Expert Opinion

Antibodies as delivery vehicles for radioimmunotherapy of infectious diseases

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The field of infectious diseases is in crisis and there is a need for strategies that can facilitate the rapid development of new antimicrobial agents. Radioimmunotherapy (RIT), a therapeutic modality originally developed for cancer treatment, has recently been suggested as a novel therapy for the treatment of a variety of infectious diseases. Because specific antibodies are used in RIT as delivery vehicles of cytocidal radiation, their molecular weight influences the nonspecific accumulation in infectious foci and blood clearance, and their affinity-specific accumulation of antibodies in infectious foci. Like the problems encountered in oncology, relevant variables in the development of RIT of infectious diseases include target antigen-shedding; delivering radionuclides to infectious foci in organs, abscesses, granulomas, heart and brain, and potential safety concerns. Dadachova and Casadevall anticipate that RIT can be developed for many types of infectious diseases, including microbes resistant to conventional antimicrobial therapy and agents of biological warfare.

Keywords: infectious diseases, monoclonal antibodies, radioimmunotherapy, scintigraphic imaging

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1. Introduction

Radioimmunotherapy (RIT) was developed for cancer treatment to take advantage of the specificity of the antigen–antibody interaction to deliver radionuclides that emanate lethal doses of cytotoxic radiation close to the target cell [1]. Radiolabelled monoclonal antibodies (mAbs) provide a valuable alternative to chemotherapy and external radiation beam by selectively delivering lethal doses of radiation to cancerous cells. Several therapeutic radionuclides are currently used in targeted radionuclide therapy (Table 1). The first US clinical trials of RIT in patients with hepatoma were performed by Order *et al.* in the mid-1980s [2]. Two decades later, RIT has been developed into a successful therapy for certain cancers as evidenced by the recent approval of mAb-based drugs, such as Zevalin® (IDEC) and Bexxar® (Corixa; anti-CD20 mAbs labelled with 90-yttrium [^{90}Y] and 131-iodine [^{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 [3] are encouraging, thus making RIT a frontline therapy in cancer.

The field of infectious diseases is currently in crisis because of an unfortunate coincidence of events that include:

- an increasing prevalence of diseases caused by highly resistant microorganisms, some of which are not susceptible to currently available antimicrobial agents
- the relative inefficacy of antimicrobial therapy in immunosuppressed individuals
- a dearth of new antimicrobial drugs in the development pipeline

To further complicate matters, new microbes are regularly identified and for many there are no available therapies, hence, there is the specter of biological weapons. For example, we recently witnessed a global outbreak of severe acute respiratory

Table 1. Therapeutic radionuclides.

Radionuclide	Type	Half-life	E_{max} (MeV)	Mean range (mm)	Imageable
^{90}Y	β	2.7 days	2.30	2.76	No
^{131}I	β, γ	8.0 days	0.81	0.40	Yes
^{177}Lu	β, γ	6.7 days	0.50	0.28	Yes
^{153}Sm	β, γ	2.0 days	0.80	0.53	Yes
^{186}Re	β, γ	3.8 days	1.10	0.92	Yes
^{188}Re	β, γ	16.9 h	2.10	2.43	Yes
^{67}Cu	β, γ	2.6 days	0.57	0.60	Yes
^{225}Ac	α, β	10 days	5.83	0.04 – 0.10	Yes
^{213}Bi	α	45.6 min	5.87	0.04 – 0.10	Yes
^{212}Bi	α	1.0 h	6.09	0.04 – 0.10	Yes
^{211}At	α	7.2 h	5.87	0.04 – 0.10	Yes
^{212}Pb	β	10.6 h	0.57	0.60	Yes
^{125}I	Auger	60.1 days	0.35	0.001 – 0.020	No
^{123}I	Auger	13.2 h	0.16	0.001 – 0.020	Yes
^{67}Ga	Auger, β, γ	3.3 days	0.18	0.001 – 0.020	Yes

Adapted from [1].
E_{max} : Maximum energy.

syndrome coronavirus infection that was associated with high mortality. In this environment, new approaches to antimicrobial therapy are needed. Specifically there is a need for strategies that can facilitate the rapid development of new antimicrobial agents, as current strategies for the development of antimicrobial drugs and vaccines take many years to yield clinically useful products.

Passive antibody therapy is a potentially useful therapeutic and preventive strategy against a variety of infectious diseases [4]. The specificity of the antigen–antibody interaction provides an attractive option for delivering microbicidal agents to sites of infection. The feasibility of RIT as an anti-infective therapy was recently demonstrated by treating murine cryptococcosis with a mAb to the *Cryptococcus neoformans* capsular glucuronoxylomannan labelled with 213-bismuth (^{213}Bi) or 188-rhenium (^{188}Re) [5,6]. Subsequently, the applicability of RIT to other fungal and bacterial infections was demonstrated [7,8]. RIT may also be potentially effective against chronically infected cells including those with viral infections [4].

