Specialists come to ILRAD to discuss parasite antigens

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Parasitic diseases cause serious illness and often death in domestic livestock and human populations in the tropics. Diseases caused by protozoan parasites include malaria, trypanosomiasis, leishmaniasis and a number of tick-borne diseases, including theileriosis and babesiosis. Research at ILRAD concentrates on two of these diseases — African animal trypanosomiasis and East Coast Fever (ECF), a particularly virulent form of theileriosis.

Specialists in immunology, biochemistry and molecular biology in many countries are carrying out research on parasites and host responses to parasite infection. A great deal of the information they are obtaining and many of the techniques they are developing are relevant to ILRAD’s research activities and goals. Several of these specialists were invited to ILRAD during the week of 13 to 17 May 1985 to review the current status of research, identify constraints to progress and explore new approaches towards improving the control of parasitic diseases.

Among the different approaches to improved disease control, ILRAD has concentrated since its establishment on developing vaccines which would prevent infection. In his opening remarks at the workshop, ILRAD’s Director General, Dr Ross Gray, told the participants, ‘... ILRAD’s approach to our two target diseases is basically immunological. We are not primarily involved with epidemiological studies, chemotherapy or vector control ... We would like to find conventional antigens which would stimulate high levels of immunity.’

Vaccines protect people or animals by stimulating their immune responses so they are ready to resist an infecting agent — virus, bacterium or parasite — when it is introduced. Most vaccines introduce the disease agent in a form in which it can stimulate immunity, but without actually causing disease: i.e. the vaccine material is either live but weakened (attenuated) or dead. For a variety of reasons, these types of vaccines have not been developed against many parasitic diseases.

The goal: a new type of vaccine
The scientists who attended ILRAD's parasite antigen workshop are working to develop new vaccines, called subunit vaccines, based on isolating and introducing a small part of the disease agent which will stimulate immunity but not cause disease. These subunits may be proteins — called antigens — from the surface coat or elsewhere in the parasite which trigger antibody production or cell-mediated responses by the host immune system.

Dr. Antony Musoke (centre), chairman of the organizing committee for the International Workshop on Parasite Antigens, with two other committee members, Dr. Onesmo ole Moi Yoi (left) and Dr. Vinand Nantulya (right).

The first step in developing a vaccine of this type is to identify a parasite antigen or antigens which can induce a protective response. Two approaches to the identification and characterization of parasite antigens were discussed at the workshop, both of which are used at ILRAD. First, antigens may be selected that react with monoclonal antibodies which have a desirable specificity, e.g. which neutralize the pathogens; the antigens identified in this way are then inoculated into host animals to determine whether they stimulate an immune response. Using the second approach, scientists incubate parasite material with sera from infected or immune animals and identify the parasite antigens which are recognized by host antibodies in the sera.

Antigens characterized in these ways must be isolated from the rest of the parasite and purified. Then several approaches may be followed to produce antigens in sufficient quantities for use as starting material in a vaccine. In general terms, antigens may either be synthesized chemically or biologically. Biological synthesis involves identifying the DNA coding for the required antigen and cloning it in a phage or plasmid expression vector. The vector can then be grown in a bacterium, yeast or other type of cell which will produce the parasite antigen molecules and sometimes secrete them into the culture medium. Scientists at ILRAD are working to clone the DNA which codes for potentially protective antigens of *Theileria parva* parasites which cause ECF and *Trypanosoma brucei*, *T congolense* and *T vivax* parasites which cause trypanosomiasis.

Because the expressed antigen molecules are very small, they need to be enlarged in some way to stimulate an immune response in a mammalian host. This can be done by crosslinking multiple copies of the antigen molecules, a process called polymerization, or by combining them into conjugates with other types of molecules. Finally, the antigens are inoculated into experimental animals together with adjuvants, substances which help the antigens induce an immune response. If these animals are then able to resist disease challenge, the experimental vaccine is ready for wider testing and hopefully for eventual introduction in the field.

**Second objective: antigens for diagnosis**
The techniques now available for diagnosing parasitic disease are often inadequate. For one thing, it is often difficult to see parasites in the bloodstream, cells or tissues of an infected animal or human patient until the level of parasitaemia is very high, and treatment, if it is to be effective, must be administered at an early stage of infection. It is also often difficult or impossible to distinguish different species, subspecies or strains of parasite by microscopic inspection or other conventional techniques. Thus, it may be impossible to determine how many different strains or species of parasite are infecting animals in a particular geographic area, information required to design an appropriate prevention or treatment regime.

