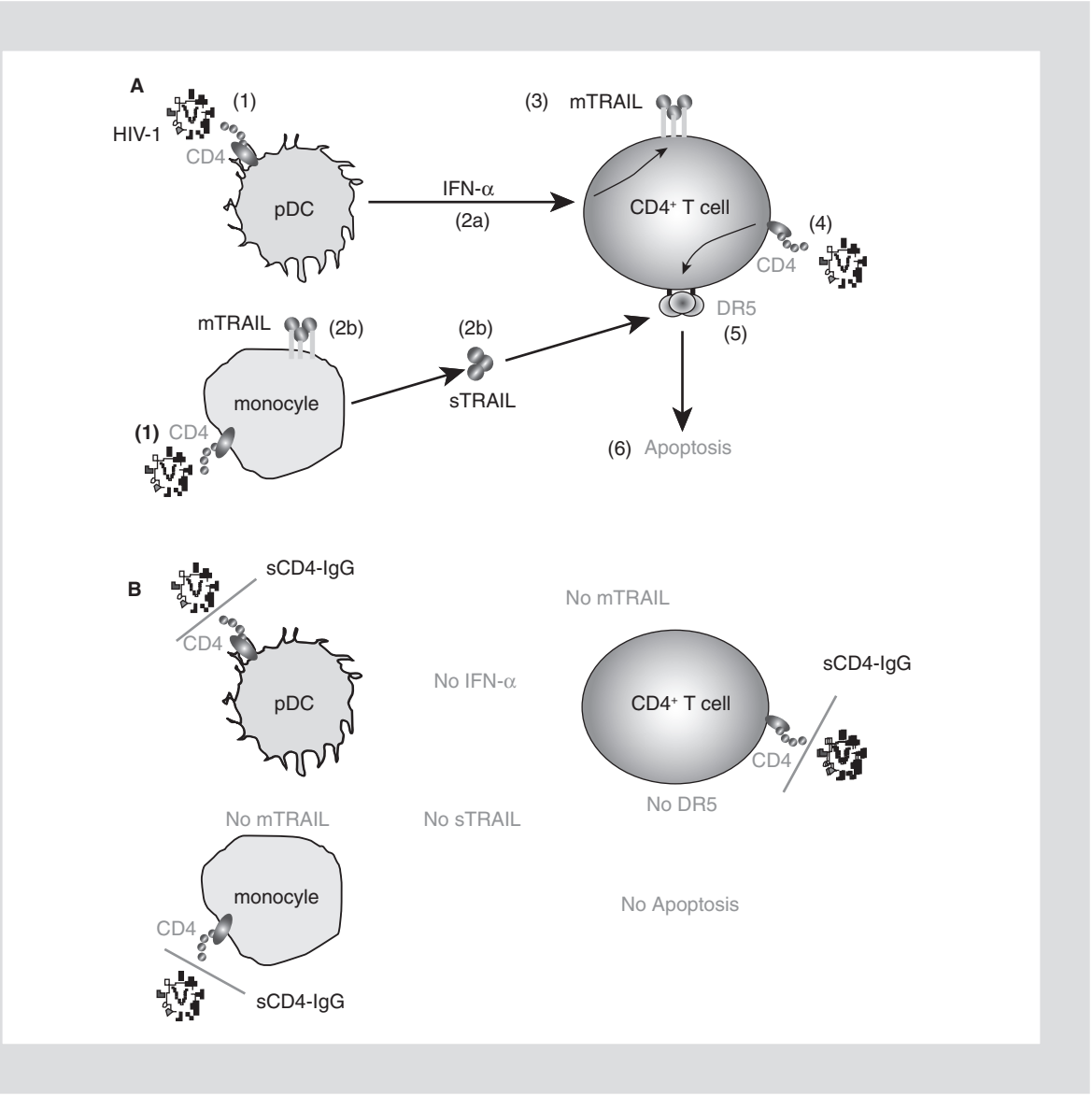


**Figure 1.** *HIV-1-induced apoptosis of CD4+ T-cells **(A)**; and inhibition of apoptosis by sCD4-IgG **(B)**. **A:** infectious or noninfectious HIV-1 gp120 binds to CD4 on plasmacytoid dendritic cells (pDC) or monocytes (1), resulting in IFNα production by pDC (2a), and in membrane TRAIL (mTRAIL) expression and soluble TRAIL (sTRAIL) production by monocytes (2b). IFNα produced by HIV-1-activated pDC binds to the IFN receptor on CD4+ T-cells and induces mTRAIL expression on these CD4+ T-cells (3). Infectious or noninfectious HIV-1 gp120 binds to CD4 on CD4+ T-cells (4), resulting in their DR5 expression (5). The binding of mTRAIL and/or sTRAIL to DR5 results in the apoptosis of CD4+ T-cells (6). **B:** sCD4-IgG blocks HIV-1-induced: IFNα production by pDC, resulting in lack of mTRAIL expression on CD4+ T-cell; mTRAIL expression and sTRAIL production by monocytes; and DR5 expression by CD4+ T-cells Other molecules that block this gp120/CD4 interaction could have the same effects as sCD4-IgG.*

pediatric[55] and adult[56] HIV-1-infected patients, whereas AMD-3100 therapy did not affect the viral loads of adult HIV-1-infected patients[57]. Recently, a study demonstrated that a tetravalent soluble PRO 542 was able to decrease the viral load of advanced HIV-1-infected patients who failed HAART[56]. Furthermore, these studies showed that there is no toxic activity of PRO 542 in patients[55,56].

Water-soluble CD4-like molecules that are smaller than CD4 could also inhibit gp120-CD4 binding. Such molecules would be more convenient for patient use because they could be administrated orally, in contrast to the currently-used sCD4-IgG or PRO 542 which are given intravenously. A 27-amino acid CD4 mimic (CD4M33) has been recently shown to bind with high affinity to the CD4 binding site of the gp120 envelope[58]. CD4M33 blocks the *in vitro* binding and infection of primary CD4+ cells by HIV-1. We consider that CD4M33 and possibly other molecules that inhibit the critical

