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Perspective

Reconsidering targeted toxins to eliminate HIV infection: You gotta have HAART

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ABSTRACT **The success of highly active anti-retroviral therapy (HAART) has inspired new concepts for eliminating HIV from infected individuals. A major obstacle is the persistence of long-lived reservoirs of latently infected cells that might become activated at some time after cessation of therapy. We propose that, in the context of treatment strategies to deliberately activate and eliminate these reservoirs, hybrid toxins targeted to kill HIV-infected cells be reconsidered in combination with HAART. Such combinations might also prove valuable in protocols aimed at preventing mother-to-child transmission and establishment of infection immediately after exposure to HIV. We suggest experimental approaches *in vitro* and in animal models to test various issues related to safety and efficacy of this concept.**

Highly active anti-retroviral therapy (HAART), involving combination treatment with drugs that block different steps in the viral replication cycle (e.g., reverse transcriptase inhibitors plus protease inhibitors), has improved dramatically the health of many individuals infected with HIV (1). Despite these advances, recent analyses of peripheral blood and lymph nodes have revealed the presence of reservoirs of resting $CD4^+$ memory T cells harboring latent replication-competent provirus (refs. 2–8; reviewed in refs. 9–11). Although such reservoirs contain exceedingly small numbers of cells, they are generated very early after primary infection and persist with no significant change after 2 years of HAART. The latently infected cells are likely to activate spontaneously at some point after termination of HAART and therefore are considered to be a major obstacle to eradication of HIV from the body. This awareness has engendered the notion of deliberately "flushing out" the reservoirs by treating HAART patients with agents that activate virus expression from latently infected cells (10–12). The idea is that the virions produced on activation will be prevented by HAART from infecting new cells; it is presumed that the newly activated cells then will be eliminated by natural mechanisms such as the cytopathic effect of the virus, immune effector mechanisms, etc. (9–11). We propose that, in considering such strategies, these natural elimination mechanisms can be accelerated aggressively by using targeted toxins that selectively kill activated HIV-infected cells. Such agents may also be useful components of cocktails aimed at preventing establishment of infection in newly exposed individuals.

Hybrid Toxins Targeted to HIV-Infected Cells

During the past decade, several types of anti-HIV hybrid protein toxins have been produced by molecular genetic and biochemical methodologies (13–15). In each case, the hybrid protein contains a binding domain that targets the agent to the HIV envelope glycoprotein (Env) expressed on the surface of the infected cell and a cytotoxic domain that actively kills the cell on internalization. The hybrid toxins are constructed by substituting the normal cell binding region of the native toxin with an Env-binding domain. The Env-binding moieties used have included the extracellular regions of CD4 as well as Fab regions of anti-Env antibodies (directed against either the external subunit gp120 or the transmembrane subunit gp41). The cytotoxic domains have been derived from natural protein toxins such as *Pseudomonas aeruginosa* exotoxin A (PE), ricin, and diphtheria toxin.

To date, only one of these hybrid toxins has been tested in humans: the genetically engineered single chain protein CD4-PE40 (soluble CD4 linked to the translocation and cell killing domains of PE). For this reason, we focus on this agent, though many of our arguments also apply to other Env-targeted hybrid toxins. CD4-PE40 displays the following properties *in vitro*: cytotoxic activity against cells expressing Envs of HIV-1, HIV-2 and simian immunodeficiency virus (SIV) (16–19), high potency and specificity for killing HIV-1-infected cells with negligible effects on major histocompatibility complex Class II-expressing cells (16, 18), requirement for HIV-1 induction in a latently infected cell line (18), suppression of spreading HIV-1 infection in an acutely infected T cell line (17) and in cultures of primary T lymphocytes or macrophages (20–22), highly synergistic activity with reverse transcriptase inhibitors (23), and potent activity against primary HIV-1 strains, including those resistant to neutralization by soluble CD4 (21, 22). These *in vitro* properties, coupled with acceptable toxicity and pharmacokinetic profiles in animal studies, supported testing this agent in HIV-infected people.

