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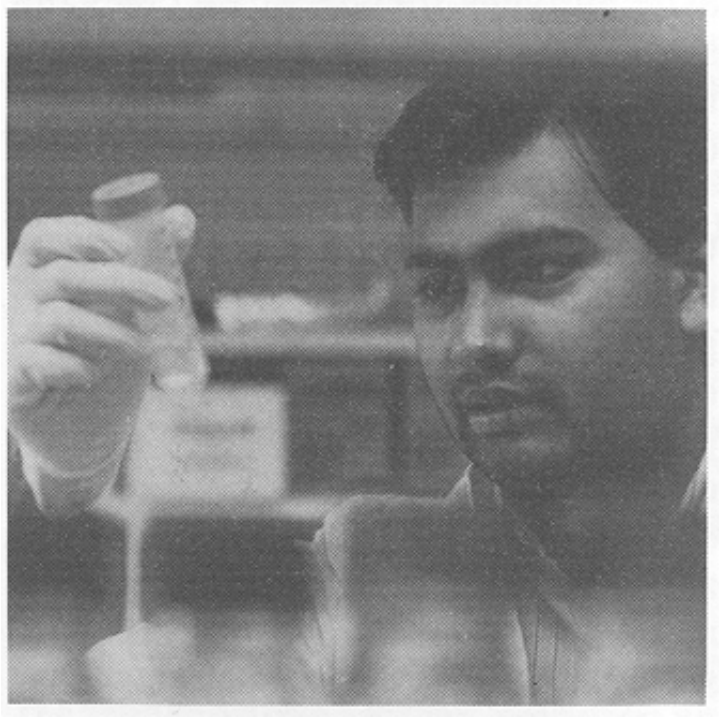
Biotechnology in the Service of Third World Agriculture

Molecular and other biological techniques are powerful tools with which to ask basic research questions. Many of these techniques may be used to find answers to seemingly intractable health and food problems in poor countries. Use of advanced biotechnologies at ILRAD is driven by the institute's research mandate, which is to develop novel methods for diagnosing and controlling diseases that cripple tropical livestock industries. In this regard, ILRAD is in a unique position to act as a technological resource and a knowledge broker for the livestock research systems of developing countries.

By providing biotechnological tools, training, advice and support, ILRAD helps national programs exploit the latest research advances to develop better ways of managing livestock disease problems. The biotechnological expertise and infrastructures being augmented today in developing countries will be of wide use in human as well as veterinary medicine. In addition, these capacities will be in place to help national scientists determine which among the technologies of the future will most appropriately address the fundamental constraints in tropical agriculture and health.

PRACTICAL APPLICATIONS of scientific knowledge generate technologies. Those that rely on living organisms or their products are known as biotechnologies. Simple biotechnologies such as those used to make alcoholic drinks and yoghurt have been in use for thousands of years. Recent advances in molecular biology, however, particularly the ability to alter the genes of organisms, have given the term new meaning. Genetic engineering is employed both to advance biological research and to construct biological products—such as vaccines, enzymes, hormones and genetic markers for diagnosing diseases—which are becoming increasingly important and commonplace in human and veterinary medicine.



Biotechnologies are used, developed, refined and modified at ILRAD on a daily basis. These tools are a prerequisite for the Laboratory's investigations into parasitic diseases of livestock and the development of improved methods for their control.

This article reviews biotechnologies that ILRAD has developed or used and transferred to institutions in developing countries to accelerate their research and improve their control of tropical livestock diseases. The biotechnologies discussed include not only methods used to manipulate biological molecules but also immunological assays, systems for cultivating cells and parasites *in vitro* and a group of techniques used in research to develop improved types of cattle.

THE RESEARCH conducted at ILRAD is designed to improve control of two disease complexes—trypanosomiasis and tick-borne diseases—that kill millions of ruminant livestock annually and cause severe production and economic losses throughout the tropical and subtropical regions of the world. The institute aims to develop better disease control methods by conducting strategic 'upstream' research. In their investigations, ILRAD staff adopt and tailor the latest molecular and immunological technologies generated in laboratories of the developed world for research on livestock disease problems of the developing world.

