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Radioimmunotherapy of Infectious Diseases

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The need for novel approaches to treat infectious diseases is obvious and urgent. This situation has renewed interest in the use of monoclonal antibodies (mAbs) to treat infectious diseases. During the last 5 years, radioimmunotherapy (RIT), a modality developed for cancer treatment, has been successfully adapted for the treatment of experimental fungal (*C. neoformans* and *H. capsulatum*), bacterial (*S. pneumoniae* and *B. anthracis*), and viral (HIV-1) infections. RIT produced none or only transient hematological toxicity in experimental animals. Investigation of radiobiological mechanisms of RIT of infections showed that microbial cells are killed by both "direct-hit" and "cross-fire" radiation. mAbs radiolabeled with either alpha- or beta-emitters stimulated apoptosis-like cell death, whereas only mAbs radiolabeled with alpha-emitter ^{213}Bi also decreased the metabolic activity of microbial cells. The success of this approach in laboratory studies, combined with earlier nuclear medicine experience in preclinical and clinical studies using radiolabeled organism-specific antibodies for imaging of infections, provides encouragement for the feasibility of therapeutically targeting microbes with labeled antibodies. We envision that first the organism-specific mAbs will be radiolabeled with imaging radionuclides such as ^{99m}Tc or ^{111}In to localize the sites of infection with single-photon emission computed tomography, followed by RIT with ^{188}Re- or ^{90}Y-labeled mAb, respectively. Also, immuno-position emission tomogrpahy might be used to image infection before treatment if such positron-emitting radionuclides as ^{86}Y (matching pair for ^{90}Y) or ^{124}I (matching pair for ^{131}I) are available. It might be possible to create a so-called "pan-antibody" that would recognize an antigen shared by a particular class of human pathogens such as fungi, for example. The availability of such antibodies would eliminate the necessity of having antibodies specific for each particular microorganism and would enormously enhance the development of RIT of infectious diseases.

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The need for novel approaches to treat infectious diseases is obvious and urgent. Currently available antibiotics have become less effective as microbes have increasingly developed resistance. In recent decades, the problem has been compounded by the emergence of many new infectious diseases, such as HIV. Simultaneously, the population of patients in whom current antimicrobial therapies are not effective because of their low immune status is expanding. These populations include HIV-infected individuals, cancer patients undergoing chemotherapy, and recipients of organ transplants. In addition, there is a threat of biological agents specifically engineered to be lethal even in immunocompetent populations.

This situation has renewed interest in the use of monoclonal antibodies (mAbs) to treat infectious diseases.[1] During the last 5 years, radioimmunotherapy (RIT), a modality developed for cancer treatment, has been successfully adapted for the treatment of experimental fungal, bacterial, and viral infections.[2-6] The success of this approach in laboratory studies, combined with earlier nuclear medicine experience on preclinical and clinical studies showing the utility of radiolabeled organism-specific antibodies for imaging of infections (reviewed in Dadachova and Casadevall[7]), provides encouragement for feasibility of therapeutically targeting microbes with labeled antibodies. In fact, the ability of a specific antibody to localize to a site of infection indicates the feasibility of using the antibody–antigen interaction to deliver microbici-

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dal radiation to sites of infection, which in turn provides strong support for the potential usefulness of this technique as a broad antimicrobial strategy.

Surprisingly, the concept of delivering microbicidal radiation by the use of highly specific microbe-targeted mAbs was not attempted until very recently. In contrast, efforts have focused on the use of radiolabeled antimicrobial peptides, chemotactic cytokines, leukotriene b4 antagonists, bacteriophages, chitinase, and fluconazole to distinguish sterile inflammation from infection and fungal infections, which are notoriously difficult to diagnose, from bacterial infections.[8-13] In addition, the use of fanolesomab, a murine immunoglobulin M (IgM) monoclonal antibody to CD15 labeled with ^{99m}Tc that colocalizes with human polymorphonuclear neutrophils at the sites of infection, was evaluated in healthy volunteers and in patients for imaging of infection and inflammation.[14,15] Here, we will summarize the therapeutic efficacy of RIT in treating infections, its toxicity, and its radiobiological mechanisms as well as outline future perspectives for combining RIT of infections with modern imaging techniques such as single-photon emission computed tomography (SPECT) and positron emission tomography.

