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Annual Scientific Report
1989

International Laboratory
for Research on Animal Diseases

P.O. Box 30709 Nairobi, Kenya

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PREFACE

ILRAD published its first *Annual Report* in 1975, and until up till 1980, the Report contained summaries of the research provided by the scientific staff. In 1981, the *Annual Report* adopted its current format, where the information provided by the scientists was used to provide a text more suitable to a wider non-specialist audience including the donor community on whom ILRAD relies for its funding. It is felt to be important that the spectrum of ILRAD's research programme, as provided by the ILRAD scientists' own annual research summaries, be given a wider circulation. To this end it was decided to publish an additional annual document, the *ILRAD Annual Scientific Report*.

This document, the third in the series, contains the individual summaries of the scientific work carried out by ILRAD staff and their collaborators during 1989. It also lists the scientific publications and the papers presented by staff at international meetings as well as the names, affiliations and research topics of visiting scientists who worked at ILRAD during the year.

The research summaries are grouped under each project area, and the project areas and their objectives are given below:

THEILERIOSIS

The theileriosis research programme is divided into three project areas with the following objectives.

TH1. Epidemiology of theileriosis

To increase knowledge of the epidemiology of bovine theileriosis and to introduce and evaluate improved immunological control methods in collaboration with national and international organisations.

TH2. Immunization with sporozoite antigens

To identify, characterise and produce antigens from the sporozoite forms of *T. p. parva* and *T. p. lawrencei* which, when suitably delivered, will induce protective immunity.

TH3. Immunization with schizont antigens

To identify, characterise and produce antigens from the intracellular forms of *T. p. parva* and *T. p. lawrencei* which, when suitably delivered, will induce protective immunity.

TRYPANOSOMIASIS

The trypanosomiasis research programme is divided into four project areas with the following objectives.

TR1. Epidemiology of trypanosomiasis

To develop a more precise understanding of the epidemiology of tsetse-transmitted and non-tsetse-transmitted trypanosomiasis so that national organisations can better apply

currently available control measures such as chemoprophylaxis and chemotherapy, use of trypanotolerant livestock and tsetse control.

TR2. Trypanosome biology and biochemistry

To obtain knowledge of important facets of the biology and biochemistry of the major pathogenic trypanosome species relevant to developing improved control measures for trypanosomiasis.

TR3. Immunology of trypanosomiasis

To determine the immunological and related mechanisms involved in the acquisition or expression of resistance to trypanosomiasis and investigate if such mechanisms can be manipulated in order to improve resistance to trypanosomiasis.

TR4. Resistance to pathogenic effects of trypanosomes

To determine the mechanisms which result in the resistance or susceptibility of livestock to the pathogenic effects of trypanosomiasis such as haemopoietic and reproductive dysfunction.

EPIDEMIOLOGY AND SOCIO-ECONOMICS

The epidemiology and socio-economics programme has the following objectives.

To identify the factors at both national and farm level which are essential for the successful delivery and acceptance of improved control measures for livestock disease.

To develop and verify predictive models for theileriosis which can be used in different locations and production systems to determine the probable epidemiological, economic, social and environmental consequences of the application of improved disease control measures including immunisation.

To develop predictive models for trypanosomiasis which can be used in different locations and production systems to determine the probable epidemiological, economic, social and environmental consequences of the application of improved disease control measures including tsetse control, chemotherapy, the use of trypanotolerant livestock and potential immunisation.

We continue to hope that this report will be of value to the scientific community at large, particularly to those scientists in both the developing and developed world, who also undertake research leading to improved control of livestock diseases.

J.J. Doyle
Director of Research

THEILERIOSIS

TH1.1 Development of DNA probes for the detection and identification of *Theileria* species and stocks in cattle and ticks

Scientists: R. Bishop, S. Morzaria, B. Allsopp*, B. Goddeeris, T. T. Dolan
Research Associates: P. Spooner, E. Gobright
Technicians: B. Sohanpal, M. Kibe, L. Juma, R. Njamunggeh, J. Kiarie

(*Department of Biochemistry, University of Cambridge, UK)

Polymerase chain reaction (PCR) technology has been applied to the dominant repetitive sequence of the *Theileria parva* genome using DNA sequence data obtained from *T.p. bovis* Boleni and *T.p. parva* Marikebuni to identify conserved sequences for use as amplification primers. The major aims of this work are to increase the efficiency of characterisation of infected lymphocyte material, attempt to detect carrier animals with low parasitaemias or schizont parasitoses, generate oligonucleotides for recognition of specific *T. parva* stocks and increase the efficiency of parasite detection in the tick vector. The primers have been used to amplify a 290 base pair sequence within the *T. parva* dominant genomic repeat from infected lymphocyte DNA samples and are currently being used in experiments to detect parasites in carrier animals and experimentally infected cattle.

In collaboration with Dr. B. Allsopp, amplification primers developed in Cambridge and more universally conserved primers identified by comparing ILRAD and Cambridge sequence data were used to apply PCR to the dominant repetitive sequence of a wide range of *T.p. parva* and *T.p. lawrencei* stocks. Application products from two potential immunisation stocks, *T.p. bovis* Boleni and *T.p. parva* Marikebuni, have been cloned and sequenced in order to develop stock-specific oligonucleotide probes. Many stocks, particularly of *T.p. lawrencei*, failed to amplify even with the improved primers. This observation, together with recent data from Southern blot analyses, suggests that although the dominant repetitive sequence may be useful for stock-specific "fingerprinting", it is evolving too rapidly to form the basis of a test which correlates with *in vivo* immunity. The potential of other sequences for use in *T. parva* characterisation has therefore been evaluated.

A synthetic oligonucleotide based on a published *Plasmodium* telomeric sequence demonstrates the existence of restriction fragment length polymorphisms (RFLP) between *T. parva* stocks when used to probe *Theileria* DNA. A *T. parva* telomeric sequence has been cloned and is undergoing sequence analysis. A *T. parva* small subunit ribosomal DNA gene cloned at ILRAD also appears useful for characterisation and preliminary Southern blot data separates *T. parva* stocks into two groups.

TH1.2 Characterisation of *Theileria parva* schizont proteins by two-dimensional electrophoresis

Scientists: M. Kishima, T.T. Dolan, L. Mutharia, B.M. Goddeeris
Research Associates: P.R. Spooner, C. Nkonge
Technician: R. Njamunggeh

Previous studies attempting to characterise *Theileria parva* stocks on schizont protein spot patterns by two-dimensional (2D) gel electrophoresis using ^{35}S-methionine-labelled schizonts demonstrated differences between the three *T. parva* subspecies. In these studies

schizont proteins of *T.p. parva* Muguga, *T.p. bovis* Boleni and *T.p. lawrencei* 7014 were compared by 2D Western blotting using sera from cattle immunised with each subspecies. In the Muguga stock, more than ten antigens with relative molecular masses (Mr) of 86 kDa, 56 kDa, 40 kDa and 32 kDa were detected. In the Boleni stock, five antigens with Mr of 83 kDa, 69 kDa and 66 kDa were detected. In the 7014 stock, two antigens with Mr of 100 kDa and 76 kDa were detected. Further studies will be done using bovine sera obtained at various intervals after immunisation.

Seven schizont protein spots with Mr of 75 kDa, 70 kDa (two spots), 60 kDa, 46 kDa, 41 kDa and 39 kDa were detected in 2D gel autoradiographs using ^{35}S-methionine-labelled cells infected with the Muguga stock. However, no responses were detected by Western blotting using immune bovine sera. Alternative staining methods will be investigated.

TH1.3 Characterisation of immunoreactive *Theileria parva* schizont antigens

Scientists: P.M. Rwambo, T.T. Dolan, A.J. Musoke
Technician: R. Njamunggeh

Monoclonal antibody (MAb) profiles have been used to characterise parasite stocks of *Theileria parva*. The current panel of MAbs recognise a variable epitope localized on a polymorphic molecule of *T. parva* schizonts. The aim of the work during the year has been to expand the panel of anti-schizont MAbs to include monoclonals that can distinguish stocks of *T.p. bovis* and *T. taurotragi*, from stocks of *T.p. parva* and *T.p. lawrencei*.

Mice immunised with purified schizonts of *T.p. bovis* Boleni or with whole infected G6 bovine lymphocytes produce cross-reactive antibodies directed at determinants on the polymorphic immunodominant molecule. Analysis of the immunoreactivity of *T.p. bovis* Boleni schizont proteins in Western blots using recovered cattle sera has identified antigens with approximate Mr 160, 70, 69, 55, 52 and 50 kilodaltons (kDa). The 69 and 70 kDa antigens are also reactive with sera from cattle infected with *T.p. parva* Muguga 3087, Mariakani 3029 and Uganda 3066 but not from cattle infected with the Marikebuni 3014 and *T.p. lawrencei* 3081 parasite stocks. The 52 kDa schizont antigen reacts only with the *T.p. bovis* Boleni antisera. To expand the specificity of MAbs to schizont proteins, the type-specific immunoreactive schizont antigens will be purified from SDS-PAGE gels and used to immunise mice for production of MAbs. Such MAbs will be useful in the characterisation of *T. parva* parasite stocks and in purification of schizont antigens for further immunological and biochemical characterisation.

TH1.4 Development of characterisation methods for *Theileria taurotragi*

Scientists: T.T. Dolan, R. Bishop, J.G. Grootenhuis, D. Wesonga*, R.A.I. Norval, S.P. Morzaria
Research Associates: P.R. Spooner, F. Mwakima
Technicians: L. Juma, J. Kiarie, R. Njumunggeh

(*National Veterinary Research Centre, Kabete, Kenya)

Theileria taurotragi is a non-pathogenic *Theileria* species infecting cattle but originating from eland. It is important because it confuses the epidemiology of *T. parva*, a pathogenic species in cattle. It is indistinguishable from *T. parva* in the vector *Rhipicephalus appendiculatus* and the two parasites cross-react serologically. Other non-pathogenic *Theileria* probably also confuse the epidemiology of *T. parva* and *in vitro* methods are urgently needed to distinguish between these *Theileria* species.

A reference stabilate (162) of *T. taurotragi* was used to infect a splenectomised eland. Ticks were fed on the eland and used to infect cattle, sheep and goats and to prepare a working stabilate (3299). The working stabilate is infective for sheep and goats. Isolates from these infections show fluorescence patterns typical of *T. taurotragi* using a panel of monoclonal antibodies raised against *T. parva*. A sheared DNA library has been constructed from infected cell DNA and is being differentially screened with radiolabelled total *T. taurotragi* infected cell DNA and bovine lymphocyte DNA to isolate parasite - specific repetitive DNA sequences. If this approach fails then attempts will be made to clone the extrachromosomal element of *T. taurotragi*, similar to that of *T. parva*, and sequencing part of it to generate a specific *T. taurotragi* oligonucleotide. Alternatively small subunit ribosomal RNA genes will be amplified using the polymerase chain reaction and the amplified products will be used to identify *T. taurotragi* specific sequences.

Monoclonal antibodies are being raised against *T. taurotragi* schizont infected cells to produce specific reagents for use in both monoclonal antibody screening and discriminatory serological tests. These tests together with DNA probes will allow the identification of *T. taurotragi* infection in cattle and ticks and discrimination between *T. taurotragi*, other non-pathogenic *Theileria* spp. and *T. parva* infections in field sera.

TH1.5 Mapping of the *Theileria parva* genome

Scientists: S.P. Morzaria, J.R. Young
Technician: T. Batavia

The main objectives for the construction of a physical map of the *Theileria parva* genome are to define the basis of *Sfi* I polymorphisms and identify deleted regions for stock characterisation, to provide a set of mapped marker loci for sexual recombination studies and to locate antigen and other genes within the map. An *Sfi* I linking library of approximately 70,000 recombinants was constructed from 4 - 6 kb partially *Sau* 3A digested *T. parva* Muguga DNA. Analysis of the minipreps from 350 clones from the library revealed that 199 had the right size DNA fragments. Further analyses by end-labelling and acrylamide and agarose-gel separation of *Sau* 3A and *Sau* 3A/*Sfi* I double digests showed 126 clones had

Sau 3A fragments containing *Sfi* I sites. These were sorted into 23 unique groups each representing one *Sfi* I site in the genome.

An incomplete map has been constructed by determining the hybridisation patterns of each of the 23 linking clones on Southern blots of *Sfi* I, *Not* I and *Sfi* I/*Not* I digested *T. parva* Muguga DNAs. Telomeric ends have been identified with an oligonucleotide of *P. berghei* telomeric fragment. Most of the ambiguities have been clarified by exploiting *Sfi* I fragment polymorphisms among stocks and allocation of linking clones to the eight *Not* I fragments of the *T. parva* genome. The results to - date show that there are four chromosomes in *T. parva* Muguga of 2715 kb, 2276 kb, 2085 kb and 1987 kb respectively. There are three extrachromosomal DNA fragments, two single copy fragments of 300 kb and 450 kb and one of multiple (10 - 15) copies of 6.5 kb.

Putative locations of five *T. parva* antigen genes, a ribosomal gene, the actin gene, the heat-shock gene, repetitive sequence and eight uncharacterised cDNA clones, have been determined on the map.

To complete the mapping of the genome, future plans are to construct a *T. parva* Muguga cosmid library and identify overlapping clones spanning the *Sfi* I sites. Further ambiguities will be sorted by probing Southern blots of *Sac* II digested *T. parva* fragments with nick-translated DNA eluted from discrete *Sfi* I fragments.

TH1.6 Investigations of improved methods for handling and storing *Theileria parva* sporozoites

Student: E. N. Kimbita
Supervisor: T.T. Dolan

The original method of stabilate preparation for ground up *Theileria* -infected ticks was developed in the early 1970's based upon the success achieved with a technique for trypanosomes. No detailed studies were conducted to explore other methods as the one first used worked. There are many unanswered questions regarding preparation, handling and use of stabilates. Is there a better cryoprotectant, freezing rate or preparation procedure? Can the sporozoites be freeze-dried and avoid cold chain problems for field immunisation? How long do sporozoites retain their infectivity at different temperatures once thawed? A series of experiments were conducted to begin to answer some of these questions.

Adult *Rhipicephalus appendiculatus*, fed on animals infected with *T. parva* Muguga as nymphs, were fed on rabbits for four days. The ticks were dissected under sterile conditions, the salivary glands ground and the sporozoites subjected to various treatments and methods of preservation.

At room temperature (24°C) the sporozoites maintained a constant infectivity for six hours. Sporozoites held at 4°C maintained the same infectivity for 48 hours. The use of DNAse at 10 µg/ml medium increased the infectivity tenfold. Experiments are underway to study the effects of DNAse in long - term storage of sporozoites. Incubation of sporozoites with 10% dimethyl-sulphoxide for 30 minutes resulted in a 100 fold reduction in infectivity. *Theileria parva* sporozoites preserved in 5% polyvinylpyrrolidone (PVP) maintained an

unchanged infectivity for up to two months. Sporozoites in 10% PVP lost 10% of their infectivity over the same period.

Foetal bovine serum, Eagles MEM, sucrose peptone water, Leibovitz-15, and saline skim milk were tested for their stabilizing abilities in the protection of sporozoites during lyophilisation. On resuscitation no infectivity was detected with any of the stabilizing media. Experiments are underway to test the infectivity of the sporozoites at each stage in the process of lyophilisation.

TH1.7 Studies of the factors affecting the infection rates of *Theileria parva* in *Rhipicephalus appendiculatus*

Scientists: R.A.I. Norval, T.T. Dolan, S.P. Morzaria, J.G. Grootenhuis
Research Associates: F. Mwakima, J. Scott
Technician: S. Mwaura

The reliable production of adults of *Rhipicephalus appendiculatus* that are highly infected with *Theileria parva* has been a consistent problem in *Theileria* research. We have now shown that the most important factor affecting infection rates is the air temperature in which cattle are held at the time of nymphal feeding. Lowest infection rates (means of 15 - 45 infected acini per female) were obtained when cattle were kept warm with infra-red lamps and highest infection rates (means of up to 200 infected acini per female), when cattle were held in a room that was cooled to ±15°C using an air conditioner. Infection rates were intermediate (means of 50 - 120 infected acini per female) when cattle were held at normal air temperatures. A likely reason for higher infection rates being obtained at the lower temperatures is that the nymphal feeding period, and hence the duration of favourable conditions for the completion of the sexual cycle of *T. parva* in the gut of the nymph, increases as the skin temperature of the host decreases.

Stocks of *R. appendiculatus* from geographically different areas have also been found to differ in their susceptibility to infection with *T. parva*. The stock in which highest infection rates have been obtained originates from Ol Pejeta Ranch, Kenya. Intermediate infection rates have been obtained in the Muguga stock and generally low infection rates in a stock from Lake McIlwaine, Zimbabwe. Under Kenyan conditions the unfed adults of southern African stocks of *R. appendiculatus*, and the closely related species *R. zambeziensis*, enter diapause and do not attach readily to hosts. Adult diapause does not occur in stocks of *R. appendiculatus* originating from East Africa.

TH1.8 Studies on pathogenic *Theileria mutans*

Scientists: S.P. Morzaria, A.J. Musoke, T.T. Dolan, V. Nene, R.A. Norval, R. Bishop
Research Associates: J. Katende, F. Mwakima, C. Nkonge
Technicians: J. Kiarie, D. Mwaengo, B. Sohanpal

Some strains of *Theileria mutans* cause disease in susceptible cattle. The disease, which is characterised by anaemia and loss in productivity, has recently been demonstrated in cattle exposed to natural tick challenge on the Zanzibar island of Unguja. The objectives of

this study are to a) characterise the pathogenic *T. mutans* Kipange stock isolated from cattle on Unguja, b) develop sensitive diagnostic tests and c) attempt to produce a vaccine against the parasite.

The *in vivo* characterisation of the Kipange stock showed that it causes high parasitaemia and chronic anaemia in splenectomised cattle but intact cattle are not clinically affected by the infection. Studies are underway to investigate the effect of *T. mutans* infection on ECF-immunised cattle.

Two monoclonal antibodies (41.1 and 41.8), which recognise different epitopes on a 32 kDa molecule have been used in immune complex and antigen capture assays. The results show that these assays together with the antibody detection ELISA, using purified *T. mutans* piroplasm antigen, are sensitive and specific in detecting acute *T. mutans* infection in cattle.

A 3.5 kb repetitive DNA fragment has been cloned from a sheared genomic DNA expression library of *T. mutans*. The 3.5 kb fragment hybridises specifically to the *T. mutans* genome and is currently being developed as a diagnostic probe for the detection of parasites in cattle and ticks. The preliminary studies show restriction fragment length polymorphism between Zanzibar and Intona stocks of *T. mutans*. Studies are planned to immunise cattle with the sporozoite and blood and stabilates for natural parasite challenge in the field. The role of 32 kDa antigen in immunity needs to be further evaluated.

TH.1.9 Identification of *Babesia bigemina*, *Anaplasma marginale* and *Theileria mutans* specific antigens for use in ELISA

Scientists: J.M. Katende, A.J. Musoke, S.P. Morzaria, T.T. Dolan
Research Associate: C. Nkonge
Technicians: J. Ngatti, A. Sore, A. Njeri, M. Omenya

The routine diagnosis of infections with *Theileria parva*, *T. mutans*, *Anaplasma marginale*, *Babesia bigemina* and *B. bovis* is based on the demonstration of parasites in Giesma-stained blood smears or the detection of antibodies in sera using whole organisms or their lysates. The microscopic examination of stained blood and lymph node smears is insensitive and cumbersome and it may be difficult to differentiate one species from another. The use of lysates of whole organisms has major disadvantages in that a multitude of antigens are being utilised, resulting in assays being unspecific and insensitive. Therefore it is desirable to generate species-specific monoclonal antibodies that can be used in assays to detect defined antigens and to use the MAbs to isolate these antigens for development of specific antibody detection assays.

Species-specific antigens have been identified using monoclonal antibodies in Western blots. The MAbs against *Anaplasma marginale* recognise an antigen of relative molecular mass of 52 kDa while the MAbs against *Babesia bigemina* identify an antigen of relative molecular mass of 44 kDa. The MAbs generated against *T. mutans* identify a 32 kDa protein. This antigen is also recognised by sera from cattle recovered from experimental tick-transmitted *T. mutans* infections. An antigen capture ELISA has been developed with two of the MAbs (Tm41.1 and 41.8) which recognise different epitopes on the 32 kDa molecule. Using this test, it is possible to detect circulating antigens or immune complexes

in sera collected from cattle during the acute or chronic phases of *T. mutans* infection. The 32 kDa protein has been purified and used in a micro-ELISA in which antibodies are detected during all stages of infection. The antibody and antigen assays are specific to *T. mutans* as no reactivities have been detected with other parasitic infections in cattle.