Reagents are being developed in several laboratories which recognize the antigens expressed by specific parasite species or strains. These include monoclonal antibodies which recognize parasite antigens and genetic probes which recognize characteristic DNA sequences which code for specific molecules.

**Discussion: how far are we on the path?**

Dr. Gray summarized very briefly ILRAD’s current position as follows: In theileriosis, despite some parasite strain variation, we can stimulate high levels of protective immunity, but we have to use whole live organisms to do so. In trypanosomiasis, we can also create good immunity, but the immunity is restricted in practice to protection against organisms of the infecting genotype. In both instances, we want something broader and better in the way of protection ... Our primary objective is to concentrate our efforts to identify, characterize and produce trypanosomal and theilerial antigens which can be used to prevent infection in livestock. Of course, these objectives might not be attainable, but we shall put our maximum effort into this job.

How do scientists see antigens? One technique used at ILRAD is illustrated by this electron micrograph showing antigen on the surface of a Theileria sporozoite. A monoclonal antibody binds to the surface antigen, electron dense gold particles are combined with protein A, and this complex binds to the antibody. The dense particles of gold appear as grains on the micrograph, indicating the location of the antigen.

The first two sessions of the workshop concentrated on the identification and characterization of potentially protective parasite antigens. Dr. I.F. Zavala from New York University (USA) described the use of monoclonal antibodies to detect possible protective malaria antigens. Dr. G.V. Brown of the Walter and Eliza Hall Institute of Medical Research (Australia) then discussed the use of sera from resistant patients in endemic areas to screen for antigens which might stimulate protection against malaria. Later during the workshop, Dr. Brown gave a more general report on progress towards the development of a malaria vaccine. Dr. G. Palmer of Washington State University (USA) described the identification of protective antigens against anaplasmosis using antisera from immunized rabbits and monoclonal antibodies. Dr. A. F. Barbet, also from
Describing research conducted at ILRAD, Dr. A. J. Musoke mentioned a number of potentially protective *T. parva* sporozoite antigens identified by analysing sera from immune cattle and rabbits and screening parasite material using monoclonal antibodies. Dr. W. I. Morrison discussed efforts to identify protective antigen(s) on the surface of *Theileria*-infected cells, and Dr. S. Z. Shapiro spoke about attempts to identify antigens which might protect against African animal trypanosomiasis using sera from resistant N'Dama cattle and wild animals and from susceptible cattle.

The next three sessions were devoted to the identification and expression of parasite antigen genes. As a preface, Dr. H. M. Geysen of the commonwealth Serum Laboratories (Australia) described a novel technique for the simultaneous synthesis of a large number of peptides which can then be screened with monoclonal antibodies to identify possible protective antigenic determinants on a protein. The technique can also be used in conjunction with a suitable antibody to generate antigenic peptides which mimic non-peptide antigens. Dr. W. Rutter of the University of California (USA) stressed the importance of choosing an appropriate vector to express parasite antigens. He found that cloning the hepatitis B surface antigen gene in yeast vectors gave a more stable gene product than cloning in bacterial systems. Dr. J. Dame of the U.S. Department of Agriculture described the construction of genomic expression libraries for malaria and other parasites using a new method which involves digestion of the DNA between genes with mung bean nuclease. Dr. J. Williams of the Imperial Cancer Research Fund (U.K.) then discussed recent developments in techniques for the construction of cDNA libraries.

Dr. F. Lemmonier of the Centre d'Immunologie Marseille (France) described recently developed techniques for transfecting cells with foreign DNA. Dr. K. Iams and Dr. R. Hall described work in progress at ILRAD to construct genomic expression libraries for *T. parva* and to identify genes from these libraries specific for different strains of the parasite, for different stages of parasite development, and for parasite antigens which could induce a protective immune response.

The induction of protective responses was the subject of the next session of the workshop. Dr. C. Jacob of the Weizmann Institute of Science (Israel) described a wide range of procedures which have been developed to convert synthetic peptides into efficient immunizing agents, and Dr. F. Audibert of the Institut Pasteur (France) summarized studies on the use of carrier molecules and adjuvants to enhance the effectiveness of peptides as vaccines.

The last workshop session was devoted to the use of antigens for the diagnosis of parasitic diseases. Dr. J. David of the Harvard Medical School (USA) discussed the use of monoclonal antibodies and DNA probes to detect *Leishmania* infections. Dr. N. Massamba of the Universite Libre de Bruxelles (Belgium) and Dr. V. Nantulya, Mr. J. Young and Mr. P. A. O. Majiwa of ILRAD described applications of these diagnostic techniques to trypanosomiasis. Dr. P. Conrad of ILRAD described applications for the detection of *Theileria* infections.