The use of RIT for tumour therapy has been studied for two decades and there is a wealth of knowledge derived from both laboratory and clinical studies regarding the delivery of radionuclides by mAbs and fragments (both murine and humanised) to cancer cells surrounding tissue and major organs [9-11]. In contrast, the application of RIT to infectious diseases is in its infancy with experience limited to animal models. Consequently, most of the information on the issues associated with the application of mAbs as delivery vehicles for RIT of infectious diseases is inferred from the literature on RIT of cancer, as well as from the reports on the use of microorganism-specific mAbs for scintigraphic imaging of infection. Although some of this information is applicable to the development of RIT as an anti-infective modality, it is also apparent that there are fundamental differences between the two disease processes that necessitate different approaches and considerations.

2. The influence on molecular weight of the antibodies on nonspecific accumulation in infectious foci

Because antibodies are serum proteins, they can exhibit long serum residence time that is a function of the molecular characteristics of the constant region or isotype. For example, in mice, IgM and IgG have serum half-lifes of 2 and 8 days, respectively [12]. The pharmacokinetics of different mAb forms, including bioengineered ones, is summarised in [10] and pharmacokinetic data from clinical applications of radiolabelled mAbs are reviewed in [13]. These studies imply that the nonspecific accumulation of mAbs in the tumours is an interplay between the mass of a mAb and its plasma clearance. When comparing RIT of infection and cancer one may deduce that certain characteristics of infection make it easier for the mAbs to deliver their cytotoxic 'payload' to target cells, which is different from the situation in oncology. A particular difficulty in RIT for cancer is the slow penetration rate of antibodies into tumour tissue, which decreases the efficiency of treatment. In contrast, pathogen-specific antibodies are likely to reach the infectious foci more rapidly given that the increased capillary permeability found at the sites of infection

allows radiolabelled antibody to easily migrate from the circulation [14]. In this regard, Rennen *et al.* [15] studied the biological distribution of 11 99m-technetium (^{99m}Tc)-labelled proteins with the molecular weight of 2.5 – 800 kDa in Wistar rats with experimental *Staphylococcus aureus* abscesses. The authors did not observe a significant correlation between abscess uptake and molecular weight of the ^{99m}Tc-labelled proteins, and concluded that the major factor determining the penetration and nonspecific uptake of radiolabelled proteins in infectious foci was the blood residence time and not the molecular weight. Some uncertainties in this study are the use of the proteins from various sources (e.g., bovine serum albumin [BSA], IgG [human], aldolase [rabbit] with some of the proteins being glycosylated). Of course, other characteristics of radiolabelled mAbs besides their molecular weight, such as charge, shape and carbohydrate content of the molecule, could affect their *in vivo* behaviour, and this would need to be determined experimentally during preclinical testing.

3. Specific accumulation of antibodies in infectious foci: the influence of affinity

The affinity of the antibody characterises the binding of a monovalent ligand (antibody in this case) to a substrate (antigen) and is calculated from the ratio of the rate constants of association and dissociation [16]. The optimal level of binding affinity for the effectiveness of RIT of cancer is a complex problem [9], as mAbs with high affinity often bind to the rim of the tumour and do not penetrate into the deeper reaches of the tumour mass, thus leaving the centre of the tumour unaffected by radiation unless the radionuclide used emits a particle with very long tissue range. In contrast, Fab′ fragments have lower affinity for the antigen than intact mAbs as a result of losing the contribution of polyvalence, and show significantly lower percentage injected dose per gram of tumour (%ID/g) than intact mAb despite having a smaller molecular weight. For example, at 48 h postinjection the tumour %ID/g for intact mAb CC49, which binds to TAG72 antigen in human colon carcinoma xenografts and its $F(ab')_2$ and Fab′ fragments, are 18, 13.9 and 2.7, respectively [17]. The experience of Dadachova and colleagues with ^{188}Re-labelled mAb 18B7, which binds to the capsular polysaccharide on *C. neoformans* and its $F(ab')_2$ and Fab′ fragments showed the same trend; when injected intravenously into mice with systemic *C. neoformans* infection, %ID/g in the lungs at 24 h postinjection was 2.5, 1.1 and 0.8, respectively (Dadachova E, Bryan RA, Moadel T, Casadevall A, unpublished observations). Given that one of the reasons behind the generation of smaller antibody fragments (better tumour penetration) may not be applicable to RIT of infections, it seems reasonable to suggest that intact mAbs with higher affinity of the antigen rather than fragments could be more effective delivery vehicles for RIT of infection. However, this supposition will have to be experimentally validated.

