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Future Vistas

Future Vistas in Alpha Therapy of Infectious Diseases

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Introduction

The field of infectious diseases is currently in crisis given that (1) there is an increasing prevalence of infections with highly resistant microorganisms that are not susceptible to existing antimicrobial agents; (2) many infections occur in immunosuppressed individuals such as patients with cancer, organ transplants recipients, human immunodeficiency virus (HIV)-infected patients in whom standard antimicrobial therapy is not very effective; and (3) there is a dearth of new antimicrobial drugs in the development pipeline as evidenced by the paucity of new drugs in the past decade. In the last decades, we witnessed such events as global outbreak of severe acute respiratory syndrome, introduction of Monkeypox virus into the United States, and emergency of extensively drug-resistant tuberculosis and other bacterial infections. In this environment, we need new approaches to antimicrobial therapy. Specifically we need new strategies that can facilitate rapid development of new antimicrobial agents. Current strategies for the development of antimicrobial drugs and vaccines take many years to yield clinically useful products. However, monoclonal antibodies (mAbs) can be made very rapidly and the linkage of radionuclides to specific mAbs provides the means to generate microbicidal antibodies.

Antibody-antigen (Ab-Ag) interaction is a powerful tool to circumvent the problem of multidrug resistance. Radioimmunotherapy (RIT) is a therapeutic modality that uses Ab-Ag interaction and utilizes Abs radiolabeled with therapeutic radioisotopes to selectively deliver lethal doses of particulate radiation to cells in cancer treatment [1]. Radiolabeled mAbs provide a valuable alternative to cancer treatments with chemotherapy or external radiation beam. Radiation is microbicidal and γ-irradiation is routinely used for the 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 *Escherichia coli, Cryptococcus neoformans* (CN), and *Mycobacterium tuberculosis* [2–4]. Despite its microbicidal properties, radiation is not used in current antimicrobial therapy. Our group proposed to use therapeutic radionuclides for the treatment of various infections [5]. We hypothesized that RIT of infections can work in several ways—in case of fungal and bacterial cells, it can kill them with "direct hit" or via "cross-fire effect". For viral infections such as HIV, the goal of RIT would be not to kill the virus that is accomplished by the antiretroviral drugs but rather to target and kill the infected hosts cells where virus propagates itself (so called viral factories). This could be accomplished by targeting viral antigens expressed on the surface of the infected host cells with the radiolabeled mAbs specific for those antigens [6]. Finally, when developing a novel approach to treatment of infectious diseases, three very important factors have to be taken into consideration as in any new drug development—efficacy, safety, and mechanism of action.

RIT of Fungal Infections

CN served as a first model organism in our laboratory for development of infection RIT. CN is an encapsulated human pathogenic fungus. CN provides an excellent model for a chronic infection, and advantages of the CN system include (1) animal models including those for pulmonary, meningeal, and latent infection; (2) the availability of very-well-characterized mAbs to CN that can be developed into RIT agents; (3) well-understood pathogenesis of infection and immune response. MAb 18B7 to CN capsular polysaccharide antigen has been used in clinical trial in patients with cryptococcal

