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Treatment of infection with radiolabeled antibodies

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The field of infectious diseases is in urgent need of new approaches to antimicrobial therapy. Radioimmunotherapy (RIT) has evolved into successful therapy for certain malignancies. Published preclinical and clinical investigations have demonstrated that radiolabeled microorganism-specific antibodies localize to tissue sites of bacterial and fungal infection. The potential of RIT as an antimicrobial treatment strategy has not been developed clinically, which could reflect lack of awareness of the difficult problems in clinical infectious diseases by the nuclear medicine community and of RIT by the infectious diseases physicians. We have recently demonstrated the feasibility of using RIT for treating murine cryptococcosis using a monoclonal antibody to *Crypto-coccus neoformans* capsular glucuronoxylomannan labeled with Bismuth-213 or Rhenium-188. Subsequently, we showed the applicability of RIT to bacterial (*Strepto-coccus pneumonia*) and viral (HIV-1) infections. Treatment did not cause acute hematologic toxicity in treated animals. The mechanisms of RIT of infection include killing of microbial cells by "direct hit" and "cross-fire" effects, promotion of apoptosis-like death, cooperation with macrophages and modulation of the inflammatory response. RIT for infection is theoretically useful for any microbe susceptible to radiation, including bacteria, fungi, viruses and parasites. The promise of this technique is based on the fact that the technology is largely in place and that the only requirements are availability of microbe-specific monoclonal antibodies and suitable radionuclides. In fact, one could anticipate that targeting microbes will be easier than targeting neoplastic cells when the enormous antigenic differences between host and microbes are taken into consideration. However, considerable basic work remains to be done to ascertain the optimal conditions for the efficacy of RIT for infection.

KEY WORDS: Radioimmunotherapy - Mycoses - Bacterial infections - Virus diseases - Antibodies - Hematology - Toxicity.

The field of infectious diseases is in urgent need of new approaches to antimicrobial therapy because of a confluence of events that have reduced therapeutic options including: 1) an increasing prevalence of diseases caused by highly resistant microorganisms, some of which are not susceptible to currently available antimicrobial agents; 2) the relative lack of efficacy of antimicrobial therapy in immunosuppressed individuals; and 3) a paucity of new anti-microbial drugs in the development pipeline. In addition new microbial diseases are being identified with increasing frequency and for many there are no available

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therapies. For example, a recent global outbreak of severe acute respiratory syndrome (SARS) coronavirus infection was associated with high mortality. There is also a credible threat of bioterrorism. In this environment, given that current strategies for development of antimicrobial drugs and vaccines take many years to yield clinically useful products, there is a need for approaches that can facilitate rapid development of new antimicrobial agents.

Radioimmunotherapy (RIT) was developed for treatment of cancer nearly three decades ago. The first clinical trials of RIT for hepatoma in the US were performed by Order *et al*. in mid-1980s.[1] RIT takes advantage of the specificity of the antigen-antibody interaction to deliver radionuclides that emanate lethal doses of cytotoxic radiation to cancer cells,[2, 3] and can provide a valuable alternative to chemotherapy and external beam radiation therapy (EBRT). RIT has been developed into a successful therapy for certain cancers as evidenced by the recent approval of monoclonal antibody (mab) therapy-based drugs such as Zevalin® and Bexxar® (anti-CD20 mAbs labeled with ^{90}Y and ^{131}I, respectively) for the treatment of relapsed or refractory B-cell non-Hodgkin's lymphoma. Recent reports on the use of RIT as an initial treatment for follicular lymphoma [4] are encouraging thus potentially making RIT first line therapy in some types of cancer.

Until relatively recently RIT was used exclusively for cancer therapy. Remarkably, its potential as an antimicrobial therapy has not been developed clinically. The reasons for this are enigmatic but could reflect the lack of awareness of the difficult problems in clinical infectious diseases by the nuclear medicine community and of RIT by the infectious diseases community. We recently attempted to cross this divide, demonstrating the feasibility of RIT as an anti-infective therapy by treating murine cryptococcosis with a mAb to *Cryptococcus neoformans* (CN) capsular glucuronoxylomannan labeled with ^{213}Bi or ^{188}Re.[5, 6] Subsequently, we showed the applicability of RIT to other fungal and bacterial infections.[7, 8] RIT may also be potentially effective against chronically infected cells including those with viral infections.[9]

Here we review the results of RIT on fungal and bacterial infections, as well as its safety and radiobiological and immune mechanisms and provide an outline for the future development of this novel treatment modality.

Feasibility of RIT for infection

In considering the feasibility of RIT of infection, the two most crucial factors for success or failure are the ability of an organism-specific radiolabeled antibody to reach the site(s) of infection in the body and the susceptibility of microbes to the radiation delivered by these antibodies.

Several groups have utilized radiolabeled organism-specific antibodies to image infections in animal models. Poulain *et al*.[10] infected Guinea pigs intravenously with *Candida albicans* and used both radioiodinated F(ab')$_2$ fragments and an intact mAb to the cell wall glycoprotein of *C. albicans* to image the sites of infection. They observed that the biodistribution of *C. albicans*-specific mAb matched the anatomic distribution of *C. albicans* infection as confirmed by the colony forming units (CFUs) per organ. There was a direct proportionality between the %ID/g organ and the number of CFUs/g in each organ. In animals with *Candida* endophthalmitis, a common complication of candidal hematogenous dissemination, the distribution of *C. albicans*-specific mAb was highly specific, in contrast to that of a radiolabeled non-specific mAb. Rubin *et al*.[11] investigated whether a radioiodinated murine mAb to Fisher Immunotype 1 *Pseudomonas aeruginosa* could detect deep thigh infections. These investigators concluded that it was feasible to image localized infections and hidden abscesses by scintigraphy with organism-specific antibodies. To investigate the feasibility of imaging tuberculomas with radiolabeled mAbs against mycobacterial antigens, researchers have used mouse [12] and rabbit [13] models of tuberculosis. They induced tubercular lesions in rabbit and injected radioiodinated anti-*M. bovis* (BCG) mAb 4-6 months later. The specific mAb localized in tuberculomas at 3 days postinjection and maximal signal-to-noise ratio between infected and non-infected tissues was observed by day 6. Huang *et al*.[14] explored the use of ^{99m}Tc-labeled mAb to *Staphylococcus aureus* to detect bacterial endocarditis in a rabbit model. The biodistribution of radiolabeled specific mAb was monitored in the infected rabbits and in normal controls. They observed that the ratio of radioactivity in the aortic valve to that in the surrounding heart tissue or blood pool was significantly higher in infected animals (>10:1) than in non-infected controls. Reaching foci of infection in the brain with radiolabeled mAbs can be challenging if the blood brain barrier (BBB) is intact. We have