Yet another option would be to use the so-called domain-deleted mAbs. Humanised mAb CC49 with a CH2 domain deletion (ΔCH2; HuCC49ΔCH2), which binds to TAG-72 antigen expressed on many carcinomas, had significantly faster blood clearance in comparison with intact HuCC49, while preserving the antigen binding affinity and having a reduced potential for causing a human anti-mouse antibody response [18]. Later studies showed that this domain-deleted mAb could be used to deliver short-lived isotopes such as ^{213}Bi with 45.6 min half-life to LS-174T human colon carcinoma xenografted tumours in mice with impressive therapeutic results [19].

4. Treating antigen-shedding infections

One theoretical impediment to using RIT in many cancers and infections alike is that patients can have high levels of circulating antigen in their blood that could interfere with RIT by binding to radiolabelled mAbs. However, high levels of microbial antigens accompany relatively few infectious diseases, possibly because the microbial burden needed to cause disease is relatively low when compared with the mass of the individual. Nevertheless, for some fungal diseases, significant amounts of antigen can be found in serum and tissue. Two examples of fungal diseases accompanied by antigenaemia are cryptococcosis and aspergillosis. However, there is experimental evidence that RIT for infection can be successful even in the presence of significant amounts of serum antigen. In this regard, Dadachova and colleagues observed that radiolabelled mAb 18B7 was therapeutic in mice even with serum antigen levels comparable with those found in patients. The most likely explanation for this effect is that the interaction of an antibody with antigen in the microbial capsule is stronger than with soluble polysaccharide. An example of this effect was observed in competition experiments, whereby very large amounts of soluble polysaccharide were needed to inhibit antibody binding to a small amount of polystyrene-absorbed polysaccharide by enzyme-linked immunosorbent assay [20]. Hence, the presence of soluble polysaccharide in the serum of these mice did not prevent the efficacy of RIT for experimental cryptococcosis. Although it remains to be determined whether soluble antigen will be a problem for RIT of other infectious diseases, Dadachova and Casadevall note that cryptococcosis is notorious for very high antigenaemia, and success in that infection provides encouragement for the applicability of RIT to other microbial diseases. Furthermore, it is noteworthy that only a few microbial antigens are released during infection and it is always possible to target those that are microbe-associated. As practically all microbial antigens are antigenically distinct from the host, this is a luxury in targeting that is not available in RIT for cancer, where suitable tumour-specific antigens are few.

In the event of soluble microbial antigen interfering with RIT in some infectious diseases, the strategies developed for the purpose of overcoming the problem of myelotoxicity could be adapted for those infectious diseases accompanied by

antigenaemia. For example, the so-called 'pre-targeting' strategy exploits the avidin/biotin system to target radioactive labels to mAbs already localised in the desirable site in the body [21]. Patients with infectious diseases could be injected with large amounts of biotinylated mAb to bind to the microorganisms at the foci of infection and to the circulating antigen in the blood (first step), followed in 24 – 48 h by avidin 'chase' to bind to biotin on the antibody and to clear the mAb–antigen complex from circulation (second step), and 24 h later by administering radiolabelled biotin.

Another strategy that can be modified to suit the special needs of RIT for infectious diseases is extracorporeal immunoadsorption (ECIA). In this approach, biotinylated and radiolabelled mAb is administered intravenously, allowing the antibody to accumulate in the tumour. Unbound circulating mAb is subsequently removed by passing the patient's blood through an extracorporeal avidin-del adsorption column [22]. Alternatively, a modification of ECIA that was used in clinical trials employed an anti-antibody adsorption column [23]. One can imagine that this approach can be modified for use in infection such that blood can be passed through the antibody column to eliminate the circulating antigen, followed by the administration of radiolabelled mAb.

5. Delivering radionuclides to infectious foci in organs, abscesses, granulomas, heart and brain

Several groups have used radiolabelled organism-specific antibodies to image infections in organs, abscesses, granulomas, heart and brain. Poulain *et al.* [24] infected guinea-pigs intravenously with *Candida albicans* and imaged them with radioiodinated intact mAb to the cell wall glycoprotein of *C. albicans* and with $F(ab')_2$ fragments. They observed that the biodistribution of *C. albicans*-specific mAb matched the anatomic distribution of *C. albicans* infection as confirmed by the colony-forming unit (CFU) per organ. There was a direct proportionality between the %ID/g organ and the number of CFU/g in each organ. In animals with *Candida* endophthalmitis, a common complication of candidal haematogenous dissemination, the distribution of *C. albicans*-specific mAb was highly specific in contrast to radiolabelled nonspecific mAb.