Efficacy of RIT in Treating Infections

RIT and Fungal Infections

We initially explored the potential efficacy of RIT against an experimental fungal infection by using *Cryptococcus neoformans* (CN).[2] CN is a major fungal pathogen that causes life-threatening meningoencephalitis in 6% to 8% of patients with AIDS. Cryptococcal infections in immunocompromised patients are often incurable because antifungal drugs do not eradicate the infection in the setting of severe immune dysfunction.[16,17] CN provided a good system to study the potential usefulness of RIT because there are excellent animal models available, well-characterized mAbs to CN antigens exist, and immunotherapy of CN infection with capsule polysaccharide-binding antibody 18B7 is already in clinical evaluation.[18] Therapeutic studies used AJ/Cr mice infected systemically with CN. Mice treated with radiolabeled CN-specific mAb 18B7 lived significantly longer than mice given irrelevant labeled IgG1 or PBS. We used a labeled irrelevant mAb (^{213}Bi- or ^{188}Re-labeled IgG$_1$ MOPC21) to control for the possibility that Fc receptor binding by the radiolabeled IgG to phagocytes at the site of infection might result in nonspecific killing of CN cells. Remarkably, 60% of mice in ^{213}Bi group were alive after treatment with 100 μCi (3.7 MBq) of ^{213}Bi-18B7 on day 75 after therapy ($P < 0.05$). In the ^{188}Re group, 40% and 20% of animals were alive after treatment with 100 (3.7 MBq; $P < 0.005$) and 50 μCi (1.85 MBq; $P < 0.05$) of ^{188}Re-18B7, respectively, whereas mice in the control groups died as the result of infection on days 35 to 40 (Fig. 1A).

Mice infected with CN and given RIT had significantly reduced fungal burden in the lungs and brain 48 hours after treatment when compared with control groups. Although there was no difference in the reduction of the fungal burden

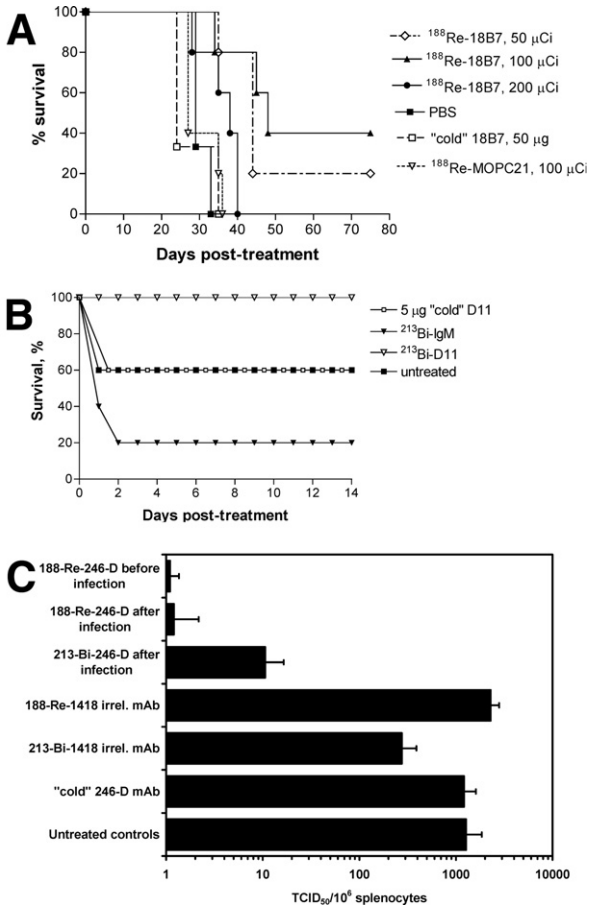

Figure 1 Radioimmunotherapy of experimental fungal, bacterial, and viral infections with ^{213}Bi- and ^{188}Re-labeled mAbs. (A) Kaplan-Meier survival curves for A/JCr mice infected IV with 10^5 *C. neoformans* cells 24 hours before treatment with 50 to 200 μCi of ^{188}Re-labeled mAbs. Animals injected with phosphate-buffered saline or 50 μg of "cold" 18B7 served as control animals. (B) RIT of *S. pneumoniae* infection with ^{213}Bi-labeled mAbs in C57BL/6 mice. Mice were infected IP with 1000 organisms 1 hour before treatment with mAbs. (C) RIT of SCID mice injected intrasplenically with JR-CSF-infected human PBMCs and treated with ^{188}Re- and ^{213}Bi-labeled human antigp41 mAb 246-D. Mice received either 20 μg of "cold" antigp41 mAb 246-D, 100 μCi (20 μg) of ^{213}Bi-1418 or 80 μCi (20 μg) of ^{188}Re-1418 as isotype-matching controls, 80 μCi (20 μg) of ^{188}Re-246-D, or 100 μCi (20 μg) of ^{213}Bi-246-D IP 1 hour after injection of PBMCs. In some experiments, mice were given 80 μCi (20 μg) of ^{188}Re-246-D IP 1 hour before injection of PBMCs.

in the lungs between the groups that received 50 and 100 μCi of ^{188}Re-18B7 (1.85 and 3.7 MBq, respectively), treatment with 200 μCi of ^{188}Re-18B7 (7.4 MBq) significantly lowered lung colony-forming units (CFUs) relative to the lower activities ($P < 0.05$). Hence, the administration of a radiolabeled antibody to CN polysaccharide prolonged survival and reduced organ fungal burden in infected mice.