previously reported that in rats with intracisternal *C. neoformans* infection there was no detectable localization of radiolabeled mAb 2H1 directed against *C. neoformans* capsular polysaccharide in the brain and cerebrospinal fluid following intravenous injection.[15] This inability of mAb 2H1 to cross the BBB was circumvented by administering mAb intracisternally, which resulted in persistent intracisternal radioactivity uptake when compared to non-specific control mAb.

At least one study has shown that microbe-specific radiolabeled mAbs can localize at the site of infection in humans. Goldenberg *et al.*[16] showed that administration of a ^{99m}Tc-labeled mAb fragment specific for *Pneumocystis carinii* localized to the lungs in 6 of 7 patients with pneumocystis pneumonia 24 h after administration. The ability of a specific antibody to localize to a site of infection indicates the feasibility of using the antibody-antigen interaction to deliver microbicidal radiation to sites of infection in form of RIT.

Radiation possesses microbicidal properties and γ-irradiation is routinely used for sterilization of medical supplies and certain foods. Ionizing radiation such as γ-rays, β- and especially α-particles from external sources can kill different strains of bacteria and fungi such as *E. coli, C. neoformans* and *M. tuberculosis.*[17-19] However, the lethal doses of external ionizing radiation for microbes are extremely high in comparison to those needed to kill mammalian cells. For example, several hundred Gy are needed to kill bacterial cells, thousands for fungal cells and around 50 000 Gy for destruction of HIV-1 viral particles.[20] In contrast, mammalian cells are killed by as few as 5-10 Gy. As in clinical RIT the peak dose rates of only 0.1 Gy/h are observed,[21] it is not possible to predict *a priori* if the amount of radioactivity delivered by antibodies to microbial cells will be sufficient to cause the death of these cells and therefore experimental proof of feasibility of RIT for major types of microorganisms, bacteria, fungi and viruses is required.

Radionuclide selection

Given that RIT of cancer has been extensively studied for at least three decades, the relationships between tumor size, curability and the tissue range of therapeutic emissions of radionuclides have been largely clarified.[22, 23] Several therapeutic radionuclides are currently utilized in targeted radionuclide therapy (Table I) [2] and RIT of infection can take advantage of the information generated in cancer studies. For example, for single cell disease (systemic infections) an isotope with a short track in tissue such as ^{213}Bi would be suitable and for the RIT of infected sites located deep inside the body, longer-lived isotopes such as ^{90}Y (half-life 2.7 days), ^{177}Lu (half-life 6.7 days) or ^{131}I (half-life 8 days) can be used with an intact mAb. Alternatively, combinations of fast-targeting domain-deleted mAbs [24] with short-lived isotopes as ^{188}Re (16.9 h) or even ^{213}Bi (45.6 min) may also prove useful.

TABLE I.—*Therapeutic radionuclides.*

Radionuclide	Type	Half-life	E_{max} (MeV)	Mean range (mm)	Imageable
^{90}Y	β	2.7 d	2.3	2.76	No
^{131}I	β, γ	8 d	0.81	0.4	Yes
^{177}Lu	β, γ	6.7 d	0.5	0.28	Yes
^{153}Sm	β, γ	2 d	0.8	0.53	Yes
^{186}Re	β, γ	3.8 d	1.1	0.92	Yes
^{188}Re	β, γ	17 h	2.1	2.43	Yes
^{67}Cu	β, γ	2.6 d	0.57	0.6	Yes
^{225}Ac	α, β	10 d	5.83	0.04-0.1	Yes
^{213}Bi	α	45.7 min	5.87	0.04-0.1	Yes
^{212}Bi	α	1 h	6.09	0.04-0.1	Yes
^{211}At	α	7.2 h	5.87	0.04-0.1	Yes
^{212}Pb	β	10.6 h	0.57	0.6	Yes
^{125}I	Auger	60.1 d	0.35	0.001-0.02	No
^{123}I	Auger	13.2 h	0.16	0.001-0.02	Yes
^{67}Ga	Auger, β, γ	3.3 d	0.18	0.001-0.02	Yes

Adapted from Milenic *et al.*[2]

Since microbial cells vary widely in size and in doubling time, one needs to carefully consider the optimal radionuclide for use in RIT of infection. Ideally the half-life of the isotope should match the doubling time of the microorganism and its emission range in tissue should parallel the microorganism's physical dimensions. In this regard, for 1 μ in diameter fast-dividing bacteria (doubling time around 20 min) short-lived radionuclides capable of delivering radiation in short "bursts" with short emission track in tissue, such as the α-emitters ^{213}Bi and ^{212}Bi (half-life 60 min) may be useful. For larger (10 μ in diameter), slower growing (doubling time 2-3 h) fungal cells, both α and β-emitters such as ^{188}Re might be effective. The nano-sized cross-sectional area of viruses can make them less susceptible to direct deactivation by radiation. Since viral replication is dependent, however, on host

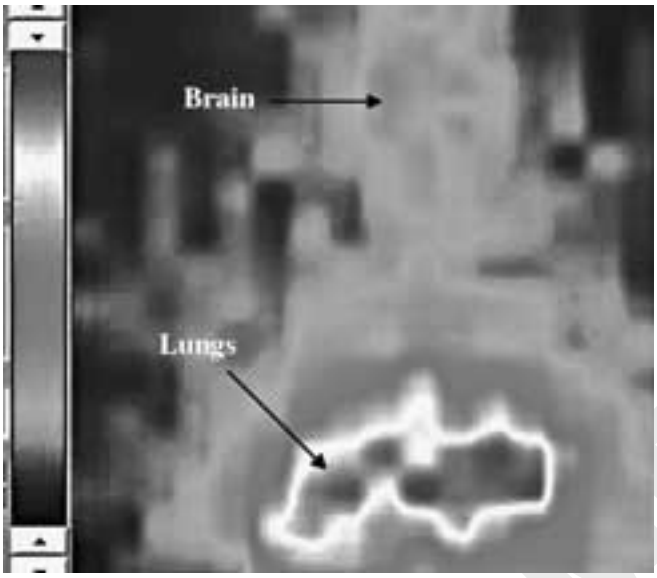

Figure 1.—Scintigraphic image of infected IV with CN A/JCr mouse 48 hr postinjection of ^{188}Re-18B7 mAb.