Rubin *et al.* [25] investigated whether a radioiodinated murine mAb to Fisher immunotype 1 *Pseudomonas aeruginosa* could detect the sites of deep thigh infections. For this purpose they induced unilateral, deep thigh infections in rats by innoculation with 2×10^8 Fisher immunotype 1 *P. aeruginosa* followed by intravenous injection of radiolabelled specific mAb or irrelevant control mAb 24 h later. Although the area of inflammation could be visualised with either the specific or nonspecific mAb at 4 h, by 48 h the signal-to-noise between abscesses and background began to disappear for the nonspecific mAb, whereas the signal-to-noise for the specific mAb-generated images continued to intensify with the differentiation between specific and nonspecific mAb-generated images becoming possible at 72 h. The authors concluded that it was feasible to image localised infections and hidden abscesses by scintigraphy with organism-specific antibodies. In this regard, abscesses were best visualised several days after injection of radiolabelled mAb. This implies that in RIT of infected sites located deep inside the body, longer-lived isotopes such as ^{90}Y (half-life 2.7 days), 177-lutetium (^{177}Lu; half-life 6.7 days) or ^{131}I (half-life 8 days) can be used with intact mAb. Alternatively, combinations of fast-targeting domain-deleted mAbs [18] with short-lived isotopes, such as ^{188}Re (16.9 h) or even ^{213}Bi (45.6 min) may also prove useful.

Extrapulmonary tuberculosis lesions are difficult to diagnose during early stages after dissemination and are characterised histologically by bacterial cells encapsulated in granulomas. The theoretical basis of attempting to image tuberculomas with radiolabelled antibody is based on the finding that mycobacterial antigen can be present in granulomas at much higher concentrations than found in the circulation. To investigate the feasibility of imaging tuberculomas with radiolabelled mAbs against mycobacterial antigens, researchers used mouse [26] and rabbit [27] models of tuberculosis infection. They induced tubercular lesions in rabbit and injected radioiodinated anti-*Mycobacterium bovis* (Bacillus Calmette–Guérin) mAb 4 – 6 months later. The specific mAb localised in tuberculomas at day 3 postinjection and maximal signal-to-noise between infected and noninfected tissues was observed by day 6. To exclude the possibility that the mAb localised in the lesions due to their increased vascularity, the authors injected control-infected rabbits with ^{99m}Tc-labelled red blood cells or radioiodinated BSA. Imaging with ^{99m}Tc red blood cells showed that the lesions were actually less vascular than the surrounding tissue and iodinated BSA did not show any significant localisation in the lesion. These results are encouraging for the feasibility of developing RIT for tuberculosis as the multi-drug resistant (MDR) *Mycobacterium tuberculosis* strains continue to emerge worldwide.

Huang *et al.* [28] explored the use of ^{99m}Tc-labelled mAb to *Staphylococcus aureus* to detect bacterial endocarditis in a rabbit model. The biological distribution of radiolabelled 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 for the infected animals (> 10:1) than in noninfected controls. The authors concluded that radiolabelled mAb was potentially useful for the detection of infected endocardial lesions.

Reaching infectious foci in the brain with radiolabelled mAbs can be challenging if the blood–brain barrier (BBB) is intact. In earlier work Goldman and colleagues found that in rats with intracisternal *C. neoformans* infection there was no detectable localisation of radiolabelled mAb 2H1 directed against *C. neoformans* capsular polysaccharide in the brain and cerebrospinal fluid following intravenous injection [29]. This inability of mAb 2H1 to cross the BBB was circumvented by administering mAb intracisternally, which resulted

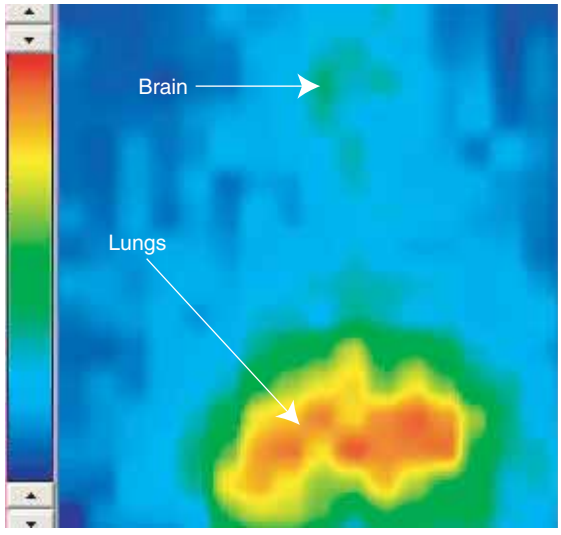

Figure 1. Immunoscintigraphic image of A/JCr mouse with systemic *Cryptococcus neoformans* infection injected with radiolabelled 18B7 mAb. Radiolabelled mAb was given 24 h postinfection and the image was obtained 48 h postinjection of radioactivity. The lungs and the brain are marked with the arrows. Red colour on a colour scale represents the highest radioactivity uptake. Reproduced from DADACHOVA E, NAKOUZI A, BRYAN R, CASADEVALL A: Ionizing radiation delivered by specific antibody is therapeutic against a fungal infection. *Proc. Natl. Acad. Sci. USA* (2003) **100**:10942-10947 [5].