gp120-CD4 binding event should be tested for HIV-1-mediated IFNα production by pDC and apoptosis of $CD4^+$ T-cells[40]. Molecules of this type should inhibit both infection and immunopathogenesis and may provide an alternative therapeutic option for people who become resistant to HAART. A list of molecules that were designed to inhibit the binding of gp120 to CD4 has been published[59]. These compounds could easily be tested *in vitro* for their ability to block the infectious or noninfectious binding of HIV-1 to cellular CD4. The inhibition of such binding should result in the failure of HIV-1 to activate IFNα, and therefore to induce expression of the apoptotic ligands TRAIL and FasL, their respective DR5 and Fas death receptors, and apoptosis of $CD4^+$ T-cells. The results of such *in vitro* testing could identify those blocking molecules that might be effective in counteracting apoptosis-mediated $CD4^+$ T-cell depletion during HIV-1 disease progression. In addition, these tests for death molecules and their receptors would detect noninfectious interactions between HIV-1 and $CD4^+$ cells that would result in $CD4^+$ T-cell apoptosis, but that would not be picked up by current virologic assays.

Our model (Fig. 1) highlights the HIV-1 gp120/cellular CD4 interactions that are essential for IFNα production and DR5 expression leading to $CD4^+$ T-cell apoptosis.

We show that the first step is the binding of infectious or noninfectious HIV-1 to CD4 present on pDC leading to IFNα production. The second step is the expression of mTRAIL by $CD4^+$ T-cells and monocytes as well as the production of sTRAIL only by monocytes. The third step is the binding of HIV-1 (infectious or noninfectious) to $CD4^+$ T-cells which induces DR5 expression on the target cells. Finally, the fourth and last step is the binding of TRAIL to DR5, which induces the apoptotic death of $CD4^+$ $DR5^+$ T-cells. This model is based on our *in vivo* and *in vitro* results[40,41,51]. The second part of the model shows that each of these steps can be inhibited by sCD4-IgG, which blocks the binding of viral gp120 to cellular CD4.

