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Radiolabeled antibodies for therapy of infectious diseases

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Abstract

Novel approaches to 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, which utilizes radiolabeled monoclonal antibodies (mAbs). 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 were performed in vitro. The armamentarium of pan-antibodies would result in reducing the dependence on microorganism-specific antibodies and thus would speed up the development of RIT of infections. We believe that the time is ripe for deploying RIT into the clinic to combat infectious diseases.

INTRODUCTION

There is a growing need for alternatives to conventional antibiotics for 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 (IFI) 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 transplants recipients.

Radioimmunotherapy (RIT) is based on the interaction between the pair of antigen-antibody to carry the 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 mAbs Zevalin® and Bexxar®, and it offers several significant advantages over naked antibody strategies. These include: 1) RIT delivers lethal radiation, such that it does not merely interfere with a single cellular pathway but

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leads to physical destruction of targeted cells via radiation induced apoptosis/autophagy/necrosis; 2) RIT is not subject to drug resistance mechanisms such as efflux through drug efflux pumps in malignant cells; 3) The effectiveness of RIT does not depend on the immunological status of the host; 4) 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; 5) RIT permits single dose or a limited number of doses to combat infections in contrast to weeks or months of standard antimicrobial therapies.

A decade ago we suggested the use of RIT for treatment of fungal pathogen *Cryptococcus neoformans* (CN) [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 pre-clinical development of RIT for infectious diseases.

FUNGAL INFECTIONS

We started with the investigation of RIT potency against *C. neoformans* (CN) fungal infection [1]. CN results in life threatening meningoencephalitis which affects people with the 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 CN antigens provided an impetus to use this pathogen for investigating RIT of infections. Importantly, immunotherapy in patients with CN with the capsule polysaccharide-binding antibody 18B7 has been already evaluated clinically [3]. Therapeutic studies employed AJ/Cr mice infected systemically with CN. A/JCr mice succumb to the systemic infection with CN, most likely because of the partial complement deficiency [4]. The survival of mice treated with radiolabeled CN-specific mAb 18B7 was significantly longer than the survival of mice treated with irrelevant labeled IgG1 or PBS. We utilized a radiolabeled irrelevant mAb (^{213}Bi - or ^{188}Re -IgG₁ MOPC21) to take into account a possibility of the radiolabeled IgG binding to Fc receptors on phagocytic cells present at the infected site which might lead to killing non-specifically of some CN cells. Interestingly, treatment with 100 μCi ^{213}Bi -18B7 resulted in 60% of mice in ^{213}Bi group surviving on day 75 post therapy ($P < 0.05$). In the ^{188}Re group 40% and 20% of animals were alive after treatment with 100 ($P < 0.005$) and 50 μCi ($P < 0.05$) ^{188}Re -18B7, respectively. In contrast, mice in control groups died from CN infection on days 35–40 (Fig. 2a). Administration of RIT resulted in significant reduction of fungal burden in lungs and brains of mice with CN 48 hrs 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 ^{188}Re -18B7. In contrast, the administration of 200 μCi ^{188}Re -18B7 lead 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 lead 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 the 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 IV via tail vein with 10^6 CN cells and were left either untreated or treated IP with: 100 μ Ci ^{213}Bi -18B7 24 hr post-infection, 100 μ Ci ^{188}Re -18B7 24 hr post-infection, or 100 μ Ci ^{188}Re -18B7 48 hrs post-infection [5]. Administration of ^{188}Re -18B7 mAb 24 hr post-infection resulted in one log reduction in the lungs CFUs ($P=0.04$) and while no reduction in CFUs was observed in the brains ($P=0.07$) (Fig. 2b). Treatment with ^{213}Bi -18B7 mAb 24 hr post-infection 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 GMS staining. Interestingly, for established infection at 48 hrs ^{188}Re -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 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 anti-fungal drug [7]. AJ/Cr mice were infected IV with 3×10^5 melanized or non-melanized CN cells. Twenty four hrs after infection the mice were given IP either: 100 μ Ci ^{213}Bi -18B7; or given amphotericin in its deoxycholate form at 1 μ g/g body weight at 24, 48, and 72 h; or given both treatments, or left untreated. Mice survival was observed for 60 days. The lungs and brains were analyzed at 60 days post infection. This analysis demonstrated that treatment with amphotericin did not significantly decrease lungs and brains CFUs in either non-melanized or melanized CN groups (Fig. 2c) ($p>0.05$). RIT administration resulted in noted decrease in fungal burden in comparison with the untreated controls or amphotericin-treated mice ($p<<0.05$). Importantly, the fungus was practically cleared from the brains of the RIT-treated non-melanized CN group (sensitivity of the detection was 50 CFUs) while in melanized CN 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 μ g/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 post treatment was provided by the subsequent amphotericin study showing a trend towards decrease in CFUs in brains and lungs which manifested itself only on day 14th of treatment with amphotericin B. Our results are in concordance with the published data showing that amphotericin was able to produce only a 1–1.5 log reduction in CFUs in immunocompetent mice such as CD-1 and Balb/c and all mice succumbed to CN infection around day 24 [8, 9]. Our results also agree