in persistent intracisternal radioactivity uptake when compared with nonspecific control mAb. Later, Dadachova *et al.* used a systemic model of *C. neoformans* infection [5] in which, apparently, the BBB barrier becomes leaky as the presence of intraperitoneally injected ^{188}Re-labelled mAb 18B7 was detected in the brain scintigraphically (Figure 1). Furthermore, when therapeutic amounts of ^{188}Re-18B7 were administered intraperitoneally to systemically infected mice, there was a considerable reduction in the number of CFUs in the brains of treated animals in comparison with untreated controls, confirming that the radiolabelled mAb, which penetrated into the brain, delivered a cytocidal amount of radionuclide to microorganisms.

At least one study has shown that microbe-specific radiolabelled mAb can localise to the site of infection in humans. Goldenberg *et al.* [30] showed that the administration of a ^{99m}Tc-labelled mAb fragment specific for *Pneumocystis carinii* localised to the lungs in six of seven patients with pneumocystis pneumonia 24 h after administration. Hence, there is published evidence that radiolabelled antibody localises to the sites of bacterial and fungal infection in several studies involving humans and experimental animals. The ability of specific antibody to localise to a site of infection indicates the feasibility of using the antibody–antigen interaction to deliver microbicidal radiation to sites of infection, which in turn provides strong support for the potential usefulness of this technique as an antimicrobial strategy.

6. Mechanism of action

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 microbicidal. The radiobiological mechanisms of cancer RIT are complex and are different from those involved in killing the cancer cells during external beam radiation therapy (EBRT). In clinical RIT peak-dose rates of only 10 rad/h (0.1 Gy/h) [31] are observed. For comparison, high-dose rate radiation, which is typical for EBRT, delivers 6 krad/h (60 Gy/h). Thus, from the viewpoint of radiation therapy, RIT delivers suboptimal doses to tumours, but it is effective by promoting apoptosis in irradiated tumour cells, 'bystander' effect (death of adjacent, nonirradiated cells) and cell cycle arrest [32-34]. In the studies of RIT of fungal infections from Dadachova and Casadevall's group, they also observed the discordance between efficacy of EBRT and RIT; human pathogenic fungi *C. neoformans* and *Histoplasma capsulatum* proved to be extremely resistant to external γ-radiation (dose lethal to 90% of organisms tested [LD_{90}] 4000 Gy) but relatively susceptible to killing by RIT with ^{188}Re- and ^{213}Bi-labelled mAbs (LD_{90} 1 – 4 Gy) [7]. Radiobiological mechanisms of microbial cells killing by radiolabelled mAbs may involve 'direct hit' (killing of a cell by radiation emanating from a radiolabelled antibody molecule bound to the microbial cell) and 'cross-fire' (killing of a cell by radiation emanating from a radiolabelled antibody molecule on an adjacent or a distant cell), cell cycle arrest, bystander effect and the ability of antibodies to catalyse the synthesis of reactive oxygen species. In fact, they have recently been able to separate direct hit and cross-fire effects by using heat-killed *C. neoformans* cells with bound ^{213}Bi- or ^{188}Re-labelled capsule-binding mAb 18B7 as a source of cross-fire radiation (Dadachova E, Bryan RA, Apostolidis C, Morgenstern A, Zhang T, Moadel T, Casadevall A, unpublished observations). 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 targeting of cancer cells the antibody is often internalised 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 internalised, thus allowing the exploration of this fundamental problem in radiobiology.

In addition, it is possible that effector functions of the naked antibody also contribute to the antimicrobial effects. In this regard, antibodies are capable of promoting phagocytosis and complement activation, which can enhance their antimicrobial effects. Radiation emitted by radiolabelled antibodies at the sites of infection may also be beneficial to the host by killing infected host cells that are exploited by microbes for intracellular replication. Furthermore, in some