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meningitis [7]. In the first series of experiments, the efficacy of RIT against CN infection was tested in A/JCr mice (NCI, NIH). This mouse strain was selected because it is very susceptible to CN infections, presumably because of partial complement deficiency [8]. Mice with partial complement deficiency succumb rapidly with disseminated infection when infected IV [9]. Nine groups of 10 A/JCr female mice were infected IV with 10^5 CN cells. Mice were treated with different doses of ^{188}Re-18B7 and ^{213}Bi-18B7 mAbs, control mAbs ^{188}Re-MOPC21 and ^{213}Bi-MOPC21, unlabeled mAb 18B7, and with phosphate-buffered saline. Mice treated with radiolabeled mAb 18B7 lived significantly longer, on average, than mice given radiolabeled MOPC21 or phosphate-buffered saline. We used irrelevant immunoglobulin 1 (IgG1) mAb ^{213}Bi-MOPC21 or ^{188}Re-MOPC21 to control for the possibility that Fc receptor binding by the radiolabeled IgG1 to phagocytes at the site of infection might result in nonspecific killing. Remarkably, on day 75 after therapy, 60% of mice in ^{213}Bi-group were alive after treatment with 100 μCi ^{213}Bi-18B7 ($P < .05$). In the ^{188}Re group, 40% and 20% of animals were alive after treatment with 100 ($P < .005$) and 50 μCi ($P < .05$) ^{188}Re-18B7, respectively. The survival of mice was dose dependent in the range of 50–100 μCi for both ^{188}Re and ^{213}Bi with significantly more mice surviving in 100 μCi-dose groups. The dose of 200 μCi, although the most efficient in eliminating the fungal load in the target organs as shown by colony-forming units (CFUs), proved to be radiotoxic as shown by the toxicity studies. The optimal treatment doses for mice appear to be in the 50–150 μCi range. Mice infected with CN and given RIT had significantly reduced fungal burden in lungs and brains 48 hour after treatment than infected mice in the control groups. Although there was no difference in the reduction of the fungal burden in the lungs between the groups that received 50 and 100 μCi ^{188}Re-18B7, treatment with 200 μCi ^{188}Re-18B7 significantly lowered lung CFUs relative to the lower activities ($P < .05$). Hence, administration of CN-specific radiolabeled mAb prolonged survival and reduced organ fungal burden in infected mice. Low levels of CFUs were detected in the lungs of surviving animals sacrificed at the completion of survival studies [10]. In the follow-up experiments, we have compared the RIT with amphotericin B, which is currently the standard of care for treatment of invasive fungal infections. Remarkably, a single injection of ^{213}Bi-labeled 18B7 completely eliminated CN infection from the lungs and brains of C57Bl6 mice, whereas 2-week course of amphotericin B was unable to significantly lower the fungal burden in these organs [11].

Currently we are developing RIT for treatment of *Blastomyces dermatitidis*. This fungal pathogen affects both companion dogs and humans, and not only immunocompromised but also healthy individuals. The experiments on evaluating safety of RIT in healthy dogs are currently ongoing and will be followed by the clinical trial of RIT in companion dogs with blastomycosis. If successful, the results will provide crucial data on efficacy and safety of RIT in large animals, which is required for FDA and Health Canada for human translation.

RIT of Bacterial Infections

Bacillus anthracis is a powerful agent for use in biological warfare, and infection with the organism is associated with a high rate of mortality, underscoring the need for additional effective therapies for anthrax. We investigated if RIT approach could be successfully used with toxin-binding mAbs for diseases caused by toxigenic bacteria. Indirect immunofluorescence studies with mAbs to protective antigen and to lethal factor revealed the surface expression of toxins on bacterial cells. Scatchard analysis of mAbs revealed high binding constants and numerous binding sites on the bacterial surface. To evaluate the microbicidal properties of these mAbs, our group radiolabeled mAbs with ^{188}Re and ^{213}Bi. In vitro, ^{213}Bi was more efficient than ^{188}Re in mediating microbicidal activity against *B. anthracis*. *B. anthracis* releases tripartite toxin which is composed of a cell-binding protein, known as protective antigen, and two enzyme components, called edema factor and lethal factor [12]. The administration of ^{213}Bi-labeled mAbs to protective antigen and to lethal factor prolonged the survival of A/JCr mice infected with *B. anthracis* Sterne bacterial cells [13]. These results indicate that RIT with mAbs that target *B. anthracis* toxin components can be used to treat experimental anthrax infection and suggest that toxigenic bacteria may be targeted with radiolabeled mAbs.

Total joint replacement is the last resort treatment for degenerative joint disease. A feared complication is prosthetic joint infection (PJI) with an incidence of 1%–2% after primary hip arthroplasty and 1%–4% after primary knee arthroplasty [14]. PJI is difficult to treat as the bacteria form a biofilm on the prosthetic material. This hinders the host immune system, but more importantly, the bacteria in a biofilm are mostly in a dormant state and therefore not susceptible to most antibiotics [15]. Currently in collaboration with Drs. van der Wal, Weinans and van Dijk from the University of Medical Center Utrecht, the Netherlands, we are investigating whether alpha-RIT directed towards wall teichoic acids in *Staphylococcus aureus,* a major pathogenic cause of PJI [16], can kill methicillin-resistant *S aureus*, thus providing the proof of principle data needed for the development of RIT into the strategy for noninvasive treatment of PJI.