## References


1. Levy J. Pathogenesis of HIV infection. Microbiol Rev 1993;57:183-289.
2. Pantaleo G, De Maria A, Koenig S, et al. CD8+ T lymphocytes of patients with AIDS maintain normal broad cytolytic function despite the loss of HIV-specific cytotoxicity. Proc Natl Acad Sci USA 1990; 87:4818-22.
3. Murray M. Tryptophan depletion and HIV infection: a metabolic link to pathogenesis. Lancet Infect Dis 2003;3:644-52.
4. Schnittman S, Greenhouse J, Psallidopoulos M, et al. Increasing viral burden in CD4+ T-cells from patients with HIV infection reflects rapidly progressive immunosuppression and clinical disease. Ann Intern Med 1990;113:438-43.
5. Finkel T, Tudor-Williams G, Banda N, et al. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nat Med 1995;1:129-34.
6. Groux H, Torpier G, Monte D, Mouton Y, Capron A, Ameisen J. Activation-induced death by apoptosis in CD4+ T-cells from HIV-infected asymptomatic individuals. J Exp Med 1992;175:331-40.
7. Dianzani U, Bensi T, Savarino A, et al. Role of FAS in HIV infection. Curr HIV Res 2003;1:405-17.
8. Dockrell D. The multiple roles of Fas ligand in the pathogenesis of infectious diseases. Clin Microbiol Infect 2003;9:766-79.
9. Estaquier J, Tanaka M, Suda T, Nagata S, Golstein P, Ameisen J. Fas-mediated apoptosis of CD4+ and CD8+ T-cells from HIV-infected persons: differential *in vitro* preventive effect of cytokines and protease antagonists. Blood 1996;87:4959-66.
10. Katsikis P, Wunderlich E, Smith C, Herzenberg L. Fas antigen stimulation induces marked apoptosis of T lymphocytes in HIV-infected individuals. J Exp Med 1995;181:2029-36.
11. Westendorp M, Frank R, Ochsenbauer C, et al. Sensitization of T-cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. Nature 1995;375:497-500.
12. Vasilescu A, Heath S, Diop G, et al. Genomic analysis of Fas and FasL genes and absence of correlation with disease progression in AIDS. Immunogenetics 2004;56:56-60.
13. Gandhi R, Chen B, Straus S, Dale J, Lenardo M, Baltimore D. HIV-1 directly kills $CD4^+$ T-cells by a Fas-independent mechanism. J Exp Med 1998;187:1113-22.
14. Badley A, Pilon A, Landay A, Lynch DH. Mechanisms of HIV-associated lymphocyte apoptosis. Blood 2000;96:2951-64.
15. Servet C, Zitvogel L, Hosmalin A. Dendritic cells in innate immune responses against HIV. Curr Mol Med 2002;2:739-56.
16. Marsters S, Sheridan J, Pitti R, et al. A novel receptor for Apo2L/TRAIL contains a truncated death domain. Curr Biol 1997;7:1003-6.
17. Pan G, Ni J, Wei Y-F, Yu G-l, Gentz R, Dixit V. An antagonist decoy receptor and a death domain-containing receptor for TRAIL. Science 1997;277:815-8.
18. Sheridan J, Marsters S, Pitti R, et al. Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. Science 1997;277:818-21.
19. Sato K, Hida S, Takayanagi H, et al. Antiviral response by natural killer cells through TRAIL gene induction by IFNα/β. Eur J Immunol 2001;31:3138-46.
20. Tecchio C, Huber V, Scapini P, et al. IFNα-stimulated neutrophils and monocytes release a soluble form of TRAIL (TRAIL/Apo-2 ligand) displaying apoptotic activity on leukemic cells. Blood 2004; 103:3837-44.
21. Ehrlich S, Infante-Duarte C, Seeger B, Zipp F. Regulation of soluble and surface-bound TRAIL in human T-cells, B-cells, and monocytes. Cytokine 2003;24:244-53.
22. Kayagaki N, Yamaguchi N, Nakayama M, et al. Involvement of TRAIL in human CD4+ T-cell-mediated cytotoxicity. J Immunol 1999; 162:2639-47.
23. Smyth M, Cretney E, Takeda K, et al. TRAIL contributes to IFN gamma-dependent natural killer cell protection from tumor metastasis. J Exp Med 2001;193:661-70.
24. Vidalain P, Azocar O, Lamouille B, Astier A, Rabourdin-Combe C, Servet-Delprat C. Measles virus induces functional TRAIL production by human dendritic cells. J Virol 2000;74:556-9.
25. Vidalain P, Azocar O, Rabourdin-Combe C, Servet-Delprat C. Measles virus-infected dendritic cells develop immunosuppressive and cytotoxic activities. Immunobiology 2001;204:629-38.
26. Herbeuval J, Lambert C, Sabido O, et al. Macrophages from cancer patients: analysis of TRAIL, TRAIL receptors, and colon tumor cell apoptosis. J Natl Cancer Inst 2003;95:611-21.
27. Griffith T, Chin W, Jackson G, Lynch D, Kubin M. Intracellular regulation of TRAIL-induced apoptosis in human melanoma cells. J Immunol 1998;161:2833-40.
28. Gura T. How TRAIL kills cancer cells, but not normal cells. Science 1997;277:768.

