

with a luminometer or a Polaroid camera (1). Plasma and Buffy coat were prepared from heparinized blood samples and used directly in the *T. brucei* bDNA assay (2).

RESULTS AND DISCUSSION

Two repetitive DNA sequences specific to the *Trypanosoma brucei* complex were chosen as targets, namely the 177 bp satellite repeat (3) and the RIME sequences (4, 5), and the appropriate oligonucleotide probes were designed and tested. Parasites were detected in biological samples with clinically relevant sensitivity (5-10 parasites/ μ L of blood), and comparable limits of detection were observed with cloned target sequences, purified *T. brucei* DNA, procyclic trypanosomes and bloodstream trypomastigotes. Analysis of the species specificity of the *T. brucei* bDNA assay with serial di-

lutions of purified DNAs revealed a strong signal from the three *T. brucei* subspecies and no signal from a variety of related organisms.

Thus, the branched DNA technology offers certain advantages over alternative molecular techniques, including the simplicity of sample preparation and of the procedure itself, the stability of the reagents, the ability to process large numbers of samples simultaneously, and freedom from cross-contamination artifacts.

REFERENCES

1. URDEA, M. S. (1993). *Clinical Chemistry* 39:725-726
2. HARRIS, E. *et al.* Manuscript in preparation.
3. SLOFF, P. *et al.* (1983). *J. Molecular Biology* 167:1-21
4. HASAN, G. *et al.* (1984). *Cell* 37:333-341
5. KIMMEL, B. E. *et al.* (1987). *Molecular and Cellular Biol.* 7:1465-1475

INTRODUCTION AND IMPLEMENTATION OF MOLECULAR TECHNOLOGY FOR THE DIAGNOSIS AND EPIDEMIOLOGY OF INFECTIOUS DISEASES IN LATIN AMERICA

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INTRODUCTION

Molecular techniques, such as the polymerase chain reaction (PCR) and nonradioactive DNA probes, can be applied to the diagnosis and epidemiology of infectious diseases in countries of limited resources. When appropriately implemented, these molecular techniques are more rapid, more sensitive, more specific, safer, and less costly than existing methods. We have developed a successful format for the transfer of molecular technology to Latin American countries through the use of on-site hands-on workshops (1).

The program is comprised of sequentially staged and progressively more complex courses which provide participants with solid experience in the theory and practice of molecular technology, epidemiology and proposal development. The courses progress from the introduction of molecular technology (Phase I) to its implementation by local scientists (Phase II and beyond) and are accessible to a wide range of participants, since prior training is not a pre-requisite. Course I is designed in consultation with local scientists, who select the pathogens to

be detected based on national health priorities. Participants in the first workshop then design the pilot studies they will conduct in Course II and collect the appropriate samples. These pilot studies form the basis of grant proposals detailing larger studies which are developed during Course II. These courses have been conducted in Nicaragua and Ecuador and are planned in a number of additional countries in the region.

MATERIALS AND METHODS

Existing methodologies (PCR and nonradioactive DNA probes) for detection of a range of pathogens were adapted for country-specific applications, allowing course participants to detect *Leishmania*, *V. cholerae*, *M. tuberculosis*, dengue virus, *Shigella* and enterotoxigenic *E. coli*, and *P. falciparum* in clinical and environmental samples. Extraction procedures were simplified for rapid processing, and PCR was carried out by manual amplification or with a thermocycler, if available. Simple but effective precautions were used to minimize the chance of cross-contamination of samples, including fre-

quent treatment of bench surfaces and micropipettor shafts with sodium hypochlorite (household bleach) and the use of separate rooms and separate sets of micropipettors for the preparation of PCR reactions, DNA extraction, and analysis of amplified products.

RESULTS AND DISCUSSION

These workshops have received enthusiastic responses from participating scientists, physicians, and medical technicians. Each course includes 20 participants from throughout the country; for instance, in the most recent Phase I course in Ecuador, participants came from 8 cities representing 15 different institutions. As a result, important contacts and collaborations have emerged between individuals and institutions, at the national, regional and international level.

As a consequence of these courses, several projects have been initiated in such areas as the molecular epidemiology of *Leishmania* in Central America and the

molecular diagnosis of tuberculosis. An ongoing study in Nicaragua funded by the European Economic Community involves the identification of Central American strains of *Leishmania* by molecular means (PCR, RAPD, RFLP) and the molecular characterization of putative hybrid strains (2). Pilot projects for the upcoming Phase II course in Ecuador planned for May, 1995, include the molecular diagnosis and epidemiology of tuberculosis, the detection of dengue virus in clinical samples and in the rapid typing of *Leishmania* strains from clinical specimens. Similar courses are currently planned in Bolivia, Honduras, Argentina and Brazil as well.

REFERENCES

1. HARRIS, E. *et al.* (1993). *Biochemical Education* 21(1): 16-22
2. BELL, A. A. *et al.* *Parasitology*, in press.

POLYCLONALS TO PEPTIDES: A SEARCH FOR THE MAGIC BULLET

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INTRODUCTION

It has been the dream of clinicians to be able to direct the therapy to a specific target organ or disease cell and produce a specific response. This response may vary from stimulation of cells in the endocrine system to cell death in cancer cells. Until the 1970s the only weapons available for this were chemical based on pharmaceuticals. These tended to have a systemic effect, for example Mustine kills cancer cells but is only slightly less toxic to normal cells. The discovery of antibodies and of their unique property of recognising a specific receptor molecule has brought with it the hope that it might be possible to deliver a drug or radioisotope to specific cell type whilst other cells would remain untouched. Hence the idea of a magic bullet guided to a specific receptor on a specific cell by the specific binding sites which will bind only with its expected target.

THE ROLE OF NUCLEAR MEDICINE

Nuclear Medicine has been at the forefront of the clinical application of biotechnology advances into patient care. Nuclear Medicine is the study of the use of radioisotopes in the diagnosis and therapy of disease. It

provides unique functional information not available with radiology. If the correct isotope is used the progress of a labelled substance can be easily followed non-invasively using a gamma camera. Therefore Nuclear Medicine is an essential partner in turning the products of biotechnology into clinically useful substances. The aim of this report is to review the progress in developing disease specific agents for diagnosis and therapy from standpoint of the Nuclear Physician.

POLYCLONAL IgG

The simplest method available which will enable imaging using antibodies is to take blood from a large number of donors these can be pooled and the labelled. The result will be a non-specific agent but one which will localise at the sites of highest antibody activity. This effectively means site of infection and inflammation.

Preparations of polyclonal human IgG have been used labelled with two metallic radioisotopes both of which can produce good images. The first of these is indium-111 (In-111) which is attached to the IgG via a diethylenetriaminepentaacetic acid (DTPA) linker. Trials in Europe and