Targeted Killing of Virally Infected Cells by Radiolabeled Antibodies to Viral Proteins

Ekaterina Dadachova[1*], Mahesh C. Patel[1,2], Sima Toussi[1], Christos Apostolidis[3], Alfred Morgenstern[3], Martin W. Brechbiel[4], Miroslaw K. Gorny[5], Susan Zolla-Pazner[5,6], Arturo Casadevall[1], Harris Goldstein[1]

1 Albert Einstein College of Medicine, Bronx, New York, United States of America, **2** Jacobi Medical Center, Bronx, New York, United States of America **3** European Commission, Joint Research Centre, Institute for Transuranium Elements, Karlsruhe, Germany, **4** National Cancer Institute, National Institutes of Health, Bethesda, Maryland, United States of America, **5** New York University School of Medicine, New York, New York, United States of America, **6** Veterans Affairs New York Harbor Healthcare System, New York, New York, United States of America

Funding: The research was supported by a pilot project grant from the Einstein/Montefiore Medical Center (MMC) Center for AIDS Research funded by the National Institutes of Health (NIH) (AI-051519) and AI60507 (ED), and AI033142, AI033774 and HL059842 (AC); European Commission (CA and AM); Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research (MWB); New York University Center for AIDS Research AI 27742, AI 36085, HL 59725 and Department of Veterans Affairs (SZP); and Lehman Brothers Foundation and NIH RO1 AI48466 (HG). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Academic Editor: H. Clifford Lane, National Institutes of Health, United States of America

Citation: Dadachova E, Patel MC, Toussi S, Apostolidis C, Morgenstern A, et al. (2006) Targeted killing of virally infected cells by radiolabeled antibodies to viral proteins. PLoS Med 3(11): e427. doi:10.1371/journal.pmed.0030427

Received: January 20, 2006
Accepted: August 22, 2006
Published: November 7, 2006

Abbreviations: HAART, highly active antiretroviral therapy; hPBMC, human peripheral blood mononuclear cell; ID, injected dose; IP, intraperitoneal(ly); mAb, monoclonal antibody; PHA, phytohemagglutinin; RIT, radioimmunotherapy; TCID50, tissue culture-infecting dose causing 50% infection

* To whom correspondence should be addressed. E-mail: edadacho@aecom.yu.edu

ABSTRACT

Background

The HIV epidemic is a major threat to health in the developing and western worlds. A modality that targets and kills HIV-1-infected cells could have a major impact on the treatment of acute exposure and the elimination of persistent reservoirs of infected cells. The aim of this proof-of-principle study was to demonstrate the efficacy of a therapeutic strategy of targeting and eliminating HIV-1-infected cells with radiolabeled antibodies specific to viral proteins in vitro and in vivo.

Methods and Findings

Antibodies to HIV-1 envelope glycoproteins gp120 and gp41 labeled with radioisotopes bismuth 213 (^{213}Bi) and rhenium 188 (^{188}Re) selectively killed chronically HIV-1-infected human T cells and acutely HIV-1-infected human peripheral blood mononuclear cells (hPBMCs) in vitro. Treatment of severe combined immunodeficiency (SCID) mice harboring HIV-1-infected hPBMCs in their spleens with a ^{213}Bi- or ^{188}Re-labeled monoclonal antibody (mAb) to gp41 resulted in a 57% injected dose per gram uptake of radiolabeled mAb in the infected spleens and in a greater than 99% elimination of HIV-1-infected cells in a dose-dependent manner. The number of HIV-1-infected thymocytes decreased 2.5-fold in the human thymic implant grafts of SCID mice treated with the ^{188}Re-labeled antibody to gp41 compared with those treated with the ^{188}Re-control mAb. The treatment did not cause acute hematologic toxicity in the treated mice.

Conclusions

The current study demonstrates the effectiveness of HIV-targeted radioimmunotherapy and may provide a novel treatment option in combination with highly active antiretroviral therapy for the eradication of HIV.

The Editors' Summary of this article follows the references.

Introduction

The HIV epidemic is a major threat to health in the developing and western worlds. Highly active antiretroviral therapy (HAART), a combination of drugs that inhibits enzymes essential for HIV-1 replication, can reduce the viremia and the onset of opportunistic infections in most patients and prolong survival [1]. However, HAART regimens are complicated and have significant toxicity [2–5]. Replication-competent virus that persists in infected cells emerges rapidly after the cessation of HAART [6–9]. The inability to eliminate HIV-1-infected cells also limits the time frame in which HAART initiated after exposure to HIV-1 can prevent infection [7]. Unless HAART is initiated within days of exposure, HIV infection of a nominal number of cells may be sufficient to develop sustained infection despite subsequent long-term HAART [10,11]. Once the clinical role of reservoir reduction is clarified, and under circumstances in which expected benefit outweighs safety considerations, a modality that targets and kills HIV-1-infected cells may become important for the treatment of acute exposure and elimination of persistent reservoirs of infected cells. To specifically kill HIV-1-infected cells, several approaches have been tried [12], including toxin-coupled antibodies that are directed at HIV-1-encoded envelope proteins and can eliminate infected cells [13,14], but none of these strategies has yet been shown to be effective in humans.

Radioimmunotherapy (RIT) takes advantage of the specificity of the antigen–antibody interaction to deliver lethal doses of radiation to target cells using radiolabeled antibodies [15]. Currently RIT is successfully used to treat of certain cancers. We demonstrated the usefulness of RIT for infectious diseases by treating murine cryptococcosis with a monoclonal antibody (mAb) to the *Cryptococcus neoformans* capsular glucuronoxylomannan labeled with bismuth 213 (^{213}Bi) or rhenium 188 (^{188}Re) [16], and extended the applicability of RIT to treatment of a bacterial infection [17]. We also recently suggested that RIT may be effective against chronically infected cells, including those with viral infections [18]. In contrast to RIT for fungal and bacterial diseases in which the target is the microbe, RIT for viral diseases targets the infected cells and consequently provides a general strategy for eliminating reservoirs of infected cells capable of producing infectious viruses. This approach could be particularly useful for treatment of drug-resistant HIV strains, which present an ever-increasing problem [19]. In the current proof-of-principle study, we examined the effectiveness of targeting and eliminating HIV-1-infected cells with radiolabeled antibodies to viral proteins.

Methods

Antibodies

Goat polyclonal antibodies against gp120 (IgG) used in preliminary in vitro experiments was purchased from Biodesign International (Saco, Maine, United States). Murine 18B7 mAb (IgG1) to *C. neoformans* [20] was used as an irrelevant control. Human mAb to gp41, 246-D (cluster I), was produced as described [21]. Human mAb 1418 (IgG1) to parvovirus B19 [22] was used as an irrelevant control for mAb 246-D, and human mAb 447 (IgG3) to the V3 loop of HIV-1 gp120 [23] was used as a positive control in the FACS studies. Antibodies were purified by protein A or G chromatography prior to labeling.

Radioisotopes and Radiolabeling of the Antibodies

Antibodies were labeled with alpha-emitter ^{213}Bi (half-life 45.6 min) obtained from ^{225}Ac generator as described in [24] or with beta-emitter ^{188}Re (half-life 16.9 h) as described in [16].

In Vitro Killing of ACH-2 Cells and Human PBMCs with Radiolabeled mAbs

The ACH-2 cell line is a latent T cell clone infected with HIV-IIIB that produces steady low levels of viruses that is markedly increased by stimulation with phorbol myristate; it was obtained through the National Institutes of Health (NIH) AIDS Research and Reference Reagent Program (Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH: ACH-2, #349, from Dr. Thomas Folks). At 48 h after stimulation with phorbol myristate, ACH-2 cells cultured in triplicate wells were treated with 0–50 μCi (0–1.85 MBq) of ^{188}Re-labeled anti-gp120 or the irrelevant control mAb 18B7; or 0–20 μCi (0–0.74 MBq) of ^{213}Bi-labeled anti-gp120 or mAb 18B7 or with matching amounts (2.5–12.5 μg) of "cold" (unlabeled) anti-gp120 mAb. Approximately 2×10^5 cells were used for each condition. The cells were incubated with radiolabeled or cold mAbs at 37 °C for 3 h, transferred into fresh cell culture medium, and then incubated in 5% CO_2 at 37 °C for 72 h. The number of viable cells was then assessed by Trypan blue dye exclusion assay.