When RIT dose dependence was investigated, the survival of A/JCr mice was dose dependent for both ^{213}Bi and ^{188}Re radioisotopes. Whereas 50 μCi (1.85 MBq) of ^{213}Bi-18B7 produced no therapeutic effect, both the 100-μCi and 200-μCi (3.7 and 7.4 MBq, respectively) doses prolonged animal

survival. Interestingly, the 200-μCi (7.4 MBq) dose of ^{213}Bi-18B7 was less efficient, possibly because it may have approached the maximum tolerated activity for this particular combination of antibody and radioisotope. In the ^{188}Re group, administration of 50 μCi (1.85 MBq) of ^{188}Re-18B7 resulted in some prolongation of survival, 100 μCi (3.7 MBq) caused significant prolongation, and 200 μCi (7.4 MBq) dose was, apparently, too toxic, with all animals dying by day 40.

The antimicrobial RIT approach was subsequently explored with another human pathogenic fungus, *Histoplasma capsulatum* (HC),[3] which is the most common cause of fungal pneumonia in immunocompromised patients.[19] HC was treated in vitro with ^{188}Re-labeled mAb 9C7 (IgM), which binds to a 17-kDa protein antigen on the surface of the HC cell wall.[20] Ninety percent of HC cells were killed with 32 μCi (1.18 MBq) of HC-specific ^{188}Re-9C7 mAb. In contrast, incubation of HC with a radiolabeled control IgM with the same specific activity produced only minimal killing within the investigated range of doses ($P < 0.01$). We also performed cellular dosimetry calculations for in vitro RIT of CN and HC[3] and compared them with the LD_{90} for external gamma radiation. Cellular dosimetry calculations showed that RIT was ~1000-fold more efficient in killing CN and ~100-fold in killing HC than gamma radiation. Thus, RIT of fungal cells with specific antibodies labeled with alpha- and beta-emitting radioisotopes was significantly more efficient in killing CN and HC than gamma radiation when based on the mean absorbed dose to the cell. These results strongly support the promise of RIT as an antifungal modality.

We also evaluated the efficacy of RIT against fungal biofilms.[4] The use of indwelling medical devices—that is, pacemakers, prosthetic joints, catheters—is rapidly growing and is often complicated by infections with biofilm-forming microbes that are resistant to antimicrobial agents and host defense mechanisms. We investigated the use of polysaccharide-specific mAbs as delivery vehicles for targeting *C. neoformans* biofilms with ^{213}Bi. ^{213}Bi-18B7 mAb (IgG1) penetrated cryptococcal biofilms, as shown by confocal microscopy, and caused a 50% reduction in biofilm metabolic activity. In contrast, when the IgM mAb 13F1 labeled with ^{213}Bi was used, there was no penetration of the fungal biofilm and no damage. Unlabeled 18B7, ^{213}Bi-labeled nonspecific mAbs, and gamma and beta types of radiation did not have an effect on biofilms. The lack of efficacy of gamma and beta radiation probably reflects the radioprotective properties of polysaccharide biofilm matrix. Our results indicate that CN biofilms are susceptible to treatment with antibody-targeted alpha radiation, suggesting that RIT could provide a novel option for the prevention or treatment of microbial biofilms on indwelling medical devices.

RIT and Bacterial Infections

Streptococcus pneumoniae (Pn), an important cause of community-acquired pneumonia, meningitis, and bacteremia, was selected to evaluate the feasibility of RIT against bacterial diseases.[5] As a specific antibody carrier, we used a human monoclonal antibody D11, which binds to pneumococcal capsular polysaccharide 8 (PPS 8), and selected a short-range alpha-emitter ^{213}Bi as the radionuclide. The experiment showed that a greater percentage of mice survived in the ^{213}Bi-D11-treated group relative to the untreated group ($P < 0.01$; Fig. 1B). In contrast, the administration of unlabeled D11 (5 μg) did not prolong survival in comparison with untreated mice ($P > 0.05$). Radiolabeled control IgM also did not have any therapeutic effect ($P > 0.05$), and the survival of the mice was actually worse than in the untreated group, which was likely attributable to the absence of target for the antibody to bind, which resulted in excessive dose to critical blood rich organs such as bone marrow. Mice in control groups died to bacteremia on day 1 to 3, whereas mice treated with 80 μCi (2.96 MBq) of ^{213}Bi-D11 mice demonstrated 87 to 100% survival. Furthermore, mice treated with ^{213}Bi-D11 were not bacteremic at 3, 6, and 10 hours after treatment, as measured by CFUs in their blood as well as on days 3 and 14 (data not shown). Treatment with radiolabeled D11 was well tolerated: no weight loss was observed in treated animals. Thus, this study established the feasibility of RIT for the treatment of bacterial infections.

