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Bovine Cytokines

The chemical messengers of the immune system can both cause and ameliorate livestock disease

The mammalian immune system is regulated by cell-to-cell contact and by protein messenger molecules secreted by cells of the immune system. Nearly 30 such protein molecules, or cytokines, have been identified to date. These both activate and inhibit functions of cells of the immune system. A group of cytokines produced in response to an infection will typically activate different and competitive kinds of immune responses. The sum of these responses makes the difference between an animal's ability to protect itself from disease and its death. Scientists at ILRAD are investigating the production of cytokines in diseased and healthy cattle to discover which help advance and which help ameliorate the development of disease.

ILRAD is working to develop improved methods of controlling two disease complexes. trypanosomiases and tick-borne diseases. These cause large production losses and fatalities in cattle and other domestic livestock in tropical countries. The molecular research approach the Laboratory is taking to solve these disease problems depends on an accurate understanding of the mammalian immune system. ILRAD scientists specifically need to know how the two main arms of the bovine immune system. B and T lymphocytes and the molecules they secrete. respond to infection by the two disease-causing parasites that are the focus of the institute's research.

African trypanosomes, which are spread by tsetse flies and cause trypanosomiasis, are extracellular parasites that circulate in the blood and body tissues of their mammalian hosts. *Theileria* parasites are intracellular, invading first the white and then the red blood cells of their hosts. Although the cells and molecules of the mammalian immune system act together in a

complex and dynamic network, they can be roughly divided according to which of two main functions they contribute. B lymphocytes and the antibody molecules that B cells secrete bind to foreign organisms circulating freely, such as trypanosomes, marking these for destruction by other cells of the immune system. T lymphocytes, in contrast, recognize and cause the destruction of the host's own cells that are infected with pathogens such as *Theileria parva* and other tick-borne parasites. Thus, although both trypanosomes and tick-borne parasites are vector-borne protozoan organisms, their occurrence in different sites as well as different lifecycle forms in their animal hosts challenges the bovine immune system in very different ways.

Some domestic animals susceptible to trypanosomiasis manage to develop effective immunity against the disease after long exposure to many different antigenic types of trypanosomes. The animals' protective immune response to trypanosome infection is believed to be mediated largely by antibodies as well as by white blood cells such as macrophages, whose function is to clear the body of foreign material by ingesting (phagocytosing) it. The principal pathogenic effect of trypanosomiasis in livestock is anaemia, which indicates that the disease adversely affects the production and/or consumption of red blood cells. In addition, trypanosome infection can cause suppression of the immune system, suggesting that infection in some way perturbs normal activation of immune cells. Remarkably, some cattle breeds indigenous in Africa, notably the N'Dama, can control both parasite numbers (parasitaemia) and the development of anaemia while infected with trypanosomes. Scientists at ILRAD are investigating the genetic and physiological basis of this tolerance of trypanosome infection and are exploring how to control trypanosomiasis in susceptible animals in ways that do not necessarily depend on eradicating the parasites.

Animals that become infected with *T. parva,* the cause of East Coast fever, and that subsequently recover spontaneously or through administration of a live parasite vaccination (the infection-and-treatment method) are immune to further challenges with the same parasite. Evidence strongly suggests that this immunity is generally mediated by cellular responses against the form of the parasite that infects lymphocytes. ILRAD researchers are studying the development and induction of protective immune responses to this and other tick-borne parasites so as to develop a safer and more economical and robust means of controlling tick-borne diseases.