The effect of radiolabeled HIV-1 glycoprotein-binding mAbs on HIV-1-infected cells was studied in vitro using human peripheral blood mononuclear cells (hPBMCs) obtained from New York Blood Center (New York, New York, United States). hPBMCs were stimulated with phytohemagglutinin (PHA) and interleukin-2 for 48 h and then infected with HIV-1 strain JR-CSF (NIH AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases, NIH: HIV-$1_{JR\text{-}CSF}$, #394, from Dr. Irvin S. Y. Chen). Although the number of ACH-2 cells infected with HIV-1 was almost 100%, only a fraction (~10%–30%) of the hPBMCs were infected with HIV-1 as determined by limiting dilution coculture technique [25]. Subsequently in the text we refer to the cells exposed to HIV-1 as "infected" cells and those that were not exposed to the virus as "noninfected" cells. At 48 h after infection, infected cells cultured in triplicate wells were treated with ^{213}Bi-labeled mAb 246-D, or ^{213}Bi-labeled irrelevant control mAb 1418, or matching amounts of cold 246-D, as above. To verify that 246-D did not bind to the surface of noninfected hPBMCs, noninfected cells were subjected to treatment with ^{213}Bi-246-D or ^{188}Re-246-D. The number of viable cells was then assessed by Trypan blue dye exclusion assay.

Flow Cytometric Analysis of mAbs Binding to Virus-Infected Cells

Binding studies of human mAbs to the surface of hPBMCs infected with HIV-$1_{JR\text{-}CSF}$ were performed as described previously [26]. Briefly, PHA-stimulated hPBMCs were infected with 1 ml of stock HIV-$1_{JR\text{-}CSF}$ virus and cultured for 13 d in medium supplemented with human recombinant interleukin-2 (20 U/ml; Boehringer Mannheim Biochemicals, Indianapolis, Indiana, United States). The cells in duplicate

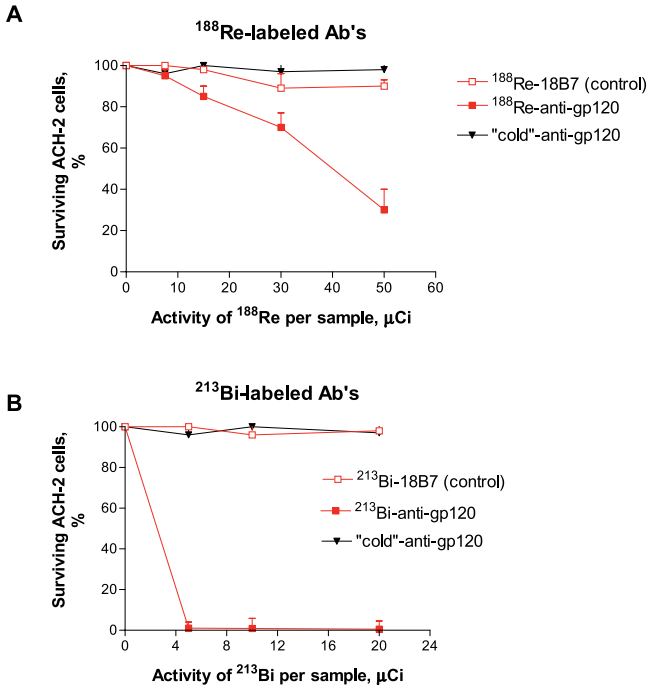


Figure 1. In Vitro Binding and Killing of ACH-2 Cells with ^{188}Re- and ^{213}Bi-Labeled Monoclonal Antibodies

(A) ACH-2 cells treated with anti-gp120 labeled with ^{188}Re. The mAb 18B7 radiolabeled with ^{188}Re was used as a irrelevant control.
(B) ACH-2 cells treated with anti-gp120 labeled with ^{213}Bi. The mAb 18B7 radiolabeled with ^{213}Bi was used as an irrelevant control.
In both experiments the number of surviving cells was determined by Trypan blue dye exclusion. Samples were done in triplicate. Every data point represents the mean of three replicates and error bars show standard error of the mean.
doi:10.1371/journal.pmed.0030427.g001

wells were incubated with each human mAb at 10 μg/ml for 1 h on ice, washed and reincubated with phycoerythrin-labeled goat F(ab′)2 anti-human IgG(γ) (Caltag Laboratories, Burlingame, California, United States). Using a FACScan flow cytometer (Becton Dickinson, Palo Alto, California, United States), live lymphocytes were selected for analysis by gating with forward and 90° scatter. The negative control consisted of cells from infected cultures stained with the conjugated anti-IgG in the absence of a human mAb.

Treatment of HIV-1 Infection in Intrasplenically Infected SCID Mice with Radiolabeled mAbs

The procedures used in animal studies described below were reviewed and approved by the Albert Einstein College of Medicine Animal Institute Committee. These experiments utilized a mouse model; first, PHA-activated hPBMCs were infected with HIV-1_{JR-CSF} as described above, then 48 h later these infected hPBMCs were injected intrasplenically (25×10^6 cells per mouse) into SCID mice. Mice were treated intraperitoneally 1 h later with one of the following: 20 μg of cold 246-D ($n = 4$); 100 μCi (3.7 MBq) (20 μg) of ^{213}Bi-1418 ($n = 5$) or 80 μCi (2.96 MBq) (20 μg) of ^{188}Re-1418 ($n = 3$) as isotype-matching controls; or 80 μCi (2.96 MBq) (20 μg) of ^{188}Re-246-D ($n = 4$) or 100 μCi (3.7 MBq) (20 μg) of ^{213}Bi-246-D ($n = 5$). In one experiment mice were given 80 μCi (2.96 MBq) (20 μg) of ^{188}Re-246-D intraperitoneally (IP) 1 h prior to infection with infected hPBMCs ($n = 4$). In dose-response experiments mice were injected IP with ^{188}Re-246-D (20 μg) with activity of 40 μCi (1.48 MBq) ($n = 4$), with 160 μCi (5.92 MBq) ($n = 4$), with 20 μg of cold 246-D ($n = 4$), or with no treatment ($n = 4$). Mice were sacrificed and spleens were harvested and processed 72 h postinjection. The number of HIV-1-infected cells present in the spleen was measured using limiting dilution quantitative coculture as described [25]. This technique measures the number of cells capable of producing infectious HIV-1.

Cells isolated from each spleen were diluted 5-fold (in the range 1×10^6 to 3.2×10^2 cells) and cultured in duplicate at 37 °C in 24-well culture plates with PHA-activated hPBMCs (1×10^6 cells) in 2.0 ml of RPMI 1640 medium containing fetal calf serum (10% vol/vol) and interleukin-2 (32 U/ml). The HIV-1 p24 antigen content of the supernatant was measured 1 wk later, using the HIV-1 p24 core profile ELISA (DuPont-NEN, Boston, Massachusetts, United States). The lowest number of added cells that infected at least half the duplicate cultures with HIV-1 was determined; this number represented the frequency of cells productively infected with HIV-1 in each spleen, reported as the tissue culture-infecting dose causing 50% infection ($TCID_{50}$)/10^6 splenocytes. For example, if coculture of 10^6 cells was positive and coculture of 2×10^5 cells was negative, we assigned this a value of 1 $TCID_{50}$/10^6 cells because 10^6 cells was the lowest number of cells that generated productive infection.

Determination of Splenic Uptake of Radiolabeled mAbs

Two groups of SCID mice (four mice per group) were used in this experiment. One group was injected intrasplenically with HIV-1-infected hPBMCs (25×10^6 cells/ mouse) and another with noninfected hPBMCs (20×10^6 cells/ mouse) as described above. After 1 h, 20 μCi (0.74 MBq) (20 μg) of ^{188}Re-246-D was given IP to each mouse, and at 3 h postinjection the animals were sacrificed, their spleens removed and weighed, radioactivity counted in a gamma counter, and the percentage of injected dose per gram (ID/g) calculated.

Determination of Platelet Counts in Mice Treated with Radiolabeled mAbs

Platelet counts were used as a marker of RIT toxicity in treated animals. For measurement of platelet counts, the blood of SCID mice (five mice per group) injected intrasplenically with HIV-1-infected hPBMCs and either treated with 100 μCi (3.7 MBq) (20 μg) of ^{213}Bi-246-D or with 160 μCi (5.92 MBq) (20 μg) of ^{188}Re-246-D IP 1 h after treatment with infected hPBMCs or untreated was collected from the tail vein into 200 μl of 1% ammonium oxalate on days 0, 3, 8, and 15 days post-therapy. Platelets were counted in a hemocytometer, using phase contrast, at 400× magnification, as described in [27].