cells it is possible to use antibodies that bind to viral antigens expressed on infected cells, thereby targeting sites of viral replication and assembly.[9]

Another area where RIT of infection can utilize the significant knowledge accumulated in RIT of cancer is in the technology of linking radionuclides to the antibodies. For labeling of the organism-specific antibodies with long-lived trivalent radiometals such as ^{90}Y or ^{177}Lu thermodynamically and kinetically stable macrocycle-based ligands such as DOTA can be used. For short-lived α-emitters ^{213}Bi and ^{212}Bi structurally modified DTPA-based ligands (for example CHXA), characterized by very favorable kinetics of metal-ligand complex formation, can be used. The transition metal ^{188}Re can be attached to the antibodies "directly" via reduction of disulfide bridges or through HYNIC or MAG3 ligands.

In our experiments to develop RIT of fungal and bacterial infections we evaluated two radioisotopes with very different emission properties: a high-energy β-emitter ^{188}Re (E_{max}=2.12 MeV) and an α-particle emitter ^{213}Bi. ^{188}Re has been used for cancer RIT, palliation of skeletal bone pain, and endovascular brachytherapy to prevent restenosis following angioplasty.[25-28] ^{213}Bi emits a high LET E=5.9 MeV α-particle with a path length in tissue of 50-80 mm. ^{213}Bi has been proposed for single-cell disorders as well as some solid tumors [29-32] and has been used to treat patients with leukemia in phase I clinical trials.[33, 34]

Infections

Fungal infection

We initially explored the potential efficacy of RIT against an experimental fungal infection using CN.[5] CN is a major fungal pathogen that causes life-threatening meningoencephalitis in 6-8% 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.[35, 36] CN provides 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.[37] In spite of high levels of circulating polysaccharide in the blood of infected mice, in both pulmonary and systemic animal models of CN infection, the radiolabeled mAb preferentially localized at the sites of infection (Figure 1). Radiolabeled with ^{213}Bi or ^{188}Re 18B7 antibody killed CN cells *in vitro*, thus converting an antibody with no inherent antifungal activity into a microbicidal molecule (Figures 2A, B).

In vitro killing encouraged us to perform therapeutic studies in AJ/Cr mice infected systemically with CN. Mice treated with radiolabeled CN-specific mAb 18B7 lived significantly longer than mice treated with irrelevant labeled IgG_1 or PBS (Figure 3). 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 non-specific killing of CN cells. Remarkably, on day 75 post-therapy, 60% of the mice in the ^{213}Bi group were alive after treatment with 100 μCi ^{213}Bi-18B7 ($P<0.05$). 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, while mice in control groups succumbed to infection between days 35-40. CN infected mice that received RIT had significantly reduced fungal burden in lungs and brains 48 h after treatment compared to infected controls (Table II). While there was no difference in the reduction of the fungal burden in the lungs between the groups that received 50 and 100