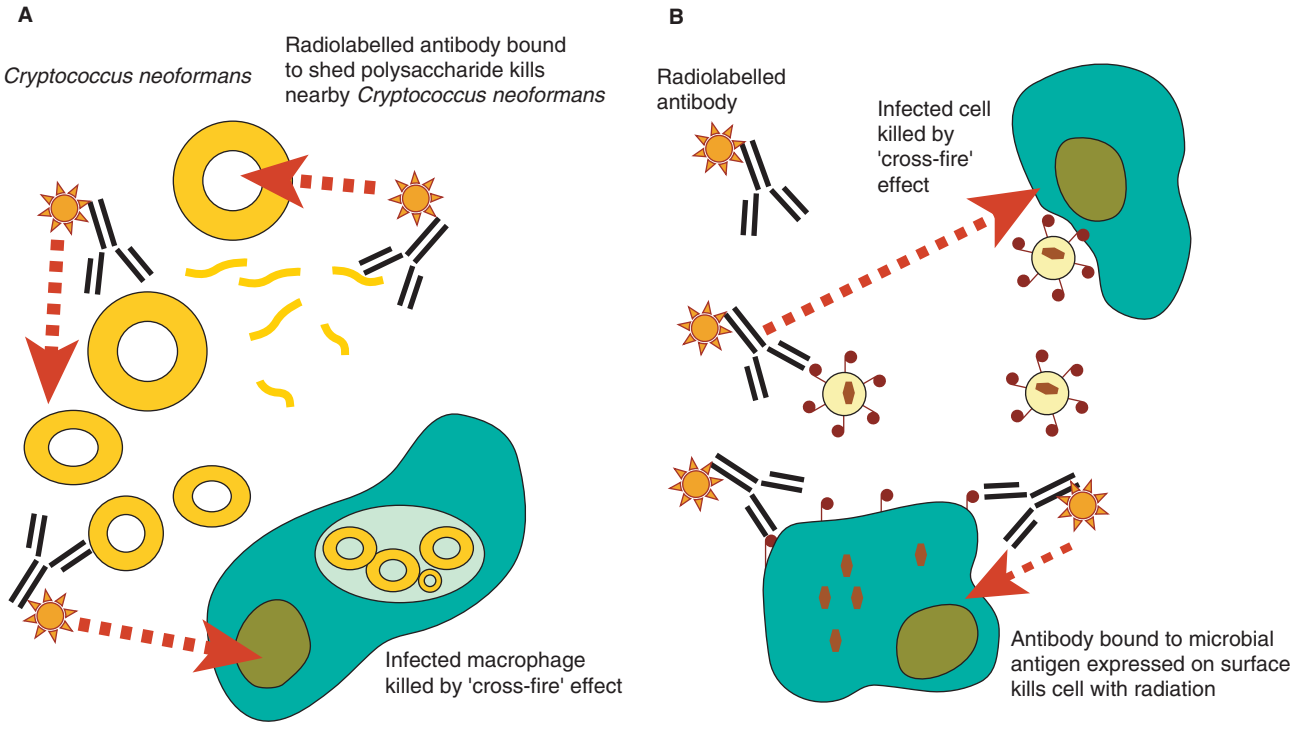

Figure 2. A schematic representation of the mechanisms by which radioimmunotherapy is effective against microorganisms. **A.** For *Cryptococcus neoformans* infection, a radiolabelled mAb binds to both capsular and shed polysaccharide, and delivers localised fungicidal radiation. Macrophages infected with replicating intracellular fungi can also be killed by a 'cross-fire' effect. **B.** The proposed mechanism by which radiolabelled antibody is effective against an intracellular pathogen, such as a virus that expresses microbial antigens on the cell surface after cellular infection. Reproduced with permission from CASADEVALL A, DADACHOVA E AND PIROFSKI L: Passive antibody therapy for infectious diseases. *Nat. Rev. Microbiol.* (2004) **2**:695-703 [4] Macmillan Magazines Ltd copyright (2004).

infectious diseases the majority of host damage is mediated by the immune response and it is possible that localised radiation would also kill inflammatory cells and reduce host-mediated damage.

RIT for infection is theoretically useful for any microbe susceptible to radiation killing, including bacteria, fungi and parasites. For viruses, their small cross-sectional area could make them less susceptible to deactivation by radiation. However, as viral replication is dependent on host cells, it is possible to use antibodies binding to viral antigens expressed on infected cells and thus target sites of viral replication and assembly. Hence, RIT is theoretically suitable for application to most, if not all, infectious diseases.