As Dr. Gray pointed out in his opening remarks, ILRAD’s goal is to develop improved control measures against two parasitic diseases of great economic importance in Africa. The primary focus is on vaccine development. This workshop on parasite antigens was held in order to put ILRAD’s work into a larger context: to expose scientists at ILRAD to new, highly relevant findings and techniques from other laboratories and to provide an outside appraisal of the techniques in use at ILRAD. In addition to the considerable value of these discussions for ILRAD’s research program, the directors of associated research institutes in the Nairobi area were invited to the workshop so that their programs could also benefit from the shared experiences of specialists from around the world.
Electron micrograph of an ultrathin frozen section of a trypanosome labeled with antibody followed by protein A and colloidal gold. The antibody binds specifically to the cross-reacting determinant in the trans-Golgi region of the parasite (Proceedings of the National Academy of Sciences of the USA, 1984, 81: 7703–7)

ILRAD and the CGIAR

The Consultative Group on International Agricultural Research (CGIAR) was established in 1971 to bring together national governments, public and private institutions, international and regional organizations, and representatives from developing countries dedicated to supporting a wide network of international agricultural research centres and programs. The basic objective is to increase the quantity and improve the quality of food production in developing countries all over the world.

The concept of a consultative group grew out of the need to build on the earlier successes of a small number of international agricultural research centres sponsored by the Ford and Rockefeller Foundations in the 1960s. It became obvious that expanded and more diversified research efforts were needed, while the costs of maintaining an expanded system were clearly beyond the means of the two foundations. The World Bank (IBRD), the United Nations Development Program (UNDP) and the Food and Agriculture Organization (FAO) of the United Nations undertook to sponsor a new kind of association for the support of international agricultural research.

The CGIAR represents an innovative and highly successful strategy for mobilizing resources to increase world food production. The group began operation in 1972 with 15 donor governments and organizations contributing about US$ 20 million in support of five international centres. In 1985, about 50 members are supporting a network of 13 international agricultural research centres with a total budget of US$171 million. The centres employ a total of 7,000 staff members, including 600 senior scientists recruited from over 50 different countries.

In addition to the donor organizations, CGIAR members include the representatives of 10 developing countries, elected by FAO regional caucuses. The group operates entirely by the common consent, shared interest and goodwill of its members. Meetings are informal, decisions are reached by consensus, and donations are on a voluntary basis between individual members and research centres.

The 13 international centres supported by the CGIAR conduct research and training activities covering a wide range of food crops, as well as livestock production. Despite
their relatively short existence, these research centres have made remarkable contributions to world food production. As might be expected, the impact of the two oldest centres — the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) in Mexico and the International Rice Research Institute (IRRI) in the Philippines — has been the most dramatic up to now. High-yielding varieties of wheat and rice derived from those developed by CIMMYT and IRRI are grown on over 55 million hectares in the developing world—one-third of the area planted to these cereals. The increases in yield achieved are sufficient to feed an estimated 300 million people, while the economic value of this additional food supply is more than US$ 5 billion a year.

The establishment of ILRAD

ILRAD was established in 1973 by a Memorandum of Agreement between the Government of Kenya and the Rockefeller Foundation, acting as executive agent for the CGIAR. This document sets out ILRAD’s mandate as follows:

The purpose of the Laboratory will be to serve as a world centre for research on ways and means of conquering, as quickly as possible, major animal diseases which seriously limit livestock industries in Africa and in many other parts of the world. The Laboratory will concentrate initially on intensive research concerning the immunological and related aspects of controlling trypanosomiasis and theileriosis (mainly East Coast fever). It may, however, eventually extend its research to other serious animal disease problems for which its facilities and expertise are appropriate . . . . In carrying forward its program, the Laboratory will develop close linkages with governmental and regional organizations undertaking research on the same or related disease problems.

The Kenya Government provided a 70-hectare site for ILRAD at Kabete, on the outskirts of Nairobi, and construction of a modern complex of laboratories and support facilities began in May 1976. The complex was inaugurated in April 1978 by President Daniel arap Moi, then Vice-President of Kenya.

ILRAD’s facilities at Kabete comprise an administration block, six large research laboratories, electron microscopy, diagnostic and radioisotope facilities, a library, conference rooms, breeding units for laboratory animals, tsetse flies and ticks, accommodation for cattle, sheep and goats, media preparation rooms, electronic and engineering workshops, a transport yard and stores. Screened isolation units are available for cattle, sheep and goats used for trypanosomiasis research. Staff housing, catering and recreation facilities are also provided.