with the clinical reports which showed that a short course of amphotericin was not able to sterilize cerebrospinal fluid or blood of patients and which also correlated the rate of sterilization with the patients survival [10]. Our observations underline the advantages of RIT which has pronounced anti-microbial effects in vivo just after one injection when compared to long and often not effective treatments with amphotericin which has long lasting side effects.

Despite radiation being a weak mutagen, an opinion that even low doses of ionizing radiation are able to create potentially dangerous cellular mutants persists within scientific community and among the lay public alike. To evaluate the possibility that RIT might select for the radiation resistance in CN cells in vivo, AJ/Cr mice were infected with CN cells recovered from mice treated previously with ^{188}Re -18B7 mAb (CN_{Re-RIT}), or with ^{213}Bi -18B7 mAb (CN_{Bi-RIT}), or with the RIT-naïve CN cells (CN_{naïve}) [11]. We treated CN-infected mice with 150 μCi ^{188}Re -18B7 or 125 μCi ^{213}Bi -18B7 24 hrs after IV infection, and then observed the mice for survival and weight loss. The number of deaths in mice infected with CN_{Re-RIT} or CN_{Bi-RIT} was the similar to that in mice infected with CN_{naïve} ($P>0.05$) (Fig. 2d). Mice given ^{213}Bi -18B7 mAb survived longer ($P=0.04$) than those given ^{188}Re -18B7 (Fig. 2d), most likely due to the higher 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 most probably shielded from the antibodies delivered radiation by 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 IFI-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 dominating 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 CN, *H. capsulatum*, *Aspergillus spp.*, *C. albicans*, *Scytalidium dimidiatum*, *Sporothrix schenckii*, *Paracoccidioides brasiliensis*, *Coccidioides posadasii*, 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 cytotoxic 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–

100% range. The ^{213}Bi -6D2 mAb to melanin eliminated 50% CN 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 out 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.