ILRAD and its research partners in developing countries employ biotechnologies both to make existing disease control methods more effective and sustainable and to find longer term solutions to these disease problems. The latter include novel vaccines, new drug treatments and genetically improved breeds of livestock.

Biotechnolgy at ILRAD

In the 1970s, a team of ILRAD scientists made a research breakthrough by developing a laboratory system for cultivating bloodstream forms of *Trypanosoma brucei,* one of the parasites that causes trypanosomiasis in cattle. Further improvements of these techniques led to the development of systems for cultivating the major species of pathogenic trypanosomes and different life cycle stages that occur in the parasite's ruminant host and tsetse vector.

The great quantity of parasites produced using these cultivation systems enables scientists to search for trypanosome genes that control the parasite's growth and differentiation from one form to another, to assess the sensitivity of different trypanosomes to trypanocidal drugs, and to determine which molecules of the parasites stimulate the animal host to produce antibodies against them. These investigations, although critically important to development of methods to control trypanosome infections, are difficult and sometimes impossible to conduct *in vivo*.

ILRAD staff also routinely generate and maintain hybridoma cell lines for the production of monoclonal antibodies, cell lines infected with life cycle stages of *Theileria parva* (a parasite that causes the tick-borne disease East Coast fever), bovine bone marrow cells, and mammalian and insect cell lines able to express foreign protein antigens. The now widespread use of cryopreservation of parasite field isolates and cultivated cell lines enables scientists to establish standardized populations of parasites and cells that may be used, for example, to produce quantified vaccine doses. Instruction on how to preserve cells using this relatively simple but critical technology is given in ILRAD's training courses for disease control personnel.

ILRAD scientists produce monoclonal antibodies for many purposes. They employ antibodies, for example, in enzyme-linked immunosorbent assays (ELISAs) to improve diagnosis and identification of disease-causing parasites. They also couple antibodies to electron-opaque labels, such as heavy metals, to follow intracellular processes at the ultrastructural level. In addition, purification of individual lymphocyte populations marked by specific monoclonal antibodies and separated electronically by a fluorescence-activated cell sorter enables scientists to identify the discrete contributions of those cells to the immune responses of ruminants.

Molecular genetics

Molecular genetics research at ILRAD has greatly expanded in recent years as the reagents and techniques for identifying, sequencing and transferring DNA among organisms have become routinely established in biomedical laboratories in developed countries. Molecular research at ILRAD falls into four categories.

1. 'Foreign' parasite and ruminant genes are expressed in host organisms such as viruses, bacteria and eukaryotic (mammalian and invertebrate) cell lines to examine the function of the protein encoded by the foreign gene.

2. Techniques to size chromosomes and amplify or hybridize DNA sequences characteristic of a given parasite are used to identify individual species or sub-groups of parasites.

3. Differences in the expression, or activation, of a given gene among the stages of a parasite's life cycle and among parasites exhibiting key differences in such important factors as drug sensitivity, infectivity and virulence are explored to identify genes directing parasite processes that are key to growth and pathogenesis.

4. The genomes of parasites and cattle are being mapped, and genetic markers are being produced, to locate genes governing important traits such as the growth of parasite populations and resistance to disease in cattle. Genetic markers are also being developed to determine what variability exists in an area of the bovine genome known as the major histocompatibility complex (MHC); this information will help scientists choose appropriate parasite antigens for use in vaccines designed to provoke immune responses in cattle that will protect them against disease.

It is probable that the different types of genetic markers and the techniques of segregation analysis of genetic material that are being developed at ILRAD will be directly and profitably applied in studies of genetic disease resistance in livestock species other than those studied at the Laboratory. The markers and techniques will also be of use in identifying and conserving indigenous breeds and genes likely to be of critical importance in increasing future livestock productivity in sustainable ways.

ILRAD is transferring to national research systems in developing countries the tools and expertise it has developed to investigate genetic disease resistance in ruminants and to make judicious decisions regarding what breeds should be conserved. For example, ILRAD training in MHC typing and molecular technologies and its transfer of gene markers has enabled a group of Tanzanian scientists from Sokoine University of Agriculture to examine genetic distances among breeds of Tanzanian cattle and to determine the possible bases of disease resistance exhibited by some breeds in that country. This enhanced national capacity for livestock research has recently been given international agency support.