Recently, we have investigated RIT of experimental *Bacillus anthracis* infection, which is a powerful agent for bioterrorism and biological warfare and underscores the need for additional effective therapies for anthrax. The administration of ^{213}Bi-labeled mAbs to anthrax toxins, namely, protective antigen and lethal factor, prolonged survival of A/JCr mice infected with *B. anthracis* germinated bacterial cells (J. Rivera, A.S. Nakouzi, A. Morgenstern, F. Bruchertseifer, E. Dadachova, A. Casadevall, unpublished observations, September 2008).

RIT and Viral Infections

The HIV-1 epidemic is a major threat to global health. Highly active antiretroviral therapy (HAART), a combination of drugs that inhibits enzymes essential for HIV-1 replication, can reduce the viremia, decrease the likelihood of opportunistic infections in most patients, and prolong survival. However, HAART regimens are complicated and have significant toxicity. Replication-competent virus that persists in infected cells provides a source of virus that emerges rapidly after the cessation of HAART. A modality that targets and kills HIV-1-infected cells combined with HAART would have a major impact on the treatment of acute exposure and elimination of persistent reservoirs of infected cells. We hypothesized that RIT could be potentially effective against chronically infected cells, including those with viral infections.[1] RIT for viral diseases would target infected cells and consequently would provide a general strategy for eliminating reservoirs of infected cells and viral cellular factories. This approach could be particularly useful for the treatment of drug-resistant HIV strains, which present an ever-increasing problem.[21]

We examined the efficacy of RIT for treatment of HIV infection in vivo with a HIV envelope-specific human antigp41 mAb 246-D radiolabeled with ^{213}Bi or ^{188}Re.[6] For these studies, human peripheral blood mononuclear cells (PBMCs) infected with HIV-1_{JR-CSF} were injected into the spleens of SCID mice and the mice were treated intraperitoneally (IP) with radio-

labeled mAbs 1 hour later. The mice were evaluated 72 hours later for the presence of residual HIV-1-infected cells by quantitative coculture.[22] The 72-hour time period was chosen to allow for sufficient time for the ^{188}Re-labeled mAb to deliver a lethal dose of radioactivity to the cells because the ^{188}Re half-life is 16.9 hours.

Treatment of mice with ^{188}Re-labeled mAb 246-D administered either before or after intrasplenic injection with HIV-1_{JR-CSF}-infected human PBMCs dramatically reduced the number of HIV-1-infected cells (Fig. 1C). Similar results were obtained after the treatment of mice with ^{213}Bi-246-D (Fig. 1C). In contrast, the administration of equivalent amounts of "cold" mAb 246-D or of a radioisotope-coupled irrelevant control mAb did not reduce the average number of infected cells detected in the SCID mouse spleens. ^{188}Re-246-D was more effective in vivo than ^{213}Bi-246-D, possibly because of the longer physical half-life of ^{188}Re (16.9 hours versus 46 minutes), which allowed the labeled mAb to reach infected cells while still carrying high activity "payload."

To investigate the dose–response effect, we treated mice with 40, 80, and 160 μCi (1.48, 2.96, and 5.92 MBq, respectively) of ^{188}Re-246-D, corresponding to 50%, 100%, and 200% of the therapeutic dose, respectively. Although 40 μCi (1.48 MBq) of ^{188}Re-246-D was not effective in killing infected PBMCs in vivo, a 160-μCi (5.92 MBq) dose completely eliminated infected cells. These results established that RIT could effectively target and kill HIV-1-infected human PBMCs in vivo. The demonstration of efficacy of RIT against HIV provided a proof-of-principle for the concept of treating viral infections by targeting viral-infected cells and this approach could be applied to other chronic viral diseases like hepatitis C.[23]

Toxicity of RIT in Treating Infections

In our studies of RIT of fungal, bacterial, and viral infections, we evaluated the hematological toxicity of radiolabeled antibodies in mice by platelet counts.[2,5,6,24] Although it was known from the published data that the platelet counts nadir usually occurred 1 week after radiolabeled antibody administration to tumor-bearing mice,[25,26] there was no information about possible toxic effects of RIT in infected animals. RIT produced only transient decreases in platelet count on day 7 after treatment in C57BL/6 mice infected systemically with Pn and treated with 80 μCi of ^{213}Bi-D11 mAb as well as in SCID mice given 160 μCi of ^{188}Re-246-D in the RIT of HIV study with counts returning to normal by day 15 (Fig. 2A and B). Also, in the RIT of HIV study, we did not observe any changes in platelet counts in mice treated with 100 μCi of ^{213}Bi-246D mAb on days 4, 8, and 15 after treatment in comparison with the infected nontreated controls, with platelet counts being stable at $(1.5 \pm 0.2) \times 10^9$ platelet/mL blood. In AJ/Cr mice systemically infected with CN, hematologic toxicity was transient for the doses of up to 150 μCi ^{213}Bi- or ^{188}Re-labeled mAbs (Fig. 2C).