BACTERIAL INFECTIONS

Streptococcus pneumoniae (Pn), an important cause of community-acquired pneumonia, meningitis, and bacteremia, was selected for evaluating the feasibility of RIT against bacterial diseases [19]. A human mAb D11 which binds to pneumococcal capsular polysaccharide 8 (PPS 8) was chosen as a delivery vehicle for RIT and was radiolabeled with a short-range alpha-emitter ^{213}Bi . The RIT administration resulted in a higher percentage of mice surviving in the ^{213}Bi -D11-treated group compared to the untreated group ($P<0.01$) (Fig. 3a). On the contrary, giving to mice 5 μg unlabeled D11 mAb did not lead to the prolongation in survival in comparison to untreated mice ($P>0.05$). Radiolabeled irrelevant IgM also did not show any therapeutic results ($P>0.05$) and, on the contrary, less mice survived its administration when compared to the untreated group. Possible explanation could be the absence of target for the irrelevant radiolabeled mAb to bind which resulted in excessive dose of radiation to the blood rich dose limiting organs such as bone marrow. Mice in control groups died from bacteremia on Days 1–3, while 87–100% of mice given 80 μCi ^{213}Bi -D11 survived. Measuring CFU's in the blood of the RIT treated mice demonstrated that they were not bacteremic at 3, 6 and 10 h post-treatment as well as 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 of 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 (IF) experiments with mAbs to protective antigen (PA; 7.5G $\gamma 2\text{b}$ and 10F4 $\gamma 1$) and lethal factor (LF; mAb 14FA $\gamma 2\text{b}$). 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 investigation 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 IP ^{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 the RIT 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, very recently we have 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 the filaments which in their 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 have chosen an alpha-emitter ^{213}Bi for this study as this radionuclide was successfully used in our previous research on RIT of 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 – the significant spore killing was 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 RIT effects 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 the classical radiobiology that the dividing cells are much more susceptible to ionizing radiation damage than the cells which 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 direction should be investigated further, possible in animal models.

HIV

The HIV epidemic remains a major world-wide 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, the patients on HAART now survive for decades. However, HAART regimens are complex, require life-long use and many have significant long term side effects such as metabolic syndrome, cardiotoxicity etc. 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 towards 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 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 which we radiolabeled with ^{213}Bi or ^{188}Re [22]. For these experiments human peripheral blood mononuclear cells (hPBMCs) were infected with HIV-1_{JR-CSF}, injected intrasplenically into SCID mice and radiolabeled mAbs were given intraperitoneally 1 h later. The mice were sacrificed 72 h after RIT and the presence of the residual HIV-infected cells was established by quantitative co-culture [23].

This time interval was chosen to provide enough time for the ^{188}Re radiolabel (its physical half-life is 16.9 hrs) 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 very 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 mice that received equivalent amounts of "cold" mAb 246-D or of a radiolabeled isotype-matched control mAb showed no reduction in the average number of infected cells detected in the spleens of SCID mice. 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 which 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 immune response, while in RIT no responses to radiolabeled human mAbs have been observed. RIT is 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 a RIT-based HIV elimination strategy [25]. 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 cell 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 to SCID mice intrasplenically. 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 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 potential bone marrow toxicity of RIT the peripheral blood of treated mice was analyzed for platelet counts. A drop in platelet numbers would be an evidence for 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]. Mosier model involves implantation of hPBMCs into SCID mice via intraperitoneal (IP) route 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 hu-PBL SCID mice led to the loss of CD4+ T cells, as it is also seen as a result of HIV infection in human hosts. Mosier model has been broadly utilized in evaluating efficacy of anti-viral 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 which are not found anywhere in a human body thus 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 of 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 its turn should translate into the highly efficacious treatment not accompanied by the 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, the US hospitals that are now regularly using RIT to treating cancer patients are fully equipped for initiating infectious diseases 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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Glossary

Radioimmunotherapy (RIT)

is a modality based on the interaction between the pair of antigen-antibody to carry the cytotoxic amounts of ionizing radiation to the vicinity of specific cellular targets.

Alpha particles emitting radionuclides

radionuclides emitting Helium atoms as a result of the radioactive decay.

Beta particles emitting radionuclides

radionuclides emitting high energy electrons as a result of the radioactive decay.

Virally infected cells

cells in which incorporation of the viral genome into the host genome results in the viral production and/or expression of viral antigens on the surface of the infected cell.

Immunotoxin

therapeutic modality wherein the bacterial or other toxins are attached to the antibody specific for a particular cellular target with the purpose of killing the targeted cell. The antibody carrier should be internalizing to deliver the toxic cargo inside the targeted cell.