Comparative studies of the genetic and immunological bases of 'trypanotolerance'—the ability of certain cattle breeds, such as the N'Dama of West Africa, to tolerate infection with trypanosome parasites—comprise a major research focus at ILRAD. These studies were made possible by the transfer to ILRAD in 1983 of N'Dama embryos obtained from The Gambia. ILRAD staff implanted the embryos in foster mothers of the Boran breed, a type of East African zebu cattle that is susceptible to trypanosomiasis. Using techniques of superovulation and embryo manipulation, ILRAD scientists subsequently produced large resource populations of disease-free N'Dama and N'Dama-Boran cross-bred animals. By implanting N'Dama and Boran embryos simultaneously into the same cow, which results in each embryo carrying lymphocytes from the other, they have also produced 'chimaeric' animals. Experimental use of these animals enables scientists to ask fundamental questions about the nature of the expression of trypanotolerance.

Biotechnology transfer through training and testing

ILRAD has established these and related technologies through widespread and substantial interactions with laboratories in developed countries (this will be the subject of a future issue of *ILRAD Reports).* Applying these technologies to research programs at its Nairobi headquarters, ILRAD serves as a source of expertise, reagents and technologies pertinent to its field of research on the African continent and, increasingly, around the world. ILRAD transfers its biotechnological expertise to relevant national control programs, research centres and university departments in Africa and other regions through training courses and individual attachments at the Laboratory and the secondment of university staff to ILRAD for graduate research studies.

Returning to their home institutions, the trainees employ the biotechnologies in their own work and impart their new expertise to colleagues and students, thus helping to pave the way for the introduction of improved control technologies when these become available. Among the preponderantly African research workers trained in this way are recent graduates from Ghana, Kenya, Nigeria and Zambia now lecturing in their national universities.

ILRAD's annual group courses introduce trainees to new technologies, particularly the ELISA, that are improving the diagnosis of parasitic diseases. ILRAD and the Food and Agriculture Organization of the United Nations (FAO) are planning to hold joint courses in the use of DNA-based diagnostic methodologies for senior researchers from developing countries. To maximize the dissemination of these powerful techniques, the participants will be selected not only for the relevance of the techniques to their research work but also for their ability and opportunity to train others in their home

institutions.

By holding courses in disease diagnosis with regional institutes, such as CIRDES (International Centre for Research for the Development of Livestock in the Subhumid Zone, in West Africa), ILRAD has broadened the application of its technologies to new regions. This has also been achieved with the help of the International Atomic Energy Agency (Vienna) and FAO, which supported the testing in ten African countries of an ELISA, based on reagents produced at ILRAD, that detects antigens of the three major pathogenic groups of trypanosomes in cattle sera. The training and problem solving in use of the assay that is included in the validation exercise make national program scientists aware of both the powers and the limitations of new technologies when used in their own institutions.

ILRAD-developed ELISAs that detect the *Trypanozoon* group of parasites have also been assessed, in collaboration with the World Health Organization (Geneva) and national laboratories in five countries, for their ability to diagnose human trypanosomiasis in Africa. Other ILRAD ELISAs are being used in Thailand and Indonesia to detect *T. evansi* infections of livestock. An antigen-capture ELISA for detecting *T. vivax* is being used in French Guyana to help determine the prevalence of *T. vivax* infections in that South American country. Through exchange visits, ILRAD staff members have helped scientists at these laboratories improve their applications of the ELISAs in different types of epidemiological studies.

An ILRAD Research Fellow from Zimbabwe, Ms. Ntando Tebele, is 'reading a sequencing gel' into a computer in this photograph. Each lane of the gel

corresponds to one of the four bases of DNA. The gene being sequenced here encodes a protein molecule of Babesia bigemina, a tick-borne parasite that causes the disease babesiosis (redwater) in African, Asian, Australian and South American cattle. This DNA sequence will be used to produce large quantities of the parasite protein it encodes, which will be employed in assays that will improve both the diagnosis of babesiosis and our understanding of how the disease is spread.

A regional training course and seminar on research into the 'Biochemistry of Protozoa', conducted jointly by the University of Nairobi, the International Union of Biochemical Societies, the International Cell Research Organization and ILRAD, was another opportunity to raise awareness among scientists in developing countries of the new research approaches made possible by newly developed biotechnologies.