Construction of thy/liv-SCID-hu Mice and Titration of HIV-1-Infected Mononuclear Cells in the hu-thy/liv Implant by Limiting Dilution Coculture

The thy/liv-SCID-hu mice. were constructed by implanting SCID mice (6–8 wk old) with human fetal thymic and liver tissue under the kidney capsules of SCID mice as described [28]. Briefly, after SCID mice were anesthetized with pentobarbital (40–80 mg/kg), approximately ten pieces of syngeneic human fetal thymic and liver tissue were implanted under the kidney capsules, and 3 mo later the size of implanted tissue had increased by more than 20-fold.

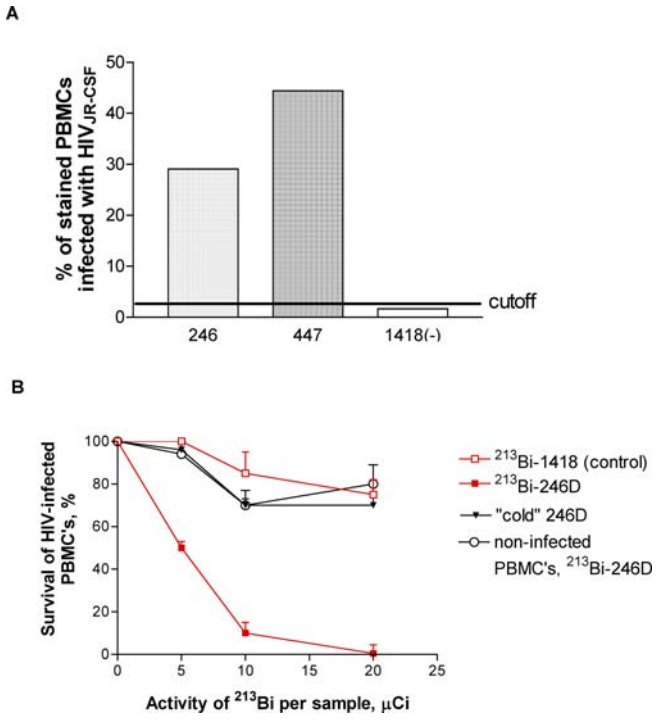


Figure 2. In Vitro Binding and Killing of HIV-Infected Human PBMCs with Human Monoclonal Antibodies

(A) Binding of 246-D human mAbs to HIV gp41 was determined by flow cytometry of hPBMCs infected with the JR-CSF strain of HIV-1. Human mAb 1418 (IgG1) to parvovirus B19 was used as an irrelevant control, and human mAb 447 (IgG3) to the V3 loop of HIV-1 gp120 (26) was used as a positive control for the FACS studies. No significant staining was detected after incubation of HIV-infected cells with the conjugated anti-human IgG in the absence of a primary human mAb. Samples were analyzed in duplicate.

(B) HIV-infected hPBMCs were treated with ^{213}Bi-246-D, cold 246-D, and ^{213}Bi-1418 (irrelevant control), and noninfected hPBMCs were treated with ^{213}Bi-246-D. The number of surviving cells was determined by Trypan blue dye exclusion assay. Samples were analyzed in triplicate. Every data point represents the mean of two (A) or three (B) replicates; error bars in (B) show standard error of the mean.

doi:10.1371/journal.pmed.0030427.g002

Biodistribution of mAb ^{188}Re-246-D in thy/liv-SCID-hu Mice

Three thy/liv-SCID-hu mice were infected with HIV-1$_{JR-CSF}$ via its thymic implant, as described above. Only one implant in each mouse (each mouse had two implants) was infected. Three thy/liv-SCID-hu mice with noninfected implants were used as controls. At 8 d postinfection mice were injected IP with ^{188}Re-246-D (60 μCi (2.22 MBq), 20 μg of mAb) and sacrificed to assay for biodistribution 18 h later. Only the infected implant was taken out to check biodistribution. The blood and major organs were removed, weighed, and counted in a gamma counter, and the percentage of ID/g was calculated.

Treatment of HIV-1 Infection in Infected thy/liv-SCID-hu Mice with Radiolabeled mAbs

Thy/liv-SCID-hu mice were infected by direct injection of HIV-1$_{JR-CSF}$ (800 TCID$_{50}$) into the human thymic implant. Selected implants were biopsied 1 wk later, and the number of HIV-1-infected thymocytes was quantified by limiting dilution coculture. One group of mice (four mice) was treated with ^{188}Re-246-D (160 μCi (5.92 MBq), 20 μg mAb) and another group (four mice) with the same activity of ^{188}Re-1418. After 72 h, the mice were sacrificed and the number of HIV-1-infected thymocytes was determined by quantitative coculture.

Statistical Analysis

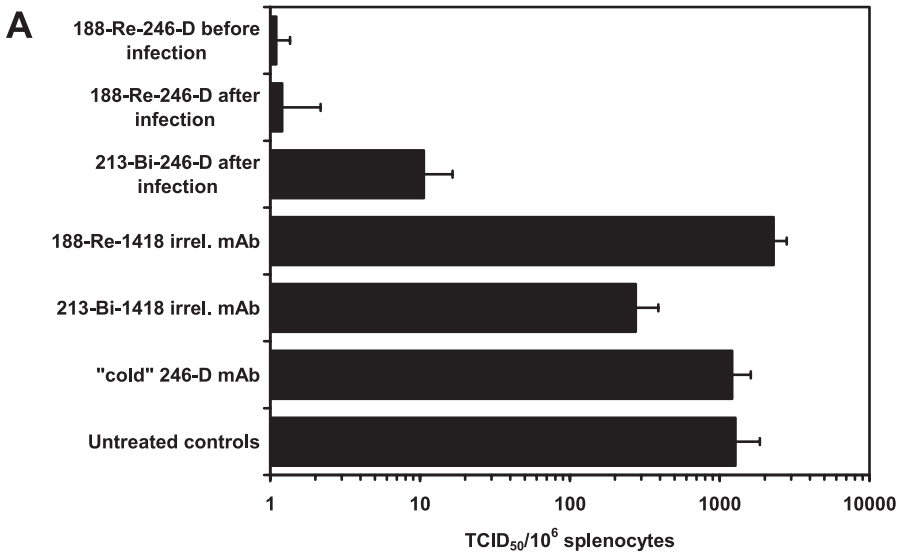
The statistical aspects of data analysis were discussed with Dr. Y. Lo, a statistician at the Einstein/Montefiore Medical Center, Center for AIDS Research. The sample sizes in animal experiments were preplanned taking into consideration availability of animals. The observers were fully blinded to the active or control nature of the experimental arm being assayed. The differences between the numbers of TCID$_{50}$/10^6 splenocytes for differently treated groups during in vivo therapy studies were analyzed by nonparametric Mann-Whitney test using Prism software (GraphPad, San Diego, California, United States). Differences were considered statistically significant when p-values were below 0.05.

Results

To determine the ability of RIT to kill HIV-1-infected cells, goat polyclonal antibodies to gp120 were labeled with radioisotopes with distinctly different emission characteristics—^{213}Bi (a radioisotope that emits alpha particles, which are He atoms with a charge of +2 and mass of 4) and ^{188}Re (a radioisotope that emits high-energy beta particles [electrons]). ^{213}Bi and ^{188}Re have different emission ranges in tissue: 50–80 μm for ^{213}Bi versus 10 mm (on average) for ^{188}Re. Both radioisotopes have been used in preclinical and clinical settings [29–31]; we used both to investigate if HIV-1-infected cells can be killed with either alpha- (^{213}Bi) or beta-radiation (^{188}Re).

The chronically HIV-1-infected ACH-2 cells were incubated with ^{188}Re-anti-gp120, ^{188}Re-18B7 (irrelevant murine mAb), or cold anti-gp120. Substantial killing of ACH-2 cells with ^{188}Re-anti-gp120 polyclonal antibody was observed compared to the minimal effect of the control Ab linked to ^{188}Re with the same specific radioactivity or with cold anti-gp120 Ab (Figure 1A). Elimination of the ACH-2 cells was not complete. By virtue of their mass, charge, and energy the relative biological effectiveness of alpha particles is significantly higher than that of beta particles emitted by ^{188}Re [32,33]. Therefore, we examined the effectiveness of the anti-gp120 linked to the alpha particle emitter ^{213}Bi for killing HIV-1-infected cells. Almost all of the ACH-2 cells were killed by 5 μCi (0.185 MBq) of ^{213}Bi-anti-gp120 compared to the minimal cytocidal activity of an irrelevant mAb linked to ^{213}Bi or cold anti-gp120 Ab (Figure 1B).

