Radiolabeled Antibodies for Therapy of Infectious Diseases

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ABSTRACT  Novel approaches to the treatment of infectious diseases are urgently needed. This need has resulted in renewing the interest in antibodies for therapy of infectious diseases. Radioimmunotherapy (RIT) is a cancer treatment modality that utilizes radiolabeled monoclonal antibodies. During the last decade we have translated RIT into the field of experimental fungal, bacterial, and HIV infections. In addition, successful proof of principle experiments with radiolabeled pan-antibodies that bind to antigens shared by major pathogenic fungi have been performed in vitro. The armamentarium of pan-antibodies would result in reducing our dependence on microorganism-specific antibodies and thus would speed up the development of RIT for infections. We believe that the time is ripe for deploying RIT in the clinic to combat infectious diseases.

INTRODUCTION

There is a growing need for alternatives to conventional antibiotics for the treatment of infectious diseases. The number of bacterial pathogens that are resistant even to the most powerful antibiotics is growing each year. HIV remains an incurable disease more than 30 years since its identification. Since 1979 there has been a >200% increase in the annual number of cases of invasive fungal infections in the United States. To exacerbate these problems, the number of patients who cannot fight infections because of impaired immunity is growing and includes HIV patients, patients who have been through cancer chemotherapy, and organ transplant recipients.

Radioimmunotherapy (RIT) is based on the interaction between the pair of antigen and antibody to carry cytotoxic amounts of ionizing radiation to the vicinity of specific cellular targets. Currently, RIT is clinically utilized in the treatment of primary, refractory, and recurrent non-Hodgkin lymphoma with the radiolabeled monoclonal antibodies (mAbs) Zevalin and Bexxar, and it offers several significant advantages over naked antibody strategies: (i) RIT delivers lethal radiation, such that it does not merely interfere with a single cellular pathway but leads to physical destruction of targeted cells via radiation-induced apoptosis/autophagy/necrosis; (ii) RIT is not subject to drug resistance mechanisms such as efflux through drug efflux pumps in malignant cells; (iii) the effectiveness of RIT does not depend on the immunological status of the host; (iv) RIT has the potential to reduce the number of doses used to combat infections with standard therapies from weeks or months to a single or limited number of doses of RIT.

A decade ago we suggested the use of RIT for the treatment of the fungal pathogen Cryptococcus neoformans (1). Since then we have evaluated the suitability of this approach to treating fungal infections for its efficacy and safety as well as expanded it to treating infections due to bacteria and viruses (Fig. 1). Here we provide a brief overview of the preclinical development of RIT for infectious diseases.

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Fungal Infections

We started with the investigation of RIT potency against *C. neoformans* fungal infection (1). *C. neoformans* results in life-threatening meningoencephalitis, which affects people with a compromised immune system and is responsible for higher mortality among individuals with AIDS in sub-Saharan Africa than tuberculosis (2). The availability of good animal models and well-characterized mAbs to *C. neoformans* antigens provided an impetus to use this pathogen for investigating RIT for infections. Importantly, immunotherapy in patients with *C. neoformans* with the capsule polysaccharide-binding antibody 18B7 has already been evaluated clinically (3). Therapeutic studies employed AJ/Cr mice infected systemically with *C. neoformans*. AJ/Cr mice succumb to the systemic infection with *C. neoformans*, most likely because of the partial complement deficiency (4). The survival of mice treated with radiolabeled *C. neoformans*-specific mAb 18B7 was significantly longer than the survival of mice treated with irrelevant labeled IgG1 or phosphate-buffered saline. We utilized a radiolabeled irrelevant mAb (213Bi- or 188Re-IgG1 MOPC21) to take into account the possibility of the radiolabeled IgG binding to Fc receptors on phagocytic cells present at the infected site, which might lead to nonspecific killing of some *C. neoformans* cells. Interestingly, treatment with 100 μCi 213Bi-18B7 resulted in 60% of mice in the 213Bi group surviving on day 75 posttherapy (P < 0.05). In the 188Re group 40% and 20% of animals were alive after treatment with 100 (P < 0.005) and 50 (P < 0.05) μCi 188Re-18B7, respectively. In contrast, mice in control groups died from *C. neoformans* infection on days 35 to 40 (Fig. 2a). Administration of RIT resulted in significant reduction of fungal burden in lungs and brains of mice with *C. neoformans* 48 h after RIT administration when compared to control groups. No difference in the percentage decrease of the fungal burden in the lungs was observed between the groups that received 50 and 100 μCi 188Re-18B7. In contrast, the administration of 200 μCi 188Re-18B7 led to pronounced reduction in lung CFUs when compared to the lower activities (P < 0.05). We concluded that giving a radiolabeled antibody to fungal polysaccharide to infected mice led to the prolongation in survival and reduction in organ fungal burden.