RIT of HIV

HIV/AIDS remains an enormous public health burden. Advances in combination antiretroviral therapy have greatly reduced mortality and morbidity but HIV remains incurable, with people living with HIV suffering numerous comorbidities and treatment-related side effects. Even with strict ART adherence, they are at significantly increased risk for cognitive deficits, cancer, osteoporosis, etc., at an earlier age than the uninfected population [17]. ART acts by blocking HIV replication steps and thus prevents infection of new cells, but it does not kill existing infected cells. Long-lived cell populations such as resting memory CD4 T cells and cells of monocytic lineage can act as reservoirs, harboring infectious HIV even in people living with

HIV with undetectable peripheral virus levels. These reservoirs are formed within the first months after HIV infection and, on interruption of ART, will reestablish productive infection [18]. In addition to cellular reservoirs, there are anatomic reservoirs created by cell-to-cell spread and the inability of drugs to reach therapeutic concentrations in various tissues. The central nervous system (CNS) is particularly vulnerable as HIV neuroinvasion that can occur as early as 4–10 days after peripheral infection, but the blood-brain barrier (BBB) restricts passage of many ART drugs [19]. The resulting sustained viral replication increases the likelihood of emergence of drug-resistant strains which can infect systemic sites. In addition, even the presence of nonreplicating virus can contribute to reinfection of the periphery and CNS when reactivated. Neurocognitive disorders affect over 50% of patients with HIV in the United States, and the prevalence will increase because of the longer life spans of HIV-infected individuals [20]. While early and aggressive treatment with ART can substantially reduce the size of the total reservoir, a stable population of latently infected CD4 T cells appears unaffected by early ART in the periphery [21]. The CNS remains a reservoir of HIV even with successful ART. In fact, recent data indicated that even ART regimens that penetrate the CNS do not decrease HIV-associated neurocognitive disorders, and often make it worse [22]. With so far unsuccessful attempts to cure HIV using bone marrow transplants [23,24], new treatments that eliminate both anatomical and cellular reservoirs of HIV are needed.

We reported on the use of RIT with radiolabeled mAb to effectively target HIV-infected cells. Our proof-of-principle experiments using RIT to eradicate HIV infection utilized human mAbs to gp41 glycoprotein as lead candidates for the RIT component [25,26]. When conjugated to an alpha-emitter ^{213}Bi, human mAb to glycoprotein 41 (gp41) 2556 safely eliminated HIV-infected human peripheral blood mononuclear cells implanted in severe combined immunodeficiency mice, with no hematologic toxicity based on platelet count [26]. RIT specifically killed HIV-infected cells from people on two different ART regimens and from ART-naïve individuals [27]. Using the same radiolabeled mAb to gp41, we demonstrated that nonactivated latently infected cells of both monocytic and lymphocytic lineages expressed low levels of gp41 on their surfaces, which were nevertheless sufficient for their targeted killing with RIT [27]. Radiolabeled 2556 mAb was able to penetrate to some extent an in vitro human BBB and selectively killed infected monocytes and peripheral blood mononuclear cells that had transmigrated across the barrier and resided in the CNS [28]. The mAbs used as homing devices in RIT are non-neutralizing and do not put selective pressure on the infectious agent. Although RIT can cause both transient and long-term myelodysplasia, neutropenia, and thrombocytopenia in patients with cancer, the overall safety record of RIT is strong [1] and is particularly important in light of the continuing high mortality and low success rates of bone marrow transplants and gene therapy approaches for treating HIV [29]. Currently we are developing novel human antibodies to gp41 with better penetration through the intact BBB that will be subsequently tested first in the state-of-the-art humanized HIV mouse model triple-knockout mice engrafted with fetal human bone marrow, liver, thymus (TKO-BLT mice) [30] followed by studies in simian/human immunodeficiency virus–infected nonhuman primates.

Conclusions

RIT was developed for the treatment of cancer decades ago. Experimental results repeatedly provide strong evidence of the potential usefulness of RIT in different microbial infections. In fact, because microbes express antigens unique and different from host antigens, simplicity in targeting them with high specificity and low cross-reactivity may potentially be achievable with fewer complications. Recent approval of several targeted radionuclide therapies in cancer arena both in Europe and the United States demonstrates that medical community has become much more willing to utilize such therapies in patients' care. We believe that the combination of immune and radiation therapy provides an exciting new strategy that may be potentially useful against a variety of intractable infectious diseases.

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