It is impractical to prepare large amounts of the antigens recognised by these MAbs and work is underway to clone the gene encoding these antigens. It is envisaged that the protein determinants identified by the MAbs will be synthesized and utilized for further development of the antibody assays.

TH2.1 Effects of irradiation on *Theileria parva* sporozoites

Student: M. Butera
Supervisors: S.P. Morzaria, T.T. Dolan

In previous investigations of irradiation of *Theileria parva* sporozoites its effects were assessed indirectly by analysing the subsequent infectivity of the parasites in cattle. There are no reports of the direct effects of irradiation on the parasite. A study was initiated in 1986 to analyse the effects of irradiation on the biology of *T. parva* sporozoites, to determine the effects on morphology, the ability to bind to and enter peripheral blood lymphocytes (PBL), the early developmental events which follow entry of sporozoites and the ability to transform and establish cell lines.

No difference was detected between the ability of irradiated (1300 rad) and non-irradiated sporozoites to bind to and enter lymphocytes. Between days one and three after infection the development of the irradiated and non-irradiated sporozoites in PBL was similar. Electron microscopic studies of these cultures revealed that both treated and untreated parasites had micronemes, developed a cytostome and the host cells developed microtubules around the parasite. However, from day four after infection significant differences in development were observed between irradiated and non-irradiated sporozoites. The majority of the cells infected with irradiated sporozoites contained intracytoplasmic particles which varied from dot-like structures to pyriform or vacuolated forms. The PBL infected with non-irradiated sporozoites had normal schizonts.

The intracytoplasmic particles and normal schizonts in cultures derived from non-irradiated sporozoites reacted with a schizont-specific monoclonal antibody (MAb) but were negative with sporozoite specific MAb. This suggested that the dot-like particles were expressing schizont antigens.

The cultures established from irradiated sporozoites showed a slower growth rate initially, than the cultures established with non-irradiated sporozoites, but once the cell lines were established, growth and infection rates were similar.

Limiting dilution cloning of cultures in which the majority of the cells contained dot-like particles was performed to determine if schizont-infected cell lines could be established. Statistical analysis showed that there was a high probability of transformed cell-lines being derived from these particles.

A comparison of cloned cell lines derived from irradiated and non-irradiated sporozoites showed that the parasite phenotypes were similar when characterised with a panel of twenty monoclonal antibodies. No differences were observed in host cell phenotype or in *in vivo* infectivity for autologous animals of these cell lines.

Studies are in progress to investigate whether genotypic differences exist between the two cell lines using a *T. parva* specific repetitive DNA probe and pulsed-field gel analysis of their genomes using rare-cutting restricting enzymes.

TH2.2 Bovine immune response to irradiated *Theileria parva* sporozoites

Scientists: A.J. Musoke, S.P. Morzaria, R.A.I. Norval, W.I. Morrison
Research Associates: S. Minja, C. Nkonge, F. Mwakima
Technician: J. Naugi

Earlier work suggested that increasing doses of irradiation killed an increasing number of *Theileria parva* sporozoites and that no immunity was induced in the animals receiving what were considered "destroyed" sporozites. The failure of the "destroyed" sporozoites to induce demonstrable immunity may have been due to the fact that only a small number of sporozoites (ten ticks per animal) was given on a single occasion. Also no attempt was made to examine the nature of the immune response in the immunised animals.

Since it is known in other parasite systems that irradiated organisms continue to synthesize proteins for a number of days before they die, we considered that the immunogenicity of irradiated *Theileria* sporozoites should be re-examined using large numbers of sporozoites coupled with several booster doses. The immune responses were examined in terms of:

(1) Kinetics, specificity and biological importance of induced antibodies
(2 Induction of cytotoxic lymphocytes
(3 Induction of T-helper cells
(4) Establishment of protective immunity

The sporozoites were irradiated lethally at 100 kr for five hr and purified by anion-exchange chromatography. The cattle were divided into three groups: Group 1 consisting of four animals was given sporozoites emulsified in Freund's complete adjuvant, Group II comprising four animals was given intact sporozoites suspended in bovine plasma albumin and Group III, with two animals, received a lysate of sporozoites in phosphate buffered saline. Each animal received the equivalent of 15,000 infected acini as primary dose. After the initial priming the cattle were given four booster doses (in Freund's incomplete adjuvant) (15,000, infected acini) and the antibody responses were measured 14 days after the fourth booster dose. The antibody responses against 85 kDa and 67 kDa were assessed by western blotting and ELISA.

The antibody titres against the 67 and 85 kDa antigens in the group of animals which received sporozoites in adjuvant ranged from 1:50 to more than 1:6000, while those titres in the group which received intact sporozoites ranged from 1:50 to 1:800. The group which received a lysate of sporozoites had the lowest response with titres ranging from 1:50 to 1:400.

The neutralization titres also followed a similar pattern, with the titres being highest in the adjuvant group (1:300) and lowest in the lysate group (1:50).

All animals were challenged with a lethal dose of sporozoites and no protection was observed. The failure of protection may be due to low antibody titres to the antigens. An electron microscopic study has shown that the majority of irradiated sporozoites do not enter lymphocytes, thus leading to a failure of antigen processing. It is also possible that high doses of irradiation may have damaged critical epitopes.

TH2.3 Analysis of T-helper cell responses in Boran cattle immunised with irradiated sporozoites of *Theileria parva*

Student: H. Pereira
Supervisor: A.J. Teale
Scientists: W.I. Morrison, A.J. Musoke, S. Morzaria

Though various methods involving the use of sporozoite preparations have been studied with a view to immunising cattle against ECF, the use of irradiated sporozoites for this purpose has not been fully investigated. The study reported here was undertaken in collaboration with others to investigate the various aspects of the immune response in animals that have been immunised with preparations of irradiated sporozoite material. Specifically, this work analyses the T-helper cell response *in vitro* to anion-exchange purified sporozoite lysate (SL) antigen and to 67 kDa antigen. The latter antigen forms a major component of the SL and antibodies to this molecule neutralise infectivity of sporozoites. The antigen was prepared by electroelution from polyacrylamide gels.

Peripheral blood mononuclear cells from three of four animals in Group A that were immunised with irradiated sporozoites plus adjuvant responded to both the 67 kDa and the SL antigens in *in vitro* proliferation assays. The fourth animal did not respond to either antigen.

In Group B, three of four animals that were immunised with irradiated sporozoites only, responded to the 67 kDa antigen but showed no response to the SL. The fourth animal in this group did not respond to either antigen.

In Group C, consisting of animals immunised with irradiated sporozoite lysate without adjuvant one animal of two has shown responses to the SL. There were no detectable responses to the 67 kDa in either animal.

Naive control animals did not respond to either of the two antigens whereas positive control animals immunised either using unirradiated sporozoite lysate or infected tick challenge responded in proliferation assays to both antigens

Animals in Group B (irradiated sporozoites) did not respond to the SL antigen. This would indicate that the animals that do respond to this antigen in Group A (irradiated sporozoites + adjuvant) and Group C (irradiated sporozoite lysate) may be recognising other antigens in addition to the 67 kDa that are present in the sporozoite lysate. These antigens, either sporozoite or tick, are either not present in the irradiated sporozoite preparation, which appears unlikely, or responses to them were below detectable levels.

TH2.4 Cell-mediated immunity against schizont antigens in animals immunised with irradiated sporozoites of *Theileria parva*

Scientists: E.A. Innes, B.M. Goddeeris, W.I. Morrison
Technicians: F. Mbwika, E. Awino, D. Ngugi

As part of a larger experiment to study the immune responses of cattle to irradiated sporozoites of *Theileria parva* (see TH2.2 and TH2.3) and to determine whether such an

immunogen could induce protective immunity, cell-mediated immune responses against schizont antigens were examined.

The cattle were immunised on six occasions at intervals of approximately one month. Four animals received irradiated sporozoite lysate in Freund's complete adjuvant (Group A); four received irradiated sporozoites in bovine plasma albumin (Group B) and two received sporozoite lysate in PBS (Group C).

The cytotoxic activity of PBM, stimulated for six days *in vitro* with autologous infected cells, was assessed using a panel of targets comprising autologous infected, autologous uninfected and BoLA mismatched infected cells. Proliferative responses of PBM stimulated by the target cells described above and by samples of purified schizonts were also measured.

Cytotoxic activity (40% target cell lysis), directed predominantly against the autologous infected target, was detected after the third inoculation in one animal in group B and one in group C. After the fourth inoculation, cytotoxic responses directed against the autologous infected target cells (target cell lysis 25%) were recorded in two of the animals from group A one from B and one from C. In one animal from group C, a cytotoxic response was recorded after the fifth immunisation.

Proliferative responses were recorded in all the experimental animals when stimulated with either autologous or BoLA mismatched infected cell lines. However, only after the fifth inoculation was a proliferative response to purified schizont antigens was detected in one animal from group C.

TH2.5 Structural organisation and encoded amino acid sequence of the gene that codes for the 67 kDa surface antigen of *Theileria parva* sporozoites

Scientists: V. Nene, K.P. Iams, A.J. Musoke
Research Associate: E. Gobright
Technician: D. Mwaengo

The amino acid sequencing was carried out in collaboration with the Wistar Institute, Philadelphia, Pennsylvania, U.S.A.

The DNA sequence of the gene that codes for the major *Theileria parva parva* Muguga sporozoite surface antigen has been determined. the gene contains an open reading frame that codes for 709 amino acid residues with a calculated molecular mass of 75,437.62 Da. The identity of the gene has been confirmed by finding two peptide sequences determined by sequencing tryptic peptides of the 67 kDa antigen within the predicted open reading frame. Peptide T18 extends from residue 366 to 380 and peptide T12 extends from residue 591 to 614. There is no significant homology of the predicted amino acid sequence of the 67 kDa antigen with protein sequences in the data base at ILRAD.

The DNA sequence of the gene has been determined by compiling sequence data from geomic and cDNA clones isolated from λ *gt*11 libraries. cDNA was constructed from

total sporozoite RNA and from mRNA isolated from infected tick salivary glands. Genomic DNA was isolated from piroplasms.

Preliminary primer extension studies suggest that transcription initiates 49-50 nucleotides upstream of the translational start codon. The transcript is punctuated by four stop codons and the polyA tail is added 42 nucleotides downstream of the first stop codon. The closest sequence to a consensus polyA signal is AATAAC which is located 36 nucleotides upstream of the polyA tail. The 1750 bp of sequence upstream of the open reading frame is 70% AT rich and is presumed to contain the promoter of the gene since we have mapped a gene that codes for a thiol proteinase to this site (see TH2.11).

The open reading frame is interrupted at amino acid residue 316 by a single intron which is 29 nucleotides long. As far as we are aware this is the shortest naturally occurring intron described. The 5' and 3' splice sites contain the consensus eukaryotic donor and acceptor site sequences.

There are no repeated amino acid sequences in the predicted sequence of the 67 kDa protein. There is a bias in codon usage in that codons with A or T at the third position are generally preferred. There are seven potential sites (Asn-X-Ser/Thr) for N-linked glycosylation and the protein is composed of 47.53% hydrophobic, 28.22% hydrophilic, 13.25% acid and 11.01% basic amino acid residues. Preliminary analyses of the purified 67 kDa protein indicate, however, that there is minimal glycosylation of the native molecule. Post-translational processing or the amino acid composition of the protein may account for the difference between the observed and calculated molecular weight of the antigen.

Secondary structure analyses of the 67 kDa antigen suggest the presence of a signal sequence at the N-terminal end. Cleavage by a signal peptidase could occur at one of three positions: between residues 16 - 17, 17 - 18 and 18 - 19. There is a hydrophobic stretch of amino acid residues at the C-terminal end of the protein that could serve as a membrane anchor sequence. These structural predictions are in keeping with the location of the 67 kDa antigen on the surface membrane of the sporozoite.

TH2.6 Recombinant 67 kDa antigen evokes *Theileria parva* sporozoite neutralising antibodies

Scientists: V. Nene, K.P. Iams, A.J. Musoke
Research associates: C. Nkonge, S. Minja
Technicians: D. Mwaengo, D. Lugo, J. Ngugi

Monoclonal antibodies (MAbs) which neutralise *Theileria parva* sporozoite infectivity *in vitro* recognise two molecules with Mr of 67 kDa and 85 kDa in *T.p. parva* Muguga. These antigens are candidate molecules for inclusion in a sporozoite vaccine.

The gene that codes for the 67 kDa molecule (p67) has been cloned and expressed as a fusion protein in *Escherichia coli*. The complete gene has been assembled using genomic and cDNA sequences. Plasmid constructs have been made using the *pGEX* expression vector system which results in the synthesis of p67 linked to the C-terminal end of a *Schistosoma japonicum* glutathione-S-transferase, Sj26. Fusion proteins can be purified

from a bacterial cell lysate in a single step by affinity chromatography using glutathione linked to agarose beads.

Three fusion protein constructs have been made. The first expresses the complete coding region of p67. The second and third constructs encode residues 9 to 316 and residues 397 to 709, respectively.

Rats have been immunised with purified fusion protein expressed by the second and third constructs. Sera from immunised animals recognise the 67 kDa antigen on immunoblots and more importantly the sera completely neutralise sporozoite infectivity. However, the neutralising capacity titrates out rapidly and sera diluted at 1:50 fail to neutralise.

Nevertheless, the results are encouraging since it appears that carbohydrate residues on the native 67 kDa antigen are not essential in evoking neutralising antibodies, at least in rats. Secondly, since both fusion proteins, which contain non-overlapping amino acid sequences, evoke neutralising antisera it appears that more than one epitope is exposed at the surface of the sporozoite.

Clearly more immunogenic preparations of the antigen are required and cattle immunisations need to be undertaken. To address these issues we are isolating the p67 recombinant antigen in bulk which will be used with a variety of adjuvants.

TH2.7 A recombinant vaccinia virus incorporating the gene which encodes the *Theileria parva* 67 kDa sporozoite surface antigen

Scientists: D.J. McKeever, V. Nene, A.J. Musoke
Technicians: D. Mwaengo, J. Nyanjui

The cloning of the gene which encodes the 67 kDa surface antigen of *Theileria parva* sporozoites has highlighted the requirement for effective antigen delivery systems for the immunisation of cattle with soluble antigens. The use of recombinant vaccinia viruses has proven an efficient means of inducing humoral and cellular immune responses to defined antigens in many other systems, and a vaccinia-based rinderpest vaccine has recently been described for cattle. As part of an overall programme directed at assessing the capacity of the 67 kDa sporozoite antigen to protect against parasite challenge, a recombinant vaccinia virus (VVp67) has been constructed which contains the gene, and a preliminary trial has been carried out in laboratory animals with this recombinant.

An adult rabbit which had been selected on the basis of sero-negativity with regard to the 67 kDa sporozoite antigen was inoculated with approximately 10^6 plaque-forming units of VVp67 by skin scarification of the ear pinna. An additional rabbit was inoculated with wild type vaccinia virus. Serum samples taken at days 7 and 14 after challenge were assayed for sporozoite neutralising activity, and for detection of the 67kDa antigen on immunoblots of sporozoite lysates. The 67 kDa antigen was detected on immunoblots by both serum samples from the rabbit inoculated with VVp67, with an increased titre being evident in the day 14 sample. In addition, significant neutralising activity was detected in the day 14 serum taken from this rabbit. Serum from the control rabbit was negative in both assays.

The results of this preliminary experiment in laboratory animals suggest that a recombinant vaccinia virus incorporating the *T. parva* 67 kDa sporozoite surface antigen might be capable of inducing an immune response to the sporozoite in cattle.

TH2.8 Analysis of bovine T-cell responses to the *Theileria parva* 67kDa sporozoite antigen

Student: H. Pereira
Supervisor: A.J. Teale
Scientists: A.J. Musoke, V. Nene

The 67 kDa surface molecule of *Theileria parva* sporozoites is an antigen to which immunised cattle produce antibodies which neutralise sporozoite infectivity. The antibodies specific for the 67 kDa molecule are cross-protective in that they not only neutralise homologous *T. parva* sporozoites but also neutralise infectivity of other *T. parva* stocks. This antigen is therefore an important potential vaccine component.

Peripheral blood mononuclear cells from four animals immunised with either DEAE-purified sporozoite lysate (SL) material or with infected ticks responded to the 67 kDa antigen in *in vitro* assays of proliferative responses. In these assays, the response to the 67 kDa antigen was 5 - 10 X higher than the response to the SL. There was also a response in these animals to DEAE-purified uninfected tick salivary gland lysate (UI). This material, however, was found to be contaminated with material in the 67 kDa range that is recognised by Tpm 12, a monoclonal antibody specific for the 67 kDa molecule. There was no response in these animals to a control antigen, keyhole limpet haemocyanin. Naive, unimmunised animals did not respond to any of the antigens studied.

In order to analyse T-cell epitopes that are recognised on the 67 kDa molecule, the responses of these animals to fusion proteins of this antigen were studied. PBM of the four animals did not respond to the control Sj26 vector antigen used in the preparation of the fusion proteins. The fusion proteins used as antigens in the assays were *pGEX* 1/BR (9 - 398), *pGEX* 3BR (9 - approx. 298) and *pGEX* 1/2.4 (397 - 709), all of which contain various parts of the 67 kDa gene. *pGEX* 1/BR + 2.4 contains the whole gene encoding the entire 67 kDa molecule (9-709). Only one animal on two of four occasions responded to the *pGEX* 3BR fusion protein. Thereafter there was no proliferation to this or any of the other fusion proteins. The three other animals did not respond to any of the fusion proteins. These assays will be repeated using fusion proteins prepared with a different vector. In addition, CNBr fragments and tryptic digests of the 67 kDa molecule will be used to study T-helper cell epitopes. T-cell clones will be used in these epitope studies. Animals of various MHC backgrounds will be immunised with the 67 kDa antigen.

TH2.9 Characterisation of the 85 kDa *Theileria parva* sporozoite antigen

Scientists: A.J. Musoke, B. Goddeeris, W.I. Morrison
Research Associates: S. Minja, C. Nkonge, G. Lamb
Technician: J. Ngugi

Previously it was reported that several of the monoclonal antibodies used for characterising *Theileria parva* stocks recognised an antigen of approximate Mr of 85 kDa in schizonts and sporozoites of *T. parva* Muguga. The antigen was shown to be located on the surfaces of these two stages of the parasite. In schizonts the antigen is polymorphic in western blots i.e. its molecular weight varied depending on the stock of parasite and also on the percentage of acrylamide used. Recent studies, however, have indicated that the antigen may not be polymorphic in the sporozoites. In three stocks, namely *T. parva* Muguga, *T. parva* Marikebuni and *T. parva* Mariakani, the molecular weight was found to be constant. This finding suggests that the antigen may be processed differently in the two stages of the parasite.

Several experiments conducted to establish whether the antigen was glycosylated have been negative, prompting the thinking that the antigen may not be glycosylated or that the carbohydrate moiety has a unique linkage.

The monoclonal antibodies as well as polyvalent antisera against the antigen were shown to neutralise the infectivity of sporozoites *in vitro* but were not as efficient as the MAbs or polyvalent serum against the 67 kDa antigen.

In collaboration with Wistar Institute, Philadelphia, Pennsylvania, USA, attempts are being made to sequence the native antigen, isolated by affinity chromatography, to complement the studies on the cloning of the gene encoding the antigen.

TH2.10 Identification of clones potentially encoding the immunodominant 82 kDa sporozoite/schizont antigen of *Theileria parva* Muguga

Scientists: K. Iams, O.K. ole-MoiYoi
Research Associate: M. Macklin

We have constructed a *Theileria parva* Muguga genomic expression library and screened it with a recovery serum obtained from cow D473 which had recently been challenged with Muguga stabilate. Serum D473 is essentially monospecific for an immunodominant 82 kDa antigen expressed in both the sporozoite and macroschizont stages of *T. parva*. We obtained eight positive clones but found that only one of the fusion proteins produced by these clones reacted with D473 antiserum on western blots. This clone was selected for further characterisation. We have now obtained the sequence of the 700 bp open reading frame of the gene which is fused to the *beta-gal* gene product. Several repetitive sequence motifs occur within the frame with the dominant one showing extensive homology through conservative amino acid substitutions with the circumsporozoite antigen of *Plasmodium*. The non-repetitive region also shows considerable homology with the *Plasmodium* merozoite antigen. Clones have also been obtained from macroschizont cDNA libraries and are being sequenced and antiserum is being raised against the fusion products to further characterize the gene.