Subsequently we investigated whether RIT was effective in a different mouse model—immunocompetent C57BL6 mice—and whether it would be effective in the setting of more established cryptococcal infection. RIT kills the microorganisms primarily by targeted
cytotoxic radiation, so it was paramount to establish its presumed independence from the immune status of the host. Additionally, showing that RIT can be efficacious in the setting of the established infections accompanied by the high fungal load would be useful for its future translation into the clinic. For this purpose C57BL6 mice were infected intravenously (i.v.) via the tail vein with 10^6 C. neoformans cells and were left either untreated or treated intraperitoneally (i.p.) with 100 μCi 213Bi-18B7 24 h postinfection, 100 μCi 188Re-18B7 24 h postinfection, or 100 μCi 188Re-18B7 48 h postinfection (5). Administration of 188Re-18B7 mAb 24 h postinfection resulted in one log reduction in the lung CFUs (P = 0.04), while no reduction in CFUs was observed in the brains (P = 0.07) (Fig. 2b). Treatment with 213Bi-18B7 mAb 24 h postinfection completely killed fungal cells in the lungs and the brains (the plating assay sensitivity was 50 CFUs) (Fig. 2b), which agreed with the results of Gomon methenamine silver (GMS) staining. Interestingly, for established infection at 48 h, 188Re-18B7 mAb efficiently decreased CFUs in the lungs (P = 0.03) and also notably decreased the number of fungal cells in the brains of treated mice (P = 0.02) (Fig. 2b). The latter observation might stem from the increased permeability of the blood-brain barrier caused by the presence of infection, which results in facilitating the entrance of the radiolabeled antibody into the brain (6). We concluded from this study that RIT demonstrated efficacy in both early and established infection in immunocompetent C57BL6 mice. In our previous work we showed RIT efficacy in complement-deficient AJ/Cr mice. The results are consistent with and supportive of the presumed RIT independence of the status of the host’s immune system. Such a finding is encouraging for the treatment of opportunistic infections in cancer and organ transplant patients.

As a part of our effort to demonstrate the value of RIT as an anti-infective strategy, we compared its efficacy with amphotericin B, which is one of the major antifungal drugs (7). AJ/Cr mice were infected i.v. with 3 × 10^5 melanized or nonmelanized C. neoformans cells. Twenty-four hours after infection the mice were given i.p. either 100 μCi 213Bi-18B7 or amphotericin in its deoxycholate form at 1 μg/g body weight at 24, 48, and 72 h, were given both treatments, or were left untreated. Mouse survival was observed for 60 days. The lungs and brains were analyzed at 60 days postinfection. This analysis demonstrated that treatment with amphotericin did not significantly decrease lung and brain CFUs in either nonmelanized or melanized C. neoformans groups (Fig. 2c) (P > 0.05). RIT administration resulted in noted decreases in fungal burden in comparison with the untreated controls and the amphotericin-treated mice (P << 0.05). Of note, the fungus was practically cleared from the brains of the RIT-treated nonmelanized C. neoformans group (sensitivity of the detection was 50 CFUs), while in the melanized C. neoformans group RIT was capable of practically clearing the infection from both brain and lungs. Our most important result was the observation of the RIT efficacy in reducing fungal burden in lungs and brains when compared to the high 1-μg/g dose of amphotericin, when the majority of mice treated with RIT were able to almost completely clear the infection. The explanation of the inability of amphotericin to decrease the fungal burden in the organs of partially complement-deficient AJ/Cr mice 3 days posttreatment was provided by the subsequent amphotericin study showing a trend toward a decrease in CFUs in brains and lungs that manifested itself only on day 14 of treatment with amphotericin B (7).