Because the HIV envelope protein gp120 is expressed extracellularly, it was the initial target for antibody-directed killing of HIV-infected cells. However, toxins coupled to gp41-specific antibodies were reported to be more effective for in vivo elimination of HIV-1-infected cells than were toxins linked to anti-gp120 [14,26,34]. An advantage of targeting gp41 over gp120 with antibodies is that gp41 is a transmembrane protein that is reliably expressed on the surface of infected cells, is not readily shed, and has a highly conserved sequence [35]. Therefore, we examined the ability of radioisotopes coupled to a human mAb specific for gp41 to target and kill HIV-1-infected hPBMCs in vitro and in vivo. For these studies, we used the human anti-gp41 mAb 246-D,



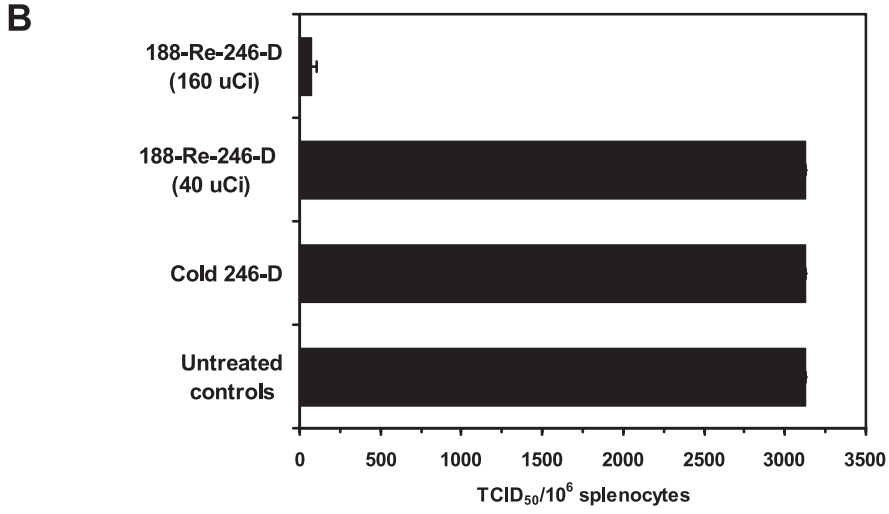


Figure 3. RIT of SCID Mice Injected Intrasplenically with HIV-$1_{JR\text{-}CSF}$-Infected Human PBMCs with ^{188}Re- and ^{213}Bi-Labeled Human Anti-gp41 mAb 246-D

(A) Mice were treated IP 1 h after infection with infected hPBMCs with either 20 μg of cold 246-D ($n = 4$); 100 μCi (3.7 MBq) (20 μg) of ^{213}Bi-1418 ($n = 5$) or 80 μCi (2.96 MBq) (20 μg) of ^{188}Re-1418 ($n = 3$) as isotype-matching controls; 80 μCi (2.96 MBq) (20 μg) of ^{188}Re-246-D ($n = 4$); or 100 μCi (3.7 MBq) (20 μg) of ^{213}Bi-246-D ($n = 5$). One group was left untreated ($n = 4$). Another group of mice were given 80 μCi (2.96 MBq) (20 μg) of ^{188}Re-246-D IP 1 h before infection with infected hPBMCs ($n = 4$).

(B) Mice were injected IP with ^{188}Re-246-D (20 μg) with activity of 40 μCi (1.48 MBq) ($n = 4$), with 160 μCi (5.92 MBq) ($n = 4$), or with 20 μg of cold 246-D ($n = 4$). A fourth group was untreated ($n = 4$). The number of HIV-1-infected hPBMCs in the spleens were then determined by limiting dilution coculture and presented as $TCID_{50}/10^6$ splenocytes. Each bar represents the mean of $TCID_{50}/10^6$ splenocytes in a given group of mice, and error bars show standard error of the mean.

doi:10.1371/journal.pmed.0030427.g003

which binds specifically to HIV-1-infected cells as demonstrated by flow cytometry of hPBMCs infected with HIV-$1_{JR\text{-}CSF}$ (Figure 2A). 246-D does not bind to the six-helix bundle that forms after gp120 CD4 intervention, but rather to an epitope, near the disulfide loop of gp41, that is an immunodominant epitope [36] and is broadly cross-reactive with genetically diverse HIV-1 strains [37,38]. When 246-D was linked with ^{213}Bi, it was very effective in eliminating HIV-1-infected hPBMCs when compared to cold 246-D and the irrelevant mAb 1418 labeled with ^{213}Bi (Figure 2B). We observed that more hPBMCs were killed by ^{213}Bi-246-D than overall percentage of infected cells in the sample. This most likely reflects a "cross-fire" effect from alpha particles emanating from adjacent HIV-1-infected cells that contained bound ^{213}Bi-mAb in the setting of cell crowding at the bottom of tissue culture wells. Importantly, in the absence of HIV-1 antigens, incubation of noninfected hPBMCs with ^{213}Bi-246-D produced no significant killing of the cultured cells (Figure 2B), and no killing of noninfected hPBMCs was observed post-treatment with ^{188}Re-246-D as well (unpublished data).

We next examined the in vivo ability of RIT with radiolabeled 246-D to eliminate HIV-1-infected cells. For these studies, hPBMCs infected with HIV-$1_{JR\text{-}CSF}$ were injected into the spleens of SCID mice and the mice were treated as indicated. We used a 80 μCi (2.96 MBq) dose for ^{188}Re-246-D

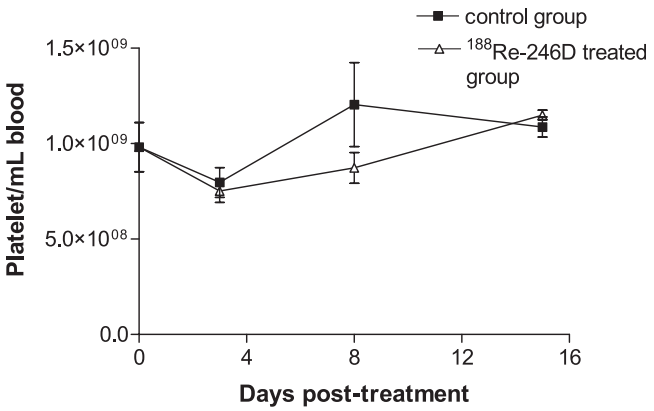


Figure 4. Effect of RIT on Platelet Counts in the Peripheral Blood

Platelet counts in the blood of SCID mice injected intrasplenically with HIV-1-infected hPBMCs and either treated with 160 μCi (5.92 MBq) (20 μg) ^{188}Re-246-D IP 1 h after infection with hPBMCs ($n = 5$) or no treatment ($n = 5$). The blood was collected from the tail vein on days 0, 4, 8, and 15 post-therapy. Each data point represents the mean of platelet counts in a given group of mice, and error bars show standard error of the mean.

doi:10.1371/journal.pmed.0030427.g004

and 100 μCi (3.7 MBq) for ^{213}Bi-246-D, because these doses were therapeutic and safe in experimental RIT of fungal and bacterial infections [17,39]. The mice were evaluated 72 h later for the presence of residual HIV-1-infected cells by quantitative coculture [25]. The 72 h time period was chosen to give the sufficient time for the ^{188}Re-labeled mAb to deliver a lethal dose of radioactivity to the cells, as ^{188}Re half-life is 16.9 h and several half-lives are required for a given radionuclide to deliver the dose to the target. Treatment of mice with ^{188}Re-labeled mAb 246-D administered either before or after intrasplenic injection with HIV-$1_{JR\text{-}CSF}$-infected hPBMCs dramatically reduced the subsequent number of HIV-1-infected cells ($p = 0.016$ and $p = 0.008$, respectively). Infected cells were also killed after treatment of mice with ^{213}Bi-246-D (Figure 3A) ($p = 0.008$). In contrast, the administration of the matching amounts of cold 246-D ($p = 1.0$) or of a radioisotope-coupled irrelevant control mAb did not reduce the average number of infected cells in the SCID mouse spleens ($p = 1.0$ and $p = 0.06$ for ^{188}Re- and ^{213}Bi-labeled control mAb, respectively). It is likely that ^{188}Re-246-D was more effective in vivo than ^{213}Bi-246-D due to the longer physical half-life of ^{188}Re (16.9 h versus 46 min), which allowed the labeled mAbs to reach infected cells while still carrying a high-activity "payload."