At the end of 1981, ILRAD acquired a 13,000-hectare ranch on the Kapiti plains about 80 kilometres from Nairobi. A breeding herd of Boran (Bos indicus) cattle is maintained on the ranch which provides nearly all the cattle required for ILRAD's research program.

Staff development has advanced steadily at ILRAD, starting with a small nucleus of scientists who assembled in Nairobi in 1975 to initiate the research program. By mid-1985, ILRAD’s staff comprised 49 senior scientific and administrative positions and 400 staff members in various support categories. Senior staff members have been recruited from many different countries, while support staff are mostly Kenyan.

With broad recognition of the high calibre and critical importance of ILRAD's research and training activities, support for the Laboratory has increased steadily since establishment. In 1985, US$ 9.3 million in core funding was provided by the World Bank, UNDP and the Governments of Australia, Belgium, Canada, Denmark, France, Germany (Federal Republic), Italy, Japan, the Netherlands, Norway, Saudi Arabia, Sweden, Switzerland, the United Kingdom and the United States of America.

Research continues closely along the lines of ILRAD's original Memorandum of Agreement. In the words of the Director General, Dr Ross Gray: ILRAD's research programs are designed to support the work of other organizations and to follow promising
avenues for disease control, particularly immunological approaches, which have not been fully pursued elsewhere .... The successful development of vaccines, which would stimulate immunity without the hazards of exposure to infection, would be a major advance in the control of these diseases.

Conferences

The International Centre of Insect Physiology and Ecology (ICIPE) is hosting a Conference on Tropical Entomology on behalf of the Council of International Congresses of Entomology. The Conference will be held in Nairobi, Kenya, from 31 August to 5 September 1986. For further information, contact:

Dr. M. F. B. Chaudhury
Conference Secretary General
P O Box 34722
Nairobi, Kenya.

The second National Conference on Haemoparasitic Diseases and their Vectors will be held in Zaria, Nigeria, from 24 to 26 February 1986. The conference is being organized to encourage the exchange of ideas and to assess current problems. Discussions will cover trypanosomiasis, tick-borne diseases and their vectors. For further information, contact:

Prof. Y. O. Aliu
Conference Coordinator
Faculty of Veterinary Medicine
Ahmadu Bello University
Zaria, Nigeria.

Locations of CGIAR research centres around the world.

Other international agricultural research centres in the CGIAR network.

CIAT: Centro Internacional de Agricultura Tropical (International Centre for Tropical Agriculture) Apartado Aereo 6713, Cali, Colombia. Major research programs: cassava, field beans, rice and tropical pastures.

CIMMYT: Centro Internacional de Mejoramiento de Maiz y Trigo (International Maize and
Wheat Improvement Centre), Londres 40, Mexico 6, D.F., Mexico. Major research programs: maize and wheat.

CIP: Centro Internacional de la Papa (International Potato Centre), Apartado 5969, Lima, Peru. Major research program: potato.

IBPGR: International Board for Plant Genetic Resources, FAO, via delle Terme de Caracalla, 00100 Rome, Italy. Major objective: to promote a network of national and international centres to collect and preserve plant genetic resources.

ICARDA: International Centre for Agricultural Research in Dry Areas, P.O. Box 5466, Aleppo, Syria. Major research programs: durum wheat, barley, faba beans, lentils and forage crops.


IFPRI: International Food Policy Research Institute, 1776 Massachusetts Avenue N.W., Washington, D.C. 20036, USA. Major objective: to analyse world food problems and determine policies which will lead to improved food production and distribution.

IITA: International Institute of Tropical Agriculture, P.M.B. 5320, Ibadan, Nigeria. Major research programs: sweet potato, cassava, yam, cowpea, lima bean, soybean, maize and rice.

ILCA: International Livestock Centre for Africa, P.O. Box 5689, Addis Ababa, Ethiopia. Major research program: livestock production and marketing systems in tropical Africa.

IRRI: International Rice Research Institute, P.O. Box 933, Manila, Philippines. Major research program: rice.

ISNAR: International Service for National Agricultural Research, P.O. Box 93375, 2509 AJ, The Hague, Netherlands. Major objective: to assist in strengthening national agricultural research programs.

WARDA: West African Rice Development Association, P.O. Box 1019, Monrovia, Liberia. Major research program: rice.

The CGIAR Secretariat is located at the World Bank, 1818 H Street N.W., Washington, D.C. 20433, USA.

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