ASSAYS DEVELOPED FOR RESEARCH ON CYTOKINES		
Level of Study	**Assay Method**	**What the Assay Detects**
cytokine codine gene		
transcribed genomic message	RNA is extracted from cells from infected animals and the cytokine message determined by hybridization using probes. cells are put on a microscope slide and intracellular message is determined by *in situ* hybridization using probes.	detects total amount of message (sensitivity can be increased by use of PCR at a price of loss of quantitative information) detects number of cells in which a cytokine gene is activated.
intracellular cytokine protein	intracellular protein in cells from infected animals is detected by antibodies using immunofluorescence	detects number of cells that have produced a cytokine
secreted cytokine protein	cytokine protein is detected in body fluids by antibodies; functional cytokine protein activity is detected by a bio-assay (information about specificity and function can be obtained in a single assay) cells from an infected animal are cultured and the cytokine product is measured by antibodies and bio-assay, as above	detects presence of cells capable of secreting a cytokine

To determine the kinds and amounts of cytokine activity induced in cattle in response to infection with trypanosomes and tick-borne parasites, ILRAD scientists use a variety of assays that detect cytokines at different levels. that of the RNA message produced by an activated cytokine gene, the cytokine protein produced when that message is transcribed, and the cytokine proteins secreted from a cell. Each of these assays has been tailored at ILRAD to answer a different kind of question.

The information obtained will improve understanding of the pathogenesis of trypanosomiasis and tick-borne diseases and will be used to develop better methods for their control. For example, a successful vaccine against East Coast fever may depend on incorporation of a cytokine gene(s) whose expression in the host will help protect the animal from the infectious agent or the development of disease. Alternatively, identification of the factor(s) that causes overproduction of cytokines. and hence cause severe disease. could lead to development of an anti-disease (rather than anti-parasite) vaccine for trypanosomiasis.

The cytokines

Knowledge of the bovine immune and blood-cell production (haemopoietic) systems has increased greatly in the last 20 years. ILRAD scientists made a major contribution in these areas by helping to determine the types and functions of the cells that make up these systems. Although the immune system of ruminants is similar to the more thoroughly studied man and mouse immune systems, bovine immune cells have had to be characterized anew, necessitating the production of many reagents, such as monoclonal antibodies, with which to identify these cell types.

Recently it has become clear that the immune system is regulated not only through direct cell-to-cell contacts but also through production of and response to intercellular protein messengers known as cytokines. Like hormones, which are secreted in an endocrine organ in response to a stimulus and which activate cells or tissues in other parts of the body, cytokines are produced by certain cell types and affect other cells that possess surface receptors for those cytokines. Unlike hormones, cytokines may be secreted and act locally rather than systemically. Furthermore, the nearly 30 cytokines identified to date express a range of activating and inhibitory functions among immune cells, induce chemotaxis, and can act as both growth promoting and growth inhibiting factors. Although cytokine activity is known to be important in the regulation and responsiveness of immune cells in both healthy and diseased animals, several different cytokines seem to perform the same function. This makes it difficult to measure specific cytokine actions and to correlate their functions with observed immune reactions. In addition, cytokine activity can elicit different and competitive sorts of immune cell responses; it is the sum of these responses that largely determines whether an animal is protected from disease or succumbs to it.

To investigate ways of inducing protective immune responses and haemopoietic control in parasite-infected cattle, ILRAD scientists have established bovine cytokine reagents such as biologically active cytokine proteins, antibodies that identify cytokines or neutralize cytokine functions, and nucleic acid probes that detect cytokine gene transcripts and measure their levels of expression. Subsequent identification of parasite factors that directly cause cytokine-mediated activity deleterious to the bovine host may open opportunities to produce 'anti-disease' rather than the more conventional anti-parasite vaccines that have been the main focus of ILRAD's vaccine research to date.