TH2.11 Cloning of a gene that codes for a thiol proteinase of *Theileria parva* and identification of thiol proteinase activity in sporozoites and piroplasms

Scientists: V. Nene, A.J. Musoke, J. Lonsdale-Eccles
Research Associates: G. Mpimbaza, S. Minja, E. Gobright
Technicians: D. Mwaengo, J. Ngugi

A gene that codes for a thiol proteinase of *Theileria parva parva* Muguga has been cloned and partially sequenced. The 3' end of the gene is located about 1750 bp upstream of the gene that codes for the sporozoite 67 kDa antigen. About one kb of genomic DNA has been sequenced and a database search of this sequence revealed that the gene codes for a thiol proteinase with homologies to cathepsin L and papain. It is possible that the cloned gene may code for an enzyme of the food vacuole of *T.p. parva* parasites, particularly those that exhibit high intraerythrocytic merogony.

Our interest in this enzyme has been stimulated by the recent reports of the toxic effects of thiol proteinase inhibitors on various pathogenic organisms.

To test whether *T.p. parva* Muguga might express such thiol proteinase activity we assayed a sporozoite preparation for hydrolytic activity against fluorometric 7-amino 4-methylcoumaryl (NHMec) peptides at acid and alkaline pH and compared this with activity in uninfected salivary glands. Minimal activity was observed in the salivary glands against any of our substrates. The sporozoite activity against Z-Phe-Arg-NHMec was approximately 70 fold greater than against Z-Gly-Gly-Arg-NHMec. Due to limited amounts of sporozoite material further studies were performed on piroplasms.

A series of NHMec peptides were tested for their susceptibility to hydrolysis by lysates of partially purified piroplasms. The best substrate was Z-Phe-Arg-NHMec followed by (H)Pro-Phe-Arg-NHMec (hydrolysis occurred at approximately 30% of the rate of Z-Phe-Arg-NHMec). Other substrates were hydrolysed at rates of approximately 10% or less of that of Z-Phe-Arg-NHMec. Hydrolysis occurred over a fairly wide pH range with a maximum at approximately pH 6.0. Thus, *T.p. parva* (Muguga) has thiol-dependent, lysosomal-like proteolytic activity that is similar to that found in other protozoan parasites.

TH3.1 Monoclonal antibodies recognising alpha and alpha/beta chain forms of bovine CD8

Research Associate: N. MacHugh
Scientists: A. Bensaid, W.I. Morrison, C.J. Howard*

(*AFRC Institute for Animal Health, Compton Laboratory, Berks, UK)

In mouse and man the CD8 antigen may be expressed on cytotoxic T-cells as a homodimer of one gene product (α) or as a heterodimer made up of two gene products (α and β chains). The CD8 antigen in cattle is also a dimeric molecule but to date the gene or genes encoding the polypeptides have not been identified. In order to determine whether the presently available monoclonal antibodies specific for CD8 distinguish between different forms of the antigen, four MAbs (IL-A17, IL-A51, CC58 and CC63) were tested by FACS analysis and immunoprecipitation on normal PBM, mouse L-cells transfected with bovine CD8 and two cloned *Theileria parva* infected T-cell lines. One of the cloned *Theileria*-infected cell lines (C197.16) has the phenotype $BoCD4^-$ $BoCD8^+$ while the other (C197.44) is $BoCD4^+$ $BoCD8^+$ and is considered to be a $CD4^+$ cell which has been induced to express CD8 by the parasite.

On FACS analysis MAbs IL-A17 IL-A51 and CC63 reacted with both *Theileria*-infected cloned T-cell lines and with the BoCD8 transfected mouse L-cells, whereas MAb CC58 only reacted with clone C197.16 ($CD4^-$ $CD8^+$). All four MAb reacted with normal bovine PBM. SDS-PAGE analysis of immunoprecipitates with CC63 run under non-reducing conditions demonstrated molecules of 64 and 68 kDa in the T-cell clone C197.16 but only the 68 kDa molecule in clone C197.44 and the BoCD8 transfectant. MAb CC58 only precipitated a 64kDa protein from clone C197.16.

Based on the likelihood that only one gene encoding the CD8 antigen has been transfected into the mouse L-cells, these results suggest that the bovine CD8 antigen is encoded by two genes and that MAbs IL-A51, IL-A17 and CC63 recognise α homodimers and α/β heterodimers whilst MAb CC58 reacts only with α/β heterodimers. Whether the epitope recognised by CC58 is on the β chain or is formed by association of the α and β chains has yet to be determined. Previous studies have indicated that activation of bovine $CD4^+$ T lymphocytes sometimes results in co-expression of CD8. The results of the present investigation indicate that these $CD4^+$ cells express only the α homodimeric form of CD8.

TH3.2 Characterisation of bovine alpha/beta T-cell antigen receptors

Scientists: A. Bensaid, S. Dunlap*, C. Baldwin, H. Clevers*, K. Iams, B. Goddeeris, J. Young.
Technicians: A. Kaushal, D. Ngugi

(*Department of Molecular Immunology, Dana Farber Cancer Institute, Boston, USA)

Studies conducted *in vivo* have shown that immune animals produce cytotoxic T lymphocytes restricted by class I molecules and often specific for the strain of *T. parva* parasite used for immunisation. It is now demonstrated that the fine specificity of T cells, in terms of antigen recognition, is due to the presence of T-cell receptors (TCRs) which are immuno-

globulin-like molecules. Therefore, the exploration of the TCR diversity should reflect the antigenic complexity of the *T. parva* antigens recognised by CTLs.

The primary structure of bovine α and β TCRs has been elucidated by screening, with human probes, a cDNA library prepared in λ *gt* 10 with RNA from peripheral blood mononuclear cells. Two selected α and β clones proved to be full-length and had sizes of 1400 and 1200 bp respectively. The predicted amino acid sequence of the bovine α constant region displayed 59.6 and 54.9% identities with human and mouse, respectively, while that of the β constant region showed 78.5 and 70.8% identities. The β and α chain V regions had between 30 and 53% identities with human and mouse V regions. Interestingly, the bovine V α region showed 65.5% identity with a human V δ region. The sequence of a second bovine V α region confirmed this result. This finding raised the possibility that bovine C δ and C α elements can rearrange with the same pool of V regions. Nucleotide sequence comparisons of the two V α regions revealed 75% identities, but the first 30 nucleotides coding for the mature protein were conserved. This information allowed us to use two pairs of oligonucleotides (20 mers) derived from the beginning of the V and C α and β regions, in an attempt to amplify TCR variable region messages by the polymerase chain reaction (PCR). Single stranded cDNA was made from two CTL clones, both restricted by the same MHC class I molecule but specific for the *T. parva* Muguga and Marikebuni strains respectively. Amplification by the PCR yielded products of 400 and 370 bp which correspond to the size of V α and β genes respectively. These amplified products have been cloned into sequencing vectors and analysis of the sequences should provide the first clue on the diversity of TCRs generated against *T. parva*.

TH3.3 Isolation of cDNA clones encoding gamma and delta chains of the bovine T-cell antigen receptor

Scientists: S. Dunlap*, A. Bensaid
Technician: A. Kaushal

(*Department of Molecular Immunology, Dana Farber Cancer Institute, Boston, USA)

A second family of T-cell receptors (TCRs) structurally similar to the α and β TCRs has been identified in humans and mice. These TCRs are composed of γ and δ chains and are expressed on a distinct subpopulation of T lymphocytes. Although the function of these γ/δ T lymphocytes is still unknown, recent studies indicate that they may interact with non-polymorphic class I MHC molecules such as CD1 in man and Q antigens in mice. In order to identify bovine γ/δ T-cells and investigate whether or not they play a role in the immunology or pathogenesis of protozoan diseases, experiments were carried out to clone cDNAs encoding the γ and δ chains.

Bovine cDNAs for the γ and δ chains of the TCR were cloned from a cDNA library prepared in λ *gt* 10 from peripheral blood mononuclear cells, using equivalent human cDNA probes. Both cDNA clones have a size of 1500 bp. The selected bovine δ chain was shown to lack part of the variable domain and displayed two frame shifts indicating that it is not a functional mRNA. Nonetheless, the bovine δ sequence displays 72% identity with the human δ constant region and therefore is a suitable probe to test material of bovine origin. The γ clone has been partially sequenced and revealed to be full-length. At the nucleotide level, this bovine cDNA shows an overall identity of 75% with a human γ chain. The predicted

amino-acid sequence of half of the V γ region shows 64% homology with a human counterpart.

TH3.4 Evidence that the population of bovine lymphocytes identified by surface expression of a 215/300 kDa antigen are gamma/delta T-cells

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Research Associates: N.D. MacHugh, G. Lamb
Technicians: A. Kaushal, E. Awino

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The monoclonal antibody IL-A29 reacts with a 215/300 kDa antigen on the surface of a discrete population of bovine lymphocytes. These cells are negative for surface Ig and for the T-cell differentiation antigens CD2, CD4, CD6 and CD8. They express low levels of CD5 which is also present on conventional T-cells and on a subpopulation of B cells. A small population (2%) of these cells is present in the thymic medulla, suggesting that they are of thymic origin. Their distribution in peripheral lymphoid tissues is distinctive, in that they are found predominantly in the marginal zones of the spleen and the peripheral cortex of lymph nodes. Numerous positive cells are also present in the skin and in the intestinal mucosa. A prominant feature of this IL-A29$^+$ cell population is that they are present in much larger numbers in young calves than in adults. Significant numbers of blasting IL-A29$^+$ cells are found in cultures of PBM stimulated with concanavalin A and in autologous and allogeneic mixed leukocyte cultures. Purified populations of IL-A29$^+$ cells have been shown to respond to concanavalin A in the presence of recombinant IL-2.

In order to identify the lineage of these cells, cell populations expressing the IL-A29 determinant, sIg or CD4 or CD8 were purified from peripheral blood by flow cytometry and RNA prepared and analysed by Northern blotting, using the available bovine CD3 and T-cell receptor (TCR) cDNA probes. The CD4$^+$ and CD8$^+$ T-cells as well as the IL-A29$^+$ cells contained transcripts of CD3 γ, δ and ε. However, while the CD4$^+$ and CD8$^+$ cells contained abundant TCR α and β message, the IL-A29$^+$ cells only contained TCR δ message. In all instances, the B-cells were negative. These results clearly indicate that the IL-A29$^+$ cells are T-cells and that they belong to the γ/δ T-cell lineage. Studies to confirm expression of TCR γ are underway. Thus the molecule identified by IL-A29 represents a novel marker for CD4$^-$ CD8$^-$ γ/δ T lymphocytes in cattle.

TH3.5 A monoclonal antibody specific for a clonotypic epitope on the bovine T-cell receptor

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Technicians: E. Awino, D. Ngugi

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The hybridoma secreting monoclonal antibody (MAb) IL-A49 was derived from spleen cells of a mouse immunised with lymphocytes from a cytotoxic T-cell clone (T20.68). This T-cell clone is specific for *Theileria*-infected lymphoblasts and is class I MHC restricted. The MAb reacts with the immunising T-cell clone but does not react with peripheral blood mononu-

clear cells, thymocytes, activated T-cells or other class I-restricted cytotoxic T-cell clones from the same animal. The cytotoxic activity of the T-cell clone T20.68 was specifically inhibited by 95% by MAb IL-A49. Immunoprecipitation from lysates of cells biosynthetically labelled with ^{35}S-amino acids and analysis by two-dimensional reducing/non-reducing SDS-PAGE, revealed two spots of approximately 45 kDa and three spots of 20, 25 and 28 kDa. These findings indicate that IL-A49 is specific for a clonotypic epitope on the bovine T-cell receptor, represented in the immunoprecipitates by the 45 kDa proteins. The additional smaller polypeptides are believed to represent components of the bovine CD3 antigen.

TH3.6 Derivation of monoclonal antibodies specific for bovine class I MHC antigens

Senior Research Fellow: P.S. Gwakisa
Scientist: A.J. Teale

Monoclonal antibodies (MAbs) provide unique advantages over alloantisera in studies of the polymorphism, structure and function of the bovine MHC (BoLA). This work was undertaken to generate and characterise anti-BoLA MAb against the products of selected MHC class I haplotypes. Work concentrated on 3 haplotypes expressing BoLA class I A-locus specificities KN8, KN12, and KN31 carried in Boran animals E54, E37 and E55, respectively. These animals are homozygous in the MHC region. Peripheral blood mononuclear cells (PBM) derived from the cattle were used to immunise mice, and immune murine spleen cells were fused with homologous murine myeloma cells (X63) to obtain hybridomas which were cultured and drug-selected using standard techniques. Several hybridoma cell lines have been raised that secrete monoclonal immunoglobulins which react with polymorphic surface antigens on bovine PBM. Six of these - IL-A89 (IgG2a), IL-A91 (IgM), IL-A92 (IgM), IL-A93 (IgG3), IL-A94 (IgG3) and IL-A95 (IgM) - have been extensively characterised in population studies.

The six MAbs have been prepared in ascitic fluid in mice and used in immunochemical, immunocytochemical and other immunoassays. Preliminary results on tissue distribution indicate that the antibodies are specific for class I BoLA determinants. Immunochemical studies revealed that IL-A92 and IL-A94 precipitate class I MHC molecules. BoLA-typing results obtained after testing approximately 300 cattle at ILRAD and in Gabon have shown that the six MAbs identify specificities defined by existing alloantisera. Further, similarity indices and correlation coefficients calculated on population data suggest that MAbs IL-A94 and IL-A95 may detect determinants on non A-locus class I products. Further application of the MAbs in sequential immunoprecipitations, sandwich ELISA and an analysis of murine cells transfected with bovine MHC genes will facilitate fine dissection of the selected haplotypes and thus provide information on the number of expressed class I MHC genes on three common haplotypes of Boran cattle.

TH3.7 An L-cell expressing genes encoding both the w10 and the KN104 class I MHC molecule

Scientists: P.G. Toye, W.I. Morrison
Research Associate: N. MacHugh
Technicians: D. Ngugi, E. Awino

Mouse fibroblasts expressing bovine class I MHC antigens may prove essential in defining parasite antigens recognised by cytotoxic T-lymphocytes (CTLs) from cattle immune to *Theileria parva*. We have previously reported the transfection, and expression, of separate genes encoding the w10 and the KN104 class I MHC molecules into mouse L-cells. It was considered important to obtain a transfected cell which expressed both MHC molecules, for two reasons.

Firstly, there is evidence that different bovine MHC molecules compete for binding and presentation of *Theileria* antigens. A cell expressing both w10 and KN104 molecules should prove useful in analysing such competition between these two molecules.

Secondly, a number of w10-specific alloreactive CTL clones (see TH3.8) recognise the w10 molecule only on those cells which also express the KN104 molecule. These CTL do not kill the transfectant which expresses the w10 molecule or $w10^+$ $KN104^-$ bovine cells. The possibility exists that these clones recognise part of the KN104 antigen presented by the w10 molecule.

A "double" transfectant was produced by transfecting genomic DNA from the L-cell expressing the w10 molecule, into the $KN104^+$ transfectant. A cell line expressing both bovine molecules was isolated by flow cytometry using MAb specific for the w10 molecule, and cloned by limiting dilution.

Cytotoxicity assays confirmed that the cell line expresses both molecules, as a w10-specific CTL clone (T28.38) and a KN104-specific clone (T28.43) killed the transfectant. However, no killing was observed with either of two CTL clones (T28.16 and T28.27) which are specific for $w10^+$ $KN104^+$ bovine cells. Thus, the reason why these cells appear only to recognise the w10 molecule when it is expressed with the KN104 molecule remains unclear.

TH3.8 Studies of the w10 and KN104 bovine class I MHC specificites with alloreactive cytotoxic T-cell clones

Scientists: W.I. Morrison, P.G. Toye, A.J. Teale
Research Associates: N. MacHugh, G. Lamb
Technician: E. Awino

As reported in 1988, two murine fibroblast cell lines expressing serologically distinct bovine class I MHC molecules have been derived by transfecting genomic DNA from a MHC-homozygous animal into mouse L-cells and selecting positive transfectants with class I-specific monoclonal antibodies (MAbs). The two transfectants express the w10 and KN104

class I specificities respectively. In order to explore further the relationship of these two MHC molecules and to examine the functional integrity of the molecules expressed in the transfected lines, a panel of alloreactive cytotoxic T-cell clones was generated and tested on the transfectants and on bovine cells of different MHC phenotypes.

Ten cytotoxic T-cell clones reactive with cells of the donor MHC-homozygous animal were studied. Based on their reactivity with bovine cells of different phenotypes and on inhibition of cytotoxicity with class I-specific MAbs, two of the clones were considered to be specific for the KN104 molecule while eight were specific for the w10 molecule. The two KN104-specific clones killed KN104$^+$ target cells from Boran and N'Dama cattle and also killed the KN104$^+$ transfected mouse cell line. The eight w10-specific clones killed w10$^+$ cells from Boran cattle (*Bos indicus*) but did not kill w10$^+$ cells from N'Dama or Friesian cattle (*Bos taurus*). Moreover, within the Boran, one clone killed all of the w10$^+$ cell lines whereas the remaining seven clones killed only those w10$^+$ cells which also expressed KN104. The w10$^+$ murine transfectant was only killed by four of the latter group of 7 clones. These findings indicate that T-cells recognise considerable heterogeneity in the w10 class I molecule. The results also indicate that the bovine class I molecules expressed in the transfected cell lines are functionally intact. The failure of some of the T-cell clones to kill the w10$^+$ transfectant is probably due to these clones recognising a self bovine peptide in association with the w10 molecule.

TH3.9 Studies of bovine Major Histocompatability Complex (MHC) class I gene structure and function

Scientists: A.J. Teale, S.J. Kemp, A. Bensaid

We have obtained, and reported previously, evidence of expression of more than one classical class I gene on an MHC haplotype of Boran (*Bos indicus*) cattle. To confirm this and to obtain detailed information of the structure of class I genes and products in cattle we have cloned class I cDNAs from a library constructed with the RNA of an animal bred for homozygosity of the MHC region. The RNA was prepared from peripheral blood mononuclear cells. The library, in λ *gt* 11, was probed with the class I gene cBoLA1 provided by Dr. R.L. Spooner of the Institute of Animal Physiology and Genetics Research, Edinburgh, UK. A number of potential clones were picked and some appropriately sized inserts were subcloned into *puc* 19 for restriction enzyme analysis and sequencing. Two clones were chosen for detailed study on the basis of poor hybridisation with each other at high stringencies. These clones, designated E98/2.1 and E98/5.1, were found to have different restriction maps. Based on sequencing information relating to the 250 bp extremities, homologies were 79% at the 5' end and 87% at the 3' end.

In comparisons with the sequences of two class I cDNAs derived from the BL-3 transformed bovine cell line (of *Bos taurus* origin) sequence homologies at the 5' end were between 79 and 87%. However, there appeared to be significant divergence in the 3' untranslated regions. E98/2.1 showed only 44% and 50% homologies with the BL3-6 and BL3-7 genes, respectively. In the same comparisons, E98/5.1 showed 45% and 44% homologies. BL3-6 and BL3-7 are 70% homologous when compared in the same manner.

We conclude that at least two non-allelic class I genes are expressed in the PBM of Boran cattle. Further, it is possible that the divergence of the *B. indicus* and *B. taurus* subspecies is reflected in the differences in non-coding regions of such class I genes.

TH3.10 Studies of bovine MHC class I gene polymorphism with allele-specific oligonucleotides

Scientists: A.J. Teale, J.R. Young, S.J. Kemp

Class I typing in cattle is routinely accomplished by the use of alloantisera and MAbs in a standard microlymphocytotoxicity assay. In order to avoid the problems associated with the requirement for viable leucocytes with this system we are giving consideration to typing methods based on DNA polymorphisms, with emphasis on the development and application of allele-specific oligonucleotides (ASOs). The sequences of five class I genes were compared in order to identify hypervariable regions with potential allele-specificity and also non-variable regions on which primers for polymerase chain reaction (PCR) amplification of bovine class I genes could be based. The sequences compared were those of cBoLA1 (sequence data provided by Dr. R.L. Spooner and colleagues, of the Institute of Animal Physiology and Genetics Research, Edinburgh, UK.), BL3-6 and BL3-7 (published sequences of cDNAs derived from the BL3 cell line) and the class I genes E98/2.1 and E98/5.1 (cloned by us from a cDNA library derived from an MHC homozygous Boran steer, E98). Relatively non-variable regions were identified towards the 5' and 3' ends of the second exons (encoding the first extracellular domain of the class I heavy chain). With primers based on these regions, conditions were optimised for amplification of class I DNA. Six potential typing oligonucleotides have now been made corresponding to variable regions of the second exons of the genes examined. Three of these have been tested for hybridisation at various levels of stringency with genomic DNA prepared from the BL3 line, the animal E98, from other Boran animals and from one N'Dama (*Bos taurus*). The ASOs designated 56 and 57 correspond to the regions encoding amino acids 21 - 26 of the second exons of the E98/2.1 and BL3-7 genes respectively. ASO 58 corresponds to the region encoding amino acids 4 - 46 of the BL3-6 gene. Typing was clearly possible with the ASOs 56 and 57 which gave good discrimination between PCR-amplified DNAs. ASO 58 was uninformative.