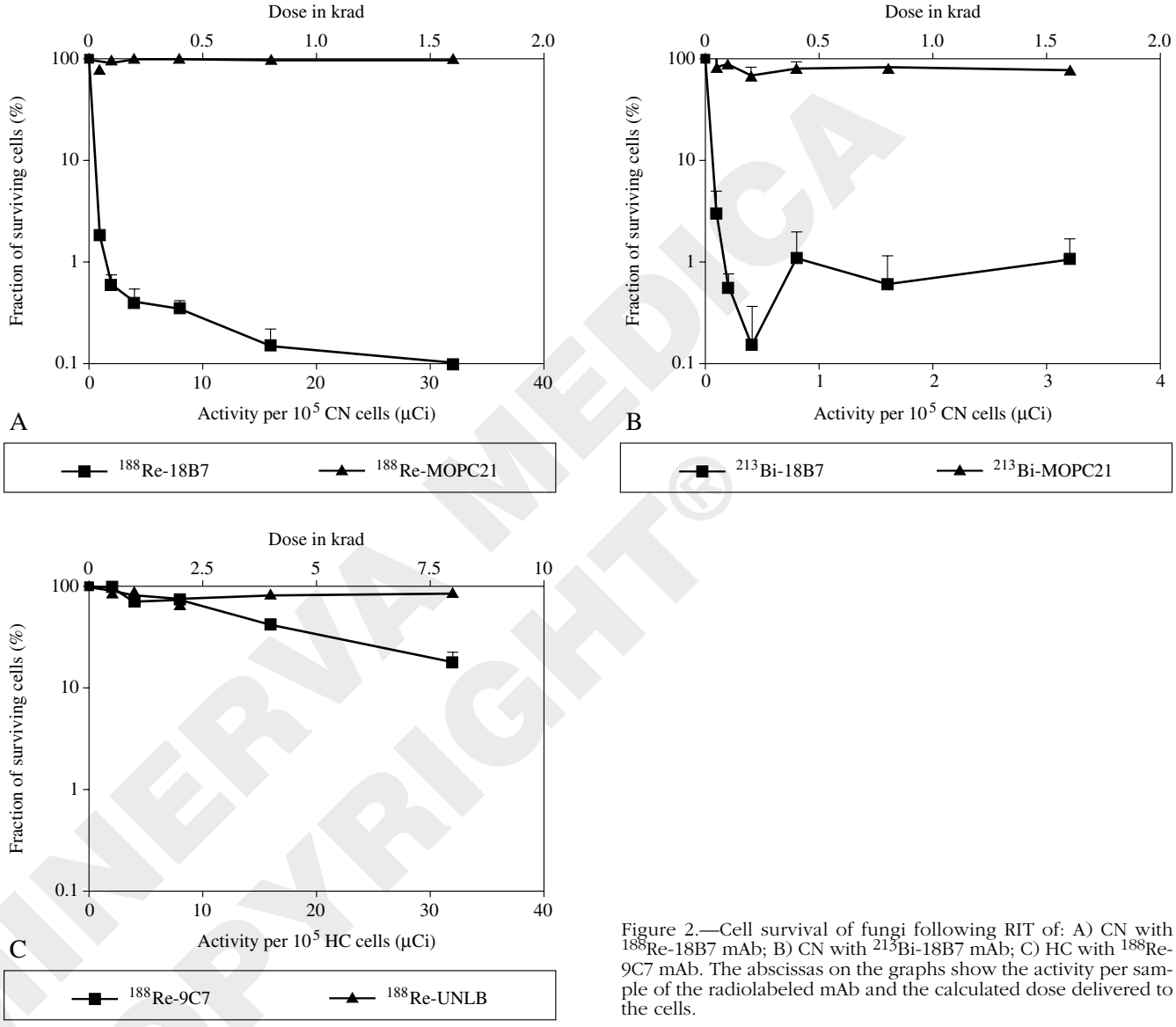

Figure 2.—Cell survival of fungi following RIT of: A) CN with ^{188}Re-18B7 mAb; B) CN with ^{213}Bi-18B7 mAb; C) HC with ^{188}Re-9C7 mAb. The abscissas on the graphs show the activity per sample of the radiolabeled mAb and the calculated dose delivered to the cells.

μCi ^{188}Re-18B7, treatment with 200 μCi ^{188}Re-18B7 significantly lowered lung CFUs relative to the lower activities ($P<0.05$). Hence, administration of CN specific radiolabeled antibody prolonged survival and reduced organ fungal burden in infected mice.

Survival of A/JCr mice treated with RIT was dose dependent for both ^{213}Bi and ^{188}Re radioisotopes. While 50 μCi ^{213}Bi-18B7 produced no therapeutic effect, both the 100 and 200 μCi doses prolonged animal survival (Figure 3). Interestingly, the 200 μCi ^{213}Bi-18B7 dose was less efficient, possibly due to the fact that it may have approached the MTA (maximum tolerated activity) for this particular combination of antibody and radioisotope. In the ^{188}Re group, administration of 50 μCi ^{188}Re-18B7 resulted in some prolongation of survival, and 100 μCi produced significant prolongation. A dose of 200 μCi was, apparently, too toxic with all animals dying by day 40.

We subsequently extended the antimicrobial RIT approach to another human pathogenic fungus,

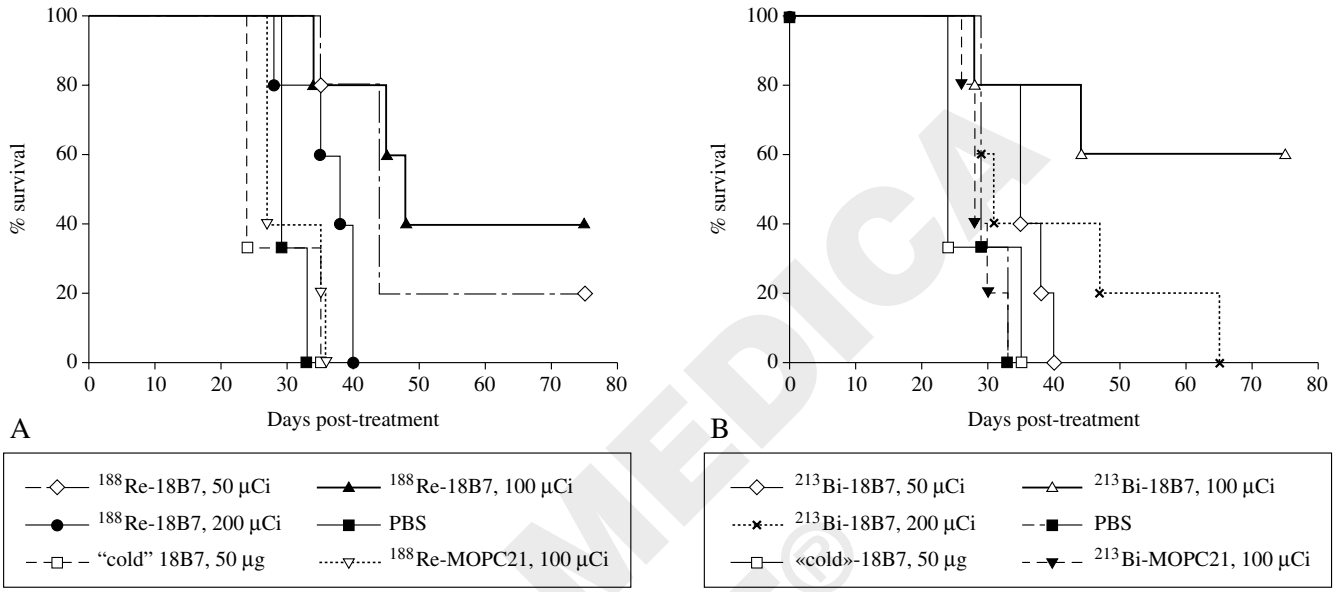

Figure 3.—Kaplan-Meier survival curves for A/JCr mice infected IV with 10^5 *C. neoformans* cells 24 h prior to treatment with: A) 50-200 µCi ^{188}Re-labeled mAb's; and B) 50-200 µCi ^{213}Bi-labeled mAb's. Animals injected with PBS (phosphate buffered saline) or 50 µg "cold" 18B7 served as controls.

TABLE II.—*CN CFUs in the lungs and the brains of A/JCr mice infected IV with 10^5 CN organisms and treated with ^{188}Re-18B7 mAb 24 h after infection*.*

Organ	No. of CFUs/g tissue, ×10^4 (SD)			
	Untreated mice and treated with unlabeled 18B7 (50 µg)	50 µCi ^{188}Re-18B7	100 µCi ^{188}Re-18B7	200 µCi ^{188}Re-18B7
Lungs	550 (47)**	11 (6) (P=0.001)	21 (5) (P=0.001)	3.3 (2) (P=0.001)
Brains	11 (5)	0.1 (0.05) (P<0.001)	0.8 (0.1) (P=0.002)	1.1 (0.1) (P=0.002)

*) Animals were sacrificed 48 h after the treatment with ^{188}Re-18B7 (2.8 188Re half-lives); the colony counts for 100 and 10 times dilutions for the lungs and brains, respectively, are given. **) P values were calculated by the Wilcoxon test, the groups treated with 188Re-18B7 were compared to combined group of untreated mice and treated with unlabeled 18B7 mAb.