We also considered the possibility that RIT of CN infection may promote lung fibrosis in treated animals. Lungs are the target organ for CN infection, and it is known from cancer field that lungs can develop fibrosis several months after treatment with external beam radiation therapy.[27] To evaluate this potential complication, we used a pulmonary model of CN in which mice are infected intratracheally (IT). In this model, CN is mostly localized to the lungs on day 5 after infection, and as a result up 10% of the injected dose/g is found in the lungs at 24 hours after treatment with radiolabeled MAbs versus 1.5% of the injected dose/g in the lungs of systemically infected mice.[2]

BALB/c mice were infected IT with 10^6 CN cells and on day 5 after infection were treated with 50 to 200 μCi ^{213}Bi- or ^{188}Re-labeled mAbs or left untreated. All mice were subsequently maintained on fluconazole to control infection (10 mg/kg in their drinking water). After 5 months, the mice were killed, and their lungs were removed, fixed with buffered formalin, sectioned, stained with hematoxylin and eosin, and analyzed histologically. There was no evidence of radiation fibrosis in the lungs of radiation-treated mice (Fig. 2E and F) compared with control animals (Fig. 2D). This lack of hematological and pulmonary toxicity can be explained by the very specific targeting of radiolabeled antibodies to the microbes/infected cells. 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. It should also be noted that in all our studies the radiolabeled mAbs were administered IP, and IP administration of the radiolabeled mAbs was reported to be better tolerated than the intravenous route.[28]

In addition, when using a radioactive therapy in patients, there is always a concern of long-term effects such as 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 infections. Nevertheless, the application of RIT to infectious diseases will require optimization of the dose to ascertain and minimize toxic effects.

Radiobiological Mechanisms of RIT in Treating Infections

Given that RIT in the treatment of infectious diseases is a relatively young field, the mechanisms by which RIT is effective are uncertain. Even in oncology, where the antineoplastic effects of RIT have been investigated for more than 25 years, the cytotoxic mechanisms are still debated. The major radiobiological mechanisms of cancer RIT are considered to be "direct-hit" and "cross-fire" effects, both of which can promote apoptosis and cell cycle redistribution.[29] We investigated the radiofungicidal effects of external gamma radiation and ^{213}Bi- or ^{188}Re-labeled mAbs on CN cells by evaluating the effect of radiofungicidal doses on cell membrane permeability, induction of apoptosis, and cellular metabolism.[30]