Establishing cytokine reagents

For one laboratory to develop all the reagents required to investigate thoroughly the bovine cytokine network would be a formidable, and probably impossible, undertaking. ILRAD staff members have thus established a wide range of collaborations (see the table on page 3) for the purpose of obtaining ready-made reagents or DNA probes and sequences that will enable them to isolate cytokine genes of interest at ILRAD and develop further cytokine reagents. The institute's approach in such collaborations is exemplified by work on the tumour necrosis factor (TNF). This cytokine was originally discovered independently by groups working on the necrosis of tumours and other groups researching disease-related wasting, or cachexia. The latter group, working with trypanosome-infected rabbits, found a serum factor responsible for

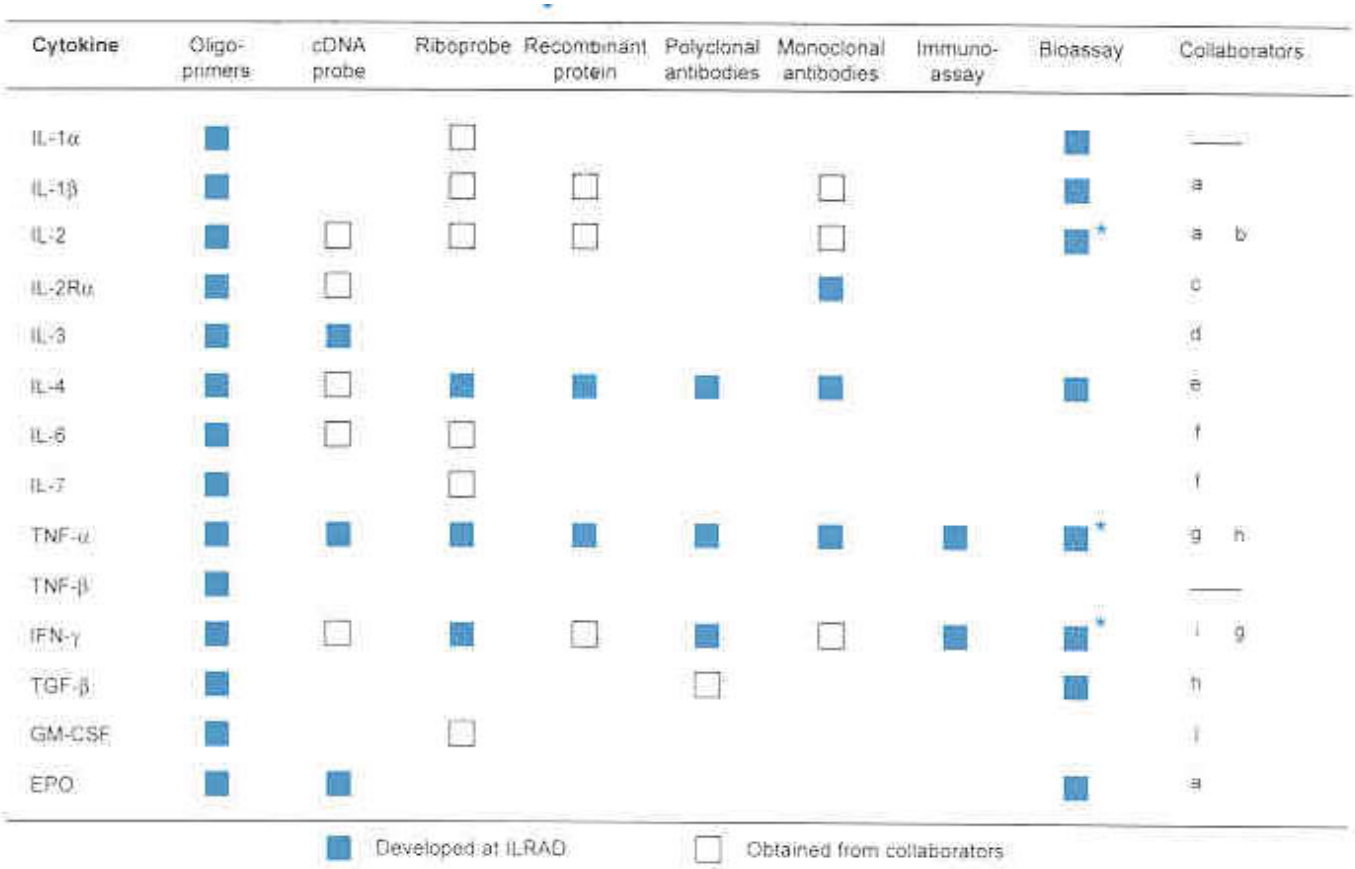
the wasting, which they termed cachectin. Purification and sequencing of the molecule revealed that this factor was the same cytokine responsible for tumour necrosis. Besides inducing cachexia, TNF might have a general disease-inducing function. Although this cytokine has been proposed to be involved in protection against both leishmaniasis and malaria, overproduction of TNF seems to be associated with disease rather than protection. In malaria, serum levels of TNF were found to correlate with severity of the disease both in experimentally infected mice and in human patients.