We conclude that this approach to BoLA class I typing is feasible. Further development requires that appropriate areas of more bovine class I genes are sequenced. However, with the primers available, it should be possible to accumulate sequence data on PCR-amplified material in a relatively short time.

TH3.11 Cloning of bovine class II MHC genes

Scientists: S.J Kemp, A.J. Teale, A. Bensaid, S. Dunlap*
Technician: J. Mwakaya

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Serological analysis of the bovine class II MHC antigens is not well advanced. It has proved difficult to generate appropriate antibodies by conventional means. If full-length cDNA from complementary MHC class II A and B chains could be cloned and sequenced, it would be

a valuable resource for a number of reasons. Transfection into mouse cells would provide a convenient immunogen for producing specific monoclonal antibodies. Transgenic mice expressing bovine class II would be expected to be tolerant of these antigens and thus would be ideally suited for the generation of monoclonal antibodies directed against allele-specific epitopes of allogeneic class II antigens. The sequence of such clones would provide part of the information needed to construct allele-specific oligonucleotides.

A pre-existing bovine cDNA library, constructed at ILRAD by C. Baldwin, K. Iams and H. Clevers from PBL of a MHC-homozygous animal, was probed with human class II probes.

A full-length DRA-like cDNA has been isolated, cloned into *puc* 19 and partially sequenced. It shows a close homology with both human and murine DRA and it is thus certainly the bovine equivalent. A DRB like cDNA was located, but sequencing showed it to be some 30 amino acids short of full length at the 5' end. Its sequence homology with the equivalent chains of other species was also more ambiguous than was the DRA. The library has therefore been re-probed and another putative DRB has been located and is currently being sequenced.

TH3.12 Further characterisation of the bovine MHC by field inversion gel electrophoresis

Scientists: A. Bensaid, A. Teale, J. Young
Technician: A. Kaushal

Previous studies performed on bovine DNA disclosed the organization of the DR and DQ class II regions. The use of mutated cells and peripheral blood lymphocytes from animals homozygous and heterozygous at the MHC level allowed us to localize DNA fragments from which functional genes are transcribed. This study was performed using the field inversion gel electrophoresis technique which permits separation of high molecular weight DNA previously digested with rare-cutter enzymes. Northern blot analysis performed with a human C4 complement probe revealed that, in some animals, the bovine C4 complement gene is located in the same 1Mbp *Not* I fragment as the class II genes. However, when using the restriction enzyme *Sfi* I, the C4 probe hybridized to a 20kb fragment while the DRA gene is contained in a 800 kb fragment. Thus, the C4 and DRA genes are linked but distant by nearly 800 kb. In contrast to the results obtained with the bovine class II genes which are located on a polymorphic *Sfi* 1 fragment, the C4 probe hybridized to the same 20 kb *Sfi* I fragment in all animals tested.

Two different 3' untranslated regions derived from cDNAs coding for class I antigens were used to probe Northern blots of *Sfi* I digested DNA from an homozygous animal. In contrast to the results obtained with an intact class I cDNA, which hybridised with six fragments, the two untranslated-region probes hybridized only to a 250 kb fragment. When double digestions were performed with *Sfi* I and *Cla* I, both untranslated-region probes revealed a 200 kb fragment. These results suggest that two different class I genes are transcribed from the same 200 kb DNA fragment. This situation is very similar to that encountered in the human MHC in which the HLA-B and C genes are separated by only 130 kb.

TH3.13 Characterisation of antigen-presenting cells in cattle

Scientists: D.J. McKeever, B.M. Goddeeris
Research Associate: N.D. MacHugh
Technician: E. Awino

Previous studies have demonstrated that bovine afferent lymph veiled cells (ALVC) are 10 to 100 times more efficient than blood monocytes at processing soluble antigens for recognition by specific T-cells, and suggest that these cells may be of major importance to the immunisation of cattle with soluble antigens. The mechanisms involved in the processing of soluble antigens by ALVC have therefore been investigated using an assay of antigen presentation which is based on the capacity of ALVC to stimulate T-cell clones specific for the VSG of *T. brucei* IITat 1.3 in the presence of antigen and bovine recombinant interleukin 2.

Responses of the clones to VSG do not require the presence of free antigen; ALVC which have been pre-pulsed in VSG and washed extensively before addition to the cultures induce maximal responses. The clones are unable to respond to VSG in the presence of glutaraldehyde-fixed ALVC, although ALVC which have been pulsed with the antigen before fixation maintain their capacity to stimulate. However, if the weak base chloroquine is present during the pulse period, the antigen-pulsed ALVC fail to stimulate the clones after fixation. Preliminary results suggest that the presence of monoclonal antibodies which recognise MHC class II antigens during the pulse period also abrogates the response of the clones to fixed ALVC.

Experiments on the MHC restriction of the clones confirm that only ALVC derived from MHC-identical or haplo-identical individuals are capable of presenting antigen to the clones. However, it has been observed that MHC-mismatched ALVC which have been pulsed with antigen and washed extensively are capable of stimulating the clones in the presence of unpulsed matched ALVC. This phenomenon does not occur when either ALVC population is fixed, suggesting that ALVC elaborate some unprocessed antigen into the culture. The results of these studies confirm that proliferation of bovine T-cells to soluble antigens requires that the antigen be processed in a pH-sensitive intracellular compartment and expressed on the cell surface in association with class II MHC molecules.

TH3.14 Mapping of T-cell epitopes on *Trypanosoma brucei* variable surface glycoprotein

Scientists: D.J. McKeever, V. Nene
Technicians: E. Awino, J. Nyanjui

The cloning of seven bovine T-cell lines specific for IITat 1.3 VSG has been described in a previous report. These lines all bear the T helper phenotype $CD2^+$ $CD4^+$ $CD8^-$, and require the presence of MHC-matched antigen-presenting cells and interleukin 2 for maximal stimulation. As part of a defined assay of antigen presentation, the clones have provided information on the processing of soluble antigens by bovine afferent lymph veiled cells (ALVC) which is the subject of an additional report. The identification of the epitopes of these clones on the VSG molecule was considered important for the study of the

processing of VSG for recognition by T-cells and the association of antigenic peptides with class II MHC molecules in cattle.

The approach which was adopted involved the cloning of a cDNA copy of the gene which encodes the IITat 1.3 VSG (the gift of Dr. J.R. Young) into the bacterial expression vector *pGEX2*. Using exonuclease III and mung bean nuclease, deletions were then made in this construct, digesting the gene from its 3' end. When expressed in *E.coli*, these deletions give rise to the production of a nested series of truncated VSG molecules in the form of fusion proteins with the glutathione-S-transferase molecule of *Schistosoma japonicum*. These fusion proteins were then purified on glutathione affinity columns, and included in cultures of ALVC with the T-cell clones. This approach has allowed the resolution of the epitope of one of the clones (T27.17) to an 80 amino acid section in the middle of the molecule. Three other clones show cross-reactivity between this section and the carboxy one-third of the molecule; protein sequence homology searches suggest that this may reflect the inclusion in one of the fusion proteins of cross-reactive translation products derived from vector sequences.

TH3.15 Endocytosis by *Theileria parva* - Infected lymphoblasts

Scientist: M.K. Shaw
Technicians: P.M. Theuri, D. Ngugi

Theileria-infected lymphoblasts grown *in vitro* divide rapidly and must acquire nutrients from the surrounding medium. In the present study the uptake of fluid phase material via endocytosis has been investigated using horseradish peroxidase (HRP) and cationized ferritin (CF). Temperature-dependent endocytosis of both HRP and CF occurs rapidly, with endocytosed markers being found within the peripheral endocytic organelles within 5 min. However, in the case of HRP, the amount of endocytosed material was usually small and even cells incubated with HRP for up to 4 hr at 37°C contained only moderate levels of endocytosed HRP. The relatively low levels of endocytosis observed in *Theileria* -infected cells may indicate that molecular uptake mechanisms play an important or predominant role in the uptake of nutrients. Alternatively, receptor-mediated uptake of specific molecules (e.g., high and low density lipoproteins and transferrin) may be important, and future work will look at the uptake of these molecules.

Although CF has been widely used as a marker for endocytosis, it may also be used to examine the behaviour of anionic sites on the cell surface. In a second series of experiments, the behaviour of CF-binding sites on the surface of *Theileria* -infected cells was being investigated. Glutaraldehyde-fixed cells treated with CF showed uniform labelling over the whole cell surface. In contrast, in living cells there was a temperature-dependent clustering of CF particles into localized regions on the cell surface. Control experiments using uninfected blood lymphocytes and Con A-stimulated lymphoblasts are currently in progress.

TH3.16 Fine structural localization of lipid material in *Theileria parva* schizonts.

Scientist: M.K. Shaw
Technicians: P.M. Theuri, D. Ngugi

During a study of the development of the intracellular schizont stage of *Theileria parva* Muguga, loose, lipid-like membranous material was consistently found associated with the schizont and localized throughout the host cell cytoplasm, particularly in close proximity to the host cell mitochondria. Although conventional fixation, dehydration and embedding procedures for biological electron microscopy result in the substantial extraction of lipids from the specimen, a number of modified protocols have been developed to reduce the extraction of lipids during specimen preparation. In the present study a number of modified fixation protocols have been used to investigate the nature of these membranous structures associated with the schizont and to study the distribution of lipid materials in general within the host cell.

The addition of either malachite green or tannic acid to the initial glutaraldehyde fixative resulted in the appearance of either numerous osmiophilic droplets or multilamellar membranous structures in both the schizont and host cell cytoplasm. By contrast, in cells post-fixed with imidazole-buffered osmium, only the membranes of the host cell mitochondria and the nuclear and mitochondria membranes of the schizonts showed any marked increase in electron density and no droplets or multilamellar structures were observed.

The addition of malachite green or tannic acid to glutaraldehyde has been shown previously to preserve certain classes of phospholipids in mammalian tissues. Thus, the present results indicate that the *Theileria* schizont contains appreciable amounts of phospholipids and that there is also a parasite-related increase in the phospholipid content of the host cell. The presence of substantial amounts of lipid within both the schizont and host cell may indicate that lipid metabolism plays a crucial role in the growth and development of the parasite and could, therefore, be a potential area for chemotherapeutic intervention..

TH3.17 Crystalline-lattice structures within *Theileria parva* schizonts

Scientist: M.K. Shaw
Technician: P.M. Theuri

During a study of the growth and development of the *Theileria parva* schizonts, a constant feature of all samples and stocks of *T. parva* examined was the presence of one or more prominent crystalline-lattice structures within the schizont cytoplasm. Similar crystalline-lattice structures appear to be a common feature of certain life cycle stages of many members of the *Apicomplexa*, although as far as is known the nature of these inclusion bodies has not been studied. The objective of the present study was to investigate the nature of these crystalline-lattice structures in *T. parva* schizonts.

The crystalline-lattice structures in *T. parva* schizonts are found free within the schizont cytoplasm and are generally round in profile measuring up to 2.3 µm in maximum diameter. In section, these structures appear to be composed of either a crystalline lattice of ± 50 nm perodicity or, of an array of tightly packed hollow spheres measuring about 40 - 50 nm in maximum diameter. Embedded within and/or closely associated with some of

the lattice structures are isometric elements of 45 nm diameter which appear either hollow or contain an electron dense core and closely resemble viral nucleocapsids.

In general, these crystalline lattice structures closely resemble viroplasms described in a wide range of animal cells. However, initial attempts to stain these structures for nucleic acids using the uranyl acetate/EDTA method have failed. Further attempts using a more sensitive enzyme-gold labelling method are in progress.

TH3.18 The structure and cytochemistry of *Theileria parva* schizont mitochondria

Scientist: M.K. Shaw
Technician: P.M. Theuri

Very little information is available concerning the fine structure, biochemistry and physiology of the intracellular *Theileria* schizonts. As part of a series of studies on the growth and development of the schizont, the fine structure of the mitochondria and the cytochemical localization of cytochrome oxidase within the schizont mitochondria have been examined.

The schizont mitochondria generally occur in pairs, with one occupying a concavity in the other. In thin section, the mitochondria present an oval to elongate profile and are composed of the usual outer and inner membranes. The inner membrane rarely exhibits the tubular invaginations usually associated with mitochondria and which occur in the host cell mitochondria.

In the initial experiments to localize cytochrome oxidase activity, it was found that brief fixation in 1% glutaraldehyde destroyed cytochrome oxidase activity in the schizont mitochondria, although activity was localized in some host cell mitochondria.

In subsequent experiments, brief fixation in 1% formaldehyde followed by incubation in either the medium of Seligman *et al.* (*J. Cell Biol.* 1968. 38, 1 - 14), or using the protocol of Angermuller and Fahimi (*Histochem.* 1982. 71, 33 - 44) resulted in the presence of DAB cytochrome oxidase reaction product within both the schizont and host cell mitochondria. In both cases, the reaction product was confined to the intramembrane space. However, in samples fixed with formaldehyde, whilst reaction product was observed in both the schizont and host cell mitochondria, the preservation of the host cell was often poor, with extensive loss of the cytoplasmic matrix. Currently, in an attempt to retain the enzymatic activity and to improve the preservation of the host cell, samples of cells fixed in a combination of 1% formaldehyde and 0.25 - 0.05% glutaraldehyde are being examined.

TH3.19 Determination of molecular mechanisms for *Theileria*-induced lymphocyte transformation

Scientists: O.K. ole-MoiYoi, K. Iams
Research Associate: M. Macklin

We have in previous years reported a marked increase in phosphorylation of infection-specific endogenous substrates in *Theileria*-infected cells. The enzymatic activity which appeared dominant, under a variety of reaction conditions in membrane and soluble fractions prepared from *Theileria*-infected lymphocytes, was cyclic nucleotide-independent, calmodulin/Ca^{2+}-insensitive, utilized either γATP or GTP as phosphoryl donors and was susceptible to modulation by polycations and polyanions. Based upon the above characteristics, its preferential phosphorylation of exogenous substrates such as casein and phosvitin and equisite susceptibility to inhibition by heparin, the enzymatic activity is considered to be casein kinase II-like. Using anti-casein kinase antibodies, we were able to show that indeed the casein kinase antigen was increased several fold in *Theileria*-infected cells. A similar increase was observed in cells infected with *T. annulata* Tova. Studies of purified schizonts indicate that in addition to increased enzymatic activity in the host cell, there is also some activity within the parasite.

A cDNA probe for the α unit of *Drosophila* casein kinase was found to hybridise with a DNA sequence within the *Theileria* genome. Using this cDNA probe to screen a *T. parva* genomic library, a *Theileria* casein kinase gene has been cloned and sequenced. The predicted amino acid sequence has the universal protein kinase domains, and the nucleotide sequence shows striking identity with other casein kinases as well with the yeast cell cycle-controlling gene products (CDC2 and CDC28) and the human analogue of CDC2 (CDC2Hs). The theilerial enzyme, however, has sequences unique to it. Ongoing work involves making fusion peptides as well as mapping the kinase gene for future determination of controlling elements and the role of this enzyme in growth as well as in *Theileria*-induced cellular transformation.

TH3.20 Phenotypic analysis of the cellular immune response of naive and immune cattle to challenge with *Theileria parva*

Scientists: W.I. Morrison, D.J. McKeever, L. Innes, S. Dunlap*
Research Associate: N.D. MacHugh
Technician: E. Awino

(*Department of Molecular Immunology, Dana Farber Cancer Institute, Boston, USA)

In susceptible cattle infected with *T. parva*, there is a marked proliferative response of uninfected lymphocytes in the regional lymph node, coinciding with the time of initial detection of parasitosis. This response has no apparent inhibitory effect on the infection and, indeed, may help to potentiate growth of the parasitised cells. The phenotype of the response has been examined by analysing cells obtained from lymph node biopsies and by cannulation of efferent lymphatic vessels. By day nine of infection, when the parasitosis in the regional lymph node was less than 5%, the lymph node and efferent lymph contained 25 - 30% blast cells. These comprised approximately equal numbers of $CD4^+$ and $CD8^+$ cells. A particularly striking observation was the presence of a large population of $CD2^-$

$CD8^+$ lymphoblasts. Cells with this phenotype are rarely detected in healthy cattle. These cells were purified by flow cytometry and RNA prepared; Northern blot analyses with cDNA probes for the T-cell receptor indicated that the cells were predominantly α/β T-cells. The purified $CD2^-$ $CD8^+$ cells were also placed in culture at limiting dilution with autologous parasitised stimulator cells and TCGF; a small number of clones thus obtained retained the $CD2^-$ $CD8^+$ phenotype, and exhibited low levels of non-specific cytotoxicity (see TH3.21).

Similar studies have been carried out with immune cattle challenged with large numbers of sporozoites. Parasitosis, not exceeding 1%, was detected transiently in the regional lymph node around days 8-9 after challenge. This was associated with a marked blast response. Efferent lymph contained about 20% blast cells; in contrast to the findings in naive cattle, there was a preponderance of $CD8^+$ lymphoblasts, which accounted for 80% of the blasts, and all of these cells expressed CD2. The $CD2^+$ $CD8^+$ lymphoblasts contained a very high frequency of specific cytotoxic T cells (see TH3.22).

Further information on the functional properties of the different cell populations stimulated in naive and immune cattle should provide insight into the role of the different responses in the pathogenesis of the disease and immunity to infection.

TH3.21 Preliminary characterisation of the effector function of $CD2^-$ $CD8^+$ cells generated in cattle infected with *Theileria parva*

Scientists: E.A. Innes, W.I. Morrison
Research Associate: N.D. MacHugh
Technician: E. Awino

A characteristic observation in the early stages of a lethal infection with *T. parva* is the appearance in the local draining lymph node of a population of blasting cells bearing the $CD2^-$ $CD8^+$ phenotype. The cells usually appear 2-3 days after the initial detection of parasite-infected cells (parasitosis < 1%). Preliminary experiments were done to characterize the effector function of the $CD2^-$ $CD8^+$ cells to achieve a better understanding of the interaction between these cells and parasitised cells, which may be important in influencing the outcome of an infection.

Efferent lymph cells and peripheral blood mononuclear cells examined on various days after infection did not significantly inhibit or stimulate the growth of *T. parva* cell lines *in vitro*. Furthermore, supernatants from purified and cloned $CD2^-$ $CD8^+$ cells did not inhibit or stimulate the growth of parasitised cell lines *in vitro*. It should be noted, however, that parasitised cell lines maintained *in vitro* may not be appropriate targets to assess the effects of test cells generated *in vivo*.

The $CD2^-$ $CD8^+$ cells were purified using a cell sorter and cultured at limiting dilution in the presence of irradiated autologous parasitised cells and T-cell growth factor (TCGF). Clones thus obtained responded better to parasitised cells plus TCGF than to TCGF alone. About half of the clones tested exhibited low levels of cytotoxic activity. However, they were not genetically restricted and, interestingly, were capable of killing autologous uninfected blast cells.

In conclusion, the $CD2^-$ $CD8^+$ cells appear to act as promiscuous killers capable of action against a range of both infected and uninfected target cells. Their precise role in the pathogenesis of theileriosis remains unclear although it is possible that they may contribute to the pan-leukopenia observed in the later stages of the disease.

TH3.22 Kinetics of cytotoxic T- lymphocyte precursor (CTL-p) populations in the lymph and blood of immune cattle following challenge with *Theileria parva*

Student: E.L.N. Taracha
Supervisor: W.I. Morrison
Scientist: D. McKeever
Research Associate: N.D. MacHugh

A limiting dilution microculture system has been applied to examine the kinetics of CTL-p populations in lymph and blood following homologous challenge of two immune cattle with *T. parva* (Muguga). This involved cannulation of efferent lymphatic vessels of the lymph node draining the site of challenge.

The frequency of CTL-p in lymph and blood was determined on days 0, 4, 7, 9, 11 and 14 after challenge. Active cytotoxic T-cells in blood and lymph were also measured on the same days using a ^{51}Cr-release assay. No active killing was demonstrated in either lymph or blood on days 0 and 4 after challenge. By day 7, cytotoxicity was demonstrated in lymph (approximately 50% at E:T ratio of 40:1) and blood (less than 10%) and had reached peak levels (60 - 70%) by day 9. By day 11, the level of killing had decreased and killing was not detectable by day 14 in either lymph or blood.