An increased membrane permeability to the dye pro-

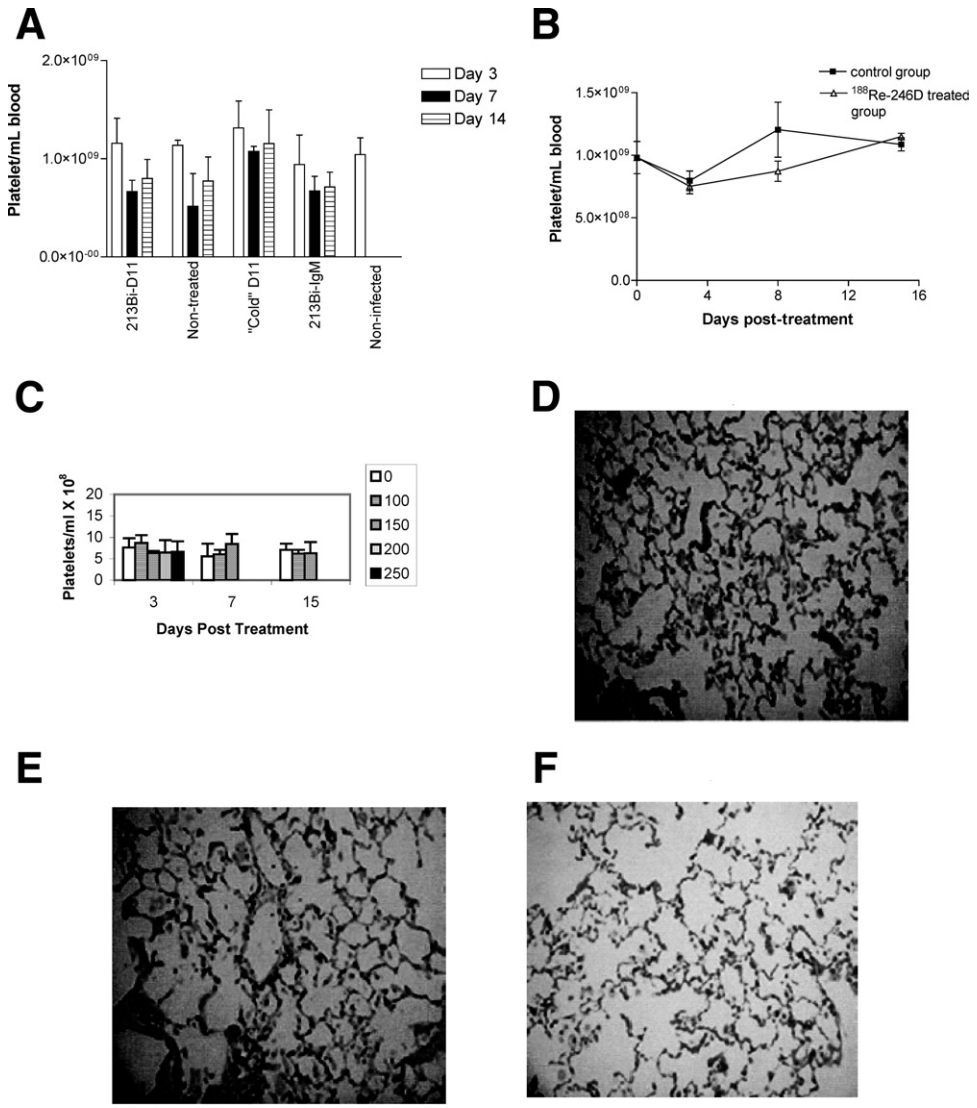

Figure 2 Toxicity of RIT in mice with experimental bacterial, viral, and fungal infections. Platelet counts in RIT-treated mice: (A) C57BL/6 mice infected IP with Pn and treated with ^{213}Bi-labeled mAb 1 hour after infection; (B) SCID mice injected intrasplenically with HIV-1-infected human PBMCs and treated with ^{188}Re-246-D mAb; and (C) *C. neoformans*-infected A/JCr mice received various doses of ^{213}Bi-18B7. A "0" indicates infected nontreated mice. Mice treated with 200 and 250 μCi of ^{213}Bi-18B7 died by day 7 after treatment. Micrographs of hematoxylin and eosin-stained lungs from BALB/c mice infected IT with *C. neoformans* and treated with radiolabeled mAbs. Mice were killed 5 months after RIT: (D) infected control group (no RIT); (E) 200 μCi of ^{213}Bi-18B7; and (F) 200 μCi of ^{188}Re-18B7.

pidium iodide (PI), which is excluded from cells with intact membranes, is considered to be a marker of cell death. Internalized PI binds to nucleic acids and undergoes a large increase in fluorescence.[31] PI staining correlates with loss of CFUs in a variety of micro-organisms, including CN treated with antifungal agents.[32] More than 95% of heat-killed CN cells were positive for PI and served as positive controls whereas untreated CN cells were negative controls. Cells stained with PI immediately or 1 hour after gamma irradiation showed no uptake of PI (not shown). The permeability increased with time between 1 and 3 hours after gamma irradiation, indicating that it was probably secondary to cell death, not a cause of death (Fig. 3A).

It seems likely that the cells in this 20% of the population are metabolically "dead" and unable to maintain membrane integrity. Cells stained 3 hours after irradiation showed dose-dependent PI staining up 300 Gy (25% PI positive), with a decrease to 10% PI positive at the highest dose (Fig. 3A). This observation suggests that membrane damage is not the primary lethal event, as 80% of the cells had lost clonogenic ability at these doses. The decrease in PI-positive cells at the highest dose may be caused by radioprotective effects from the shed capsule.[33] Treatment of CN with ^{188}Re-18B7 did not make the cells PI permeable (Fig. 3B). Treatment with ^{213}Bi-18B7 mAb led to about 7% of the cells becoming PI permeable, at a dose that caused 80% loss of CFUs (Fig. 3C). Higher doses of ^{213}Bi-18B7 mAb actually decreased the permeability.