Our results are in accordance with the published data showing that amphotericin was able to produce only a 1 to 1.5 log reduction in CFUs in immunocompetent mice such as CD-1 and BALB/c, and all mice succumbed to C. neoformans infection around day 24 (8, 9). Our results also agree with the clinical reports that show that a short course of amphotericin was not able to sterilize cerebrospinal fluid or blood of patients and that correlated the rate of sterilization with patient survival (10). Our observations underline the advantages of RIT, which has pronounced antimicrobial effects in vivo after just one injection, compared to long and often ineffective treatments with amphotericin, which has long-lasting side effects.

Despite radiation being a weak mutagen, the belief that even low doses of ionizing radiation are able to create potentially dangerous cellular mutants persists within the scientific community and the lay public alike. To evaluate the possibility that RIT might select for the radiation resistance in C. neoformans cells in vivo, AJ/Cr mice were infected with C. neoformans cells recovered from mice treated previously with 188Re-18B7 mAb (CNRe-RIT), with 213Bi-18B7 mAb (CNBi-RIT), or with the RIT-naive C. neoformans cells (CNnaive) (11). We treated C. neoformans-infected mice with 150 μCi 188Re-18B7 or 125 μCi 213Bi-18B7 24 h after i.v. infection and then observed the mice for survival and weight loss. The number of deaths in mice infected with CNRe-RIT or CNBi-RIT was similar to that in mice infected with CNnaive (P > 0.05) (Fig. 2d). Mice given 213Bi-18B7 mAb survived longer (P = 0.04) than those given 188Re-18B7 (Fig. 2d), most likely due to the higher...
FIGURE 2 RIT of experimental fungal infections with $^{213}\text{Bi}$- and $^{188}\text{Re}$-labeled mAbs. (a) Kaplan-Meier survival curves for A/JCr mice infected i.v. with $10^5$ C. neoformans cells 24 h prior to treatment with 50 to 200 $\mu$Ci $^{188}\text{Re}$-labeled mAbs. Animals injected with phosphate-buffered saline or 50 $\mu$g cold $^{188}\text{Re}$-18B7 served as controls. (b) RIT of C57BL6 mice infected i.v. with $10^6$ C. neoformans cells: CFUs in the brains and the lungs of RIT-treated and control mice. Mice were treated i.p. with either 100 $\mu$Ci $^{213}\text{Bi}$-18B7 24 h postinfection, 100 $\mu$Ci $^{188}\text{Re}$-18B7 24 h postinfection, or 100 $\mu$Ci $^{188}\text{Re}$-18B7 48 h postinfection or were left untreated and sacrificed 75 days posttreatment. The detection limit of the method was 50 CFUs. No CFUs were found in the brains and lungs of mice treated with 100 $\mu$Ci $^{213}\text{Bi}$-18B7, which are presented in the graph as 40 CFUs/organ. The asterisks show the groups in which the CFUs were significantly different from the untreated controls. (c) Comparison of RIT and amphotericin B efficacy toward melanized C. neoformans in vivo. CFUs in the lungs and brains of mice infected with melanized C. neoformans. A/JCr mice were infected i.v. with $3 \times 10^5$ C. neoformans cells and were given either 100 $\mu$Ci $^{213}\text{Bi}$-18B7 RIT or amphotericin B at 1 $\mu$g/g body weight on days 1, 2, and 3 postinfection or combined treatment or left untreated. The detection limit of the method was 50 CFUs. No CFUs were found in the brains and lungs of mice infected with melanized C. neoformans cells and treated with RIT, which are presented in the graph as 40 CFUs/organ. (d) Median survival of A/JCr mice infected i.v. with $5 \times 10^5$ C. neoformans and treated 24 h later with 150 $\mu$Ci $^{188}\text{Re}$-18B7 or 125 $\mu$Ci $^{213}\text{Bi}$-18B7 mAb. CNnaive, cells from ATCC; CNRe RIT, cells recovered from mice treated with $^{188}\text{Re}$-18B7 mAb; CNRe RIT, cells recovered from mice treated with $^{213}\text{Bi}$-18B7 mAb; Re RIT/CNnaive, mice infected with CNnaive and treated with $^{188}\text{Re}$-18B7; Bi RIT/CNnaive, mice infected with CNnaive and treated with $^{213}\text{Bi}$-18B7; Re RIT/CNRe RIT, mice infected with CNRe RIT and treated with $^{188}\text{Re}$-18B7; Bi RIT/CNRe RIT, mice infected with CNRe RIT and treated with $^{213}\text{Bi}$-18B7. (e, f) RIT with $^{213}\text{Bi}$-4E12 antibody to Hsp60: (e) RIT of C. albicans; (f) RIT of C. neoformans. Each experiment was performed three times, and the results shown are from one typical experiment. The CFUs for each antibody dose were plated in triplicate. doi:10.1128/microbiolspec.AID-0023-2014.f2
killing power of $^{213}$Bi-emitted alpha particles when compared to $^{188}$Re-emitted beta particles. In general, the interaction of fungal cells with particulate radiation resulted in the loss of the cells’ ability to divide (1, 12), which could provide an explanation for the non-emergence of radiation-resistant phenotypes post-RIT. The residual cells that replicated post-RIT were probably shielded from the antibodies that received radiation from a biofilm, an abscess, or a host cell.