Histoplasma capsulatum (HC),[7] the most common cause of fungal pneumonia in immunocompromised patients [38] by treating HC *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.[39] The dependence of the RIT-treated HC cell survival on the amount of radioactivity added to the cells is presented in Figure 2C. Ninety percent of HC cells were killed with 32 µCi 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). The significantly higher killing associated with the specific antibody almost certainly reflects higher radiation exposure for HC as a consequence of antibody binding to the HC cell wall. We also performed cellular dosimetry calculations for *in vitro* RIT of CN and HC [7] and compared them with the LD90 for external γ radiation. The cellular absorbed doses delivered by radiolabeled antibodies are shown in Figure 2. Cellular dosimetry calculations showed that RIT was ~1 000-fold more efficient in killing CN and ~100-fold in killing HC than γ radiation. Thus, RIT of fungal cells using specific antibodies labeled with α- and β-emitting radioisotopes was significantly more efficient in killing CN and HC than γ radiation when based on the mean absorbed dose to the cell. These results strongly support the concept of using RIT as an antifungal modality.

Bacterial infection

Streptococcus pneumoniae (Pn) is an important cause of community-acquired pneumonia, meningitis,

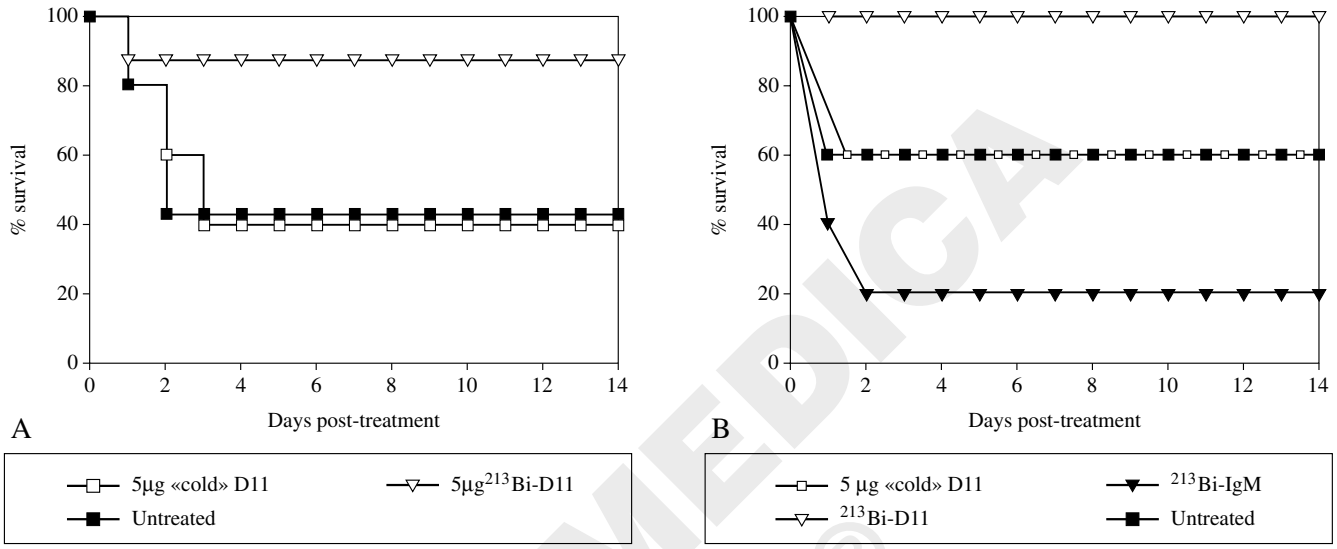

Figure 4.—RIT of Pn infection with ^{213}Bi-labeled mAb's in C57BL/6 mice. 8-10 mice per group were used. Mice were infected IP with 1,000 organisms 1 h before treatment with mAb's. Results of two experiments are presented.

and bacteremia. The problem of pneumococcal disease is exacerbated by increasing drug resistance. Furthermore, patients with impaired immunity are at high risk for invasive pneumococcal infections. Our earlier encouraging results with RIT of CN and HC provided the impetus for applying RIT to an experimental bacterial infection. However, in contrast to fungal infections which are usually chronic, disseminated bacterial infections can progress rapidly and bacteria replicate much faster than fungi. For example, the replication rate of Pn and *C. neoformans* are 20 min and 3 h, respectively. Cryptococci have average diameters that are approximately 10 times larger than pneumococci [40] and, consequently, present significantly larger targets for RIT than bacterial cells. It remained to be seen if the sensitivity of a microorganism towards particulate radiation depended on the amount of DNA per volume matter. In addition, these two microbes elicit different inflammatory responses with neutrophils predominating in pneumococcal infections and macrophages predominating in cryptococcal infections. Consequently, the success of RIT against experimental cryptococcal infection could not necessarily be extrapolated to bacteria from the prior fungal study.

We investigated the feasibility of applying RIT approach to the treatment of Pn infection by evaluating the susceptibility of Pn to radiolabeled antibody *in vitro* and in an animal infection model.[8] For the specific antibody carrier we utilized a human mab D11, which binds to pneumococcal capsular polysaccharide 8 (PPS 8), and selected a short range α-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 ($P<0.01$) (Figure 4). In contrast, administration of unlabeled D11 (5 µg) did not prolong survival in comparison to untreated mice ($P>0.05$). Similarly, the radiolabeled control IgM did not have any therapeutic effect ($P>0.05$). Mice in control groups succumbed to bacteremia on day 1-3, while mice treated with 80 µCi ^{213}Bi-D11 demonstrated 87-100% survival. Furthermore, mice treated with ^{213}Bi-D11 were not bacteremic at 3, 6 and 10 h post-treatment as measured by CFUs in their blood as well as on days 3 and 14 (data not shown). Treatment with radiolabeled D11 was very well tolerated—no weight loss was observed in treated animals. Thus, this study established the feasibility of RIT for the treatment of bacterial infections.