CTL-p were detected in both lymph and blood at comparable frequencies of about 1:5000 and 1:6000, respectively, on days 0 and 4. By day 7, the frequency of CTL-p in lymph had increased to more than 1:150 while that in blood remained relatively unchanged. Between day 9 and 11, the frequency of CTL-p increased to more than 1:40 in lymph and to 1:1000 to 1:625 in blood. By day 14, CTL-p were present at 1:625 in blood, but by the sixth week, the frequency had decreased 3 - 5-fold.

In order to determine which subset of cells in lymph was responsible for the CTL response, lymph cells, collected on day 9 of challenge, were fractionated using a fluorescence-activated cell sorter, into $CD8^+$ and $CD8^-$ populations and into $CD8^+$ large cells (blasts) and $CD8^+$ small cells. Frequency analysis of CTL-p in these sorted subpopulations indicated that $CD8^+$ large cells were the major mediators of the CTL response.

TH3.23 Analysis of parasite strain - specificity of the cytotoxic T- lymphocyte response in seven cattle immunised with *Theileria parva* Muguga

Student: E.L.N. Taracha
Supervisor: W.I. Morrison
Scientist: B.M. Goddeeris

The parasite strain specificity and MHC restriction of cytotoxic T- lymphocytes (CTLs) and their relationship with protection to heterologous challenge were examined in seven cattle immunised with *T. parva* Muguga. The strain specificity of CTL responses was monitored in individual cattle using a limiting dilution microculture system to determine the frequency of cytotoxic T- lymphocyte precursors (CTL-p) specific for cells infected with Muguga or with a cloned population of *T. parva* Marikebuni 214. In the same way, the MHC restriction of the response was determined using target cells which were haplo-identical to the effectors. The immune status of the animals was then tested by challenging them with a lethal dose of *T. parva* Marikebuni 214 sporozoites.

In two of the cattle, the majority of the CTLs elicited killed target cells infected with either parasite population. The remaining animals elicited CTLs, the majority of which were specific for the Muguga stock of the parasite. There was no obvious correlation between the parasite strain - specificity of CTL-p and MHC phenotype. The two cattle, in which the CTL-p were cross-reactive, were solidly immune to challenge with Marikebuni 214; they showed transient parasitosis only for one day. By contrast, the other five cattle in which a majority of CTL-p was specific for Muguga-infected target cells, showed fever of up to 41°C for 11 - 19 days with 4 - 15% parasitosis detected over a period of time ranging from 12 to 22 days. They then recovered.

The results indicate a correlation between the strain specificity of the CTL response and protection to heterologous challenge. These findings provide further supportive evidence that class I MHC-restricted CTL responses play a role in immunity to infection with *T. parva*.

TH3.24 Immune responses of cattle inoculated with non-infective, irradiated, autologous cells infected with *Theileria parva*

Scientist: W.I. Morrison
Technician: E. Awino

The capacity to induce immunity to *T. parva* by inoculation of animals with non-infective parasitised cells would provide a valuable system to define protective immune responses against the parasite in the absence of an active infection. Since MHC-restricted T-cell responses are believed to be important in immunity, it was decided to examine the responses of cattle inoculated with viable autologous parasitised cells rendered non-infective by γ-irradiation.

Two experiments were carried out in which cattle that received three or four doses each of 2×10^8 to 5×10^8 irradiated (20,000 rads) autologous parasitised cells were monitored for generation of cytotoxic T-cells and for protection against challenge with sporozoites. Class I-restricted $CD8^+$ cytotoxic T-cells specific for parasitised cells could be

generated *in vitro* from the PBM of these animals after three or four doses of irradiated cells. Despite this finding, however, the animals showed only partial and variable protection. In those animals in which protection was observed, control of parasitosis was not evident until about 13 days after challenge (as compared to 9 - 10 days in animals immunised by infection and treatment). Two of three cattle which received similar doses of allogenic irradiated cells also showed some evidence of protection. These findings suggest either that responses in addition to cytotoxic T-cell responses are required for immunity, or that the cytotoxic T-cells generated in these cattle were of an inappropriate specificity to provide protection *in vivo*.

TH3.25 Parasite strain specificity of cytotoxic T- cell clones generated from an animal immunised with a cloned population of *Theileria parva* Marikebuni

Scientists: B.M. Goddeeris, W.I. Morrison
Technician: D. Ngugi

The Marikebuni stock of *T. parva* is of major interest in studies of cytotoxic T- lymphocye (CTL) responses, as it protects cattle against a wide range of other stocks of *T. parva*. Previous studies of CTL induced with this parasite stock demonstrated CTL clones which killed target cells infected with different stocks. However, recent studies with parasite-specific MAbs and cDNA probes have demonstrated considerable genetic heterogeneity within the Marikebuni stock. With the availability of cloned populations of Marikebuni, it was decided to re-examine the specificity of the CTL response in an animal immunised with a cloned population of Marikebuni.

An MHC-homozygous animal positive for the w10 and KN104 class I specificities, was immunised with *T. parva* Marikebuni clone 214 by infection and treatment. Following immunisation, cytotoxic T-cell clones were obtained and their MHC restriction and parasite strain specificities determined by screening on cell lines of different MHC phenotypes, infected with *T. parva* Muguga or Marikebuni 214. The capacity of MAbs specific for the w10 and KN104 class I specificities to inhibit the cytotoxic activity of the clones was also tested.

Two groups of parasite-specific CTL clones were shown to be restricted by the w10 and KN104 class I molecules, respectively. The MHC restriction of a further two specific clones could not be determined. Many of the clones killed target cells infected with Marikebuni 214 but did not kill targets infected with the Muguga parasite. These results differ from previous findings that CTL clones generated from an animal immunised with uncloned *T. parva* Marikebuni killed target cells infected with either Marikebuni or Muguga. Further studies are underway to determine whether this difference in parasite specificity is due to the antigenic properties of this Marikebuni clone or to an influence of the genotype of the immunised animal.

TH3.26 A bovine class I MHC molecule plus a *Theileria parva* antigen resemble mouse H-2K^d

Scientist: B.M. Goddeeris
Technician: D. Ngugi

During the course of studies of the specificity of *Theileria*-specific cytotoxic T- lymphocyte clones (CTL) generated from an animal immunised with *T. parva* Marikebuni clone 214 (see TH3.25), one clone (T33.22) was found to kill a mouse hybridoma cell line. Subsequently, cell lines originating from mouse strains of different MHC haplotypes (a, b, k, qpl) were tested. Only mouse cell lines with the H-2^d haplotype were killed. Moreover, preincubation of the H-2^d targets with a MAb specific for the H-2K^d molecule inhibited the cytotoxic activity of the CTL clone; MAb specific for the H-2D^d or H-2L^d molecules had no influence on the cytotoxic activity.

These results indicate that a bovine CTL clone which exhibits self-restricted cytotoxicity for a cloned *T. parva* parasite can express simultaneously xenoreactivity for the mouse class I H-2K^d product. These results add to those in mouse studies where CTL recognising antigen (H-Y, influenza virus) in the context of self MHC molecules, may also exhibit cytotoxicity for particular allogenic MHC molecules. Thus, populations of T-cells recognising allogeneic or xenogeneic MHC molecules include T-cells specific for antigen in association with self class I MHC molecules.

TH3.27 Phenotypic and functional characterization of cloned populations of lymphocytes exhibiting non-specific cytotoxic activity

Scientists: B.M. Goddeeris, S. Dunlap*, A. Bensaid, C. Baldwin, H. Clevers*, W.I. Morrison
Research Associates: A. Kaushal, N. MacHugh,
Technician: D. Ngugi

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During the course of studies to define the specificity of cytotoxic T- lymphocyte (CTL) clones generated from an animal immunised with *T. parva* Marikebuni clone 214 (see TH3.25), two clones (T36.30 and T36.78) with non-specific cytotoxic activity were identified. These clones could be maintained in culture by periodic restimulation with autologous parasitised cells in medium containing T-cell growth factor. In comparison with the specific clones, they exhibited relatively low levels of killing (30 - 40%) in a four-hour ^{51}Cr-release assay. They killed autologous parasitised and non-parasitised lymphoblasts as well as parasitised cells from allogeneic animals with a range of different class I MHC phenotypes. Their cytotoxic activity could not be inhibited by MAbs specific for class I MHC. On the contrary, addition of anti-class I MAbs to the cytotoxicity assays increased the level of killing by about twofold.

The two clones were further analysed for cell surface phenotype with MAbs specific for T-cell differentiation antigens, and for expression of CD3 and T-cell receptor (TCR) mRNA by Northern blotting. Clone T36.30 expressed the CD2 antigen but was negative for CD4, CD5, CD6 and CD8; clone T36.78 was positive for CD8 but negative for the other T-cell antigens. Messenger RNA for the CD3 γ and δ chains was not detected in either of the clones. They were also negative for TCR α mRNA. However, both clones contained

mRNA for TCR-δ and a truncated form of TCR-β message considered to be non-functional. Since cell surface expression of TCR molecules cannot occur in the absence of CD3, the results indicate that these non-specific cytotoxic lymphocytes utilise molecules other than conventional T-cell receptors in recognition of their target cells.

TH3.28 Serum factors and macrophages in recovery from *Theileria parva* infection

Scientists: T.T. Dolan, A.J. Musoke, D. McKeever
Research Associates: P.R. Spooner, C. Nkonge, N. McHugh
Technician: L. Juma

Among the responses of cattle recovering from *Theileria parva* infection are the production of serum factors, which cause the intracellular degeneration of schizonts, and a marked increase in macrophage-like cells in peripheral lymph nodes. The nature of the serum factors and the identity or role of the macrophage-like cells are unknown. These responses are detected in recovery from primary infection but are not detected following challenge of immune cattle.

Further observations have been made on the phenomenon of anti-theilerial schizont activity in recovery sera. No activity was detected against sporozoites (inhibition of infectivity) or piroplasms (no obvious morphological deterioration) and no activity was detected in sera from two *T. parva*-susceptible buffalo. Fractionation of the sera on a sepharose 6B column and assaying the activity of fractions against *T. parva* schizonts *in vitro* showed that the activity was present in the alpha-2-macroglobulin fraction. This confirmed an earlier study using Amicon filtration where the activity was retained by membranes of 300,000 kDa and above and suggested that the factor might be of low molecular weight associated with high molecular weight plasma proteins. No activity was detected following isoelectric focusing.

In a separate experiment, the efferent lymph was collected from the prescapular drainage area of a calf following infection with a diluted dose of *T.p. lawrencei* stabilate. Blasting cells increased to 12% at the time of schizont detection on day 11 and to 40% on day 12. On day 12 the lymph contained a large number (34.7%) of $CD2^{-}CD8^{+}$ lymphocytes (see also TH3.20). By day 17 macrophage-like cells were detected in increasing numbers. On days 18 and 19 these cells constituted about 10% of the lymph node cells and there was a rapid degeneration and disappearance of schizonts. These cells stained positively with the IL-A24 antibody, which reacts with cells of the monocyte/granulocyte series. No anti-theilerial activity was detected in the serum at this time. An attempt will be made to isolate these cells and assess their effects upon established *T. parva* cell lines *in vitro*.

TH3.29 Refinement of methods for purifying and quantifying schizonts from *Theileria parva* - infected cells

Scientist: B.M. Goddeeris
Research Associate: J. Magondu
Technician: D. Ngugi

Identification of the target antigens of *Theileria* -specific cytotoxic T- lymphocytes (CTL) is of prime importance as these antigens might be used in vaccines against the disease. Approaches to identification of the relevant parasite antigens and the genes encoding them require access to purified schizonts from infected cells.

A simple method has been developed to obtain high yields of purified schizonts from cultured parasitised cells. Lysis of the host cells is accomplished with hemolysin from *Aeromonas hydrophila*. One volume of the lysed cell suspension is then added to four volumes of a 10% metrizamide solution (290 mosm/kg), to achieve lysis of the host cell nuclei. Free schizonts are separated from the lysate by centrifugation on Nycodenz (1.15 mg/ml; Nyegaard & Co. A/S) at g_{max} = 4,300 for 30 min. Free schizonts are collected from the interface and washed twice in RPMI 1640 medium supplemented with 10% bovine serum albumin.

Cytospins of the schizont preparations stained with Giemsa were found to contain intact free schizonts and were free of unlysed host cells and host cell nuclei. Aliquots of the purified schizonts were also stained with carboxy-fluorodiacetate (20 µg/ml), and counted with a fluorescence-activated cell sorter. From these data, the total number of schizonts in each preparation could be calculated. On the four occasions that schizonts were counted, yields were 21%, 33%, 36% and 80%.

Total RNA was isolated from a schizont pellet by the acid guanidinium thiocyanate-phenol-cloroform extraction method and electrophoresed in a formaldehyde-agarose mini-gel. A band at 3.3 kb detected in the parasite RNA preparation was absent in RNA of uninfected bovine lymphocytes. Conversely a band of 4.7 kb observed in bovine lymphocyte RNA was absent from the parasite RNA. Northern blots of the schizont RNA reacted with a parasite-specific cDNA probe but not with a bovine MHC cDNA probe. These results further substantiate the purity of the schizont preparations.

TH3.30 *Theileria* -specific antigens recognized by immune T lymphocytes

Scientists: D.J. Grab, L. Innes, C. Baldwin, W. Brown, K. Iams
Technician: Y. Verjee

The aim of this project is to identify antigens on the surface of *Theileria* -infected cells, which are recognised by T lymphocytes from *Theileria* -immune cattle. Studies have continued to focus on a soluble antigen which, when given to antigen-presenting cells induces antigen-specific proliferation of $CD4^+$ T lymphocytes from immune cattle. A protocol has been developed whereby the antigen is purified from a 100,000 xg supernatant fraction obtained from infected cells by chromatography on DEAE-cellulose, hydroxyl apatite, and TSK sizing columns. Fractions containing the antigenic material are recognised by *Theileria* -specific, $CD4^+$ T-cell clones derived from immune animals but not by peripheral blood T cells from

naive animals. These T-cell clones also recognise glutaraldehyde-fixed autologous *Theileria* -infected lymphocytes, indicating that the antigen recognised in the 100,000 xg supernatant fraction is associated with the cell membrane of infected cells. The fractions containing the antigenic material stimulate proliferation of the *Theileria* -specific T-cell clones at a protein concentration of less than 10ng/ml. However, because of the small amounts of antigen that are derived from a billion cells, we have not been able to visualize the protein(s) on SDS-PAGE using conventional staining methods. If the cells are metabolically labeled for several days in the presence of tritiated amino acids, the purified antigen, as visualized by autoradiography of SDS-PAGE gels, to have an apparent molecular weight (Mr) of about 10,000. There is also a minor component at 6,500 Mr. A stockpile of frozen infected cells is currently being made so that sufficient antigen can be generated for both antibody production and further biochemical analysis.

TH3.31 Identification of schizont antigens recognised by *Theileria* -specific T cells

Scientists: E.A. Innes, B.M. Goddeeris, D.J. McKeever, D.J. Grab
Technicians: F. Mbwika, J. Nyanjui

A technique is being developed using nitrocellulose bound antigen in an attempt to directly identify the schizont proteins recognized by *Theileria* -immune T cells.

Schizonts were purified from infected cells and the protein components separated by polyacrylamide gel electrophoresis in the presence of SDS. The separated proteins were transferred to nitrocellulose and bands were located on the unstained filter by staining a representative strip using a total protein stain or alternatively the bands were stained with aurodye, a colloidal gold solution which is reported to be non-toxic for T cells. Bands excised from the filter were processed to give a solution of finely dispersed floccules of nitrocellulose containing the protein antigens.

Polyclonal T-cell lines were prepared *in vitro* from four MHC-homozygous animals immune to *T. parva*.

Proliferative responses were observed with the immune T cells when stimulated with unfractionated purified schizonts denatured by sonication or freeze-thawing. However the response to nitrocellulose-bound antigen was less reproducible. The main problem being a high background proliferation when using the control nitrocellulose fraction which did not contain any bound antigen. Despite this high background, proliferative activity of approximately 20 - 25,000 cpm above background was observed with nitrocellulose fractions which had relative molecular weights of 180, 116 and 85 kDa with a second peak of activity observed using fractions at 26 kDa and below. The positive control, which comprised unfractionated schizonts spotted directly onto nitrocellulose, induced a proliferative response in the T cells of 40,000 cpm above background.

The results to date indicate that this technique will be useful for identifying the *Theileria* antigens which stimulate specific proliferative T lymphocyte responses.

TH3.32 Further biochemical and immunological characterisation of the polymorphic, immunodominant molecule

Scientists: P.G. Toye, B.M. Goddeeris
Technician: J. Nyanjui

The anomalous migration rate of the polymorphic immunodominant molecule (PIM) through polyacrylamide gels and its location on the surface of sporozoites and schizonts, suggests that this molecule may be heavily glycosylated. Determination of the extent of glycosylation is important, in terms of both the immunogenicity of this molecule and interpretation of putative sequence data.

To acertain the extent of glycosylation of PIM, schizont-infected cells were grown in the presence of tunicamycin, which inhibits the attachment of carbohydrates to the native protein. In addition, lysates of infected cells and PIM purified by immunoprecipitation were treated with periodic acid or endoglycosidase F to remove the sugar residues. Treated and untreated samples were analysed by Western blotting using MAb 40.2 (specific for PIM) or immune serum B213. No differences in migration rate or antibody reactivity were detected following any of the treatments.

Attempts were made to label infected cells biosynthetically with tritiated N acetylglucosamine, an essential component of N-linked carbohydrates. No labelled proteins were immunoprecipitated with MAb 5 from a lysate of labelled cells.

These results suggest that PIM is not heavily glycosylated, and that the epitope recognised by MAb 5 is not carbohydrate.

Western blot assays using MAbs 7 and 12 revealed immunological heterogeneity among the family of PIM molecules. The MAbs were used to probe lysates of cells infected with schizonts of *T. parva* Muguga, *T. parva* Uganda, *T. parva* Mariakani, *T. parva lawrencei*, *T. parva bovis* Boleni and four cloned cell lines derived from *T. parva* Marikebuni, each of which expresses a PIM of different Mr.

The most striking result was that neither MAb recognised the PIM of *T. bovis* Boleni. This observation is the more interesting as the Mr of the Boleni PIM is the same as that of *T. parva* Uganda and *T. parva* Mariakani, and reveals immunological heterogeneity between PIMs of the same size. It should also be noted that both MAbs reacted very weakly with *T. parva* Muguga and two of the *T. parva* Marikebuni clones, D232 clone 12 and D247 clone 1.

TH3.33 Isolation of *Theileria parva* genes which encode proteins which are immunologically cross-reactive with the polymorphic immunodominant molecule

Scientists: P.G. Toye, K. Iams
Research Associate: M. Kibe
Technician: J. Nyanjui

In order to isolate the genes encoding the polymorphic immunodominant molecule (PIM), λ*gt* 11 libraries of genomic DNA from *T. parva* Muguga and *T. parva* Marikebuni were screened with antiserum from the immune animal, B213. This antiserum recognises predominantly the PIM on Western blots of schizont antigens. One clone from each library showed positive reactivity, and was purified for further characterisation.

Antibodies eluted from these phages after incubation with B213 antiserum were observed to bind only to PIM in Western blots of schizont material. This result suggested that the phages contained DNA encoding part or all of the PIM. Restriction enzyme digestion of both inserts yielded similar patterns, indicating that the inserts were similar if not identical.

However, subsequent analysis of these DNA clones has revealed that these genes probably do not encode the PIM. Firstly, sequence analysis of the Muguga insert revealed an open reading frame encoding a protein of predicted Mr 52,000. The estimated Mr for the Muguga PIM is 85,000, and we have repeatedly failed to find evidence that the PIM is glycosylated (see TH3.32). Secondly, although fusion proteins which include the product of the open reading frame reacted with sera from several immune cattle, they were not recognised by MAb 5 or antisera from rats immunised with purified PIM. Thirdly, an antiserum from a rabbit inoculated with these fusion proteins failed to react with PIM on Western blot assays of schizont antigens.

In summary, it appears that we have isolated genes whose products, if they are not the PIM antigen, cross-react immunologically with it.