To further its research on the development of drug resistance in trypanosome populations, ILRAD is producing monoclonal antibodies to trypanocidal drugs and developing tissue culture and drug uptake assays. It is hoped that use of the drug quantification assays, in combination with the trypanosome antigen detection assays described above, will lead to development of simple and reliable tests that can be used in the field to detect drug resistance in trypanosome populations in all countries afflicted by trypanosomiasis.

Biotechnology transfer through collaborative research

Scientists from ILRAD and national institutes regularly apply the Laboratory's experimental technologies to answer important research and control questions that arise in the field. In Kenya, for example, a trial was conducted by staff of the Kenya Agricultural Research Institute, the Overseas Development Administration (UK) and the Centre for Tropical Veterinary Medicine (UK) to test the efficacy of the infection-and-treatment method in immunizing cattle against *Theileria parva.* The trial disclosed that an unusual *Theileria* parasite was infecting the Kenyan cattle. The parasite was not transmitted by the expected vector (the brown ear tick) and antibodies to the parasite reacted weakly with other *Theileria* species. In collaboration with scientists from Japan's National Institute of Animal Health, ILRAD staff analysed the parasite using DNA probes made to hybridize with ribosomal DNA sequences, which conclusively demonstrated that the unusual parasite was *Theileria buffeli.*

Another collaborative effort that made an important contribution to understanding the epidemiology of East African theileriosis was a demonstration made using molecular technology that some apparently healthy cattle previously infected with *Theileria parva* remain carriers of the parasite. To identify such carrier cattle in the absence of obvious parasitaemia, scientists previously had to resort to the laborious method of xenodiagnosis through experimental infection of ticks. In this study, ILRAD scientists used fragments of *T. parva* genes already under study at the institute to look for similar parasite DNA sequences in whole blood samples from cattle. Using the polymerase chain reaction, a technique that amplifies a given piece of DNA many million-fold in a matter of hours, the ILRAD scientists detected parasite DNA in many of the blood samples, demonstrating that those cattle, although healthy, remained carriers of *T. parva,* and thus might pass on the parasite to other animals.

ILRAD scientists have also developed probes that react with highly repetitive DNA sequences in trypanosomes. Use of these probes at the Nairobi-based International Centre for Insect Physiology and Ecology (ICIPE) enabled scientists at that institute to determine the extent of diversity in apparently identical populations of *Trypanosoma congolense* transmitted by tsetse flies in different areas of Kenya. Using a new probe produced to investigate the character of unknown trypanosome parasites, ILRAD

scientists have discovered new genetic types of *T. congolense.*

These DNA probe techniques have now been modified to obviate the need for radioactive materials. The simpler and less hazardous techniques are being tested in Kenya for their sensitivity by ICIPE scientists examining the vector transmission of trypanosomes.

The application of these new technologies to research in the field both tests and extends the relevance of upstream science: ILRAD is able to verify the effectiveness of a technology in solving a problem outside the laboratory and national research and control institutions are able to use the technology to obtain more sophisticated answers to their questions and more accurate epidemiological data in disease surveillance and vaccination trials.

New technologies are best transferred from one scientist to another when those scientists work side by side in a research project. With the joint publication of results, such collaboration has the satisfying bonus of linking scientists from different disciplines and institutes for the benefit of future research.

Assistance to allied programs

The routine work conducted at ILRAD employs a range of current biotechnologies, many of which are not available to scientists in national agricultural research systems. Where appropriate, ILRAD provides facilities and reagents to developing country scientists working on the research and control of parasitic diseases. Such assistance in the past has included chromosome separation and sizing technology for studies of the epidemiology of *Trypanosoma evansi* conducted by scientists at the Kenya Trypanosomiasis Research Institute; irradiation procedures for schistosome vaccine trials conducted by members of the Institute for Primate Research (Kenya) and their international collaborators; DNA probes to detect trypanosomes in flies and cattle in a tsetse control campaign in Uganda; synthesis of oligonucleotides for genetic research in malaria conducted by workers at the Kenya Medical Research Institute; and characterization and preservation of important stabilates for vaccination against theileriosis for scientists and disease control workers in the Kenya Agricultural Research Institute, the Department of Veterinary Services in Zimbabwe, the governments of Burundi, Zanzibar and Zambia, and the Vaccine Production and Control Laboratory in Malawi (the latter distributes this live vaccine throughout areas of Africa affected by theileriosis).