To study TNF production in cattle, a collaboration was established with CIBA-GEIGY (Switzerland), which provided ILRAD with purified recombinant TNF. The ILRAD researchers then produced monoclonal antibodies to TNF, which they shared with their Swiss colleagues. Further, a bioassay based on the rupture (lysis) of murine tumour cells by TNF was introduced and optimized for use with bovine TNF. This bioassay was also employed to help select monoclonal antibodies that neutralize TNF activity and antibodies that are used to capture and assay TNF in serum.

Collectively, these reagents have enabled ILRAD scientists to measure TNF production in trypanosome-infected cattle. Circulating cytokines, however, represent an excess of cytokine synthesis; these levels give little indication of the local production and effect of a given cytokine during disease. Staff therefore resorted to *ex vivo* measurements of TNF activity. Peripheral blood mononuclear cells were removed from cattle before and during infection with trypanosomes of different pathogenicity and TNF was titrated in the supernatant of the cultured cells. With cells from animals infected with a *T. vivax* stock causing acute anaemia, TNF production rose to a peak 17 days after infection and thereafter decayed. No TNF production was detected in similar cultures of cells taken from animals infected with a less acute stock of *T. congolense.* These results suggest a correlation between TNF production and the severity of anaemia in trypanosomiasis.

Despite careful experimentation, it is probable that *ex vivo* measurements of cytokines do not always mirror local cytokine production in infected animals. ILRAD scientists therefore evaluated expression of TNF at the genetic level in infected cattle. On the basis of the DNA sequences for TNF genes in other mammals, oligonucleotides. short, single strands of DNA. were designed and synthesized. Using these in the polymerase chain reaction (PCR), a complementary DNA sequence (cDNA) coding for bovine TNF was isolated and cloned. Recombinant TNF protein was produced in bulk in the bacterium *Escherichia coli.* The recombinant TNF is biologically active and reacts with the monoclonal antibodies. It is being used to produce other antisera for research. By using cDNA on riboprobes specific for TNF, ILRAD scientists were unable to detect specific TNF messenger RNA sequences in monocytes isolated from *T. congolense*-infected cattle. Using the more sensitive PCR amplification assay, cDNA sequences for bovine TNF were detected in cells from animals infected with both *T. vivax* and *T. congolense.* The level indicates that the earlier failure to detect TNF during the *T. congolense* infection was due to insufficient sensitivity of the immunological methods employed. Nevertheless, the level of transcription was demonstrated to be elevated and to occur earlier in *T. vivax* than in *T. congolense*-infected cattle. This result supports the earlier correlation made between TNF production and anaemia.

Bovine cytokines at ILRAD



The table above shows the extent of ILRAD's development of cytokine reagents for research on cattle disease. The cytokines are denoted by their standard abbreviations: TGF is transforming growth factor, GM-CSF is granulocyte/macrophage-colony stimulating factor and EPO is erythropoletin, a hormone, rather than cytokine, affecting production of red blood cells. cDNA is synthetic DNA having a complementary sequence to the messenger RNA transcribed from a (in this case, cytokine) gene.

A riboprobe is a nucleic acid sequence complementary to specific messenger RNA that in this case can be used to detect or assay that messenger RNA. For the bioassays marked with an asterisk (*), antibodies that neutralize the effect of the cytokine have been produced so that the assays can quantify cytokines as well as detect their presence or absence.