Fungal cells undergo apoptosis, or programmed cell death.[34]

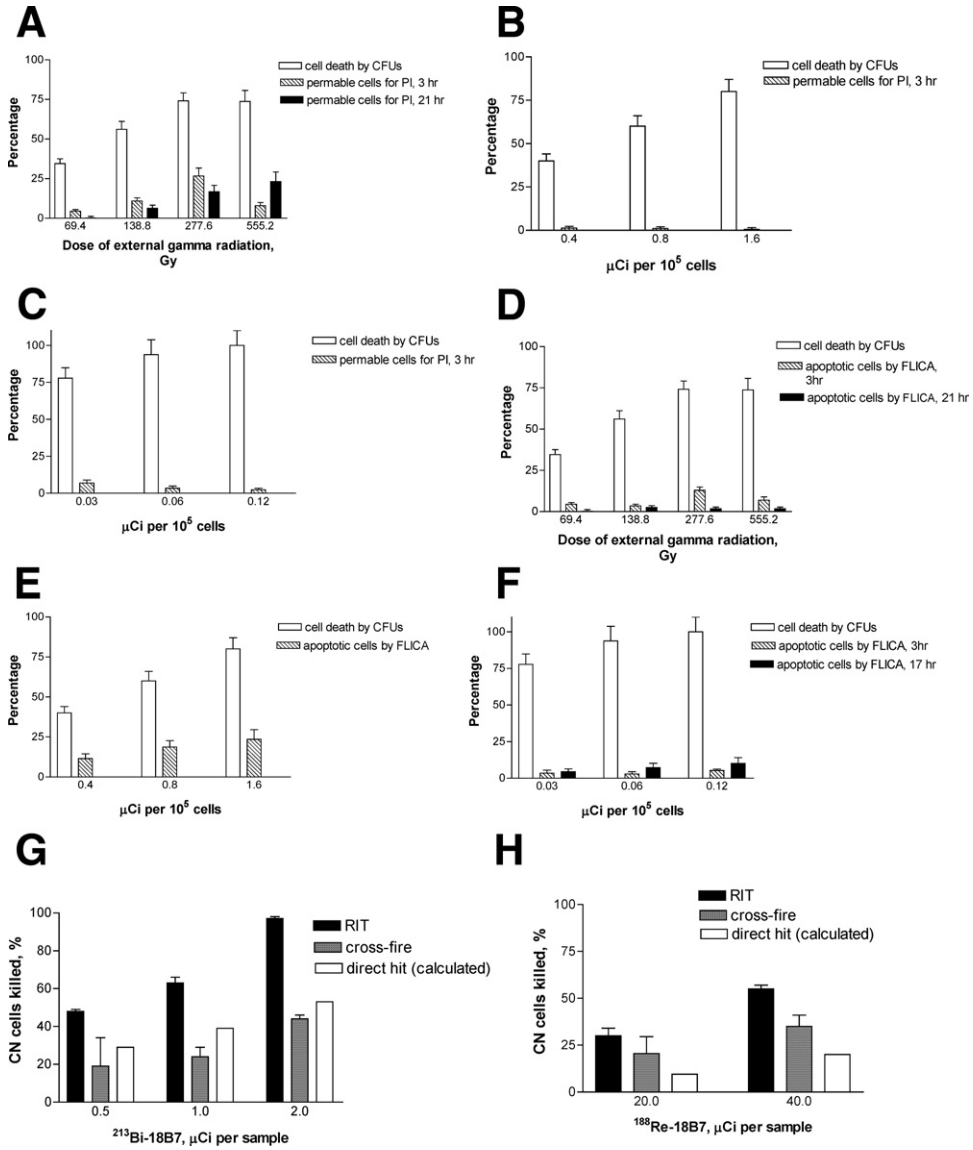

Figure 3 Contribution of different radiobiological effects to RIT of *C. neoformans* with ^{213}Bi-18B7 and ^{188}Re-18B7 mAbs. Shown are CFUs and PI permeability for (A) external gamma radiation; (B) ^{188}Re-18B7; and (C) ^{213}Bi-18B7. Shown are CFUs and apoptosis levels by FLICA: (D) external gamma radiation; (E) ^{188}Re-18B7; and (F) ^{213}Bi-18B7. Shown are contribution of "cross-fire" and "direct-hit" toward killing of *C. neoformans* cells: (G) "cross-fire" and "direct hit" for ^{213}Bi-18B7; and (H) "cross-fire" and "direct hit" for ^{188}Re-18B7. The contribution of "direct hit" toward cell killing was calculated by subtracting percentage of cells killed by "cross-fire" from percentage of cells killed by RIT.