To investigate the dose-response effect, the mice were treated with 40 and 160 μCi of ^{188}Re-246-D, corresponding to 50% and 200% of the therapeutic dose, respectively. While 40 μCi (1.48 MBq) ^{188}Re-246-D was not effective in killing HIV-infected hPBMCs in vivo ($p = 1.0$), 160 μCi (5.92 MBq) dose almost completely eliminated the HIV-infected cells ($p = 0.03$) (Figure 3B).

To further investigate the specificity of radiolabeled mAb binding to gp41 on HIV-infected hPBMCs we compared the splenic uptake of ^{188}Re-246-D in mice injected intrasplenically with noninfected hPBMCs and HIV-1-infected hPBMCs. The uptake expressed as percentage of ID/g of spleen (with standard deviation) was 8% ± 4% and 57% ± 10% ID/g for noninfected and infected hPBMCs, respectively. This result indicates that the ^{188}Re-246-D was targeted in vivo to HIV-1-infected cells.

We evaluated the hematological toxicity of radiolabeled 246-D during HIV-1 infection in the SCID mice by platelet counts. The platelet count nadir usually occurs 1 wk after radiolabeled antibody administration to tumor-bearing animals [40,41]. We did not observe any changes in platelet counts in mice treated with 100 μCi (3.7 MBq) of ^{213}Bi-246-D on days 4, 8, and 15 post-treatment compared to nontreated infected controls; platelet counts were stable at 1.5×10^9 (± 0.2×10^9) platelets/ml of blood (unpublished data). For mice given 160 μCi of ^{188}Re-246-D—the highest dose used in this study—a slight nonsignificant drop in platelet count was noted on day 7 post-treatment, but counts returned to normal by day 15 (Figure 4). This lack of hematologic toxicity likely reflects the very specific targeting of HIV-1-infected hPBMCs by radiolabeled mAb, since gp41 antigen is expressed only on infected cells in the mouse. In fact, one of the advantages of using RIT to treat infections as opposed to cancer is that, in contrast to tumor cells, cells expressing microbial antigens are antigenically very different from host tissues and thus provide the potential for exquisite specificity and low cross-reactivity. This safety profile is compatible with our previous data showing that RIT was also relatively nontoxic in mouse models of experimental fungal and bacterial infection [17,39]. A large therapeutic window is available because the therapeutic effect was achieved with activities significantly lower than reported maximum tolerated activity for ^{188}Re-labeled (800 μCi (29.6 MBq) for intravenous injection [42]) and ^{213}Bi-labeled IgGs (in excess of 1,000 μCi (37 MBq) when given IP [42]). In this regard, IP administration of the radiolabeled mAbs utilized in our study has proved to be better tolerated than the intravenous route [42], and it helps to circumvent the problem of often relatively low specific activity of radiolabeled mAbs (in mCi/ml) by allowing the administration of higher volumes than during intravenous administration.

To further examine the in vivo capacity of RIT to target and eliminate HIV-1-infected lymphocytes, we utilized our well-characterized thy/liv-SCID-hu mouse model [43–45]. This system uses SCID mice implanted under their kidney capsules with fetal human thymic and liver tissue that develops into a structure closely resembling a human thymus; this thymic implant is readily infectible with clinical isolates of HIV-1. This mouse model can be used to evaluate the in vivo capacity of candidate anti-HIV agents to inhibit HIV-1 infection [43,45,46]. Initially we measured the biodistribution of IP-injected ^{188}Re-246-D in thy/liv-SCID-hu mice with HIV-infected and noninfected thymic implants (Table 1). The uptake of 246-D in infected implants was higher than in noninfected implants; however, the overall uptake in the infected implants 18 h postinjection was modest, most likely due to the relatively poor vascularization of the implants. We have previously demonstrated that HIV-1-infected thy/liv-SCID-hu mice develop HIV plasma viremia, which may correlate with the higher uptake of 246-D in the blood as well as spleen, liver, and kidneys of the HIV-infected mice.

Because uptake of the radiolabeled anti-gp41 mAb in infected implants of thy/liv-SCID-hu was modest after IP injection, the therapy experiments were performed using intraimplant injections of radiolabeled mAbs to accommodate the predominantly murine vasculature of the implant,

Table 1. Biodistribution of ^{188}Re-246-D mAb in thy/liv-SCID-hu Mice Uninfected and Infected with HIV Examined 18 h after Intraperitoneal Injection

Organ	Uptake in HIV-Infected Mice, % ID/g[a]	Uptake in Noninfected Mice, % ID/g[a]
Blood	10.7 (0.5)	6.7 (0.4)
Implant	2.45 (0.08)	1.85 (0.08)
Kidneys	10.9 (0.5)	9.3 (0.1)
Liver	3.1 (0.4)	1.8 (0.4)
Spleen	3.3 (1.0)	1.6 (0.5)

Each value is the mean for three mice per group.
[a]Standard deviation of the mean is given in parentheses
doi:10.1371/journal.pmed.0030427.t001

which may contribute to decreased transport of the radiolabeled antibody. Sufficient blood supply to the tissue treated with RIT is crucial for successful treatment, as it has been established that early in RIT development antibodies mostly localize along the blood vessels [47]. As shown in Figure 5A, there was a 2.5-fold reduction in the number of HIV-1-infected thymocytes in the human thymic implant grafts of mice treated with ^{188}Re-246-D compared to those treated with the ^{188}Re-control mAb. To directly confirm the anti-HIV effect of the ^{188}Re-anti-gp41 treatment, we compared the level of infection in the human thymic implants of a biopsy taken before treatment with the degree of infection after treatment. The mean number of HIV-infected thymocytes was reduced more than 95% by ^{188}Re-anti-gp41 in three of the four treated mice (Figure 5B). Taken together, these results demonstrate that RIT using ^{188}Re-anti-gp41 mAb potently and selectively eliminates HIV-1-infected cells.

Discussion

We have demonstrated that the antibodies to HIV-1 envelope glycoproteins gp120 and gp41 labeled with radioisotopes ^{213}Bi and ^{188}Re selectively killed chronically HIV-1-infected human T cells and acutely HIV-1-infected human peripheral blood mononuclear cells (hPBMCs) in vitro. Treatment of SCID mice harboring HIV-1-infected hPBMCs in their spleens with a ^{213}Bi- or ^{188}Re-labeled monoclonal antibody to gp41 viral protein resulted in a greater than 99% elimination of HIV-1-infected cells in a dose-dependent manner. The number of HIV-1-infected thymocytes decreased 2.5-fold in the human thymic implant grafts of SCID mice treated with the ^{188}Re-labeled antibody to gp41 compared with those treated with the ^{188}Re-control mAb. The treatment did not cause acute hematologic toxicity in the treated mice.

In cancer treatment, the success of FDA-approved drugs such as Zevalin and Bexxar (anti-CD20 mAbs labeled with yttrium 90 [^{90}Y] and iodine 131 [^{131}I], respectively) in the treatment of relapsed or refractory B cell non-Hodgkin lymphoma is proof of the enormous potential of RIT for targeted elimination of malignant cells and for treatment of patients who have failed all standard therapeutic regimens. Recent encouraging reports on the use of RIT as an initial treatment for follicular lymphoma [48] are making RIT a first-line anticancer therapy. This clinical experience using RIT creates a favorable environment for the introduction and use of RIT for treating HIV-1-infected patients. The effectiveness of RIT for HIV-1 infection is enhanced because the majority of long-lived infected cellular targets are lymphocytes, which are among the most radiosensitive cells in the body.

RIT of HIV-1 has five marked advantages over the immunotoxin approach. (1) The antibody used for radiation delivery does not need to be internalized to kill the cell. (2) Not every infected cell in the body needs to be targeted by the antibody because of the "cross-fire" effect. Consistent with this mechanism, ^{188}Re-labeled mAbs may be more effective in vivo than in vitro (Figures 1A and 3A). In vitro, the "cross-fire" radiation is largely deposited on the two-dimensional surface represented by the cell layer at the bottom of a tissue culture well, whereas in vivo there are many infected cells in the nearby three-dimensional space such that "cross-fire" radiation is more effective. In this regard, beta-emitting radionuclides, which kill cells preferentially by "cross-fire" [15], have been successfully used for therapy of leukemia, a single-cell disease [49], and in preclinical RIT of systemic fungal infections [16,39,50]. (3) In contrast to immunotoxins, the radioisotope linked to the antibody is unlikely to elicit significant immune responses that would limit subsequent use. (4) RIT is potentially less toxic than immunotoxins, because the chemistry of linking different radioisotopes including ^{188}Re and ^{213}Bi to the antibodies has been well developed, and the exceptional stability of radiolabeled mAbs in vitro and in vivo has been confirmed [15,42,51]. (5) The availability of many isotopes differing in half-life and radiation type [15] offers great versatility for designing RIT. Nevertheless, combination therapy consisting of RIT and immunotoxins may provide greater advantages than either modality alone.