The following scientists or companies provided ILRAD with reagents: (a) American Cyanimid Co., USA; (b) Institute of Animal Health, Compton, UK; (c) Dr. R. Reid, Washington State University. USA; (d) Moredun Research Institute, Edinburgh, UK; (e) Dr. D. Dobbelaere, University of Bern, Switzerland; (f) Dr. A. Burny, Free University of Brussels, Belgium; (g) Ciba-Geigy, Switzerland; (h) GenenTech Inc., USA; (i) Dr. M. Carrington, University of Cambridge, UK; (j) Collagen Corporation, USA.

Cytokines influencing lymphocyte function

Another cytokine, interleukin 4 (IL-4), was first described as a B-cell growth factor (BCGF). It is predominantly a product of helper (CD4+) T lymphocytes and is involved in the regulation of immune responses in several ways. IL-4 has pleiotropic effects on many cells of different origin, including B cells, T cells, macrophages and mast cells. IL-4 also has potent anti-tumour activity and affects haemopoiesis. These diverse effects of IL-4 in vivo can contribute to both beneficial and detrimental immune responses. For instance, IL-4 is responsible for IgE-mediated immunological responses to helminth infections. On the other hand, administration of IL-4 to mice that normally develop a protective Th 1 (T helper cell) response to a lethal infection with another protozoan parasite, *Leishmania major,* renders these animals more susceptible to disease. Administration of antibodies against IL-4 to mice infected with *L. major* enables these animals to clear the infection. Their immune response is dominated by

production of another cytokine, interferon gamma.

These observations indicate that IL-4 is likely to be a key cytokine in influencing cellular immune responses to parasitic diseases such as trypanosomiasis. A bovine IL-4 cDNA probe, a gift from Dr. D. Dobbelaere (University of Bern), was used at ILRAD to produce recombinant IL-4 in *E. coli* as a fusion protein with glutathione Stransferase (IL-4 GST). The fusion protein is biologically active when tested in a T-cell growth factor proliferation assay. The recombinant IL-4 GST has been used as an immunogen to raise polyclonal and monoclonal antibodies. Cattle infected with trypanosomes have been shown to have a large increase in CD5+ B-cells in the blood and spleen. The assay and DNA probes can now be used to analyse the possible role IL-4 plays in this intriguing increase as well as in T-cell-dependent responses to trypanosome antigens.

Cytokines in immunosuppression

Immunosuppression has been reported to occur in mice experimentally infected with trypanosomes. Activated macrophages cause a shift in the T-cell cytokine pattern: the production of interleukin 2 (IL-2) remains unaffected, whereas interferon-gamma (IFN gamma) is increased and expression of the interleukin 2 receptor (IL-2R) is drastically suppressed. Other cytokines and their receptors were required to evaluate the equivalent situation in cattle. Interferon gamma was supplied to ILRAD as a recombinant cytokine by Dr. J. Peel of CIBA-GEIGY. The messenger RNA encoding the bovine IL-2R has been cloned as complementary DNA by Dr. R. Reeves and his colleagues at Washington State University and the protein produced in a bacterial expression system by Drs. D. Dobbelaere and I. Roditi, at the University of Bern. At ILRAD, the IL-2R gene was transfected into mouse cells which then expressed the bovine receptor protein. This modified cell was used to screen for monoclonal anti-bodies, two of which were found to block the interaction between recombinant IL-2 and its receptor. Using these reagents, lymph node cells of infected cattle were shown to inhibit the production by normal lymph cells of IL-2 and their expression of IL-2R without impairing the production of IFN-gamma. Macrophages were shown to play a central role in this alteration of T-cell cytokine profile. Importantly, the suppressive macrophage activity can be induced by incubating normal macrophages with a trypanosome lysate *in vitro.* This assay system therefore provides an opportunity to identify individual trypanosome proteins that affect macrophage function and immune responsiveness through the cytokine network.