TRYPANOSOMIASIS

TR1.1 ILRAD contribution to the African Trypanotolerant Livestock Network (health aspects)

Scientist: E. Authié
Collaborating Scientists: ILCA scientists

Research in the African Trypanotolerant Livestock Network falls into four areas, namely, epidemiology of trypanosomiasis, criteria of trypanotolerance, genetics of trypanotolerance, health and productivity responses to interventions. Sites are located in Côte d'Ivoire, Ethiopia, Gabon, Zaïre, The Gambia and Senegal. The research activities include:

1. Evaluation of effects of tsetse control on health and productivity of livestock maintained under traditional management in Côte d'Ivoire. 2. Studies on the relative importance of resistance to the development of anaemia and ability to control parasitaemia in determining trypanotolerance of N'Dama cattle. In Gabon and Zaïre a close association was shown between ability to maintain the PCV level and productivity performances. Repeatability and heritability of PCV measurements in cattle under natural challenge are being evaluated. 3. Evaluation of associations between particular BoLA and LCA phenotypes and trypanotolerance of N'Dama in Gabon and Zaïre. 4. Studies on drug resistance and on the use of trypanocides. A collaborative research program has been set up as part of the third phase of the Boundiali project, in order to assess the prevalence and level of drug resistance in northern Côte d'Ivoire. Resistance to diminazene in *Trypanosoma congolense* was shown to occur at the Ethiopian site. Studies are on-going both at ILRAD and in the field to characterise this resistance and establish effective strategies for trypanosomiasis control.

TR1.2 ILRAD contribution to the African Trypanotolerant Livestock Network (vector aspects)

Research Associate: S.G.A. Leak
Collaborating Scientists: ILCA Scientists and Network field site personnel

The relationships between estimates of tsetse challenge and trypanosome prevalence in trypanotolerant and susceptible cattle were examined at sites of the African Trypanotolerant Livestock Network (ATLN). Estimates of tsetse challenge were determined as the product of tsetse relative densities, their trypanosome infection rates and the proportions of feeds taken by them from cattle. Trypanosome prevalence in both trypanotolerant and susceptible breeds of cattle was estimated from monthly examination of groups of ear-tagged cattle. Regression equations between estimates of trypanosome prevalence and tsetse challenge were fitted using an arcsin transformation of trypanosome prevalence and a $\log_{10}$ transformation of tsetse challenge. Highly significant relationships between estimates of tsetse challenge and trypanosome prevalence were found for sites with trypanotolerant cattle ($P>0.0001$) and sites with susceptible cattle ($P>0.01$). Mean trypanosome prevalence at a given level of tsetse challenge was at least twice as high in susceptible as in trypanotolerant cattle. The slopes of the regression lines for the two groups of cattle were significantly different ($P>0.05$). The curves obtained for the two groups of cattle illustrate the necessity in tsetse control campaigns for a major reduction of tsetse populations to take place before significant decreases in trypanosome incidence in livestock can be achieved.

The importance of *fusca* group tsetse was examined and evidence from the ATLN indicates that *G. tabaniformis* can be an important vector of pathogenic *Trypanosoma* species in cattle.

In Côte d'Ivoire a tsetse control campaign using cypermethrin-impregnated traps has been in progress since January 1988. Initial results indicate a 95% reduction in relative density of *G. palpalis* and a 97% reduction in relative density of *G. tachinoides* compared with the respective mean densities for 1987.

TR1.3 Therapeutic and prophylactic activity of isometamidium chloride against drug-resistant *Trypanosoma congolense* in Boran cattle

Scientists: I.A. Sutherland*, S.K. Moloo, A.S. Peregrine
Student: V. Codjia
Technician: S. Kemei

(*Veterinary School, University of Glasgow, UK)

The recommended route of administration for isometamidium chloride (ISMM) is intramuscular. However, recent reports from the field have indicated that the intravenous route may be more efficacious in eliminating ISMM-resistant infections. Experiments were therefore conducted in Boran (*Bos indicus*) cattle to determine which route was more efficacious under controlled laboratory conditions. Three *T. congolense* clones were used: IL3270, IL3035 clone 1 and IL1180. Previous studies had shown that these clones express high, moderate and low levels of resistance, respectively, to the therapeutic action of ISMM. All three clones were transmitted to cattle via infective *G.m. centralis*.

Both IL3270 and IL3035 clone 1 expressed a high level of resistance to the prophylactic activity of ISMM: five cattle treated intramuscularly with 1.0 mg kg^{-1} ISMM all became infected when challenged twenty eight days later with IL3270. Similarly, five cattle treated with 0.5 mg kg^{-1} ISMM all became infected when challenged twenty eight days later with IL3035 clone 1.

Intravenously administered ISMM, at doses as low as 0.25 mg kg^{-1}, was shown to eliminate infections of IL1180. In contrast, 0.5 mg kg^{-1} failed to eliminate IL3270 infections and 1.0 mg kg^{-1} failed to eliminate IL3035 clone 1 infections. Thus, intravenously administered ISMM was efficaceous in eliminating fully sensitive infections, but this route was not efficacious at doses of up to 1.0 mg kg^{-1} in eliminating infections that express significant levels of resistance. Moreover, the intramuscular route appeared to be the preferred route for attempting to treat ISMM-resistant infections with ISMM.

TR1.4 *In vitro* studies on the uptake of isometamidium by clones of *Trypanosoma congolense*

Scientists: I.A. Sutherland*, J.D. Lonsdale-Eccles, A.S. Peregrine
Technician: S. Kemei

(*Veterinary School, University of Glasgow, UK)

Uptake of isometamidium by clones of *Trypanosoma congolense* from across the African continent has been quantitated by fluorescence spectrophotometry, utilising isometamidium's innate fluorescence when excited at 380 nm. The studies have shown that, first, uptake of isometamidium appears to be temperature and time dependent, and is also dependent on the drug concentration in the surrounding medium. Second, fluorometry has been shown to be a reliable method for determination of both the presence and level of resistance to isometamidium: differential uptake occurs between isometamidium-resistant and isometamidium-sensitive populations. Third, we have been unable to observe efflux of isometamidium from resistant trypanosomes but have observed efflux, to a certain extent, in sensitive clones. Finally, preincubation in SHAM/glycerol results in a drop in fluorescence in susceptible clones but an increase in fluorescence in resistant clones. Thus, uptake of isometamidium by sensitive clones may be an active process and energy dependent. Similarly, reduced uptake of isometamidium by resistant clones may also be energy dependent.

TR1.5 Resistance to isometamidium and diminazene in *Trypanosoma congolense*

Scientist: S.K. Moloo
Research Associate: S.B. Kutuza
Technicians: I. Okumu, C. Sabwa

Investigations were conducted on the sensitivity to isometamidium chloride (Samorin®) and diminazene aceturate (Berenil®) of derivatives of three of the *Trypanosoma congolense* stocks isolated between 1978 and 1983 from Zebu cattle in the Bobo-Dioulasso region of Burkina Faso. Boran cattle were used in the drug-sensitivity tests and were infected using *Glossina morsitans centralis*. The results showed that *T. congolense* stock IL2466 isolated in 1978 was sensitive to the standard therapeutic dose of isometamidium chloride (0.25 mg kg^{-1}) and of diminazene aceturate (active ingredient, 3.5 mg kg^{-1}). However, *T. congolense* stock IL2468 isolated in 1982 was resistant to both the prophylactic (0.5 and 1.0 mg kg^{-1}) as well as the therapeutic doses of isometamidium chloride (up to 1.0 mg kg^{-1}) although the sensitivity to the therapeutic dose of diminazene aceturate (3.5 mg kg^{-1}) was not affected. The *T. congolense* stock IL2856 isolated in 1983 was highly resistant to the therapeutic action of diminazene aceturate (up to 10.5 mg kg^{-1}) as well as the prophylactic (up to 1.0 mg kg^{-1}) and therapeutic action of isometamidium chloride (up to 2.0 mg kg^{-1}). The infection rates of the drug-resistant stocks of *T. congolense* in *G.m. centralis* when goats were used as reservoir hosts were as high (range, 22.3 - 56.3%) as those of the drug sensitive stock (49.5%). The resistance trait in the two stocks remained stable after their cyclical development in the tsetse vectors. The rate of transmission of the drug-resistant stocks to mice by the infected tsetse was also high (mean 81.3%).

TR1.6 Biodynamics of Isometamidium-resistant *Trypanosoma congolense*

Scientists: A.S. Peregrine, M.A. Gray*, S.K. Moloo, N.B. Murphy
Research Associate: J. Scott
Technician: S. Kemei

(*Kenya Trypanosomiasis Research Institute, Kikuyu, Kenya)

Studies have been conducted on clones of *Trypanosoma congolense*, previously characterised for their sensitivity to isometamidium (ISMM) *in vivo* and which span the entire spectrum of resistance to ISMM. Boran (*Bos indicus*) calves were infected with each of the clones and groups of teneral *G .m centralis* were fed on them to determine infection rates in tsetse. The studies showed that although clones of a homogeneous genetic background may produce significantly different infection rates, there appeared to be no correlation between tsetse infectivity and expression of resistance to ISMM. In addition, genetically homogeneous clones which produce similar infection rates in tsetse may differ considerably in their transmission rates to mice. Finally, the duration of infection in a cow before teneral tsetse first feed on the animal appears to have no effect on the resultant infection rate.

T. congolense IL1180 and IL1180/100X (a derivative of IL1180 selected for resistance to ISMM and a hundred times more resistant *in vivo* to ISMM than IL1180) were characterised *in vitro* for their sensitivity to ISMM, diminazene and homidium. A metacyclic culture system showed that IL1180/100X was two-hundred times more resistant to ISMM and homidium, and ten-times more resistant to diminazene, than IL1180. Thus, selection for resistance to ISMM appeared to be accompanied by cross-resistance to homidium at a similar magnitude to the increased resistance to ISMM, and slight, but significant, cross-resistance to diminazene.

Finally, nine subclones were derived from *T. congolense* clone IL3270, which expresses a high level of resistance to ISMM. All nine subclones were characterised in mice for their sensitivity to ISMM. Seven of the nine subclones expressed a significantly lower level of resistance to ISMM than the parental clone and amongst the subclones there was significant variation in expression of resistance to ISMM. Thus, gene amplification with unequal cross-over between repeats may be a possible basis for resistance to ISMM in *T. congolense*, and resistance to ISMM may not be a stable trait.

TR1.7 Characterisation of Ethiopian field isolates for sensitivity to trypanocides

Student: V. Codjia
Supervisor: A.S. Peregrine
Collaborating ILCA Scientists: M. Woudealew, J. Rowlands, G. d'Ieteren

The African Trypanotolerant Livestock Network has been conducting a field project in Ghibe Valley, southwestern Ethiopia, for the last three years. Recent analyses of data from the project have indicated that there may be a significant problem with resistance to diminazene aceturate (Berenil®). Since the history of the project site has indicated that the purported resistance may be true resistance to diminazene and not quinapyramine cross-resistance, 49 field isolates were obtained from animals in the project. Fifteen of these isolates are being examined in the present study. Each isolate is being characterised *in vivo* for its

sensitivity to diminazene, isometamidium, homidium and quinapyramine. Preliminary studies in cattle have shown that certain *T. congolense* isolates are resistant to intramuscular treatment with 7.0 mg kg^{-1} bodyweight diminazene aceturate. In addition to determining whether this is true diminazene resistance, the prevalence of the resistance phenotypes is currently being defined.

TR1.8 Identification of biochemical markers for drug resistance in *Trypanosoma congolense*

Scientists: A.S. Peregrine, M. Kishima, L. Mutharia*
Technician: S. Kemei

(*Department of Biology, University of Guelph, Ontario, Canada)

Trypanosoma congolense clone IL1180 expresses a high level of sensitivity to isometamidium. Over an eighteen-month period a set of derivatives of IL1180 have been produced which express increasing levels of resistance to isometamidium and are all tsetse-transmissible. The resistance has been increased by repeated subcurative treatments of infected mice and at present a derivative has been produced which is 300 times more resistant to isometamidium than IL1180.

IL1180 and two derivatives which are 50 times and 200 times more resistant to isometamidium have been examined by 2-dimensional SDS-PAGE using the ISODALT system. Analyses of the three populations have demonstrated that with increasing levels of resistance to isometamidium there appear to be alterations in expression of eight proteins: five proteins of Mr 45 to 70 kDa decrease in intensity with increasing levels of resistance. By contrast, three proteins of Mr 35 to 92 kDa decrease in intensity with increasing levels of resistance.

Further studies will be conducted to determine the exact molecular weight and pI of each protein. In addition, *in vitro* ^{32}P-incorporation studies will be undertaken to determine whether the pI shifts observed are due to differences in the level of protein phosphorylation. Finally, other, unrelated, clones of *T. congolense* from across Africa will be examined by 2D-SDS-PAGE to determine whether the protein changes observed in the IL1180 derivatives are consistent with expression of resistance to isometamidium.

TR1.9 *In vitro* isolation of trypanosomes from mammalian hosts and tsetse

Scientists: R. Kaminsky, E. Zweygarth*, S.K. Moloo
Technicians: F. Chuma, J. Kabata

(*Kenya Trypanosomiasis Research Institute, Kikuyu, Kenya)

A simple and rapid method for the initiation of *Trypanosoma brucei brucei* bloodstream form cultures from the mammalian host has been developed. Blood samples from infected hosts were aspirated with a five or ten µl Eppendorf pipette and then each was transferred into a well of a culture plate (24-well plate). The blood volume was deposited in the corner at the

bottom of the wells. Bloodstream form cultures were successfully initiated from mice and cattle with low parasitaemia in wells with and without feeder layer cells.

A tube-isolation technique was used to initiate insect form cultures of *T.b. brucei* from infected blood samples. Blood (0.3 - 1.0 ml) was mixed with five ml medium in a tube and incubated at 27°C. After sixteen hours the blood cells had formed a pellet and the supernatant containing most of the trypanosomes was transferred to a T-25 flask for further transformation and propagation of trypanosomes. The tube-isolation was successful when blood samples were obtained from infected mice (high or medium parasitaemia), but unsuccessful with blood from infected cattle (low parasitaemia).

Initiation of insect form cultures of *T. congolense* was achieved when one to five proboscides or one midgut of infected tsetse was placed in a tube containing 0.3 - 0.5 ml medium. When trypanosomes were detected in the medium, samples were transferred to T-25 flasks for further propagation. Initiation of cultures was always quicker from midguts than proboscis. Cunningham's medium (SM-based) proved to be superior to MEM-based medium. Established procyclic populations were maintained in SM medium. When transferred to MEM-based medium, procyclics transformed to epimastigotes and metacyclics. The same technique was applied to initiate *T. vivax* insect form cultures from infected proboscides. However, after initial attachment and growth of *T. vivax* epimastigotes, no further growth occurred in SM, MEM or Iscove's based medium.

TR1.10 Expression of drug resistance in *Trypanosoma brucei brucei in vitro*

Scientists: R. Kaminsky, E. Zweygarth*
Research Associate: J. Scott
Technician: F. Chuma

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The susceptibility of *Trypanosoma brucei brucei* stocks and clones to diminazene aceturate (Berenil®) and isometamidium chloride (Samorin®) was determined *in vivo* and *in vitro*. Two stocks were resistant in mice to both drugs. Differences in drug induced growth inhibition occurred between drug-resistant and drug-sensitive stocks when bloodstream trypanosomes were incubated for 24 hr at 37°C in 4% CO_2 in air in a feeder-layer-free culture system. Diminazene concentrations which inhibited growth of trypanosomes by 50% (EC_{50}) differed more than 50-fold between drug-resistant and drug-sensitive stocks, but there were only minor differences in EC_{50} values for isometamidium. However, differences in growth inhibition due to isometamidium occurred when trypanosomes were incubated with drug concentrations (0.001 - 0.05 µg/ml) below the EC_{50} values. Cultivation periods of up to nine months did not have an effect on the susceptibility pattern *in vitro*.

Trypanosome stocks which were propagated *in vitro* in the presence of drugs for a period of ten days differed greatly in their viability. In contrast to drug-sensitive trypanosomes, drug-resistant stocks could be maintained without loss of viability in the presence of 0.01µg/ml diminazene aceturate or 0.0001 - 0.001µg/ml isometamidium chloride.

Four clones which were derived from the drug-resistant stock CP 547 expressed similar susceptibility to the drugs as the parental stock. However, one clone from the multidrug-resistant stock CP 2469 showed intermediate susceptibility to isometamidium.

Growth of procyclic forms was inhibited after incubation for 48 h in the presence of 0.001 - 1.0µg/ml isometamidium chloride. Procyclic forms of the drug-resistant stock CP 547 were less affected by the drug than procyclic forms of susceptible stocks. However, no differences in drug-induced growth inhibition occurred between procyclic forms of the drug-resistant stock CP 2469 and procyclics of susceptible stocks.

TR1.11 Effect of Isometamidium chloride on insect forms of *Trypanosoma vivax*

Scientists: R. Kaminsky, E. Zweygarth*, S.K. Moloo
Technician: F. Chuma

(*Kenya Trypanosomiasis Research Institute, Kikuyu, Kenya)

Isometamidium chloride-resistant and -sensitive *Trypanosoma vivax* stocks from infected cattle or mice were adapted to an *in vitro* system in which bloodstream trypomatigotes transformed into insect forms. The latter were continuously propagated without feeder layer cells, in a semidefined liquid medium at 27°C. The effect of isometamidium chloride (Samorin®) on *T. vivax* was assessed by monitoring viability of trypanosomes and production of metacyclic forms. Populations of epimastigotes of *T. vivax* IL1392 showed reduced growth and died after ten days when cultivated in the presence of 1 ng/ml isometamidium chloride and after six days in the presence of 10 ng/ml drug. In contrast, populations of the isometamidium-resistant *T. vivax* CP 2171 and CP 2331 continued to grow for seventeen days in the presence of 1 ng/ml isometamidium chloride without loss of viability. The production of metacyclics was inhibited in cultures of *T. vivax* IL1392 when incubated in medium containing 1 or 10 ng/ml isometamidium chloride. Epimastigotes of *T. vivax* CP 2171 and CP 2331 produced metacyclic forms in the presence of 1 ng/ml isometamidium chloride but not 10 ng/ml drug. In contrast to the drug-sensitive *T. vivax* IL1392, the *T. vivax* CP 2171 infection rates in *Glossina morsitans centralis* were not reduced when they were fed for three days on an isometamidium-treated Boran steer.

TR1.12 The effect of Isometamidium and diminazene on procyclic forms of *Trypanosoma congolense*

Student: I.E. Elrayah
Supervisor: R. Kaminsky

Cultures of insect forms of drug-resistant and drug-sensitive *Trypanosoma congolense* stocks and clones were initiated from bloodstream form trypanosomes harvested from mammalian hosts. The cultures were maintained *in vitro* at 27°C.

Growth of procyclic *T. congolense* stocks/clones was inhibited after incubation for 48 hrs with 0.1µg/ml diminazene aceturate or 0.0001µg/ml isometamidium chloride. Differences in drug-induced growth inhibition between drug-sensitive and drug-resistant

stocks/clones were not detected. The procyclic forms of either, drug-sensitive and drug-resistant trypanosomes were propagated *in vitro* in the presence of 0.01 or 0.1 µg/ml diminazene without loss of growth for up to twenty days. In the presence of 0.0001 or 0.001 µg/ml isometamidium, the trypanocidal effect of the drug was detected in cultures of drug-sensitive and drug-resistant trypanosomes after six to eight days and cultures died after ten to twelve days. In a pulse incubation assay the trypanocidal effect of isometamidium was detected after seven days when procyclic forms of drug-sensitive and drug-resistant *T. congolense* were incubated with 0.001 or 0.01 µg/ml drug for 24 hrs and propagated thereafter without drug.

It was not possible to distinguish reliably between drug-sensitive and drug-resistant *T. congolense* stocks/clones using the three described assays with procyclic forms.

TR1.13 Possible molecular mechanisms of drug resistance in African trypanosomiasis

Student: H.R. Jamnadass
Supervisor: N.B. Murphy

A 6 kb extrachromosomal type DNA element has been identified in a drug resistant *Trypanosoma brucei* field isolate. Results indicate that there is an apparent loss or reduction in the amount of the 6 kb element when trypanosomes are grown under non-selective conditions. Re-applying selection leads to an apparent increase in copy number of this element. This suggests that this element might encode the drug-resistant determinant and gene amplification is one possible mechanism. By using gel-purified DNA from the 6 kb region as a radiolabelled probe, genomic restriction fragments have been identified in the *T. brucei* genome. Attempts are now being made to clone these fragments, to identify the encoded product and finally to establish whether such products confer drug resistance.