7. Advantages and disadvantages of radioimmunotherapy of infections

RIT of infections has certain advantages when compared with standard antimicrobial treatment strategies that rely on antibiotics or naked microbe-specific antibodies. The most important advantage of RIT over antibiotics is that the antigen–antibody interaction is not subject to multidrug resistance mechanisms, thus making it suitable for use against any of the many existing types of MDR microbial strains. Furthermore, it is highly unlikely that its use would result in the emergence of radiation-resistant strains as radiation damages microbes by multiple mechanisms. The antibodies used as delivery vehicles for radionuclides do not need to be protective as required in naked antibody therapy. In fact, the only requirement for an antibody to be a suitable reagent for RIT is that it binds to the targeted microbe. Although RIT could conceivably select for microbes not expressing the target antigen, this problem could be avoided by targeting an essential component of the microbial cell, such as the cell wall. Another advantage of RIT relative to naked mAb therapy is that the latter can be complicated by the occurrence of prozone-like effects at high mAb concentrations [35]. As the amounts of mAb administered during RIT are low, prozone-like phenomena are not expected to occur. As our experience has shown, microbes are susceptible to different forms of particulate radiation [5-8], so either β- or α-emitters can be used for the radiolabelling of mAbs. Importantly, RIT can kill not only microbial cells but also cells harbouring microbes, such as infected macrophages, via the cross-fire effect (Figure 2).

Certain features of infectious diseases make them more amenable for treatment with RIT than malignancies. As previously discussed, pathogen-specific antibodies are more likely

Table 2. Summary of the potential benefits and limitations of mAbs and their forms in radioimmunotherapy for infectious diseases .

mAb form	Advantage	Disadvantage
Intact		
Murine	Relatively easy to generate Suitable for use in many infectious diseases that occur once and require short treatment time Minimal toxicities associated with mAb alone Multiple isotypes available Effector functions associated with different isotypes (e.g., IgG2a mediates ADCC, IgG3 mediated CMC) Can be chemically modified to alter *in vivo* properties Genes can be cloned for genetic engineering	Immunogenecity in humans reduces half-life and suitability for repeated use Slow clearance from blood compartment could contribute to radiation toxicity of the treatment
Human	Nonimmunogenic Suitable for prolonged and repeated administration Multiple isotypes available mAb may be generated that possess several types of effector function (e.g., IgG1 isotype mediates ADCC)	Slow clearance from blood compartment could contribute to radiation toxicity of the treatment Difficult to generate and produce Difficult to obtain an appropriate isotype
Enzymatic fragments		
$F(ab')_2$	Smaller molecular weight Rapid clearance from blood compartment Improved target-to-normal tissue ratios due to rapid clearance from blood compartment Reduced immunogenecity	Reduced affinity if derived from IgM or IgA Difficult to generate by enzymatic methods Lower percentage delivered to the infected site as a result of more rapid clearance from blood compartment and reduced affinity
Fab′	Smaller molecular weight Reduced immunogenecity Rapid clearance from blood compartment High target-to-normal tissue ratios due to rapid clearance from blood compartment	Reduced affinity by loss of avidity Renal uptake Low percentage delivered to the infected site as a result of more rapid clearance from blood compartment and reduced affinity Difficult to generate by enzymatic methods
Genetically engineered forms		
Chimeric	Reduced immunogenecity Longer serum half-life than murine Introduce effector functions (e.g., CMC, ADCC)	Slow clearance from blood compartment Immunogenecity Reduced affinity Anti-idiotypic antibody responses Difficult to generate and produce
Humanised	Reduced immunogenecity Produce a mAb with desired effector functions CDRs can be manipulated to increase affinity	Slow clearance from blood compartment Reduced affinity and/or changes in fine specificity Difficult to generate and produce
Single chain	Single gene is introduced into a cell Does not rely on proper heavy–light chain association	Difficult to generate and produce Slow clearance from blood compartment
Domain deleted	Minimal renal uptake Rapid clearance from blood compartment Early maximal targeting High target-to-normal tissue ratios Reduced immunogenecity Good percentage delivered to the target	Possible reduction in affinity Generation and purification can be challenging
Hypervariable domain region peptides	Rapid clearance from blood compartment Easily produced May be synthesised with specific labelling sites	Low percentage delivered to the target Reduced affinity/avidity Only possible with linear CDR sequences May crossreact with normal tissues
Fv fragments (single chain, double strand)	*In vivo* stability Rapid clearance from blood compartment Reduced immunogenecity Early maximal targeting High target-to-normal tissue ratios	Increased renal uptake Low percentage delivered to the target

Adapted from [10].

ADCC: Antibody-dependent cellular cytotoxicity; CDR: Complementarity-determining region; CMC: Complement-mediated cytotoxicity; mAb: Monoclonal antibody.

Table 2. Summary of the potential benefits and limitations of mAbs and their forms in radioimmunotherapy for infectious diseases (continued).

mAb form	Advantage	Disadvantage
Multivalent Fv's	Increased valency improves affinity Improved percentage delivered to the target	Increased residence time in blood compartment
Minibodies	High target-to-normal tissue ratios Similar to advantages of domain-deleted mAb form	
Bispecific	High target-to-normal tissue ratios	Generation and production can be difficult

Adapted from [10].