29. Liabakk N, Sundan A, Torp S, Aukrust P, Froland S, Espevik T. Development, characterization and use of monoclonal antibodies against sTRAIL: measurement of sTRAIL by ELISA. J Immunol Methods 2002;259:119-28.
30. Stylianou E, Bjerkeli V, Yndestad A, et al. Raised serum levels of interleukin-18 is associated with disease progression and may contribute to virologic treatment failure in HIV-1-infected patients. Clin Exp Immunol 2003;132:462-6.
31. Katsikis P, Garcia-Ojeda M, Torres-Roca J, Tijoe I, Smith C, Herzenberg L. Interleukin-1 β converting enzyme-like protease involvement in Fas-induced and activation-induced peripheral blood T-cell apoptosis in HIV infection. TRAIL can mediate activation-induced T-cell death in HIV infection. J Exp Med 1997;186:1365-72.
32. Lum J, Pilon A, Sanchez-Dardon J, et al. Induction of cell death in HIV-infected macrophages and resting memory CD4 T-cells by TRAIL/Apo2l. J Virol 2001;75:11128-36.
33. Jeremias I, Herr I, Boehler T, Debatin K. TRAIL/Apo-2-ligand-induced apoptosis in human T-cells. Eur J Immunol 1998;28:143-52.
34. Miura Y, Misawa N, Maeda N, et al. Critical contribution of TRAIL to apoptosis of human CD4+ T-cells in HIV-1-infected hu-PBL-NOD-SCID Mice. J Exp Med 2001;193:651-60.
35. Lichtner M, Maranon C, Vidalain P, et al. HIV-1-infected dendritic cells induce apoptotic death in infected and uninfected primary CD4 T lymphocytes. AIDS Res Hum Retroviruses 2004;20:175-82.
36. Badley A, Dockrell D, Simpson M, et al. Macrophage-dependent apoptosis of CD4+ T lymphocytes from HIV-infected individuals is mediated by FasL and TNF. J Exp Med 1997;185:55-64.
37. Huang Y, Erdmann N, Peng H, Zhao Y, Zheng J. The role of TRAIL in neurodegenerative diseases. Cell Mol Immunol 2005;2:113-22.
38. Huang Y, Erdmann N, Zhao J, Zheng J. The signaling and apoptotic effects of TRAIL in HIV-1 associated dementia. Neurotox Res 2005;8:135-48.
39. Miura Y, Koyanagi Y, Mizusawa H. TRAIL induces neuronal apoptosis in HIV-encephalopathy. J Med Dent Sci 2003;50:17-25.
40. Herbeuval J, Boasso A, Grivel J, et al. TRAIL in HIV-1-infected patients and its *in vitro* production by antigen-presenting cells. Blood 2005;105:2458-64.
41. Herbeuval J, Hardy A, Boasso A, et al. Regulation of TRAIL on primary CD4+ T-cells by HIV-1: role of type I IFN-producing plasmacytoid dendritic cells. Proc Natl Acad Sci USA 2005;102:13974-9.
42. Mouton Y, Alfandari S, Valette M, et al. Impact of protease inhibitors on AIDS-defining events and hospitalizations in 10 French AIDS reference centers. Federation National des Centres de Lutte contre le SIDA. Aids 1997;11:F101-5.
43. Palella F Jr., Delaney K, Moorman A, et al. Declining morbidity and mortality among patients with advanced HIV infection. HIV Outpatient Study Investigators. N Engl J Med 1998;338:853-60.