Haemopoietic cytokines and growth factors

Research on how cytokines regulate haemopoiesis should lead to a better understanding of the decrease in all blood cell types, particularly of red cells, that accompanies trypanosome infection. Interleukin 3 (IL-3) is a haemopoietic cytokine, produced by activated T lymphocytes, activated mast cells and granulocytes. IL-3 acts on early progenitor cells and thus influences the maturation of all haemopoietic lineages. This cytokine can also modulate the growth and effector functions of mature cells such as macrophages, lymphocytes and mast cells. In humans and mice, IL-3 cannot be readily detected systemically in healthy individuals or in normal bone marrow. The cytokine is detectable, however, in the circulation in graft-versus-host disease, a condition characterized by widespread activation of T lymphocytes. A role for IL-3 in the so-called 'stress haemopoiesis', which follows infection, has been suggested and IL-3 has been shown to have marked effects on platelet recovery. In other infectious diseases, a deleterious effect of IL-3 has been demonstrated. In mice that have developed leishmaniasis, for example, IL-3 promotes the disease by antagonizing the protective effects of IFN-gamma.

Despite these wide-ranging effects, IL-3 is highly species-specific in its activity. Complementary DNA sequences of human and mouse IL-3 show very low homology (only 29% at the amino acid level, for example). To clone the bovine counterpart at ILRAD, oligonucleotide primers based on the sequence of IL-3 cDNA from sheep (courtesy of the Moredun Research Institute, in Scotland) were used. The bovine cDNA clone, obtained by amplification using the polymerase chain reaction (PCR) technique, shows high homology with the ovine IL-3 sequence (85% at the amino acid level; 90% at the nucleotide level). Experiments are in progress to express the cDNA. Bovine-specific IL-3 DNA probes and the recombinant protein will be important tools to assess the role of IL-3 in T-Cell activation and in

the anaemia associated with trypanosome infections of cattle.

Several other cytokines have recently been found to influence haemopoiesis positively. They include IL-1, IL-4, IL-6, IL-7 and stem cell factor. The latter has been shown to be an important regulator of early haemopoietic cell development. Stem cell factor has a profound effect on the erythroid lineage in co-stimulation assays with erythropoietin. Cloning and production of soluble stem cell factor at ILRAD is being approached in a manner similar to that used to produce TNF and IL-3. Recombinant bovine stem cell factor will be a valuable tool for use in in vitro cultivation systems of bovine bone marrow as well as in making assessments of the effects of trypanosome infection on development of bovine blood cells. To evaluate TNF alpha, TGF beta and IFN gamma, which have all been described as suppressors of red blood cell production, ILRAD scientists have produced specific sets of primers. These are being used in reverse-transcription PCR amplification reactions to analyse cytokine RNA expression and to localize activation of lymphocytes or bovine stem cells critical for protective or compensatory responses to disease. Recent research results from laboratories in Europe suggest that trypanosomes may respond directly to cytokines such as IFN gamma or may require these chemical messengers as growth factors. Cytokines may thus not only be critically important to immune and pathological processes of the host, but also be fundamental elements of host-parasite interactions.

This article is based on reports written by Bea Mertens and Maarten Sileghem, which were consolidated by Peter Gardiner.

Experiments with twin calves offer evidence that cytotoxic T cells can clear *T. parva* infections

DISEASES CAUSED by apicomplexan blood parasites are a significant factor in the progress of human and agricultural development in the Third World. Probably the most devastating of these diseases are the human malarias and the theilerioses of cattle. Caused by parasites of the genera *Plasmodium* and *Theileria*, respectively, these diseases have many features in common. In each case infection is initiated by the bite of an arthropod vector and characterized by invasion of host cells by the parasite. Expansion of the parasite within infected cells is accompanied by its differentiation to a stage infective for red blood cells. Invasion of erythrocytes following lysis of the infected cell allows the completion of the parasites' life cycle through the infection of another arthropod.

