THREE-STEP IMMUNOSCINTIGRAPHY WITH THE AVIDIN-BIOTIN SYSTEM: STATE OF THE ART AND FUTURE PROSPECTIVES

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INTRODUCTION

Specific targeting of radioactive agents to tumor cells has been a major goal of the in vivo use of monoclonal antibodies (Mab) for diagnostic and therapeutic purposes. However, only a relatively small amount of the injected dose of Mabs is bound to the tumor, while Mab conjugated to radioisotope keep circulating in the blood stream and in normal tissue. Also circulating Mabs, especially when bound to a beta emitting isotope, are obviously undesirable and should be limited in order to protect normal tissue such as bone marrow.

Antibody fragments, such as F(ab')₂ or Fab have a faster blood clearance than whole antibodies, to the extent of allowing the use of short-lived radionuclides such as ⁹⁹mTc with good diagnostic sensitivity. This improvement, however, does not yield tumor to background ratios high enough to allow low-risk therapeutical applications.

Strategies of tumor pre-targeting, where Mab and radiolabel are administered separately have been proposed to reduce the background noise due to circulating antibodies (1, 2, 3, 4). A 3-step immunoscintigraphy (3-S-ISG) using the avidin-biotin system has been used in cancer patients (5).

MATERIALS AND METHODS

Injection of biotinylated Mab (first step) is followed by avidin (second step) in order to precipitate circulating biotinylated Mab and at the same time to target the tumor cells allowing adequate homing in of the subsequently administered labelled biotin (third step). From early 1990 to November 1993 we have studied and followed up 127 patients according to the protocol described above using different Mabs specific for different tumors including colon and lung cancer, gliomas, melanomas and apudomas.

RESULTS

The method has shown to be safe, reliable and of clinical utility since an overall sensitivity of 88% with 94% specificity and 84% accuracy was demonstrated. Moreover, 38 unknown lesions in 25 patients were localized and 21 were confirmed in their follow-up. Of these patients, 16 had not evidence of disease at the time of 3-S ISG but only increased tumor markers. The immunoresponse against biotinylated mouse IgG (HAMA) and avidin (HAAR) was evaluated in 73 patients. None developed HAMA after the injection of 1-2 mg of whole biotinylated IgG and 8/73 patients developed a weak HAAR response. However, radioactivity delivered per gram of tumor was in the range of 0.01-0.001% i.d., still below the optimal dose for radioimmunotherapy. This was probably due to the fact that avidin blood clearance is very fast with a T1/2 of 82 minutes. Thus, we are now developing a recombinant avidin molecule in order to reduce the immunogenicity and improve its pharmacokinetic in view of a therapeutical application of this approach to cancer treatment.

DISCUSSION

Tumor pretargeting methods with the 3-step approach have been shown to offer several advantages over the administration of directly labelled Mabs. In particular since the label is a small molecule, with a fast blood clearance background radioactivity levels are drastically reduced and imaging can be performed shortly after injection of the radiolabel. The 3-step protocol is designed to remove the excess circulating biotinylated antibodies as cold complexes and this is obtained prior to label injection.

The use of unlabelled, unfragment antibodies also avoids their damage by autodisolysis and by enzyme treatments. Given that more than one molecule of avidin can bind to a single polybiotinylated Mab molecule localized on the tumor, and that up to three radioactive biotin molecules can bind to an avidin molecule this approach is also designed to provide an amplification of the signal from the tumor.

Moreover, one can use any Mab from a panel, or Mab mixtures: the second and third steps of the three-step protocol would be common to all studies and the use of a cocktail would enhance the possibilities of targeting more tumor cells by using different tumor antigens as target. For successful diagnosis as well as therapy the tumor must be covered as much as possible in avidin.
These protocols have now been optimized and can potentially be applied widely in the majority of solid tumors including breast, lung, colon, ovary and others malignancies.

Techniques employed in genetic engineering will be able to provide chimeric proteins made up of antibody fragments and recombinant avidin in order to obtain an antibody molecule conjugated with a modified avidin tetramer of low immunogenicity.

REFERENCES


A NONRADIOACTIVE, BRANCHED DNA-BASED TECHNIQUE FOR DETECTION OF TRYPANOSOMA BRUCEI SPP. IN BLOOD

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INTRODUCTION
Since their recent advent, molecular diagnostic methodologies have proven to be very useful and offer a number of advantages over microscopic, biochemical, and immunologic procedures for the detection of pathogens. To date, target amplification methods such as the polymerase chain reaction (PCR) have been the most commonly applied molecular techniques. The branched DNA (bDNA) Signal Amplification Assay is an alternative hybridization based system for the sensitive and rapid detection of agents of infectious disease. This molecular technique amplifies the signal from a target molecule rather than the target itself, and thus avoids artifactual problems that have hampered other molecular diagnostic methodologies (1).

We have developed a nonradioactive bDNA-based assay for detection of Trypanosoma brucei in clinical samples. T. brucei gambiense and T. brucei rhodesiense are the etiologic agents of sleeping sickness, and the disease is considered a major health problem in many African countries for humans as well as for cattle, which are infected with T. brucei. The accurate diagnosis of African sleeping sickness by direct blood examination is problematic due to the wave-like fluctuations in levels of parasites present in biological samples, while immunologic tests are hampered by the trademark antigenic variation of T. brucei used by the parasite to avoid the host immune response. We have developed a sensitive and specific diagnostic assay for African trypanosomiasis, regardless of the stage of infection, variable antigen type (VAT), or subspecies of T. brucei.

MATERIALS AND METHODS
In the branched DNA assay, crude lysates of samples are denatured and hybridized in solution to two sets of oligonucleotide probes (50 mers). One set serves to capture the target sequence from the organism of interest by hybridizing to both the target sequence and oligonucleotide probes bound to microtiter dish wells. The other set of probes contains regions complementary to both the target sequence and to branched DNA molecules and serves to amplify the signal. Each of the 45 branches in this amplifier structure then hybridizes to an alkaline phosphatase-labeled probe. Finally, the complex is detected by the addition of an enzyme-triggerable chemiluminescent substrate, and light emission is measured.