TR1.14 Evaluation of antigen-trapping-ELISA for diagnosis of animal trypanosomiasis

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Chemotherapy is an integral factor in the present control strategy for African trypanosomiasis. For chemotherapy to be rationally and effectively applied, however, simple and reliable methods for diagnosis are essential. We have recently reported the development of enzyme immunoassays which show encouraging results in the diagnosis of the disease in livestock. The assays are based on monoclonal antibodies against antigens specific for *T. congolense*, *T. vivax* or *T. brucei / T. evansi*. In a limited analysis of sera from twenty camels with parasitologically confirmed *T. evansi* infection, eighteen were antigen-positive. Essentially similar results were obtained when camel sera from a *T. evansi*-endemic area in Mali were examined for circulating antigens. Analysis of sera from pigs on a farm in Thailand with a suspected *T. evansi* outbreak also gave satisfactory results, in that 66.7%

of the animals were antigen-positive, a sensitivity rate that compared well with that recorded for the antibody-detection CATT test (45.6%) or the indirect haemaglutination test (47.8%). The assays have also been evaluated for diagnosis of bovine trypanosomiasis. Initial results indicate that the sensitivity of the assays for antigens of *T. congolense*, *T. vivax* and *T. brucei*, in field sera from cattle may be of the order of 80 - 90%, that a high proportion of the cases had mixed infections and that a significant number of parasitologically negative animals were infected as shown in the tests for antigen. The assays are easy to perform and the results can be read visually.

TR1.15 Identification of gene fragment(s) encoding *Trypanosoma vivax* species-specific antigen

Scientists: R. Masake, V. Nantulya, O.K. ole-Moi Yoi
Technician: J. Makau

A species-specific diagnostic monoclonal antibody (TV 27) recognizes four bands of proteins in Western blots using freshly prepared *Trypanosoma vivax* lysates. The smallest of these protein bands was well demarcated from the neighbouring bands. This protein was subsequently electro-eluted from SDS-PAGE gels. Rabbits were immunised with this eluate to raise polyvalent antiserum (RT-491). RT-491 was found to react strongly with the immunising protein band of relative molecular mass 8 kDa and weakly with the other proteins corresponding to those recognised by the monoclonal antibody. This antiserum is specific for *T. vivax* as demonstrated by its non-reactivity with *T. congolense* and *T. brucei brucei* lysates. RT-491 serum was then used to screen a *T. vivax* DNA expression library. Twenty-eight of about 200,000 clones reacted with RT-491. The majority of these clones were positive on antibody select. *T. vivax* DNA inserts were released from these clones by enzyme digestion. An insert of 800 bp (VM 4.5.1) was radiolabelled and used to probe the Southern blots obtained from the aforementioned digested clones. All the clones were positive while λ*gt* 11.2 and protein markers were negative. We are currently sequencing the 800 bp DNA insert to determine the nucleotide sequence of the DNA fragment encoding *T. vivax* species-specific antigen.

TR1.16 Characterisation of trypanosome isolates from cattle in Uganda using DNA probes reveals a predominance of mixed infections

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The primary objective of this work was to characterise trypanosome isolates collected in cattle from Uganda using species-specific DNA probes available at ILRAD. A total of fifty nine trypanosome isolates were examined : twenty were from natural infections in cattle and fifteen, obtained from the Uganda Trypanosomiasis Research Organisation, were

stabilates obtained after one passage in mice. The other twenty-four isolates were from natural infection in tsetse flies.

Most of the twenty isolates from cattle were identified as either *T. brucei* or *T. congolense* by microscopical methods based upon parasite motility and morphology. Using DNA probe hybridisation, several mixed infections were detected. This technique revealed one cow which was infected with *T. brucei*, *T. congolense* (both Savannah - and Kilifi-type) and *T. vivax*. Overall *T. congolense* (Kilifi-type) was more prevalent than *T. congolense* (savannah-type) and was identified as far as north-western Uganda along the Sudan border. This genotype was first isolated in cattle from Kilifi on the Kenyan coast. Infections from five cattle did not show hybridisation signals to any of the available probes. The presence of *T. congolense* forest type, which was not examined, cannot be ruled out.

All the fifteen trypanosome stabilates, which had been prepared from mouse-passaged material, were *T. brucei*. During field surveys, many cattle were found to have mixed infections of *T. brucei* and *T. vivax* by parasitological examination. Following passages in mice, none of the *T. vivax*, which was identified by microscopical examination in mixed natural infections, was detected by DNA probe hybridisation.

Most samples from tsetse flies did not show any hybridisation signals, except three which were all *T. vivax* (East African) type. It is likely that these particular results were due to the DNA being degraded as the samples were, unavoidably, kept at 4°C for over four days.

TR2.1 The endosomal/lysosomal system of African trypanosomes and their digestive processes

Scientists: J.D. Lonsdale-Eccles, D.J. Grab
Research Associates: P. Webster, G. Mpimbaza
Technician: Y. Verjee

African trypanosomes can internalise large extracellular molecules by a process called endocytosis. The parasites contain some of the enzymes necessary for the digestion of the molecules and our work has been directed towards ascertaining whether these enzymes are indeed located within the endosomal system, and how they express their activity. The work has focused on the uptake of fluorescently labelled large and small molecules that are potential substrates.

In mammalian cells, endocytosis can be inhibited at discrete points of the endocytosis pathway by lowering the cells to specific temperatures. At 0°C trypanosomes exhibited no movement and essentially no protein uptake or peptide hydrolysis. Using Z-Phe-Arg-NHMec as a probe for hydrolytic activity in intact cells, parasites exhibited a continuous 2.6 fold increase in hydrolysis for each 10°C rise in temperature. These results accord with a reported increase for endocytosis in some mammalian cells but also suggest that the concept of specific temperature blocks to endocytosis may not be completely appropriate in trypanosomes although this needs to be confirmed by alternative techniques.

Because we believe the hydrolytic activity against Z-Phe-Arg-NHMec is localised within the endosomal/lysosomal systems, we have begun to analyse these organelles. For example, as expected, when the parasites were pretreated with tyloxapol, we observed a shift in the apparent density of the organelles containing Z-Phe-Arg-NHMec hydrolytic activity when measuring only the lysosomal fraction of the trypanosome extracts. This is suggestive of an endosomal/lysosomal location of the enzyme. However, analysis of the total extract of trypanosomes pretreated with tyloxapol showed a doubling of the Z-Phe-Arg-NHMec activity in the parasites as well as a destabilisation of the lysosomal membranes. This may be the mechanism by which tyloxapol exerts its trypanocidal activity.

TR2.2 To identify and characterise trypanosomal hydrolases and their modes of regulation

Scientists: J.D. Lonsdale-Eccles, D.J. Grab
Research Associate: G. Mpimbaza

Recent studies on the thiol-dependent proteases of African trypanosomes, which appear to be localised in the endosomal/lysosomal system of these parasites, suggest that exogenous factors in serum might regulate the activity of these lysosomal enzymes. Analysis, by electrophoresis in fibrinogen containing sodium dodecyl sulphate polyacrylamide gels (Fbg-SDS-PAGE), of trypanosomal extracts incubated with serum reveals the appearance of additional bands of proteolytic activity. As previously noted, the moiety in serum is abundant in human, rabbit, rat, and bovine sera. Normal rat serum has been the focus in these studies.

We have purified this moiety by chromatography through a variety of resins. Of particular interest is the chromatographic profile of the moiety on Amicon Blue-A beads. The moiety fails to bind to this resin although serum albumin has a tight affinity. Notwithstanding this, the moiety has electrophoretic properties, molecular weight profiles and an N-terminal amino acid sequence which suggest that it is rat serum albumin. It does not react with antibody to bovine serum albumin but it does react with antibody to rat serum albumin. However, neither purified rat serum albumin nor bovine serum albumin were able to effect the same activation. Antiserum prepared against the purified moiety reacted similarly with rat serum albumin or purified factor. Changes in the electrophoretic mobility of the bands of activated protease have been observed in the presence of antiserum against the moiety and antiserum against albumin, but the nature of these changes are not yet clear. It remains to be determined whether the moiety that we are examining is a serum albumin variant, a serum albumin associated molecule, or a molecule that has co-purified with albumin.

TR2.3 The purification and characterisation of the biochemical properties of a thiol protease of African trypanosomes

Student: Z. Mbawa
Supervisor: J.D. Lonsdale-Eccles

We have purified and partially characterised a thiol-dependent protease from *Trypanosoma congolense* that we call trypanopain Tc. It is a typical cysteine protease. It is rapidly inactivated by cystatin and E-64. Its activity is enhanced by reducing agents and it hydrolyses many proteins. Subsite specificity experiments show that the enzyme prefers Z-Phe-Arg-NHMec as a substrate, hence making it cathepsin L-like. However its activity differs in some respects from cathepsin L; e.g, it does not hydrolyse the connective tissue protein elastin. Hydrolysis of Z-Arg-Arg-NHMec, the recommended substrate for cathepsin B, was not detected. It failed to hydrolyse the aminopeptidase H substrate Arg-NHMec. Its high affinity for concanavalin A-sepharose suggests that the molecule is glycosylated. The enzyme activity peaks at pH 6.0 but has a broad pH activity range in the alkaline region which is typical of lysosomal thiol proteases from protozoa.

The Michaelis constant (Km) value for trypanopain Tc with Z-Phe-Arg-NHMec is 0.7μM. Depending on the mammalian species, the Km for the hydrolysis of this substrate by cathepsin B and L are 75 - 150μM and 0.7 - 7μM respectively. Thus, trypanopain Tc is similar to cathepsin L isolated from rabbit, rat and human liver. However, as described above, there are differences between cathepsin L and trypanopain Tc. For example, cathepsin B is rapidly inactivated by Z-Phe-Ala-CHN_2, whereas cathepsin L is more rapidly inactivated with Z-Phe-Phe-CHN_2. Trypanopain Tc is more rapidly inactivated by Z-Phe-Ala-CHN_2 than Z-Phe-Phe-CHN_2, which suggests that trypanopain Tc resembles cathepsin B more than L in this instance. The nature of these differences can only be dissected by a detailed kinetic and structural comparative study of the enzymes.

Polyclonal antibodies against the purified enzyme react with proteins from crude lysates of bloodstream forms of *T. brucei* and *T. vivax*. This observation supports the conclusion that, like *T. congolense* and *T. brucei*, *T. vivax* contains trypanopain activity.

TR2.4 The three-dimensional structure of the endocytic organelles in *Trypanosoma brucei* and *T. congolense*

Research Associate: P. Webster
Technicians: Y. Verjee, P.M. Theuri, S. Wasike

The organelles of African trypanosomes involved in endocytosis can be revealed ultrastructurally using horseradish peroxidase (HRP) and colloidal gold coupled to bovine transferrin (Au-Tf) or bovine serum albumin. At 0°C the markers bind to the cell surface but do not enter the flagellar pocket and are not internalised. Upon warming to 37°C, the markers are found in the flagellar pocket and appear to enter all the intracellular endocytic organelles within 5 min. Serial sectioning of resin-embedded cells reveals a pseudo three-dimensional view of these organelles. The organelles involved are of three types: (1) small vesicles and cisternae (20 - 25 nm in diameter), (2) large tubular networks (200 nm diameter) similar to handsomest of mammalian cells, and (3) large lysosome-like vesicles. These organelles, located between the flagellar pocket and the nucleus, are also associated with one face of the Golgi apparatus. In pulse/chase experiments HRP is not detected in intracellular organelles after 410 min but Au-Tf is seen in residual bodies. No exocytosis of Au-Tf from the flagellar pocket has been detected.

The data suggest that the processes of endocytosis in African trypanosomes may be similar to the endocytic processes found in mammalian cells.

TR2.5 Occurrence of endocytosis and intracellular sorting in different life-cycle stages of African trypanosomes

Research Associate: P. Webster
Scientist: W.R. Fish
Technicians: Y. Verjee, P.M. Theuri, S. Wasike

Horseradish peroxidase (HRP) and colloidal gold-labelled proteins enter many of the endocytic organelles of bloodstream forms of *Trypanosoma brucei* and *T. congolense*. However, the colloidal gold markers are excluded from substantial parts of the pathway that contain HRP. Morphometric studies reveal that HRP enters organelles that account for approximately 5% of the total cell volume while transferrin-gold enters organelles that comprise approximately 2% of the total cell volume. In addition, large colloidal gold particles are excluded from organelles that contain smaller gold particles. Antibodies raised against the variable surface glycoprotein, when applied to thawed cryosections, label structures from which endocytosed colloidal gold coupled to bovine serum albumin is excluded. Endocytosis occurs in two *in vitro* propagated forms of trypanosomes, similar to those found in the insect vector (*Glossina* spp.). The mammal-infective metacyclic forms are like bloodstream forms in that they endocytose HRP and colloidal gold markers but exclude colloidal gold from approximately 3% of the endocytic organelles. Estimation of the flagellar pocket volumes of bloodstream form *T. brucei* shows that this organelle occupies 0.5 - 1.4% of the total cell volume. The flagellar pocket volume of *T. congolense* varies between life-cycle stages, with a fractional volume of 4.4.% for bloodstream forms, 2.3% for metacyclic forms and 1.4% for procyclic forms. Endocytosis of HRP, but not of protein-gold markers, occurs in procyclic (uncoated) forms. Differences in the fractional volume of the

flagellar pocket between life-cycle stages may reflect different rates of endocytosis related to the environment in which the parasite finds itself.

TR2.6 The biochemical identification of flagellar pocket and endosome-derived membranes in subcellular fractions of *Trypanosoma brucei*

Scientists: D.J. Grab, K.J. Lindqvist, W.R. Fish, J.D. Lonsdale-Eccles
Research Associates: G. Mpimbaza, P. Webster
Technician: Y. Verjee

By incubating trypanosomes with the fluid phase marker horseradish peroxidase (HRP) at defined temperatures, the compartmentalisation of the marker can be manipulated, e.g., its accumulation in the flagellar pocket vs. the endosome/lysosome system. After disruption of HRP-loaded trypanosomes in the French Press, a crude flagellar pocket/endosome membrane fraction was isolated on discontinuous Percoll gradients and subjected to further purification on continuous sucrose gradients. It was found that the density of the flagellar pocket vesicles generated during the procedure have a density of 1.11 g/ml, whilst those vesicles derived from the endosome system are significantly lighter. Free-flow electrophoresis has also been successful in separating these components according to charge. The fractions are currently being analysed for various marker enzymes to further delineate their cellular origins.

TR2.7 Identification of a transferrin binding protein in *Trypanosoma brucei*

Scientist: D.J. Grab, M. Marsh*
Research Associate: P. Webster
Technician: Y. Verjee

(*Chester Beatty Research Laboratories, London, UK)

Studies were focused on the biochemical identification of transferrin-binding proteins in *Trypanosoma brucei*. This was accomplished by incubating iodine-labelled diferric-transferrin with *T. brucei* in transferrin-free medium followed by solubilisation of the trypanosomes with detergent. The solubilised extract was subjected to chromatography on Sephacryl columns. Free transferrin eluted approximately with BSA. A small percentage of the iodinated protein was bound in a high molecular weight complex and a transferrin-binding protein, roughly half the size of a mammalian transferrin-receptor, was immunoprecipitated. In addition, it has been found that the parasite's receptor may also bind much more tightly to its ligand.

TR2.8 The Interaction of *Trypanosoma brucei* with antibodies to the variable surface glycoprotein

Research Associate: P. Webster
Scientist: S.J. Black
Student: D.C.W. Russo

Modulation of polyvalent IgMs and IgGs, monoclonal IgGs and Fab_1 monomers of monoclonal IgGs specific for exposed epitopes on *Trypanosoma brucei* variable surface glycoproteins (VSGs), was monitored by both immunofluorescence and immunocytochemistry. All antibodies, and antibody fragments, were uniformly distributed over the parasite surface after incubation with the organism at 0°C and were moved via the flagellar pocket into intracellular organelles upon warming to 37°C. Removal of single layers of bound antibody, or Fab fragments, from the cell surface at 37°C, as determined by immunofluorescence, was complete within twenty minutes and occurred in the presence or absence of protein synthesis. Parasites which had altered the original distribution of surface bound antibody remained fully covered with VSGs of the original antigen type.

TR2.9 The vacuolar ATPase of African trypanosomes

Scientists: W.R. Fish, N. Murphy, J.R. Young, R.L. Cross*, P. Vogel*
Research Associates: M. Macklin, P. Webster
Technicians: C. Muriuki, A. Muthiani

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Biochemical investigations of clathrin-coated vesicles (CV) of *Trypanosoma brucei brucei* revealed that the ATPase activity present in the CV appeared to be entirely due to that of a vacuolar ATPase (V-ATPase). These studies included responses to characteristic inhibitors and an active site directed photoaffinity probe. The latter labelled only one protein band and the Mr was similar to that of the catalytic subunit of V-ATPase's from other sources. Labelling was almost completely eliminated in the presence of excess MgATP.

A synthetic consensus oligonucleotide probe was designed from known DNA sequences of the catalytic subunit of the V-ATPase from other organisms. An 83 bp piece of DNA was cloned from a *T. congolense* IL3000 metacyclic cDNA library that, at the DNA and amino acid level, is closely homologous to the corresponding sequence of other organisms. The clone has been used to screen for further recombinants and the purification and sequencing of the latter is ongoing. It is probable that one of these clones contains a full-length insert (approx. 2 kb). Screening of the same library with a consensus oligonucleotide to a second subunit has allowed the isolation of a further five clones. Finally, the synthesis of two consensus oligonucleotides for the membrane bound proton-pore proteolipid of the V-ATPases has been accomplished and these will be used in the polymerase chain reaction to quickly obtain sufficient DNA to sequence. The latter subunit is small in size and a reasonable proportion of the sequence might be obtained by such methodology

TR2.10 Identification and characterisation of a trypanosome filament-forming protein

Student: W. Endege
Supervisor: O.K. ole-MoiYoi

African trypanosomes have a very highly organised cytoskeletal system composed of the microtubular and the other filamentous systems. Of interest is what happens to the composition of these structures as the parasite differentiates from the long, slender, bloodstream forms to the short, stumpy forms, divides or attaches. What elements determine this transition? This may be an area that could offer great potential as a chemotherapeutic target. There is evidence, particularly in nematodes, that imidazoles have antiparasitic activity which is directed against certain components of the cytoskeleton.

Cytoskeletal ghosts were prepared from *Trypanosoma brucei* by extraction with buffers of low and high salt concentration containing Triton X-100. These ghosts were highly enriched in microtubular and 10 nm filament-like proteins. Because of the apparent presence of intermediate filament-like proteins in these ghosts, we isolated apparent intermediate filaments using a method modified from that of O'Shea *et al.* (*Biochem. J.* 195: 345 - 356, 1981) and obtained an Mr 80 kDa acidic protein (pI 5.0 - 5.5), which can be polymerised *in vitro* into 10 nm filaments, protofilaments and sheet-like structures. Rabbit antibodies to this protein recognised a single band of M_r 80 kDa (Tp80) on Western blots. We have also demonstrated the presence of this protein in *T. congolense* and in *T. vivax*, as well as in procyclic and stumpy forms of *T. brucei*.

Using immunofluorescence and electron microscopy, IgG to Tp80 was observed to label the flagellum and kinetoplast regions of the parasite, as well as weakly labelling the cell cytoplasm. The antibody labelling, visualized with protein A gold on thin cryosections of *T. brucei* by electron microscopy, showed intense labelling of the paraflagellar rod and basal bodies. Some labelling of the cytoplasm and nucleus was also observed. On cryosections of dividing trypanosomes this antibody labelled an area bounded by one already divided flagellar pocket and basal bodies and an elongated, but not yet divided, kinetoplast. The antibody labelled the side of the kinetoplast proximal to the flagellar pocket, but not the opposite side. These results suggest that Tp80 may play a role in cytoplasmic filamentous structures involved in the anchoring of the paraflagellar rod and the base of the flagellum to the cell body. It is possible that Tp80 might also be involved in the process of division of the basal body and kinetoplast during cell replication.

TR2.11 A comparison of the isoenzymes of *Trypanosoma (Duttonella) vivax* isolates from East and West Africa

Student: A.I. Fasogbon
Supervisor: P.R. Gardiner

The phenotypic diversity of some *Trypanosoma (Duttonella)* vivax isolates from East and West Africa was compared by isoenzyme analysis. Thirteen stocks and clones were subjected to isoenzyme analysis for thirteen enzymes. The Ugandan and West African stocks showed similar banding patterns, which differed from those of the Kenyan stocks. Two stocks associated with a severe haemorrhagic form of disease, IL2337 (Galana,

Kenya) and IL3067 (Bamburi, Kenya), showed a high degree of similarity in banding patterns. One of the Kenyan stocks, MID 627, differed in most of its banding patterns from all other stocks and clones. The similarity indices for all possible pairs of stocks and clones were calculated and the values obtained were in agreement with the similarities or differences in the banding patterns judged empirically.