Disappointing Results in Phase 1 Clinical Trials

The high hopes from the promising preclinical findings were dashed in the initial Phase I trials with HIV-infected patients (24, 25). The toxin produced dose-limiting hepatotoxicity; at the low doses that were tolerated (10 μg/kg), the peak plasma levels of CD4-PE40 remained below concentrations shown to be efficacious *in vitro*. The significant but reversible hepatotoxicity greatly diminished enthusiasm for CD4-PE40 in par-

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Abbreviations: HAART, highly active anti-retroviral therapy; Env, HIV envelope glycoprotein; PE, *Pseudomonas aeruginosa* exotoxin A; SIV, simian immunodeficiency virus; CD4-PE40, soluble CD4 linked to the effector domains of *Pseudomonas aeruginosa* exotoxin A.

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ticular and for Env-targeted toxins in general. The CD4-PE40 clinical program was terminated.

A New Context Suggests a New Concept: Testable Hypotheses

We propose that recent developments with HAART present new opportunities for exploring the therapeutic utility of Env-targeted hybrid toxins to help eradicate residual HIV-infected cell reservoirs. We hypothesize a plausible mechanism for the CD4-PE40 hepatotoxicity observed in HIV-infected people and suggest that this problem may not occur in patients with the very low viral loads achieved by HAART. We present rationales based on *in vitro* data suggesting that Env-targeted toxins might accelerate the elimination of infected cell reservoirs beyond the rates caused by natural mechanisms. These agents may also be useful components of drug cocktails aimed at preventing postexposure infection and mother-to-child transmission. Of most importance, many of these notions are subject to experimental testing *in vitro* and in animal models.

The first issue concerns the hepatotoxicity with low doses of CD4-PE40 observed in Phase I clinical trials. This problem was unexpected because preclinical toxicity studies in rodents and monkeys indicated much higher tolerated doses. Furthermore, it is now clear that hepatotoxicity is not a general property of PE derivatives in humans; several anticancer clinical trials conducted with PE-based immunotoxins have revealed striking antitumor responses without hepatotoxicity (26). Why, then, was hepatotoxicity encountered with low doses of CD4-PE40 in clinical trials with HIV-infected people? We propose that the HIV infection, particularly the high virus load, is the culprit. HIV-infected individuals likely produce free gp120 that is shed from virions or infected cells. Shedding of gp120 has been studied extensively *in vitro*; furthermore, evidence for free gp120 in sera of infected individuals has been reported, although precise measurements are confounded by the formation of immune complexes as well as by the association of released gp120 with circulating $CD4^+$ T lymphocytes (reviewed in refs. 27–29). We hypothesize that, in HIV-infected patients treated with CD4-PE40, some of the chimeric toxin associates with shed gp120. Because gp120 is glycosylated extensively and contains highly diverse oligosaccharide chains (30), the complex likely would be a substrate for the human hepatocyte asialoglycoprotein receptor, which internalizes glycoproteins containing terminal galactose or N-acetylglucosamine residues (31). The result would be the serious side effect of hepatocyte killing. Moreover, gp120/CD4-PE40 complexes bound to anti-gp120 antibodies might also contribute to liver damage.

According to this hypothesis, hepatotoxicity should not be a major problem in HAART patients because the low viral loads presumably would produce minimal amounts of free gp120. Even on induction of virus expression from latently infected memory T lymphocytes, the newly produced free gp120 is unlikely to be problematic because the number of such cells is much smaller than the number of virus-producing T lymphocytes in patients before HAART (ref. 3; also T.-W. Chun, personal communication); moreover, the newly produced gp120 will have accumulated only during the relatively short period after induction, in contrast with the prolonged duration of gp120 production before therapy. We also suggest that gp120-mediated toxicity would not be problematic when given along with HAART to newly exposed individuals because they should not yet have produced significant amounts of free gp120.