By providing these resources and services and by opening new research opportunities through the introduction of new biotechnologies, ILRAD enhances the research capacity of national programs. Regarding vaccine characterization, ILRAD also provides national systems with the tools needed to implement improved control programs for theileriosis.

Biosafety and property rights

ILRAD scientists offer national program staff advice and support in determining the optimal research avenues for tackling problems constraining agricultural productivity and development. With other international agricultural research centres, ILRAD has contributed to CGIAR and donor initiatives on biotechnology. The Laboratory is currently providing animal health and other data to the International Biotechnology Service in the Hague, which is compiling a database on biotechnologies relevant to agriculture in developing countries. ILRAD staff members also provide expert advice to donor agencies wishing to facilitate technological transfer to developing countries, particularly in Africa. With its host nation, Kenya, ILRAD assists national planners in priority setting, program formulation and implementation schemes for biotechnology in

agriculture. Further assistance is provided more directly to national committees for livestock development. Senior ILRAD scientists have also recently attended biotechnology planning meetings in India, the Netherlands, Sweden, Uganda and the UK to assist in national and regional planning seminars for the appropriate investment and use of biotechnologies related to animal health.

Institutions that employ biotechnology must ensure the proper containment and use of molecularly engineered microoganisms. ILRAD's advanced research capabilities have necessitated that the institute considers how best to handle engineered organisms safely and effectively in developing countries, some of which have not yet formulated safety regulations for this class of biological agents. Similarly, ILRAD's work with novel antigens and vaccine vectors created by molecular engineering—which not only may have profound animal health benefits but also may be commercially valuable—has demanded that the institute develop a policy governing intellectual property rights over its discoveries for the benefit of potential recipients in the farming communities of developing countries.

ILRAD's experiences in developing appropriate policies for biosafety and intellectual property rights have allowed the institute to contribute both to national debates and to guidelines on these subjects prepared in the wider agricultural context by the Consultative Group on International Agricultural Research (Washington, D.C.). These aspects, which form the regulatory conditions for employment of the new and powerful methodologies of modern science, are increasing in importance as a wide range of biotechnologies are being assembled to tackle the problems of livestock disease and improving animal health in developing countries.

This article is based on a report written by Peter Gardiner, ILRAD's Information and Planning Officer.

The next article on identification of genes that control trypanosome proliferation, is based on a report and work done by ILRAD scientists Noel Murphy and Roger Pellé.

Decoding the language of cellular interactions

SINGLE-CELLED trypanosome parasites, the cause of the animal and human disease complex known as trypanosomiasis, multiply in their animal host by binary fission. This cell division is controlled at different stages of the parasite's life cycle. A group of scientists at ILRAD is working to reveal the molecular mechanisms that govern trypanosome cell division with the aim of finding ways to attack the parasites without harming the cells of their animal hosts. Discovering these mechanisms may allow staff to devise artificial signals that will stop trypanosomes from proliferating. This would give the immune system of the animal host sufficient time to gain control over the infection and to clear the parasites from the body.

Trypanosomes have become adapted to many of Africa's wild ruminants and to a few indigenous domestic livestock breeds, such as the N'Dama cattle of West Africa. In these natural hosts, parasite numbers are controlled. Such a state of host-parasite equilibrium is almost certainly maintained through an exchange of signals between the host and the infecting parasite population and among the parasites themselves. In most cattle in Africa, however, and particularly in exotic animals, these signalling events clearly go awry because untreated trypanosome infections eventually kill the animal host.

To discover how signals regulate trypanosome growth, researchers first need to identify several kinds of molecules, such as signalling molecules between host and parasite, parasite receptors, and parasite molecules that trigger proliferation. The development of novel techniques to perform such molecular investigations is essential to this research. With the new tools in hand—tools based on genes and the protein molecules they encode—the scientists will then attempt to devise immunological or chemical ways of interfering with the signalling processes so as to stop the development of disease.