ADCC: Antibody-dependent cellular cytotoxicity; CDR: Complementarity-determining region; CMC: Complement-mediated cytotoxicity; mAb: Monoclonal antibody.

to reach the infectious foci rapidly due to increased capillary permeability at the sites of infection. In most infectious diseases, it is not necessary for the treatment used to kill every microbe to achieve a therapeutic benefit because the immune system may effectively control infection when the inoculum is reduced by microbicidal radiation. In contrast, incomplete eradication of malignant cells would result in tumour recurrence and possible metastatic spread. Furthermore, in contrast to tumour cells, microbial cells are antigenically very different from host tissue, thus providing the potential for abundant antigen–antibody interactions with the fungal cell and low crossreaction with tissue.

As RIT of cancer is more than two decades old, the relationships between tumour size, curability and the tissue range of therapeutic emissions of radionuclides have been largely clarified [36]. However, considerable basic work remains to be carried out to ascertain the optimal conditions for the efficacy of RIT of infection. In fact, it is likely that the development of RIT for each infectious agent would encounter specific development issues given the variability inherent in microbes and their interactions with the host. Another area where RIT can take advantage of the information generated in cancer studies is dependent on the criteria devised for the selection of radionuclides for cancer RIT [37]. In contrast to its earlier days when ^{131}I was practically the only therapeutic radioisotope available, multiple radioisotopes are now available with decay characteristics that are useful for either the therapy of large tumour lesions (abscesses in case of RIT of infection) or for single-cell disease (systemic infections) (Table 1). As the field of RIT of infection evolves, bioengineered delivery vehicles, such as humanised and chimeric whole antibodies; domain-deleted and single-chain antibodies; hypervariable domain region peptides; Fv fragments and their multimeric forms; minibodies; and bispecific antibodies that are currently under investigation in cancer RIT (Table 2), can also be exploited for RIT of infectious diseases. In this regard, the use of a bispecific antibody, which is first monovalently bound to its target antigen and subsequently crosslinked by the divalent hapten with a second bispecific mAb to form a divalent binding bridge to the tumour antigen, offers additional opportunities for improved delivery of radioactivity to the tumour, while reducing the dose to bone marrow [38]. Dadachova and Casadevall envisage that by analogy with RIT of cancer, patients can be initially imaged with the organism-specific antibody labelled with a diagnostic isotope such as 99mTc or 111In to obtain dosimetry information prior to RIT administration. In general, the acceptance of RIT for certain lymphomas combined with the existence of a technical infrastructure to support this type of therapy have created a favourable environment for the development of RIT of infections in the clinical setting.

The disadvantages of RIT of infection are its high cost relative to conventional antimicrobial therapy. Antibody therapies have always been expensive because immunoglobulins are produced in either living donors or cell culture. Adding to the aforementioned costs of developing high-specificity antibodies and the manufacturing costs to include the requirements for a cold chain, intravenous administration and a precise diagnosis prior to use [4]. With regards to the potential acute toxicity to a patient, the data accumulated in clinical RIT of cancer indicate that the primary toxicity of high-dose RIT is likely to be bone marrow suppression. Important determinants of the extent and duration of myelosuppression include bone marrow reserve (based on prior cytotoxic therapy and extent of disease involvement), total tumour (infection) burden, spleen size and radioimmunoconjugate stability [9]. In case of infectious diseases accompanied by shedding of the antigen, significant amounts of antigen–antibody complex may accumulate in the liver and spleen, thus, potential toxicity to these organs should also be taken into consideration. Clearly, the application of RIT to infectious diseases will require optimisation of the dose to minimise toxicity and additional clinical development. However, the fact that the initial studies in mice suggest that RIT of infection is relatively well tolerated [5,6,8] and may have a significantly higher therapeutic index than RIT of cancer is encouraging.

In addition, when using a radioactive therapy in patients there is always concern of long-term effects such as neoplasms arising from radiation-induced mutations. Although this risk is relatively low and certainly outweighed by the benefits of treating life threatening infections, one needs to be aware of these potential concerns. In addition, since infectious disease specialists have little or no experience with radiation therapy, a significant education effort may be needed to gain acceptance of this therapeutic modality.

8. Expert opinion

RIT involves the application of established technology developed for the treatment of malignancies to infectious diseases. The development of RIT for infectious diseases is potentially easier than its application to tumour therapy given antigenic- and tissue-perfusion differences between sites of microbial infection and tumour infiltration. Nevertheless, considerable preclinical and clinical development work is likely to be required to learn how to optimally use RIT for infection. RIT for infectious diseases may be of particular value:

- 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
- for the treatment of latent infection in organ transplant patients
- for the treatment of infectious diseases caused by highly resistant microorganisms for which therapeutic options are currently very limited
- for infectious diseases where there is no known treatment
- for protection against biological warfare agents.

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