Finally, in an effort to develop broader antifungal therapy that did not rely on pathogen-specific mAbs, we investigated the targeting of antigens shared by major invasive fungal infection-causing fungi (pan-fungal antigens) to deliver RIT without the need for specific mycological diagnosis or concerns about drug resistance. We explored the possibility of targeting common cell-wall-associated antigens, which also happen to be the dominate virulence factors for these fungal pathogens. The majority of fungal cells, in both yeast and hyphal forms, display beta-glucans on their cell surface. Cassone and colleagues generated a mAb to beta-glucans in Candida albicans, C. neoformans, and Aspergillus fumigatus in animal models (13–15). Heat shock protein 60 (Hsp60) is a major regulator of virulence in Histoplasma capsulatum, and mAbs directed to this protein are protective in murine histoplasmosis (16). We established that a mAb to H. capsulatum Hsp60 also bound other pathogenic fungal species but did not react with human Hsp60 (16). Melanin is present in the cell wall of diverse human fungal pathogens, and a mAb 6D2 to fungal melanin was shown to bind C. neoformans, H. capsulatum, Aspergillus spp., C. albicans, Scytalidium dimidiatum, Sporothrix schenckii, Paracoccidioides brasilensis, Coccioidoides posadassi, and Blastomyces dermatitidis (17). To explore the feasibility of using RIT to target these pan-antigens we utilized mAbs 4E12, an IgG2a to fungal HSP60; 2G8, an IgG 2b to beta-(1,3)-glucan; and 6D2, an IgM to melanin, and radiolabeled them with $^{213}$Bi (18). C. neoformans and C. albicans were used to evaluate the cytocidal effects of these radiolabeled mAbs. $^{213}$Bi-labeled mAbs to HSP60 (Fig. 2e,f) and to the beta-(1,3)-glucan each decreased the viability of both fungi in the 80 to 100% range. The $^{213}$Bi-6D2 mAb to melanin eliminated 50% of C. neoformans cells but did not kill C. albicans. Treatment with unlabeled mAbs and with radiolabeled isotype-matching control mAbs resulted in no killing. These results point to the possibility of developing RIT against fungal pathogens by targeting shared fungal antigens. This approach could be utilized against fungal infections for which current therapies are not working well.