Toxicity

Based on data accumulated in RIT of cancer, the primary toxicity of RIT of infection is likely to be bone marrow suppression. Important determinants of the

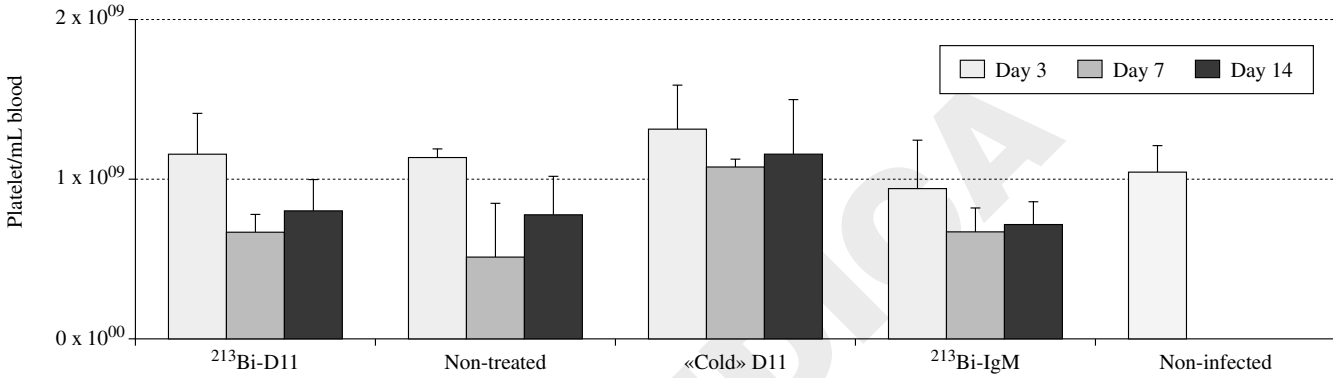

Figure 5.—Platelet counts in RIT-treated mice. C57BL/6 mice infected IP with Pn and treated with ^{213}Bi-labeled mAb's 1 h postinfection.

extent and duration of RIT-induced myelosuppression are bone marrow reserve (based on prior cytotoxic therapy and extent of disease involvement), total infection burden and spleen size.[41, 42] We note that RIT of NHL is effective in patients who have received several unsuccessful courses of chemotherapy and consequently, have depleted bone marrow reserves and weakened immunity not unlike immunocompromised patients susceptible to infections. Nevertheless, the application of RIT to infectious diseases will require optimization of the dose to ascertain and minimize toxic effects.

In our studies of RIT of fungal and bacterial infections we evaluated the hematological toxicity of radiolabeled antibodies in mice by platelet counts.[5, 6, 8] The platelet count nadir usually occurs 1 week after radiolabeled antibody administration to tumor-bearing animals.[43, 44] For example, in C57BL/6 mice infected systemically with Pn and treated with 80 µCi ^{213}Bi-D11 mAb only a transient drop in platelet count was noted on post-treatment day 7 with counts returning to pretreatment levels by day 15 (Figure 5). This lack of hematologic toxicity can be explained by the very specific targeting of radiolabeled antibodies to the microbes. In fact, one of the advantages of using RIT against infection, 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 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 IV route.[45]

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. Although infectious disease specialists have little or no experience with radiation therapy, the efficacy of RIT coupled with a significant education effort should facilitate their acceptance of this therapeutic modality.[46]

Radiobiological and immune mechanisms of RIT of infection

The mechanism of antimicrobial action of RIT presumably reflects the delivery of radionuclide to a location in close proximity to a microbe such that the emitted radiation is cytotoxic to the microbe or to the infected host cell. The radiobiological mechanisms of cancer RIT are complex and are different from those involved in killing the cancer cells using EBRT. In clinical RIT, peak dose rates of only 0.1 Gy/h [21] are observed. In comparison, high-dose rate radiation, typical for EBRT, delivers 60 Gy/h. Thus, from the viewpoint of radiation therapy, RIT delivers suboptimal doses to tumors but it is effective by promoting apoptosis in irradiated tumor cells, "bystander" effect (death of adjacent, non-irradiated cells) and cell cycle arrest.[47-49] In our studies of RIT of fungal infections we also observed the discordance between efficacy of external beam and RIT - human pathogenic fungi *C.*