To test this model of CD4-PE40-mediated hepatotoxicity, we propose that effects of the agent be compared in animals with high vs. low levels of free gp120; we predict that hepatotoxicity will be much less severe in the latter case. There are several experimental paradigms in which this question can be examined, including comparison of CD4-PE40 hepatotoxicity in uninfected vs. chronically HIV-infected severe combined immunodeficient-hu mice or SIV-infected rhesus macaques. Perhaps more important is to use these systems to compare animals with the normal high viral loads occurring during chronic infection vs. the reduced loads achieved with potent antiviral therapy, e.g., HAART in HIV-infected severe combined immunodeficient-hu mice (32) or reverse transcriptase inhibitor therapy in SIV-infected macaques (33). A related analysis would compare in chronically infected animals the effects of hybrid toxins targeted to gp120 (e.g., CD4-PE40 and gp120-targeted immunotoxins) versus those targeted to gp41; according to our model, the latter agents would not produce hepatotoxicity even in animals with high virus load because gp41 is not released spontaneously from the membrane. In another approach, uninfected animals can be given CD4-PE40 without or with soluble gp120 to test directly whether hepatotoxicity depends on both proteins. Together, these experiments should provide important insights into whether the hepatotoxicity of CD4-PE40 is associated with high viral load, and in particular with free gp120.

HAART therapy also provides opportunities to test the therapeutic potential of Env-targeted hybrid toxins to eradicate residual infected cells. The idea is to augment their natural rates of decay, which are presumed to reflect the viral cytopathic effect and host effector mechanisms (9–11). Several previous *in vitro* studies are promising in this regard. CD4-PE40 (but not soluble CD4) markedly inhibited the spread of infection in various target cell types (17, 20–23), including primary T lymphocytes and macrophages acutely infected with primary HIV-1 strains; the interpretation of these findings is that the toxin accelerates the killing of infected cells beyond the rates associated with the viral cytopathic effect. The results with macrophages are particularly striking because these cells are refractory to HIV-mediated killing during productive infection and are thought to represent an important viral reservoir with markedly slower decay kinetics compared with $CD4^+$ T lymphocytes (9). Also of note are the promising *in vitro* results indicating highly synergistic effects of CD4-PE40 and reverse transcriptase inhibitors (23). CD4-PE40 plus 3′-azido-3′-dideoxythymidine or 2′,3′-dideoxyinosine completely inhibited acute virus replication and prevented virus-mediated killing of the $CD4^+$ target T cell population; moreover, continuation of the culture after cessation of drug treatment indicated that the infection had been eliminated completely. By contrast, each agent alone suppressed virus replication during the treatment period, but the protective effects were reversed on drug removal. These results highlight the potential value of combination treatment involving a drug(s) that inhibits HIV replication plus another that selectively kills the infected cells. Taken together, these earlier studies provide impetus for considering Env-targeted toxins to augment HAART, particularly in the context of protocols to deliberately activate virus production from latently infected cell reservoirs. These agents, in combination with other antiretrovirals, may also diminish the frequency of postexposure infection and mother-to-child transmission.

We propose additional lines of *in vitro* and *in vivo* study. Experiments can be designed to optimize the *ex vivo* activation of latently infected T lymphocytes obtained from HAART patients and to test *ex vivo* whether an Env-targeted toxin in combination with continued HAART promotes or accelerates killing of the activated cells (similar to the studies noted above with acutely infected T cells). As an *in vivo* parallel to the previous *in vitro* success with combination treatment, we propose examination of the combined effects of HAART and Env-targeted toxins in HIV-infected severe combined immunodeficient-hu mice or SIV-infected macaques. Regarding efforts to deliberately activate latently infected cells, *in vitro* experiments would guide the choice of the most promising

modes of activation. Such experiments would suggest whether Env-targeted toxins in the presence of HAART can eradicate virus from infected animals. Finally, the ability of the targeted toxins to augment other antiretrovirals in preventing infection can be examined in the HIV/severe combined immunodeficient-hu mouse and the SIV/macaque models. Favorable results in these *in vitro* and *in vivo* systems would set the stage for safety and efficacy trials of Env-targeted toxins as components of therapeutic and prophylactic protocols against HIV.

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