We have recently shown that RIT of infection works through the combination of cell-killing mechanisms such as "direct hit" and "cross-fire," promotion of apoptotic death of targeted cells, generation of reactive oxygen species and modulation of the inflammatory response [50]. All of these mechanisms require certain levels of radioactivity to manifest themselves. It is likely that in our study the threshold dose at which those mechanisms become effective in killing HIV-infected cells is in the range 40–80 (1.48–2.96 MBq) μCi. There are numerous published data on the existence of threshold RIT doses below which no therapeutic effects on tumors in animals are observed and above which the effects quickly reach maximum [42, 52]. However, determination of

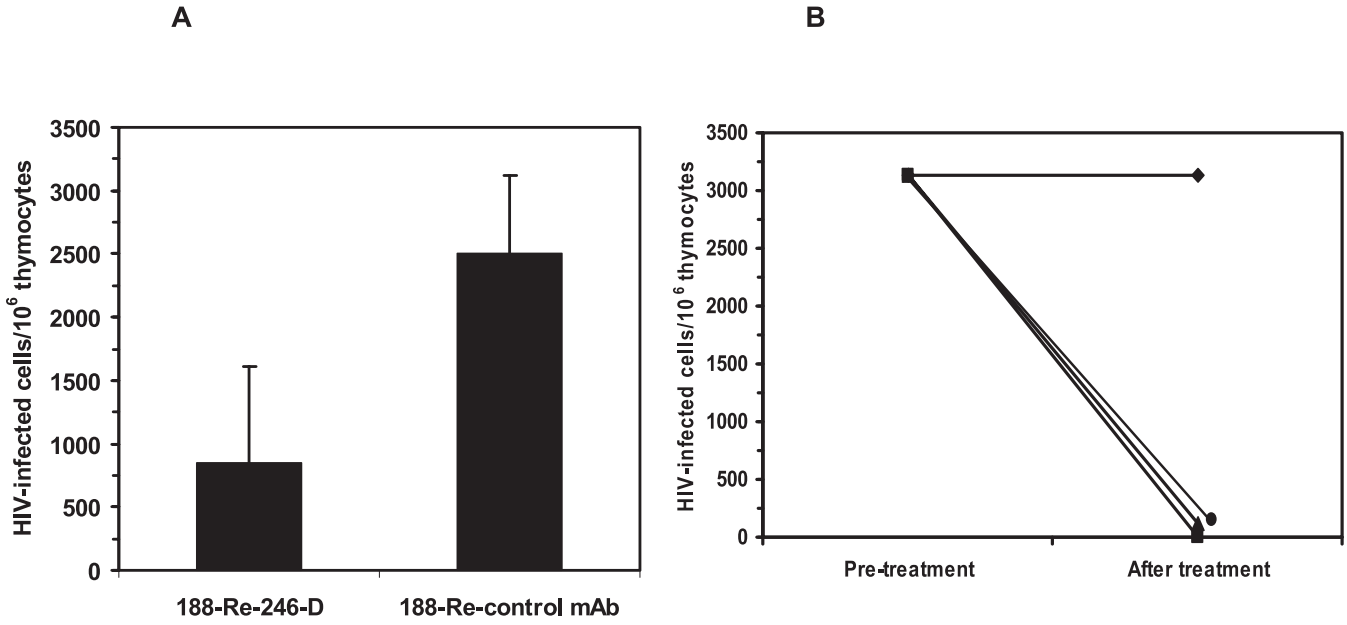


Figure 5. RIT Using Anti-gp41 mAb ^{188}Re-246-D Reduces HIV Infection in the Human Thymic Implants of thy/liv-SCID-hu Mice

One week after thy/liv-SCID-hu mice were infected with HIV-1, they were treated by intraimplant injection of two doses of either ^{188}Re-246-D ($n = 4$) or ^{188}Re-1418 control mAb ($n = 4$) given on two consecutive days ($p = 0.11$).

(A) The number of HIV-infected thymocytes was determined 3 d later. The bars show mean number (error bars show standard error of the mean) of HIV-infected thymocytes/10^6 thymocytes in the human thymic implants of thy/liv-SCID-hu mice treated with ^{188}Re-246-D or ^{188}Re-1418 control mAb.

(B) Comparison of the number of HIV-infected thymocytes in the human thymic implants in each of the four treated thy/liv-SCID-hu mice before treatment was initiated and after treatment with ^{188}Re-246-D.

doi:10.1371/journal.pmed.0030427.g005

the threshold doses in RIT is not considered to be of practical importance as in the clinic this therapy is administered as maximum tolerated dose (MTD) to maximize the therapeutic effect. These results establish that RIT can effectively target and kill HIV-1-infected hPBMCs in vivo.

Based on the data accumulated in clinical RIT of cancer, the primary toxic effect of RIT applied to infections is likely to be bone marrow suppression. Important determinants of the extent and duration of myelosuppression include bone marrow reserve (based on prior cytotoxic therapy and extent of disease involvement), total infection burden, and spleen size [53,54]. Nevertheless, RIT of non-Hodgkin lymphoma is effective in patients who have received several unsuccessful courses of chemotherapy and consequently have depleted bone marrow reserves and weakened immunity—not unlike some patients with advanced HIV-1 infection. The application of RIT to infectious diseases will require optimization of the dose to ascertain and minimize toxic effects.

In addition, when using a radioactive therapy in patients there is always a concern of long-term effects such as the possible subsequent development of neoplasms arising from radiation-induced mutations. However, this risk should be extremely low after short-term exposure and would likely be outweighed by the benefits of treating or preventing HIV infections [55]. It is also extremely unlikely that particulate radiation utilized in RIT will cause mutation of the HIV virus, the genomic information of which is carried by two positive RNA strands and not by DNA, and ionizing radiation is a weak mutagen compared to chemical mutagens [56]. Furthermore, HIV replication already has an inherently high rate of viral mutation.

Acutely exposed individuals lack HIV-specific antibodies that could reduce the effectiveness of RIT by competitively binding to gp41, so RIT could be used for treatment initiated after HIV exposure. However, the effectiveness of treatment in patients with established HIV infection may be reduced by competition between the radionuclide-linked antibody and the patient's own antiviral antibodies directed to the same antigen on HIV-1-infected cells. If this competition occurs, it could be circumvented by increasing the RIT antibody dosage or by coupling the radionuclides to very high affinity antibodies likely to bind more effectively to the gp41 than self-antibodies. The latter approach would be enhanced by using cocktails of mAbs against noncompeting epitopes and, following the well-known radiation oncology approach of dose fractionation, each antibody in the antibody cocktail would carry a partial dose (one-half or one-third, depending on the composition of the cocktail). Our group is currently investigating the role of competition between radiolabeled mAb 246-D and cold polyclonal human anti-gp41 antibody binding to HIV-1 gp41 and killing HIV-1-infected cells.

If advanced to clinical use, RIT might be most effective when used in combination with HAART [57], which blocks virus replication in newly infected cells. The major therapeutic use of RIT will likely be as an adjunct with HAART to prevent HIV infection when administered to individuals within the first days of exposure to HIV. Even if infection is not prevented in some cases, initial treatment of patients soon after infection may reduce the initial number of HIV-1-infected cells and thereby reduce viral set point. RIT also may be a useful adjunct for protocols designed to "flush out" quiescent, latently infected lymphocytes by the administra-

tion of factors, such as valproic acid, that promote HIV replication [58]. Moreover, since viral resistance is often a result of mutations introduced during replication, a therapy that reduces the number of HIV-producing cells may reduce the emergence of drug-resistant strains. To avoid selection of cells harboring antibody-resistant viruses, a cocktail of mAbs to various epitopes on the HIV envelope may be desirable. We envision that the availability of RIT could provide a novel treatment option that may hasten the day when curative regimens are available for the eradication of HIV-1 infection.

Acknowledgments

A. Casadevall and H. Goldstein share senior authorship of this paper. We thank Ms. C. Williams (New York University) for excellent technical assistance; Dr. R. A. Bryan and Ms. R. Jones (Albert Einstein College of Medicine) for performing platelet counts; and Dr. Y. Lo (Einstein/Montefiore Medical Center, Center for AIDS Research) for statistical consultation.