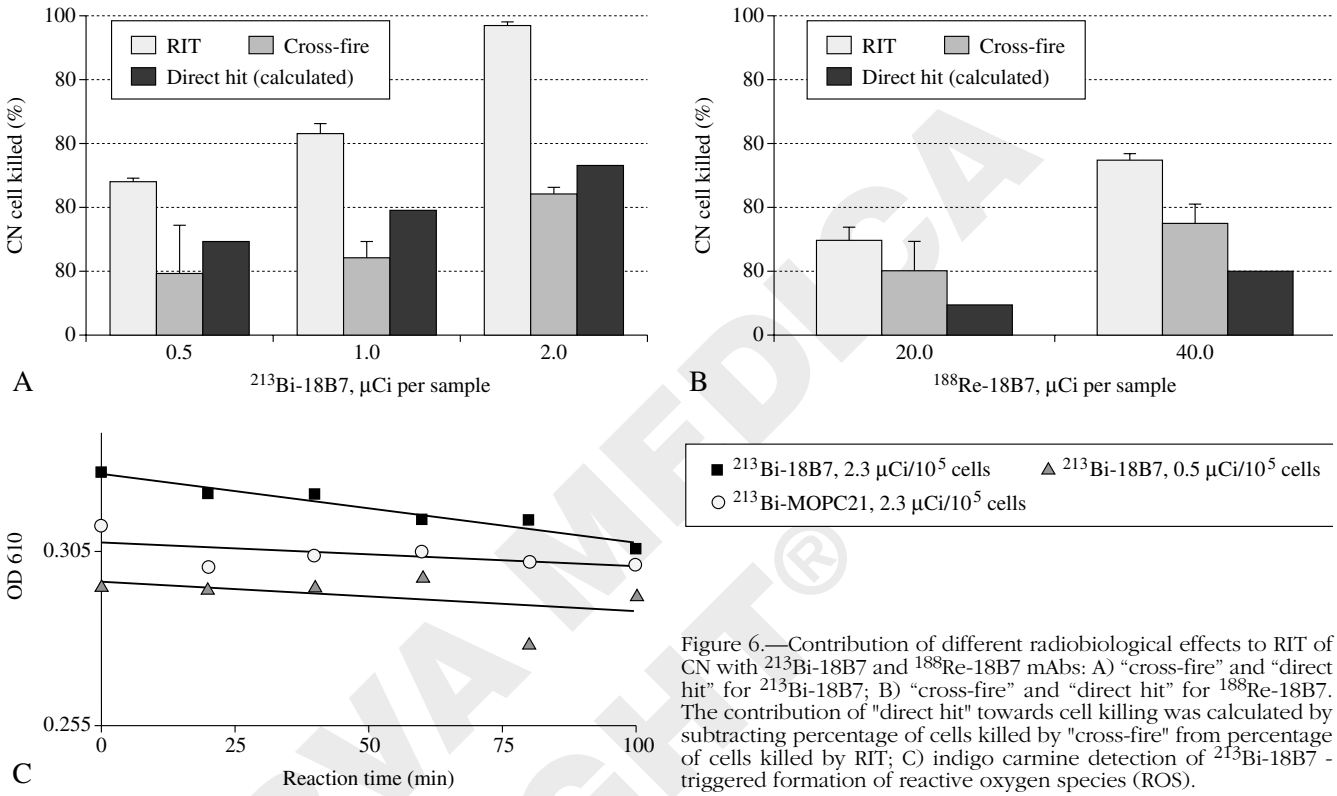

Figure 6.—Contribution of different radiobiological effects to RIT of CN with ^{213}Bi-18B7 and ^{188}Re-18B7 mAbs: A) "cross-fire" and "direct hit" for ^{213}Bi-18B7; B) "cross-fire" and "direct hit" for ^{188}Re-18B7. The contribution of "direct hit" towards cell killing was calculated by subtracting percentage of cells killed by "cross-fire" from percentage of cells killed by RIT; C) indigo carmine detection of ^{213}Bi-18B7 - triggered formation of reactive oxygen species (ROS).

neoformans and *H. capsulatum* proved to be extremely resistant to external g radiation (LD_{90}=4 000 Gy), but relatively susceptible to killing by RIT with ^{188}Re- and ^{213}Bi-labeled mAbs (LD_{90}=1-4 Gy).[7]

Radiobiological mechanisms of microbial cell killing by radiolabeled mAbs might involve "direct hit" (killing of a cell by radiation emanating from radiolabeled antibody molecule bound to the microbial cell) and "cross-fire" (killing of a cell by radiation emanating from a radiolabeled antibody molecule on an adjacent or a distant cell), cell cycle arrest, "bystander" effect, and ability of antibodies to catalyze the synthesis of reactive oxygen species (ROS). We have recently performed a detailed study of the radiobiological and immune mechanisms of RIT of CN infection *in vitro* and *in vivo*.[50]

To elucidate the contribution of "direct hit" and "cross-fire" effects to RIT of CN we compared the fungicidal activity of a mAb conjugated to ^{213}Bi and ^{188}Re—isotopes with different emission ranges in tissue—50-80 μm for ^{213}Bi *versus* 2.4 mm for ^{188}Re. In cancer RIT ^{213}Bi is assumed to kill by "direct hit", while ^{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" towards 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 (Figure 6A). No killing of CN cells by unlabeled mAb 18B7 was observed. Previously we had shown that radiolabeled irrelevant mAbs were unable to kill CN cells.[6] For ^{188}Re-18B7 "cross-fire" effect was responsible for most of CN killing (Figure 6B). This system per-