TRYPANOSOMES are primitive organisms whose processes and gene products controlling cell division are likely to differ substantially from those in their animal host. Past ILRAD research suggests that a central gene regulating cell division in the trypanosome, cdc2, differs significantly from the corresponding host gene. Such genes and others that are central to the control of trypanosome proliferation and whose protein products are accessible to immunological or chemical attack represent target candidates on which to base control strategies. Trypanosomes also differ from their host in having a single mitochondrion, which necessitates the coordination of parasite processes involved in mitochondrial and nuclear division. (Other organisms, having multiple mitochondria, do not need to coordinate these events.)

To identify signals produced during particular stages in the trypanosome's life cycle, scientists look for genes that are 'expressed'—activated to produce the proteins encoded by the genes—only during particular stages. To speed up identification of such 'developmentally regulated' genes, ILRAD staff developed a novel technique for generating 'fingerprints' of genes specifically expressed during any of the developmental stages. This technique is sufficiently powerful to disclose important genes, many of which are weakly expressed, together with genes switched on or off in response to external stimuli. The fingerprinting technique will replace a tedious and expensive method of subtractive enrichment. Use of the new technique is enabling ILRAD staff to follow host-parasite and parasite-parasite interactions at the molecular level and to discover how these interactions influence gene expression. The technique is thus accelerating the pace of research on processes that regulate trypanosome proliferation and differentiation.

With tools available to identify the genes that control the establishment and maintenance of a trypanosome infection as well as those that control the numbers of infecting parasites, and with the development of systems for transfecting genes in trypanosomes (see the January 1992 issue of *ILRAD Reports),* ILRAD scientists are now in position to determine how interference with cell-to-cell signalling processes affects parasite growth and to exploit this knowledge to develop methods that will control parasite numbers and the development of disease.

New ILRAD technique to fingerprint expressed genes

Short oligonucleotide primers (10-mers) of arbitrary sequence are used in the polymerase chain reaction (PCR) technique to generate genomic fingerprints for characterizing and differentiating parasites and for mapping loci of genes that confer identifiable phenotypes. ILRAD scientists reasoned that use of such primers on complementary DNA (cDNA) might generate fingerprints characteristic of expressed genes. To test this, they reverse transcribed messenger RNA (mRNA) from actively dividing and non-dividing bloodstream forms of *Trypanosoma brucei* into cDNA, and used this as a template for arbitrary primer amplification. A small amount of cDNA (1–20 ng) was mixed with a single 10-mer primer and the PCR carried out. The fingerprints of DNA fragments generated were examined by agarose gel electrophoresis. Reproducible fingerprints were generated with different primers and some primers generated numerous polymorphisms for the different parasite forms used. Using this technique, ILRAD scientists identified a gene that is only transiently

expressed as the parasite differentiates from an actively dividing to a non-dividing form.

Staff developed this technique further for use with small numbers of parasites by exploiting the fined 5'- and 3'-ends found in all trypanosome mRNAs to amplify the total mRNA populations into cDNA. Arbitrary primers were then used on this cDNA to generate PCR products, which were again analysed by agarose gel electrophoresis. The DNA fragments showing differences among stages were purified. Using this approach, the scientists identified amplified cDNA fragments that hybridize specifically with transcripts of slender and stumpy mammalian bloodstream forms and procyclic insect forms together with cell-cycle specific products. These results demonstrate the power of this PCR-based technique, now referred to as RADES (randomly amplified developmentally expressed sequences). This ILRAD technique represents a significant advance in parasite biology: it enables scientists for the first time to look at differences among large numbers of parasite samples, it reduces the time needed to identify genes by 500 to 1,000-fold, and it reduces the amount of parasite material needed by at least ten-million-fold. Modifications to the technique, such as the use of pairs of arbitrary primers, will open up a multitude of new research approaches of critical importance in molecular investigations.

Identification of novel invariant antigens of trypanosomes

The study of invariant membrane proteins of trypanosomes is important for several reasons. Many trypanosome membranes are accessible to components of the extracellular milieu. Their proteins thus represent a group of potential target molecules that may be exploited for either immunoprophylaxis or chemical intervention. These molecules are also specific to areas of certain membranes. This enables them to serve as potential markers for characterization of subcellular compartments, making them invaluable tools for examining trypanosome biochemistry and cell biology.