44. Gulick R, Mellors J, Havlir D, et al. Treatment with indinavir, zidovudine, and lamivudine in adults with HIV infection and prior antiretroviral therapy. N Engl J Med 1997;337:734-9.
45. Dyrhol-Riise A, Voltersvik P, Olofsson J, Asjo B. Activation of CD8 T-cells normalizes and correlates with the level of infectious provirus in tonsils during HAART in early HIV-1 infection. Aids 1999;13:2365-76.
46. Badley A, Dockrell D, Algeciras A, et al. *In vivo* analysis of Fas/FasL interactions in HIV-infected patients. J Clin Invest 1998;102:79-87.
47. Dyrhol-Riise A, Ohlsson M, Skarstein K, et al. T-cell proliferation and apoptosis in HIV-1-infected lymphoid tissue: impact of HAART. Clin Immunol 2001;101:180-91.
48. Gougeon M, Montagnier L. Programmed cell death as a mechanism of CD4 and CD8 T-cell deletion in AIDS. Molecular control and effect of HAART. Ann N Y Acad Sci 1999;887:199-212.
49. Locatelli G, Cancio R, Spadari S, Maga G. HIV-1 reverse transcriptase inhibitors: current issues and future perspectives. Curr Drug Metab 2004;5:283-90.
50. Tozser J. HIV inhibitors: problems and reality. Ann N Y Acad Sci 2001,946:145-59.
51. Herbeuval J, Grivel J, Boasso A, et al. CD4+ T-cell death induced by infectious and noninfectious HIV-1: role of type 1 interferon-dependent, TRAIL/DR5-mediated apoptosis. Blood 2005;106:3524-31.
52. Yamamoto J, Barre-Sinoussi F, Bolton V, Pedersen N, Gardner M. Human alpha- and beta-interferon but not gamma- suppress the *in vitro* replication of LAV, HTLV-III, and ARV-2. J Interferon Res 1986; 6:143-52.
53. Allaway G, Davis-Bruno K, Beaudry G, et al. Expression and characterization of CD4-IgG2, a novel heterotetramer that neutralizes primary HIV-1 isolates. AIDS Res Hum Retroviruses 1995;11:533-9.
54. Donzella G, Schols D, Lin S, et al. AMD3100, a small molecule inhibitor of HIV-1 entry via the CXCR4 coreceptor. Nat Med 1998;4:72-7.
55. Shearer W, Israel R, Starr S, et al. Recombinant CD4-IgG2 in HIV-1-infected children: phase 1/2 study. The Pediatric AIDS Clinical Trials Group Protocol 351 Study Team. J Infect Dis 2000; 182:1774-9.
56. Jacobson J, Israel R, Lowy I, et al. Treatment of advanced HIV-1 disease with the viral entry inhibitor PRO 542. Antimicrob Agents Chemother 2004;48:423-9.
57. Hendrix C, Flexner C, MacFarland R, et al. Pharmacokinetics and safety of AMD-3100, a novel antagonist of the CXCR-4 chemokine receptor, in human volunteers. Antimicrob Agents Chemother 2000; 44:1667-73.
58. Martin L, Stricher F, Misse D, et al. Rational design of a CD4 mimic that inhibits HIV-1 entry and exposes cryptic neutralization epitopes. Nat Biotechnol 2003;21:71-6.
59. Vermeire K, Schols D. Anti-HIV agents targeting the interaction of gp120 with the cellular CD4 receptor. Expert Opin Investig Drugs 2005;14:1199-212.