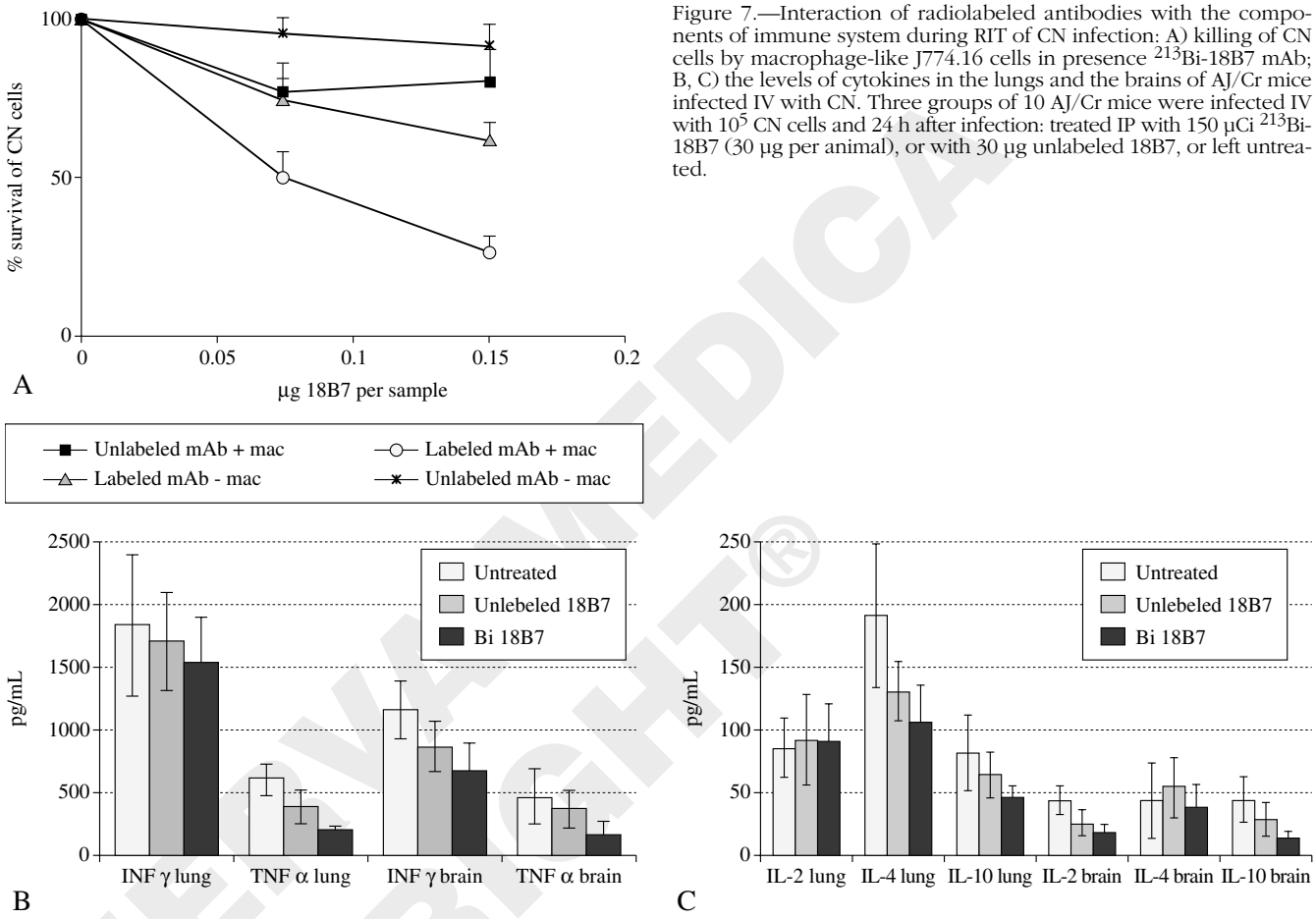

Figure 7.—Interaction of radiolabeled antibodies with the components of immune system during RIT of CN infection: A) killing of CN cells by macrophage-like J774.16 cells in presence ^{213}Bi-18B7 mAb; B, C) the levels of cytokines in the lungs and the brains of AJ/Cr mice infected IV with CN. Three groups of 10 AJ/Cr mice were infected IV with 10^5 CN cells and 24 h after infection: treated IP with 150 µCi ^{213}Bi-18B7 (30 µg per animal), or with 30 µg unlabeled 18B7, or left untreated.

mits experiments to elucidate precise mechanisms of cell killing in RIT that have not been performed either for microbial or cancer cells. In RIT targeting of cancer cells the antibody is often internalized after binding, adding significant complexity to the experiment. One of the advantages of the *C. neoformans* system is that the capsule is outside the cell wall and the antibody is not internalized, thus allowing exploration of this fundamental problem in radiobiology.

Exposing the mixture of CN cells and unlabeled 18B7 mAb to UV light initiated formation of singlet molecular oxygen $^1O_2$* required for mAb-catalyzed production of ROS[51] (results not shown). Radiation from radiolabeled mAb in solution which causes hydrolysis of water also triggered the antibody-catalyzed ROS generation. For ^{213}Bi-18B7 ROS formation was dose-dependent with ROS formation observed at 2.3 µCi/10^5 cells and none for 0.5 µCi/10^5 cells (slope not significantly different from zero, P=0.37) (Figure 6C). No ROS was observed for control ^{213}Bi-MOPC21 (P=0.2) suggesting a need for Ag-Ab interaction in this process (Figure 6C). However, ROS are unlikely to contribute significantly to fungal cell killing since no significant oxidant formation was observed for the dose of 0.5 µCi/10^5 cells which was fungicidal.

As CN can reproduce within macrophages, we investigated the interaction of macrophage-like J774.16 cells with ^{213}Bi-labeled mAb 18B7. Macrophages remained viable in the presence of radiolabeled mAb but the efficacy of fungal killing by J774.16 cells was significantly enhanced by ^{213}Bi-18B7 mAb in comparison with unlabeled mAb (Figure 7A). This cooperation was impressive given that the efficacy of CN

killing by mAb ^{213}Bi-18B7 alone in DMEM or in mouse serum was reduced when compared to killing in PBS (results not shown). Thus, synergy with macrophages in CN killing may be one of the immune mechanisms of RIT of infection.

As mAb-mediated protection in the setting of CN infection may be in part due to changes in cytokine expression,[52, 53] we also investigated whether RIT affected IL-2, IL-4, IL-10, TNF-α, and IFN-γ expression in treated mice. These cytokines are representative of Th1- and Th2-related cytokines important in the effective immune response to CN infection.[52, 54, 55] RIT was associated with changes in lung and brain cytokine expression (Figure 7B, C). There was a significant ($P<0.05$) reduction in the expression of TNF-α, INF-γ, IL-10 (brains and lungs), IL-4 (lungs), and IL-2 (brains) in RIT-treated mice relative to the untreated controls. There was a trend toward increased IL-2 in the lungs of RIT-treated mice ($P=0.06$); also unlabeled mAb 18B7 caused some increase in IL-4 in the brains of the mice. It is conceivable that local radiation could have affected inflammatory cell number or location thus resulting in an alteration in cytokine expression. Given the hypothesis that death in mice with CN infection can be caused by host-inflammatory damage,[54] the decrease in the levels of cytokines in RIT-treated animals raises the tantalizing possibility of benefit from reduced inflammatory damage.

Future perspective

RIT for infection is theoretically useful for any microbe susceptible to radiation killing including bacteria, fungi, viruses and parasites. The promise of RIT for infection is based on the fact that the technology is largely in place and that the only requirements are the availability of microbe-specific mAbs and radionuclides. In fact, it is conceivable that targeting microbes will be easier than targeting neoplastic cells because of the enormous antigenic differences between host and microbes. However, considerable basic work remains to be done to ascertain the optimal conditions for the efficacy of RIT for infection. It is likely that development of RIT for each infectious agent would encounter specific development issues given the variability inherent in microbes and their interactions with the host. RIT for infectious diseases may be of particular value: 1) in special populations such as immunosuppressed patients infected with *C. neoformans* or with other AIDS-associated opportunistic infections that are refractory to treatment with standard antimicrobial agents; 2) for treatment of latent infection in organ transplant patients; 3) for treatment of infectious diseases caused by highly resistant microorganisms for which therapeutic options are currently very limited; 4) for infectious diseases where there is no known treatment; 5) for protection against biological warfare agents.

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