We investigated whether radiation increased levels of fungal caspase, as measured by FLICA (*fl*uorochrome-*l*abeled *i*nhibitor of *ca*spase) binding, a membrane-permeable substrate that binds to caspases induced during early apoptosis. Earlier, we validated this technique for use with CN by comparing the FLICA results with those obtained using APO-BrdU terminal deoxynucleotidyl transferase dUTP nick end labeling apoptosis detection kit.[35] Gamma-irradiated cells were about 10% FLICA positive at 3 hours (Fig. 3D), whereas 20% and 5% of CN cells exposed to ^{188}Re-18B7 or ^{213}Bi-18B7 mAbs, respectively, became FLICA positive (Fig. 3E and F). The number of FLICA-positive ^{213}Bi-18B7 mAb-treated cells that were stained was greater at 17 hours than at 3 hours, indicating an ongoing process of apoptosis induction. Apoptosis is a dynamic process, and cells pass through several stages, not staying at any one stage for a long time. The decrease seen at 21 hours for the gamma-radiation treated cells may indicate that, at 21 hours, the cells have finished the stage of apoptosis during which the caspases are available to bind the fluorescent inhibitors. This is in contrast to the increase with time observed for ^{213}Bi-18B7 mAb-treated cells and may reflect a difference in pathways of cell death induced by the different forms of radioactivity.

We concluded that gamma, beta and alpha radiation affected cells via different pathways. Gamma radiation had more effect on the cell membrane than ^{213}Bi-18B7 or ^{188}Re-18B7. All forms of radiation stimulated apoptosis-like cell death with gamma radiation and ^{188}Re-18B7 mAb having

more pronounced effect than ^{213}Bi-18B7 mAb. ^{213}Bi-18B7 mAb delivered "directly" decreased the metabolic activity of fungal cells, while the other forms of radiation did not. Clonogenic survival proved to be the most practical measure of assessing RIT efficacy, by virtue of reflecting a combination of multiple mechanisms leading to fungal cell death. Cells that remain alive after RIT treatment, but do not replicate, may or may not contribute to the disease.

To elucidate the contribution of "direct hit" and "cross-fire" effects to RIT of CN, we compared the fungicidal activity of a mAb radiolabeled with ^{213}Bi or ^{188}Re isotopes with different emission ranges in tissue, that is, 50 to 80 μm for ^{213}Bi versus 10 mm for ^{188}Re. In cancer RIT, ^{213}Bi is assumed to kill by "direct hit" and ^{188}Re through "cross-fire." In principle, every cell with bound radiolabeled mAb molecules can be killed by a "direct hit" and simultaneously serve as a source of "cross-fire" radiation. By measuring the killing of the cells in RIT and in "cross-fire" experiments, we can calculate contribution of "direct hit" toward cell killing by subtracting percentage of cells killed by "cross-fire" from percentage of cells killed by RIT. To observe "cross-fire," we had to ensure that the cells that served as the sources of "cross-fire" radiation could not be killed themselves by "direct hit." Consequently, we used heat-killed CN cells. Experiments with ^{213}Bi-18B7 showed that although most fungal cells were killed by "direct hit," "cross-fire" effect also contributed to the fungicidal effect of RIT (Fig. 3G).

No killing of CN cells by unlabeled mAb 18B7 was observed. For ^{188}Re-18B7, the "cross-fire" effect was responsible for most of CN killing (Fig. 3H). This system permits experiments to elucidate precise mechanisms of cell killing in RIT that have not been performed either for microbial or cancer cells. In RIT of cancer cells the antibody is often internalized after binding, adding significant complexity to the experiment. One of the advantages of the CN system is that the capsule is outside the cell wall and that antibody is not internalized, thus allowing exploration of this fundamental problem in radiobiology. Knowledge of the radiobiological mechanisms of RIT will allow creation of more effective protocols for RIT of opportunistic fungal infections.

In summary, because microbial cells are foreign to the human body, they contain antigens that are not expressed by human tissues and this provides a major contrast to cancer RIT because tumor-associated antigens are also expressed on normal tissues. Consequently, the theoretical therapeutic index of RIT for microbial diseases should be significantly greater than for neoplastic diseases. This exquisite specificity promises exclusivity of targeting which should translate into high efficacy of treatment and low toxicity. In cancer RIT often the matching pairs of radionuclides are used for imaging of the disease followed by therapy with the example being ^{111}In-^{90}Y matching pair of radionuclides utilized in Zevalin regimen. Likewise, we envision that first the organism-specific mAbs will be radiolabeled with imaging radionuclides such as ^{99m}Tc or ^{111}In to localize the sites of infection with SPECT followed by RIT with ^{188}Re- or ^{90}Y-labeled mAb, respectively. Also, immuno-positron emission tomography might be used in the imaging of infection before treatment if positron-emitting radionuclides such as ^{86}Y (matching pair for ^{90}Y) or ^{124}I (matching pair for ^{131}I) are available. It might be possible to create a so-called "pan-antibody" that would recognize an antigen shared by a particular class of human pathogens such as fungi, for example. An example of such "pan-antibody" is a mAb 6D2 that initially was developed against fungal melanin and also binds to synthetic, invertebrate (cuttlefish), murine, and human melanins.[36] The availability of such antibodies would eliminate the necessity of having antibodies specific for each particular microorganism and would enormously enhance the development of RIT of infectious diseases.

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