In this study, two novel invariant membrane proteins of *Trypanosoma vivax* were identified and partially characterized with the help of monoclonal antibodies generated against whole, formalin-fixed parasites. *Trypanosoma vivax* is an important disease-causing parasite in livestock from Africa and South America and has a much wider geographical distribution than the more thoroughly studied *Trypanosoma brucei* and *Trypanosoma congolense.*

A protein with a 35-kilodalton (kDa) mass was identified and shown to localize to the parasite surface membrane. Preliminary biochemical characterization demonstrated its association with both membrane and soluble fractions of bloodstream forms of *T vivax .* The molecule exhibited some biochemical characteristics similar to the 3'-nucleotidase found in the surface membrane of *T vivax* and having the same apparent molecular mass and partial membrane association. However, the 3'-nucleotidase bound to concanavalin A-Sepharose and the immunoreactive 35-kDa molecule did not.

The second molecule, a 65-kDa protein (gp65), was the main focus of the study. This molecule was shown to be associated with the membranes of tubulo-vesicular structures within *T. vivax.* These structures were shown by immunoelectron microscopy to comprise an intermediate part of the endocytic pathway, the system by which trypanosomes take in many essential nutrients. These organelles are probably similar in function to early endosomes that have been described in mammalian cells. The gp65 was found to be an integral membrane glycoprotein with both N- and O-linked oligosaccharide side chains.

To determine the accessibility of gp65 to binding by antibodies, protease protection and cross-liking studies were carried out. Results from these suggest that gp65 exhibits

properties of a transmembrane protein with both trypsin cleavage sites and NH2-cross-linking sites on the lumenal face of the membrane. The gp65 molecule also appears to have cleavtion with both membrane and soluble 1 age sites for the endoproteinases Glufractions of bloodstream forms of T. C and Asp-N on the cytoplasmic face *vivax.* The molecule exhibited some of the membrane. However, some of biochemical characteristics similar to the cytoplasmic sites appear to be protected from cleavage by both enzymes, suggesting that this portion may be bound to other molecules. The gp65 is able to form higher molecular weight complexes, possibly dimers or trimers, by disulphide linkage. A monoclonal antibody to the antigenic epitope of gp65 also reacted with a 22 kDa molecule (p22). This was assumed to be a product of the gp65, resulting from limited enzyme hydrolysis, which remained attached to intact gp65 by disulphide linkages. The p22 is contained within the membrane and probably has a membrane spanning region but does not have the endoglycosidase H- or O-glycosidase side chains detected on the entire gp65 molecule.

Both the 35- and the 65-kDa invariant proteins of *T. vivax* are accessible to external agents and therefore merit further study for their potential as targets for immunoprophylaxis or chemotherapeutic agents. In addition, gp65 is the first putative marker for an endosomal compartment of these trypanosomes. This molecule therefore has potential use in studies of endocytosis, a process that appears to be critically important for the survival of African

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Summary of a Ph.D. thesis abstract submitted in 1992 to the Department of Microbiology and Immunology, University of Western Ontario, London, Ontario, Canada.

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IIRAD was founded in 1973 to conduct research into better ways of controlling livestock diseases. The current primary goal of the Laboratory is to develop safe, effective and economical methods to control the most important parasitic diseases constraining anima! production in Africa: Trypanosomiasis, transmitted to animals by the bite of a tsetse fly, and tick-borne diseases, particularly East Coast fever. An international staff of about 50 scientists conducts basic research, much of it aimed at the development of vaccines, in the fields of biochemistry, cell biology, electron microscopy, epidemiology, genetics, immunology, molecular biology, pathology, parasitology and the socioeconomics of animal disease control.

ILRAD is one of 18 international agricultural research centres sponsored by the Consultative Group on International Agricultural Research (CGIAR). The secretariat of the CGIAR is located in the World Bank headquarters, in Washington. D.C. The CGIAR is an informal umbrella organization of 40 national governments, international organizations and private foundations that together provide about US$230 million annually to the 18 centres for research, training and advisory services. The CGIAR aims to help farmers in developing countries increase their production of staple food crops, livestock, fish and trees in ways that

improve the nutrition and well-being of low-income peoples and the management of natural resources.