**FIGURE 3** RIT of bacterial infections. (a) S. pneumoniae, $^{213}$Bi-labeled mAbs in C57BL/6 mice. Mice were infected i.p. with 1,000 organisms 1 h before treatment with mAbs. (b) RIT of B. anthracis Sterne infection with $^{213}$Bi-labeled mAbs. Mice were infected 1 h prior to labeled-mAb treatment. Survival experiment was repeated three times with similar results. Controls include unlabeled mAbs given in the same amounts (15 μg) as radiolabeled mAbs. doi:10.1128/microbiolspec.AID-0023-2014.f3
tion could be the absence of a target for the irrelevant radiolabeled mAb to bind to, which resulted in an excessive dose of radiation to the blood-rich dose-limiting organs such as bone marrow. Mice in control groups died from bacteremia on days 1 to 3, while 87 to 100% of mice given 80 μCi $^{213}$Bi-D11 survived. Measuring CFUs in the blood of the RIT-treated mice demonstrated that they were not bacteremic at 3, 6, and 10 h post-treatment or on days 3 and 14. RIT with radiolabeled D11 did not cause any weight loss in treated animals. In summary, this proof of principle study was the first to demonstrate the ability of RIT to treat experimental bacterial infection.

Later we investigated RIT for experimental Bacillus anthracis infection. B. anthracis is a potential agent for bioterrorism and biological weapons, which underscores the necessity for additional, different mode of action therapies for anthrax (20). The surface expression of toxins on bacterial cells was demonstrated by indirect immunofluorescence experiments with mAbs to protective antigen (7.5G γ2b and 10F4 γ1) and lethal factor (mAb 14FA γ2b). Scatchard analysis of mAbs binding to the bacterial surface demonstrated high binding constants and multiple binding sites on the surface of bacteria, which provided the impetus for RIT studies. The mAbs to the toxins were radiolabeled with either $^{188}$Re or $^{213}$Bi for investigating the microbicidal potential of RIT. $^{213}$Bi-labeled mAbs were more efficient in vitro than $^{188}$Re-labeled mAbs in killing B. anthracis Sterne bacterial cells. Giving i.p. $^{213}$Bi-labeled mAbs 10F4 and 14FA to A/JCr mice lethally infected with B. anthracis cells significantly prolonged their survival (Fig. 3b). Our results point to RIT's utility in treating experimental anthrax infection with mAbs targeting B. anthracis tri-partite toxin components and suggest that toxigenic bacteria may be targeted with radiolabeled mAbs to its toxins.

Finally, we recently investigated the potential of RIT against germinating B. anthracis spores (21). B. anthracis spores are covered by an impenetrable two-layered exosporium, which is composed of a basal layer and an external hair-like nap. The nap consists of filaments, which in turn are composed of trimers of a collagen-like glycoprotein BclA. BclA is considered to be an immunodominant antigen on the spore surface. The antibodies to BclA are highly specific and can specifically identify B. anthracis spores among the spores produced by other Bacillus species. We investigated whether EA2-1 mAb to BclA armed with $^{213}$Bi would be capable of sterilizing B. anthracis spores. We chose an alpha-emitter, $^{213}$Bi for this study, as this radionuclide was successfully used in our previous research on RIT for bacterial pathogens such as S. pneumoniae and B. anthracis.

First, we confirmed the previous reports that the spores were completely resistant to the external gamma radiation. Our initial RIT experiments demonstrated that dormant spores were not killed by $^{213}$Bi-EA2-1 mAb either. Only when the dose of $^{213}$Bi-EA2-1 mAb reached 300 μCi was the significant spore killing observed. However, this killing was not mAb-specific, as the isotype control mAb labeled with 300 μCi $^{213}$Bi showed the same results. Our next step was to examine the effects of RIT on the germinating versus dormant spores. The reasoning for that was that the spores become pathogenic in a host when they start germinating and dividing, which leads to the development of anthrax disease. In addition, it is known from classical radiobiology that dividing cells are much more susceptible to ionizing radiation damage than cells that are not dividing. Thus, the germinating spores might present a better target for RIT. The experiments showed that 75 and 150 μCi $^{213}$Bi-EA2-1 killed significant numbers of germinating spores, while the matching activities of the isotype matching control mAb did not. We concluded from this study that while dormant spores are resistant to both external radiation and RIT, the germinating spores are RIT-susceptible, and this line of study should be investigated further, possibly in animal models.