The most important form of theileriosis in sub-Saharan Africa is East Coast fever, caused by *Theileria parva.* This disease threatens over 25 million cattle in eastern, central and southern Africa and is a major hindrance to agricultural development in the region. A major difference between East Coast fever and malaria is the stage of the parasite that gives rise to clinical disease. In malaria, sporozoites inoculated by an infected mosquito invade a small number of liver cells and differentiate to schizonts with no adverse effects on the host. The parasite undergoes a preliminary expansion within the infected hepatocyte before being released as merozoites. These invade red blood cells and undergo further expansion; it is this event that is associated with clinical disease. In contrast, sporozoites of *T. parva* invade a subset of white blood cells known as lymphocytes but their differentiation to schizonts is associated with uncontrolled proliferation of the infected cell. Severe clinical disease is therefore present before significant invasion of erythrocytes by the merozoite stage of the parasite.

Cattle that recover from East Coast fever, either naturally or as the result of treatment, are solidly immune to challenge with the same isolate of the parasite. It has been established for some time that this immunity is directed at the schizont-infected cell and that it is mediated by immune cells rather than antibodies. In a series of experiments conducted on immune cattle at ILRAD, the dominant bovine cellular immune response to *T. parva* has been determined to be parasite-specific cytotoxic T lymphocytes. These killer cells are, like those seen in viral diseases of mouse and man, restricted in function to infected cells bearing self antigens of the major histocompatibility complex. Cytotoxic T cells are found in the blood of immune cattle under challenge about the time that the parasite is cleared, and the capacity of an immune animal to resist challenge with a different isolate of the parasite depends on whether its

cytotoxic T cells can recognize that isolate. In spite of these observations, until recently scientists have been unable to provide direct evidence that cytotoxic T cells can actually clear an established infection with *T. parva*.

QUESTIONS OF THIS NATURE have been answered in several murine disease model systems by inoculation of immune cells into naive animals and establishment by subsequent challenge that immunity is conferred on the recipients. Such experiments have been impractical in cattle due to the large numbers of cells that are required for transfer in this species. Recently, lymphatic cannulation techniques have been applied in the study of bovine immune responses to *T. parva*.

In general, immune responses occur in the lymph node that drains the site of challenge. Lymphatic cannulation allows collection of the cells leaving a responding node over long periods of time, so that the precise kinetics of the immune response can be determined. These studies revealed that during the peak of the nodal response to *T. parva,* as many as 1 in 32 of the cells leaving the node can be a parasite-specific cytotoxic T cell. Since up to 10^{11} (one hundred billion) cells leave the node during a 24-hour period, this clearly allowed the collection of enormous numbers of parasite-specific cytotoxic T cells and raised the possibility that transfer experiments might be conducted in cattle.

To achieve this, a method was developed at ILRAD to prepare large numbers of cytotoxic T cells by killing non-cytotoxic T-cell populations in overnight collections of lymph fluid by complement mediated lysis. This method involves the use of a panel of monoclonal antibodies generated at ILRAD that defines subpopulations of lymphocytes, in conjunction with factors present in rabbit serum that give rise to lysis of cells coated with antibody. This technique allowed the preparation of cytotoxic T-cell fractions to levels of purity as high as 85%.

To avoid problems with graft rejection, adoptive transfer experiments require identical donor and recipient animals. Identical twin calves were therefore generated at ILRAD using embryo splitting techniques. Three sets of these calves were used to determine whether parasite-specific cytotoxic T cells could clear naive calves of established *T. parva* infections. The calves were infected with a lethal dose of the parasite such that the peak of the immune response of the donor twin would coincide with emergence of parasitosis in the naive recipient. In two experiments of this kind, as many as 10^{10}purified *T. parva*-specific cytotoxic T cells were transferred to lethally infected calves over a four-day period. In both cases, the recipient calves resisted challenge, while untreated control calves developed severe clinical signs that required treatment. In a third experiment, in which cytotoxic T cells were depleted from the inoculum by complement mediated lysis before transfer, no protection was observed in the recipient.