Author contributions. AC provided the idea for this study. ED, MCP, AC, SZP and HG designed the study. ED, MCP, ST, SZP, AC, and HG analyzed the data. ED, MCP, MWB, MKG, SZP, AC, and HG contributed to writing the paper. CA and AM supplied $^{225}Ac/^{213}Bi$ radionuclide generators. MWB synthesized and provided the bifunctional chelating agent that was used for these studies. MKG did the following experiments for the study: binding of the human monoclonal antibodies 246, 447, and 1418 to hPBMCs infected with HIV-$1_{JR\text{-}CSF}$. SZP and MKG produced and provided human monoclonal antibodies 246-D, 447, and 1418.

Competing Interests: AC and ED are consultants for Pain Therapeutics on a project unrelated to the work described in the manuscript. The relationship with the company involves monetary compensation for our advice, reimbursement for company related travel, and share options. The patent application for the treatment of HIV/AIDS with RIT has been licensed by Albert Einstein College of Medicine to Pain Therapeutics.

References

1. Saag MS, Schooley RT (1998) Antiretroviral chemotherapy. Curr Clin Top Infect Dis 18: 154–179.
2. Barbaro G (2006) Metabolic and cardiovascular complications of highly active antiretroviral therapy for HIV infection. Curr HIV Res 4: 79–85.
3. Levy AR, James D, Johnston KM, Hogg RS, Harrigan PR, et al. (2006) The direct costs of HIV/AIDS care. Lancet Infect Dis 6: 171–177.
4. Smith KY (2002) Selected metabolic and morphologic complications associated with highly active antiretroviral therapy. J Infect Dis 185: S123–127.
5. Grinspoon SK (2005) Metabolic syndrome and cardiovascular disease in patients with human immunodeficiency virus. Am J Med 118: 23S–28S.
6. Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, et al. (1997) Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc Natl Acad Sci U S A 94: 13193–13197.
7. Chun TW, Engel D, Berrey MM, Shea T, Corey L, et al. (1998) Early establishment of a pool of latently infected, resting CD4+ T cells during primary HIV-1 infection. Proc Natl Acad Sci U S A 95: 8869–8873.
8. Chun TW, Davey RT Jr., Ostrowski M, Shawn Justement J, Engel D, et al. (2000) Relationship between pre-existing viral reservoirs and the re-emergence of plasma viremia after discontinuation of highly active anti-retroviral therapy. Nat Med 6: 757–761.
9. Blankson JN, Persaud D, Siliciano RF (2002) The challenge of viral reservoirs in HIV-1 infection. Annu Rev Med 53: 557–593.
10. Markowitz M, Vesanen M, Tenner-Racz K, Cao Y, Binley JM, et al. (1999) The effect of commencing combination antiretroviral therapy soon after human immunodeficiency virus type 1 infection on viral replication and antiviral immune responses. J Infect Dis 179: 527–537.
11. Vigano A, Trabattoni D, Schneider L, Ottaviani F, Aliffi A, et al. (2006) Failure to eradicate HIV despite fully successful HAART initiated in the first days of life. J Pediatr 148: 389–391.
12. Hamer DH (2004) Can HIV-1 be cured? Mechanisms of HIV-1 persistence and strategies to combat it. Curr HIV-1 Res 2: 99–111.
13. Saavedra-Lozano J, Cao Y, Callison J, Sarode R, Sodora D, et al. (2004) An anti-CD45RO immunotoxin kills HIV-latently infected cells from individuals on HAART with little effect on CD8 memory. Proc Natl Acad Sci U S A 101: 2494–2499.
14. Pincus SH, Fang H, Wilkinson RA, Marcotte TK, Robinson JE, et al. (2003) In vivo efficacy of anti-glycoprotein 41, but not anti-glycoprotein 120, immuno-toxins in a mouse model of HIV infection. J Immunol 170: 2236–2241.
15. Milenic DE, Brady ED, Brechbiel MW (2004) Antibody-targeted radiation cancer therapy. Nat Rev Drug Discov 3: 488–498.
16. Dadachova E, Nakouzi A, Bryan R, Casadevall A (2003) Ionizing radiation delivered by specific antibody is therapeutic against a fungal infection. Proc Natl Acad Sci U S A 100: 10942–10947.
17. Dadachova E, Burns T, Bryan RA, Apostolidis C, Brechbiel MW, et al. (2004) Feasibility of radioimmunotherapy of experimental pneumococcal infection. Antimicrob Agents Chemother 48: 1624–1629.
18. Casadevall A, Dadachova E, Pirofski L (2004) Passive antibody therapy for infectious diseases. Nat Rev Microbiol 2: 695–703.
19. Little SJ, Holte S, Routy JP, Daar ES, Markowitz M, et al. (2002) Antiretroviral-drug resistance among patients recently infected with HIV. N Engl J Med 347: 385–394.
20. Casadevall A, Cleare W, Feldmesser M, Glatman-Freedman A, Goldman DL, et al. (1998) Characterization of a murine monoclonal antibody to Cryptococcus neoformans polysaccharide that is a candidate for human therapeutic studies. Antimicrob Agents Chemother 42: 1437–1446.
21. Xu JY, Gorny MK, Palker T, Karwowska S, Zolla-Pazner S (1991) Epitope mapping of two immunodominant domains of gp41, the transmembrane protein of human immunodeficiency virus type 1, using ten human monoclonal antibodies. J Virol 65: 4832–4838.
22. Gigler A, Dorsch S, Hemauer A, Williams C, Kim S, et al. (1999) Generation of neutralizing human monoclonal antibodies against parvovirus B19 proteins. J Virol 73: 1974–1979.
23. Conley AJ, Gorny MK, Kessler JA 2nd, Boots LJ, Ossorio-Castro M, et al. (1994) Neutralization of primary human immunodeficiency virus type 1 isolates by the broadly reactive anti-V3 monoclonal antibody, 447–52D. J Virol 68: 6994–7000.
24. Apostolidis C, Molinet R, Rasmussen G, Morgenstern A (2005) Production of Ac-225 from Th-229 for targeted alpha therapy. Anal Chem 77: 6288–6291.
25. Ho DD, Moudgil T, Alam M (1989) Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. N Engl J Med 321: 1621–1625.
26. Zolla-Pazner S, O'Leary J, Burda S, Gorny MK, Kim M, et al. (1995) Serotyping of primary human immunodeficiency virus type 1 isolates from diverse geographic locations by flow cytometry. J Virol 69: 3807–3815.
27. Miale JB (1982) Laboratory medicine hematology. St. Louis (Missouri): CV Mosby. 864 p.
28. Kollmann TR, Kim A, Pettoello-Mantovani M, Hachamovitch M, Rubinstein A, et al. (1995) Divergent effects of chronic HIV-1 infection on human thymocyte maturation in SCID-hu mice. J Immunol 154: 907–921.
29. Knapp FF Jr. (1998) Rhenium-188—A generator-derived radioisotope for cancer therapy. Cancer Biother Radiopharm 13: 337–349.
30. Hoher M, Wohrle J, Wohlfrom M, Hanke H, Voisard R, et al. (2000) Intracoronary beta-irradiation with a liquid (188)re-filled balloon: Six-month results from a clinical safety and feasibility study. Circulation 101: 2355–2360.
31. Hassfjell S, Brechbiel MW (2001) The development of the alpha-particle emitting radionuclides 212Bi and 213Bi, and their decay chain related radionuclides, for therapeutic applications. Chem Rev 101: 2019–2036.
32. Wheldon TE (1994) Targeting radiation to tumors. Int J Radiat Biol 65: 109–116.
33. Casarett AP (1968) Radiation biology. Upper Saddle River (New Jersey): Prentice-Hall. 367 p.
34. Till MA, Zolla-Pazner S, Gorny MK, Patton JS, Uhr JW, et al. (1989) Human immunodeficiency virus-infected T cells and monocytes are killed by monoclonal human anti-gp41 antibodies coupled to ricin A chain. Proc Natl Acad Sci U S A 86: 1987–1991.
35. Till MA, Ghetie V, May RD, Auerbach PC, Zolla-Pazner S, et al. (1990) Immunoconjugates containing ricin A chain and either human anti-gp41 or CD4 kill H9 cells infected with different isolates of HIV, but do not inhibit normal T or B cell function. J Acquir Immune Defic Syndr 3: 609–614.
36. Gorny MK, Zolla-Pazner S (2000) Recognition by human monoclonal antibodies of free and complexed peptides representing the prefusogenic and fusogenic forms of human immunodeficiency virus type 1 gp41. J Virol 74: 6186–6192.
37. Nyambi PN, Mbah HA, Burda S, Williams C, Gorny MK, et al. (2000) Conserved and exposed epitopes on intact, native, primary human immuno-deficiency virus type 1 virions of group M. J Virol 74: 7096–7107.
38. Nyambi PN, Nadas A, Mbah HA, Burda S, Williams C, et al. (2000) Immunoreactivity of intact virions of human immunodeficiency virus type 1 (HIV-1) reveals the existence of fewer HIV-1 immunotypes than genotypes. J Virol 74: 10670–10680.
39. Dadachova E, Bryan RA, Frenkel A, Zhang T, Apostolidis C, et al. (2004) Evaluation of acute hematological and long-term pulmonary toxicity of radioimmunotherapy of Cryptococcus neoformans infection in murine models. Antimicrob Agents Chemother 48: 1004–1006.
40. Behr TM, Behe M, Stabin MG, Wehrmann E, Apostolidis C, et al. (1999) High-linear energy transfer (LET) alpha versus low-LET beta emitters in radioimmunotherapy of solid tumors: Therapeutic efficacy and dose-