HIV

The HIV epidemic remains a major worldwide health problem. Highly active antiretroviral therapy (HAART), a combination of drugs that inhibits enzymes essential for HIV replication, can decrease the viremia to almost undetectable levels and decrease the likelihood of opportunistic infections in the majority of patients. As a result, patients on HAART now survive for decades. However, HAART regimens are complex and require lifelong use, and many have significant long-term side effects such as metabolic syndrome, cardiotoxicity, etc. A replication-competent virus that “hides” in latently infected cells serves as a source of viremia that emerges rapidly after the discontinuation of HAART. A modality that specifically targets and eliminates HIV-infected cells in patients on HAART could be a major contributor toward the eradication of persistent HIV cellular reservoirs. We hypothesized that RIT could be able to kill virally infected cells, RIT for viral diseases would target viral antigens on infected cells and consequently would provide a completely different approach for treating HIV.
Initially, we studied the efficacy of RIT against HIV infection in SCID mice using an HIV envelope-specific human mAb 246-D to gp41 that we radiolabeled with $^{213}$Bi or $^{188}$Re (22). For these experiments human peripheral blood mononuclear cells (hPBMCs) were infected with HIV-1JR-CSF and injected intrasplenically into SCID mice, and radiolabeled mAbs were given i.p. 1 h later. The mice were sacrificed 72 h after RIT, and the presence of the residual HIV-infected cells was established by quantitative coculture (23). This time interval was chosen to provide enough time for the $^{188}$Re-radiolabel (its physical half-life is 16.9 h) on the mAb to deliver a lethal cytotoxic dose to the infected cells. Administration of $^{188}$Re-armed 246-D mAb before or after intrasplenic injection with HIV-infected hPBMCs significantly decreased the numbers of HIV-infected cells in mice (Fig. 4a). Similar results were obtained with $^{213}$Bi-246-D (Fig. 4a). In contrast, control SCID mice that received equivalent amounts of “cold” mAb 246-D or a radiolabeled isotype-matched control mAb showed no reduction in the average number of infected cells detected in their spleens. These results established the feasibility of using RIT to specifically target and eliminate HIV-infected hPBMCs in vivo and provided a first experimental proof for the concept of fighting viral infections by targeting virally infected cells with the radioactively armed mAbs to viral antigens. We anticipate that the same approach could be useful for treatment of other chronic viral infections, e.g., hepatitis C (24).

It should be noted that the antibodies used in RIT do not neutralize the virus and consequently are not expected to exert selective pressure on the virus. Only the epitopes on the viral proteins that are conserved throughout all HIV strains and clades, which is consistent with their role in the maintenance of envelope protein structure, are chosen as targets for RIT. As a result, even in case of a mutation, such epitopes will probably be present on mutated viral particles and, as a result, on HIV-infected cells. In this regard RIT has certain advantages over immunotoxins to eradicate infected cells. In immunotoxins a mAb is conjugated to immunogenic toxin and thus can elicit an immune response, while in RIT no responses to radiolabeled human mAbs have been observed. RIT is a highly versatile modality due to the availability of the radionuclides with various emissions and decay schemes. Recently we identified a human mAb 2556 as a superb reagent for the development of an RIT-based HIV elimination strategy.