These experiments provide the first direct evidence that cytotoxic T cells can clear *T. parva* infections in cattle. In addition, because of the similarities between malaria and *Theileria* infections, they provide support for the belief that parasite-specific cytotoxic T cells may play a role in the control of human malaria. Current efforts are focused on the identification of *T. parva* components that provoke cytotoxic T cell responses so that they can be incorporated in an improved sub-unit vaccine for East Coast fever.

This article was written by ILRAD scientist Declan McKeever.

Two genes encode an important trypanosome protein (PH.D.Thesis)

AFRICAN TRYPANOSOMES are protozoan parasites that cause trypanosomiasis in people and their domestic animals. A promising approach to developing new therapeutic agents for this disease is to identify and characterize cellular processes and structures that occur in trypanosomes but not in cells of the parasite's host animal. Interventions targeted against these processes or structures would interfere little with host cells.

The paraflagellar rod, which makes up a major part of the trypanosome's flagellum, is one such unique structure. The function of this rod is still unknown; it is a complex lattice of

filaments with ultrastructural characteristics unrelated to any of the major filamentous systems of the host cells..

Trypanosome cytoskeletal, cellular ghosts were prepared from bloodstream forms of *Trypanosoma brucei brucei* and highly enriched for microtubules and 10-nm filaments. *AT. brucei* paraflagellar rod protein (Tcp75) was purified and a similar protein (Tcp75) identified in *Trypanosoma congolense.* The protein molecule does not assemble into filaments *in vitro* but forms protofilaments and sheet-like structures.

Antibodies were raised in rabbits against Tcp75. The protein was discovered to be highly conserved among trypanosome species and life-cycle stages, indicating that it may perform functions important for trypanosomal survival.

Two genes that code for paraflagellar rod proteins of *T. congolense* metacyclic trypanosomes were identified by immunoscreening a bacteriophage lambda gt 11expression library using the antibody raised against the Tbp75 protein. Both genes were identified from the complementary DNA (cDNA) library due to serological cross-reactivity of their fusion proteins.

The products of these genes (G1 and G2) have a high homology (63%) at the amino acid level. Interestingly, the genes do not hybridize at the DNA level, as one would expect. Further analysis showed that copies of each of the genes are arranged in tandem within the parasite genome. There are about four to six copies of each gene in the *T. congolense* genome. Both genes are differentially transcribed in the *T. brucei* ILTat 1.1 and *T. congolense* IL3000 developmental forms.

In summary, these studies show that an acidic soluble protein, Tcp75, is a major component of the paraflagellar rod in trypanosomes. There are at least two distinct, actively transcribed genes that code for this protein in *T. congolense* IL3000 metacyclics. The constitutive expression of Tcp75 in all the parasite's developmental forms suggests that its functions are required for the parasite's survival.

Biochemists and molecular parasitologists researching the biology of trypanosomes could now focus efforts on identifying trypanocidal drugs targeted at the paraflagellar rod. A rational approach would be to investigate *in vivo* the pathways involved in the biosynthesis of the paraflagellar rod subunit proteins and their assembly into the highly ordered filamentous lattice that makes up the paraflagellar rod.

Wilson Endege
Kenyan ILRAD Research Fellow.
Summary of a Ph.D. thesis abstract submitted in 1992 to the Faculty of Science,
Institute for Molecular Biology,
Vrije Universiteit Brussel, Belgium
ILRAD Ph.D. supervisors:
Onesmo ole-MoiYoi and John Lonsdale-Eccles.

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Telephone: + 254 (2) 632-311.
Telefax: + 254 (2) 631-499.
Telex: 22040 ILRAD.
Electronic mail: (BT Tymnet) CGI017.

ILRAD 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 of controlling the most important parasitic diseases constraining animal production in Africa: trypanosomiasis, transmitted to animals by the 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.