limiting toxicity of 213Bi- versus 90Y-labeled CO17-1A Fab′ fragments in a human colonic cancer model. Cancer Res 59: 2635–2643.
41. Sharkey RM, Blumenthal RD, Behr TM, Wong GY, Haywood L, et al. (1997) Selection of radioimmunoconjugates for the therapy of well-established or micrometastatic colon carcinoma. Int J Cancer 72: 477–485.
42. Milenic D, Garmestani K, Dadachova E, Chappell L, Albert P, et al. (2004) Radioimmunotherapy of human colon carcinoma xenografts using a 213Bi-labeled domain-deleted humanized monoclonal antibody. Cancer Biother Radiopharm 19: 135–147.
43. Goldstein H, Pettoello-Mantovani M, Bera TK, Pastan IH, Berger EA (2000) Chimeric toxins targeted to the human immunodeficiency virus type 1 envelope glycoprotein augment the in vivo activity of combination antiretroviral therapy in thy/liv-SCID-hu mice. J Infect Dis 181: 921–926.
44. Goldstein H, Pettoello-Mantovani M, Katopodis NF, Kim A, Yurasov S, et al. (1996) SCID-hu mice: A model for studying disseminated HIV infection. Semin Immunol 8: 223–231.
45. Pettoello-Mantovani M, Kollmann TR, Katopodis NF, Raker C, Kim A, et al. (1998) Thy/liv-SCID-hu mice: A system for investigating the in vivo effects of multidrug therapy on plasma viremia and human immunodeficiency virus replication in lymphoid tissues. J Infect Dis 177: 337–346.
46. Pettoello-Mantovani M, Kollmann TR, Raker C, Kim A, Yurasov S, et al. (1997) Saquinavir-mediated inhibition of human immunodeficiency virus (HIV) infection in SCID mice implanted with human fetal thymus and liver tissue: An in vivo model for evaluating the effect of drug therapy on HIV infection in lymphoid tissues. Antimicrob Agents Chemother 41: 1880–1887.
47. Kennel SJ, Falcioni R, Wesley JW (1991) Microdistribution of specific rat monoclonal antibodies to mouse tissues and human tumor xenografts. Cancer Res 51: 1529–1536.
48. Kaminski MS, Tuck M, Estes J, Kolstad A, Ross CW, et al. (2005) 131I-tositumomab therapy as initial treatment for follicular lymphoma. N Engl J Med 352: 441–449.
49. Burke JM, Jurcic JG, Scheinberg DA (2002) Radioimmunotherapy for acute leukemia. Cancer Control 9: 106–113.
50. Dadachova E, Bryan RA, Apostolidis C, Morgenstern A, Zhang T, et al. (2006) Interaction of radiolabeled antibodies with fungal cells and components of immune system in vitro and during radioimmunotherapy of experimental fungal infection. J Infect Dis 193: 1427–1436.
51. Dadachova E, Nosanchuk JD, Shi L, Schweitzer AD, Frenkel A, et al. (2004) Dead cells in melanoma tumors provide abundant antigen for targeted delivery of ionizing radiation by a mAb to melanin. Proc Natl Acad Sci U S A 101: 14865–14870.
52. McDevitt MR, Ma D, Lai LT, Simon J, Borchardt P, et al. (2001) Tumor therapy with targeted atomic nanogenerators. Science 294: 1537–1540.
53. Knox SJ, Meredith RF (2000) Clinical radioimmunotherapy. Semin Radiat Oncol 10: 73–93.
54. Hernandez MC, Knox SJ (2003) Radiobiology of radioimmunotherapy with 90Y ibritumomab tiuxetan (Zevalin). Semin Oncol 30: 6–100.
55. Dadachova E, Casadevall A (2005) Antibodies as delivery vehicles for radioimmunotherapy of infectious diseases. Expert Opinion Drug Deliv 2: 1075–1084.
56. Hall E (2000) Radiobiology for the radiologist. 4th Ed. New York: Lippincott, 368 p.
57. Berger EA, Moss B, Pastan I (1998) Reconsidering targeted toxins to eliminate HIV infection: You gotta have HAART. Proc Natl Acad Sci U S A. 95: 11511–11513.
58. Lehrman G, Hogue IB, Palmer S, Jennings C, Spina CA, et al. (2005) Depletion of latent HIV-1 infection in vivo: A proof-of-concept study. Lancet 366: 549–555.

Editors' Summary

Background. In a person infected with HIV, the symptoms of AIDS can be delayed or controlled with drug combinations such as highly active antiretroviral therapy (HAART). However, at the moment there is no cure for HIV infection or AIDS; HAART has to be taken for life and has unpleasant side effects, and the HIV virus can become resistant to some of the drugs. Even in people for whom HAART is successfully controlling disease, HIV remains at very low levels in white blood cells, and is capable of infecting more cells if treatment is stopped for some reason or becomes ineffective because the virus has developed resistance. One possible approach that could potentially eradicate HIV in an infected person is to inject antibodies, targeted against elements of the HIV particle, joined to a radioactive "tag." The idea is that the antibodies would bind to HIV particles at the surface of infected white blood cells, and the radioactivity would then kill the infected cell. This strategy, called "radioimmunotherapy," has been successfully used to develop treatments for certain cancers.

Why Was This Study Done? The researchers wanted to find out whether radioimmunotherapy had any potential for treating HIV infection. As the first step, they needed to find out whether radioactive antibodies targeted against HIV proteins could kill HIV infected cells in animals, and also whether the animals suffered any serious side effects as a result. This is an early step in developing new treatments that would need to show promising results before the approach would be tried in humans.

What Did the Researchers Do and Find? The researchers first did some experiments on HIV-infected white blood cells in vitro (i.e., test tube experiments), and second in vivo on HIV-infected PBMCs in the spleens of mice. They found that in vitro, HIV-infected white blood cells were successfully killed by radioactive antibodies that had been developed against specific proteins in the HIV particle that are routinely displayed at the surface of infected cells. Two different types of antibody, and two different types of radioactive tag, were tried. Both antibodies were very effective in targeting HIV infected cells, but one type of radioactive tag (bismuth 213) was better than the other (rhenium 188). Then, SCID mice were infected intrasplenically with HIV-infected PBMCs and treated with the radioactively tagged antibodies (these particular mice had a deficient immune system, which means that they tolerate transplanted HIV-infected human PBMCs that serve as in vivo targets for the radioactive antibodies. The number of HIV-infected human PBMCs was reduced in the treated mice compared with control animals, which were treated with antibodies not joined to a radioactive tag. The greater the antibody dose, the greater the proportion of HIV-infected human PBMC that were killed. Finally, the researchers also looked at whether the antibody treatment damaged platelets in the infected mice, and they saw a drop in platelet numbers only for the mice receiving the highest dose of antibodies.

What Do These Findings Mean? These results provide preliminary support for the idea that radioimmunotherapy might be an approach for treatment of HIV. They argue that additional experiments in animals are warranted. If those continue to be promising, it will be critical to find out whether the radioactively labeled antibodies are safe in humans, and whether they are effective. The researchers who did this study feel that the strategy, if eventually shown to be safe and effective, would likely be of most value in preventing HIV infection very shortly after someone is exposed to the virus or treating HIV-infected patients not responsive to anti-retroviral therapy.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.0030427.

- The Medline Plus (provided by the US National Library of Medicine) encyclopedia has an entry on HIV/AIDS
- The AIDSMap provides patient information on HIV/AIDS treatment and care
- The US Centers for Disease Control and Prevention has resources on HIV/AIDS treatment, including current treatment guidelines