**FIGURE 4** RIT of SCID mice injected intrasplenically with JR-CSF HIV-infected human PBMCs and treated with $^{188}$Re- and $^{213}$Bi-labeled human anti-gp41 mAbs 246-D (a) or 2556 (b). (a) Limiting coculture results for 246-D mAb. Mice received either 20 μg cold anti-gp41 mAb 246-D, 100 μCi (20 μg) $^{213}$Bi-1418 or 80 μCi (20 μg) $^{188}$Re-1418 as isotype-matching controls, 80 μCi (20 μg) $^{188}$Re-246-D, or 100 μCi (20 μg) $^{213}$Bi-246-D i.p. 1 h after injection of PBMCs. In some experiments mice were given 80 μCi (20 μg) $^{188}$Re-246-D i.p. 1 h prior to injection of HIV-infected PBMCs. (b) PCR data for RIT with 50, 100, and 200 μCi $^{213}$Bi-2556 mAb. The cold 2556, untreated mice, and matching activities of the irrelevant 1418 mAb were used as controls. doi:10.1128/microbiolspec.AID-0023-2014.f4
mAb 2556 is a human mAb to a conserved domain of HIV gp41 glycoprotein and was able to outperform the endogenous antibodies in HIV-positive serum for binding to gp41. This latter quality is very important because it means that the antibody target site is not likely to be obscured by endogenous antibodies produced by infected individuals.

To investigate the feasibility of killing HIV-infected cells with radiolabeled 2556 mAb in vivo we used two HIV mouse models—a splenic model (22) and the Mosier model (26). As in the previous study (22), human mAb 1418 (IgG1) to parvovirus B19 (27) was used as an irrelevant isotype-matched control. As in our previous study, HIV-infected hPBMCs were given intrasplenically to SCID mice. After 1.5 h, these mice were given a single injection of $^{213}$Bi-2556 or control mAbs. The radioactivity doses were 50, 100, and 200 μCi per animal (1 mg of mAb 2556/kg body weight). Three days following treatment, the mice were sacrificed, their spleens were harvested, and the viral load was determined by real-time PCR for HIV-1 DNA. The results demonstrated that $^{213}$Bi-2556 killed HIV-infected hPBMCs much more effectively than isotype-matching control mAb armed with the same amounts of radioactivity or cold mAb 2556 (Fig. 4b). To investigate the potential bone marrow toxicity of RIT, the peripheral blood of treated mice was analyzed for platelet counts. A drop in platelet numbers would be evidence of an undesirable effect of the radiolabeled mAb on the bone marrow, indicating hematologic toxicity (28). There was no difference in platelet counts between RIT-treated and control mice, consistent with no significant acute hematologic toxicity.

Subsequently $^{213}$Bi-2556 was further evaluated in the SCID mouse model described by Mosier (26). The Mosier model involves i.p. implantation of hPBMCs into SCID mice, resulting in activation of T cells, thus providing a cellular population easily infected by HIV and mimicking the widespread lymphocyte activation observed in chronic HIV infection. HIV infection of humoral SCID mice led to the loss of CD4+ T cells, as also seen as a result of HIV infection in human hosts. The Mosier model has been broadly utilized in evaluating the efficacy of antiviral drugs (29, 30). Similar to the splenic model, RIT led to a several log reduction in viral load in groups treated with 25, 50, and 100 μCi $^{213}$Bi-2556 relative to controls ($P < 0.05$). These results are encouraging for further development of RIT as a backbone strategy for HIV eradication. We are currently planning a pilot clinical trial of RIT in HIV-infected patients on HAART.

CONCLUSIONS

The foreign nature of microbial cells results in their display of antigens that are not found anywhere in the human body, making RIT for infectious diseases more specific than RIT for cancer, since tumor-associated antigens are also sometimes found on normal tissues. As a consequence, the specificity of RIT for infections at least theoretically should be much more pronounced than in cancer, given higher selectivity and specificity for target cells. This exquisite specificity will lead to precise targeting, which in turn should translate into a highly efficacious treatment not accompanied by side effects. Additionally, the technology for linking radionuclides to mAbs is well established, so the lessons learned in the development of RIT in oncology could be readily applied to infectious diseases. Also, U.S. hospitals that are now regularly using RIT to treat cancer patients are fully equipped for initiating infectious disease RIT since the two use the same approach and differ only in the type of antibody used. An added bonus that would occur with some isotopes is the possibility for imaging patients receiving RIT to ascertain the targeting of radiolabeled mAbs to tissue and the anatomical extent of infection. We believe a combination of need together with the presence of a mature technology means that the time is ripe for deploying RIT into the clinic to combat infectious diseases.

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