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Fig. 1. Mechanisms of RIT efficacy against infections: a) direct targeting of microbial cells with the radiolabeled organism-specific antibodies; b) killing of virally-infected host cells by targeting viral antigens expressed on the surface of infected cells.

Fig. 2.

RIT of experimental fungal infections with ^{213}Bi - and ^{188}Re -labeled mAbs: a) Kaplan-Meier survival curves for A/JCr mice infected IV with 10^5 *C. neoformans* cells 24 hr prior to treatment with 50–200 μCi ^{188}Re -labeled mAbs. Animals injected with PBS or 50 μg "cold" 18B7 served as controls; b) RIT of C57BL/6 mice infected IV with 10^6 CN cells: CFUs in the brains and the lungs of RIT-treated and control mice. Mice were treated IP with either: 100 μCi ^{213}Bi -18B7 24 hr post-infection; 100 μCi ^{188}Re -18B7 24 hr post-infection; 100 μCi ^{188}Re -18B7 48 hrs post-infection; or left untreated and sacrificed 75 days post-treatment. Detection limit of the method was 50 CFUs. No CFUs were found in the brains and lungs of mice treated with 100 μCi ^{213}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 towards melanized CN in vivo. CFUs in the lungs and brains of mice infected with melanized CN. A/JCr mice were infected IV with 3×10^5 CN cells and 24 hr later either given 100 μCi ^{213}Bi -18B7 RIT or amphotericin B at 1 $\mu\text{g}/\text{g}$ body weight on Days 1, 2 and 3 post-

infection or combined treatment or left untreated. Detection limit of the method was 50 CFUs. No CFUs were found in the brains and lungs of mice infected with melanized CN cells and treated with RIT which are presented in the graph as 40 CFUs/organ; d) median survival of AJ/Cr mice infected IV with 5×10^4 CN and treated 24 hrs later with 150 μCi ^{188}Re -18B7 or 125 μCi ^{213}Bi -18B7 mAb; CN_{naive} - cells from ATCC; CN_{passaged} - cells recovered from untreated AJ/Cr mice; CN_{Re RIT} - cells recovered from mice treated with ^{188}Re -18B7 mAb; CN_{Bi RIT} - cells recovered from mice treated with ^{213}Bi -18B7 mAb; Re RIT/ CN_{naive} - mice infected with CN_{naive} and treated with ^{188}Re -18B7; Bi RIT/ CN_{naive} - mice infected with CN_{naive} and treated with ^{213}Bi -18B7; Re RIT/CN_{Re RIT} - mice infected with CN_{Re RIT} and treated with ^{188}Re -18B7; Bi RIT/CN_{Bi RIT} - mice infected with CN_{Bi RIT} and treated with ^{213}Bi -18B7; (e, f) RIT with ^{213}Bi -4E12 antibody to Hsp60: e) RIT of *C. albicans*; f) RIT of *C. neoformans*. Each experiment was performed three times, the results shown are from one typical experiment, the CFUs for each antibody dose were plated in triplicate.

Fig. 3. RIT of bacterial infections: a) *S. pneumoniae*, ^{213}Bi -labeled mAbs in C57BL/6 mice. Mice were infected IP with 1,000 organisms 1 hr 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 3 times with similar results. Controls include unlabeled mAbs given in the same amounts (15 μ g) as radiolabeled mAbs.

Fig. 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) 213Bi-1418 or 80 μCi (20 μg) 188Re-1418 as isotype-matching controls, 80 μCi (20 μg) 188Re-246-D, or 100 μCi (20 μg) 213Bi-246-D IP 1 hour after injection of PBMCs. In some experiments mice were given 80 μCi (20 μg) 188Re-246-D IP 1 h prior to injection of HIV-infected PBMCs; b) PCR data for RIT with 50, 100 and 200 μCi 213Bi-2556 mAb. The cold 2556, untreated mice and matching activities of the irrelevant 1418 mAb were used as controls.