From the results obtained, the *T. vivax* isolates used could be divided into two groups typical of East Africa and West Africa, with the Ugandan stocks tending to be closer to the West African group. Some rodent-infective or rodent-adapted stocks were also examined but it was not possible, due to the limited number of stocks used, to deduce whether the observed similarities in banding patterns were as a result of their rodent infectivity or geographical location. Currently, some renaturation studies are being carried out on a group of isoenzymes, the peptidases, of *T. vivax*, which have been observed to differ from those of *T. brucei brucei* and *T. congolense*. It will be of interest to correlate the existence of these enzymes with the particular phenotypic characteristics of individual *T. vivax* stocks and the disease syndromes they cause.

TR2.12 *Trypanosoma vivax*: Investigation of variable surface antigens

Scientists: P.R. Gardiner, M.W. Clarke*, V. Nene
Technician: R. Thatthi

(*University of Western Ontario, Canada)

There are important differences between the biological behaviour of *Trypanosoma vivax*, and that of *T. congolense* and *T. brucei*, which affect the induction of immunity in ruminant hosts to the former parasite. The "infection and treatment" methods fail to induce immunity to homologous stocks of *T. vivax* parasites, but self cure of long term infections (even syringe induced) can induce such immunity. Further, we have demonstrated by serological means that VATs reoccur during infections of ruminants . The inference is that immunity to *T. vivax* infections is based on a bloodstream VAT repertoire, which may be limited, and not on a restricted set of metacyclic VATs, as is the case in *T. brucei* and *T. congolense*.

We are thus attempting to determine if the recurring variants are structurally identical or not, if metacyclic trypanosomes express a restricted set of VSGs in this species, and the relationships between the VSGs of *T. vivax* and the VSGs of other species of African trypanosomes.

A cDNA library has been prepared from IIDat 2.1 oligo-dT-purified mRNA and screened with polyclonal rabbit antisera to the IIDat 2.1 VSG. Sequencing of *Bal*31-digested fragments of the VSG cDNA, cloned into M13, is in progress. This strategy will be used to (a) examine the structural homology between early and recurring variants that we have identified during infections of goats, and (b) examine the structural diversity amongst the VSGs of *T. vivax* metacyclics grown *in vitro*.

Other structural studies have suggested differences between the organisation of the myristilated C-terminal domain of *T. vivax* VSGs and those of *T. brucei* VSGs. ^{3}H-myristate-labelled *T. brucei* VSGs can act as a substrate for the endogenous phospholipase-C in *T. brucei* lysates, releasing the labelled fatty acid moiety of the cross-reacting determinant.

It was previously shown ,however, that *T. vivax* lysates had little or no capacity to similarly liberate label from the *T. brucei* substrate. In the inverse experiment, *T. brucei* (and *T. vivax* lysates), commercial phospholipase-C and fresh bovine serum (as a source of phospholipase-D) were unable to release ^{3}H from labelled IIDat 2.1 VSG. It is suggested, therefore, that the anchor of the *T. vivax* VSG, although similar to that of *T. brucei* and *T. congolense* VSGs, is not identical.

TR2.13 Investigation of surface membrane proteins of *Trypanosoma vivax*

Student: B. Burleigh
Supervisor: P.R. Gardiner

Rabbit antiserum raised against a membrane-enriched fraction of *Trypanosoma vivax* IIDat 1.2 reacted with molecules other than VSG on Western blots. These molecules, having approximate molecular weights of 69, 46, 39, 35 and 31 kDa, are also present in a *T. vivax* clone bearing an immunologically non-cross reactive VSG and in a clone of *T. brucei*. Since earlier studies involving cell surface biotinylation revealed that a 39 kDa molecule was labelled, this band as well as a 35 kDa protein were excised from a preparative SDS-PAGE gel and electroeluted. Rabbits were immunised with these putative membrane proteins in attempts to obtain a more specific antiserum.

Membrane bound 3'-nucleotidase activity has been previously demonstrated on the surface of procyclic and bloodstream forms of *T. b. rhodesiense*. Using a staining technique for the detection of phosphohydrolases in SDS-PAGE gels, 3'-nucleotidase activity has been demonstrated in lysates of *T. vivax*. The enzyme has a molecular weight of 36 kDa. Similar gels run with subcellular fractions of *T. brucei* reveal enzyme activity in the membrane fraction. There are two bands of activity, one corresponding to the molecular weight of the *T. vivax* enzyme and the other slightly lower. The electroeluted 35 kDa protein of *T. vivax* was also positive for 3' nucleotidase activity in the gel system. Therefore, the antisera raised against the 35 kDa protein may be useful for more specific localisation of the enzyme by immunocytochemistry on frozen thin sections, affinity purification of the enzyme, and investigations of the degree of surface exposure by immunofluorescence.

Recent efforts have been directed toward the production of monoclonal antibodies to *T. vivax* surface proteins. BALB/c mice have been immunised with formalin-fixed, three-day uncoated culture forms of *T. vivax* 1392. Selected monoclonal antibodies recognising invariant cell surface molecules will be used to screen a λ *gt* 11 expression library of *T. vivax* IIDat 2.1 cDNA, and to affinity purify proteins. These steps will enable the structural and functional relationships between *T. vivax* invariant surface proteins to be elucidated.

TR2.14 Further studies on the axenic *in vitro* cultivation of *Trypanosoma vivax* insect forms

Research Associate: I.D. Gumm
Scientist: W.R. Fish

Axenic insect form cultures of *Trypanosoma vivax* were initiated from bloodstream forms of East African *T. vivax* (EATv) stock CP 2331 obtained from steers with a late parasitaemia, from West African *T. vivax* (WATv) stock IL1392 and from clone IIDat 1.2 obtained from mice with a mid or late parasitaemia. Iscove's medium supplemented with foetal bovine serum (FBS), L-proline, L-glutamine, hypoxanthine, adenosine, pyruvate and 2-mercaptoethanol was used. Continuous production of infective metacyclics was maintained for 6 - 12 months and the identity of the trypanosomes confirmed as *T. vivax* by DNA probe and isoenzyme analyses.

Attempts to culture other mouse-adapted EATv stocks (IL2699) or clones (IL2931), in the presence or absence of mammalian feeder layer cells, were not successful. In contrast to CP 2331, IL2699 and 2931 are not transmissible by tsetse flies.

A characteristic of *T. vivax*, either from cultures or tsetse flies, is the production of low numbers of metacyclics. A high percentage of those derived *in vitro* are immature, i.e., lacking a terminal kinetoplast and a detectable surface coat. Preliminary results from axenic insect form cultures of CP 2331 and IIDat 1.2 indicate that the yields and "maturity" of metacylics are influenced both by culture manipulation and by the concentrations of supplements used in the medium. If the epimastigotes, which attach to and multiply on the plastic of the culture flask, are not physically scraped from the plastic when they reach confluency, very few metacyclics are produced. In addition, an increase in the concentration of L-glutamine or 2-mercaptoethanol produced a subsequent increase in the number of metacyclics and percentage of "mature" forms. An increase, however, in the concentration of FBS, L-proline or hypoxanthine caused a decrease in the number of metacyclics and percentage of mature forms. Presumably due to the non-physiological nature of the supplements themselves, or their concentrations, the effects were transitory and eventually resulted in the cultures dying out. The differentiation of epimastigotes to metacyclics seems to be related to nutritional stress.

TR2.15 Isolation of developmentally regulated genes in bloodstream forms of *Trypanosoma brucei*

Scientist: N.B. Murphy
Technician: E.O. Omolo

This project involves the isolation and characterisation of genes involved in the slender to stumpy switch in bloodstream form trypanosomes and ultimately those genes which specify the pleomorphic nature of some trypanosome isolates. The search for genes expressed in pleomorphic strains and absent or not expressed in monomorphic strains of trypanosomes may help in identifying those genes crucial to the switch. In addition, since stumpy forms are non-dividing, genes involved in the regulation of cell division should also play a role.

The approach of subtractive hybridisation and enrichment of stage-specific cDNA by metal chelate chromatography was carried out and a library of stumpy-enriched cDNA sequences cloned in λ*gt* 10. Analysis on RNA blots indicates that at least two of the sequences being analysed are indeed developmentally regulated. Limited sequence analysis with a subsequent search of the database, however, has not revealed any potential function for these genes. In addition, these sequences appear to be part of multigene families thus complicating their analysis.

One clone which is expressed in slender forms, but not in stumpy forms, has been identified in studies on the *T. brucei* cytoskeleton. This gene encodes a 17 kDa protein which appears to be associated with an 80 kDa cytoskeletal protein. Hybridisation analysis indicates that this sequence is more highly conserved than tubulin in the different trypanosome species. So far no potential homologues of this sequence have been identified in the databases.

Oligonucleotides have been constructed which are specific for kinases based on the conserved amino acid sequences in the catalytic domains of these enzymes. Using the polymerase chain reaction on total genomic DNA from *T. brucei* and *T. congolense* with mixed oligonucleotides for both strands, some genomic sequences have been specifically amplified which should correspond to some of the kinases involved in cell cycle control.

TR2.16 Transfection studies in trypanosomes

Scientist: N.B. Murphy
Technician: E.O. Omolo

Trypanosomes are not, at present, amenable to traditional methods of genetic analysis, which have been powerful tools in studies of differentiation, drug resistance and gene regulations in other organisms. Expression of transfected DNA sequences, and a means of assaying expression, are required for most of these applications. In constructing potential vectors for the transfection of trypanosomes, the following potential requirements have been considered: promoter, enhancer, mini-exon splicing signal(s), ribosome binding site, transcription termination signal and polyadenylation signals(s). None of these have been unequivocally determined in trypanosomes. Two approaches have been taken in attempts to transfect trypanosomes. One explores the possibility of inducing transient expression in order to assess the viability of the system. The other hopes to achieve integrative transfection for stable expression, rather than autonomously replicating vectors, in order to overcome our lack of knowledge of the elements involved.

Results so far indicate that it is possible to transfect and express foreign DNA in trypanosomes, specifically, the bacterial neomycin-resistance gene. The various assumptions made in the construction of the vectors appear to have held, although there have been some unexpected findings. It is clear that more work is required in order to elucidate the mechanisms involved in the apparent expression of the neomycin resistance determinant. The results from attempts to generate stable transfectants indicate that the vector construct contains most of the elements required for expression and replication, although the construct is lost in the absence of selection. Further modifications of this vector should help in generating a more useful vector expressing higher levels of resistance to the amino glycoside drugs.

TR2.17 The characterisation of the protein kinases of African trypanosomes and the determination of their role in modulation of trypanosome metabolism and cell cycle transitions

Scientists: J.D. Lonsdale-Eccles, O.K. ole-MoiYoi
Student: T. Aboagye-Kwarteng

Protein phosphorylation is a major mechanism by which cellular functions are regulated. Of particular importance is the role of a complex network of protein phosphorylation/ dephosphorylation in signal transduction and amplification. In spite of a wealth of information concerning casein-kinases (CKs) in higher eukaryotes, the biological functions of these enzymes are largely unknown. However, recent sequence data shows that casein kinase-II (CK-II) is closely related to cell-cycle controlling enzymes encoded by genes such as yeast CDC 2 and CDC 28. Changes in the activity of poly (A) polymerases, phosphorylase kinase, RNA polymerase-I & -II and topoisomerase-I & -II have been effected by CKs. It has also been shown that CK-II may phosphorylate nuclear oncogenes such as c-myc, c-fos, and c-myb, all of which are activated upon exposure of cells to growth factors. Thus, CKs may play important roles in the regulation of gene expression.

The importance of protein kinases in the regulation of gene expression of African trypanosomes has been exemplified recently by our observations of changes in protein phosphorylation between the rapidly dividing slender and non-dividing stumpy forms of the parasites. The phosphorylation of these proteins appears to be effected by enzymatic activity that is similar to mammalian CK-II. In order to define the enzyme(s) better, *T. brucei* CK activity has been partially purified and characterised. However, a number of the enzymatic, chemical, and inhibition characteristics of the *T. brucei* enzymes are sufficiently different from their mammalian counterparts that the classification of the activity as CK-II may not be appropriate.

TR2.18 A feeder-cell-layer-free system for cultivating bloodstream forms of *Trypanosoma brucei*

Scientists: H. Hirumi, M.K. Shaw
Research Associate: K. Hirumi
Technician: J.R. Wando

Bloodstream forms (BSFs) of *Trypanosoma brucei brucei* (GuTat 3.1) and *T. b. gambiense* (IL2343), obtained from infected Balb/c mouse blood, were continuously propagated *in vitro* in the absence of feeder cell layers at 37°C until the experiment was terminated on day 86, using a BSF-supporting medium (HMI-9). The medium was modified from Iscove's DMEM (Dulbecco's modified Eagle's minimum essential medium, GIBCO, Scotland) by supplementation with 0.05 mM bathocuproine sulphonate, 1.5 mM L-cysteine, 1 mM hypoxanthine, 0.2 mM 2-mercaptoethanol, 1 mM sodium pyruvate, 0.16 mM thymidine, 10% (v/v) heat-inactivated foetal bovine serum (FBS) and 10% (v/v) Serum Plus™ (SP) (Hazleton Biologics, Lenexa, Kansas). Each primary culture was initiated by placing 3.4 - 4.0 x 10^6 BSFs isolated from the infected mice in a 25 cm^2 T-type flask containing 5 ml of the medium. The cultures were maintained by replacing the medium every 24 hrs for 5 - 7 days. During this period, many BSFs died. However, from day 4 onwards, long slender BSFs increased in number. On days 5 - 37, trypanosome suspensions were pooled and cell debris was

removed by means of diethylaminoethyl cellulose (DE52) column chromatography. Bloodstream forms were then collected by centrifugation, resuspended in fresh medium at 7 - 9 x 10^5/ml, and transferred to new flasks. Subcultures were maintained by readjusting the BSF density to 7 x 10^5/ml every 24 hr By this method, 2 - 3x10^6 BSFs/ml were obtained consistently every 24 hr for more than 80 days. Trypanosomes propagated in this system retained the morphological characteristics of long slender BSFs seen in the infected mouse blood, were covered with an electron-opaque surface coat, and were infective to mice.

TR2.19 A low serum containing medium for cultivating *Trypanosoma brucei* bloodstream forms in the absence of feeder layers

Scientist: H. Hirumi
Research Associate: K. Hirumi
Technician: J.R. Wando

In order to establish a serum-free culture medium for cultivating bloodstream forms (BSFs) of *Trypanosoma brucei* in the absence of feeder cell layers, the bloodstream form-supporting medium HMI-9, was further modified. The modification was carried out by alternating the amounts of two supplements in the medium: (1) heat-inactivated foetal calf serum and (2) Serum Plus™(SP). According to the manufacturer, the latter (Lot No. 14001004) contained a low level of FBS proteins (13 µg/ml), specific growth promoting factors, transport proteins, hormones, a high level of glucose (12.24 mg/ml) and low amounts of haemoglobin (10 µg/ml) and endotoxin (5.8 ng/ml) with a pH of 7.32 and osmolarity of 378 mOsm. To minimise the amount of FBS in culture medium, concentrations of FBS were reduced gradually at 5 - 7day intervals by alternating the amounts of FBS (10% v/v) and SP (10%, v/v) in the original HMI-9 medium, with (1) 5% FBS and 15% SP (HMI-10), (2) 2% FBS and 18% SP (HMI-14), and (3) 20% SP (HMI-18). Bloodstream forms of *T. b. brucei* GuTat 3.1, as well as of *T.b. gambiense* IL2343, which were propagated *in vitro* using HMI-9 medium in the feeder-layer-free system, were adapted step by step to these FBS-reduced media. After being adapted, consistent daily yields of 2 - 3 x10^6 BSFs/ml were obtained in HMI-18 medium every 24 hr by adjusting the daily seeding density to 9 x10^5 BSFs/ml (3ml/flask). Bloodstream forms also could be cultivated with HMI-18 in 24-well culture plates with 2% CO_2 in air using a similar method to that employed in the flask culture. The final maintenance medium (HMI-18) developed in this study still contained a low level of FBS proteins in SP. Thus, further improvement will be needed to establish a completely serum-independent culture system. Nevertheless, differing results which often have been due to variability between FBS batches have been minimised by using HMI-18 containing no additional FBS.

TR2.20 *In vitro* propagation of *Trypanosoma congolense* bloodstream forms in a feeder layer-free system

Scientists: H. Hirumi, M.K. Shaw
Research Associate: K. Hirumi
Technician: J.R. Wando

Bloodstream forms (BSFs) of *Trypanosoma congolense* (IL3000) have been continuously cultivated *in vitro* until the experiment was terminated on day 66 in the absence of feeder

cell layers at 34 - 36°C, using the bloodstream form-supporting medium HMI-9. Trypanosomes obtained from two different sources were used to initiate the cultures: (I) metacyclic forms (MCFs) which were propagated *in vitro* at 27°C using glucose-free Eagle's MEM supplemented with 10 mM L-proline, 2 mM L-glutamine and 20% (v/v) foetal bovine serum, harvested on day 68 and separated from epimastigotes by means of DE52 column chromatography, and (II) BSFs isolated from infected mouse blood. The MCFs placed in 25 cm^2 T-type flasks rapidly attached to the bottom of the flasks and transformed to BSFs during the initial 24 hr and continued to proliferate forming (1) surface-attached (AT) populations and (2) detached (DT) populations of BSFs in culture fluid. Every 4 - 5 days, AT-BSFs were re-suspended in fresh medium by gentle scraping and then were subcultured in new flasks. The best growth was achieved by seeding with 1 x 10^6 AT-BSFs/ml (3ml/flask) and incubating at 36°C: the number of generation/24 hr n = 2.6; the population doubling time, g = 9.2 hr. On the contrary, population doubling times in subcultures seeded with 3 x 10^6 and 5 x 10^6 AT-BSFs/ml were 13.9 and 16.2 hr respectively. Subcultures, which were seeded with 3 x 10^6 AT-BSFs/flask and maintained by replacing the DT-BSF containing medium every 24 hr at 34°C for 5 days, produced a total of 7.1 x 10^7 BSFs/flask during 24 hr on day 5. This method was also successfully applied to the direct cultivation of BSFs isolated from infected mouse blood. BSFs propagated in this system were morphologically similar to those grown *in vivo*, covered with an electron-opaque surface coat and were infective to mice. This did not, however, support the growth of BSFs of three other tested stocks (IL1180, IL2079 and IL3266), indicating that further improvement of the method is needed to cultivate BSFs of *T. congolense* in general in a feeder-layer-free system.

TR2.21 Further study on *In vitro*-induced monomorphic bloodstream forms of *Trypanosoma brucei*

Scientists: T.Yanagi, H. Hirumi, S.K. Moloo

We have reported earlier that monomorphic bloodstream form (mBSF) populations could be experimentally induced from clonal, pleomorphic (pBSF) populations of *Trypanosoma brucei brucei* GuTat 3.1 by treating the pBSFs *in vitro* with bovine haemoglobin (BHb) (ILRAD, *Ann. Sci. Rep.*, TR2.9, 1988). The BHb-treated BSFs, primary cultures of which were started from infected tsetse salivary glands, showed only monomorphism in rodent hosts, killed the hosts within a short time without developing parasitaemic waves, and became non-infective to tsetse. In further studies, primary cultures of pBSFs were initiated from infected Balb/c mouse blood, treated with murine erythrocyte lysates (MEL) and then maintained at 26°C for nine months, as reported earlier. Under such culture conditions, the trypanosomes retained the morphological characteristics of the long-slender BSF (L-BSF) and did not transform to procyclic forms (PCFs) even after additional MEL-treatments at 26°C. Two goats, inoculated with 1 x 10^5 and 5 x 10^5 MEL-treated BSFs intravenously and subcutaneously, respectively, developed infection six days after inoculation. Thereafter, low levels of parasitaemia were consistently detected until the experiment was terminated at the time when the PCV decreased to 20% on day 50. All trypanosome populations, examined every 3 - 4 days throughout the course of experiment, consisted of L-BSFs. All ten Balb/c mice, inoculated intraperitoneally with 5 x 10^4 MEL-treated BSFs, developed infections showing only mBSFs. In all, 178 *G.m. centralis*, which were fed either on the infected mice on day seven or by means of membrane feeding of culture fluids containing the MEL-treated BSFs, no infection was detected on examination of their guts, labia, hypopharynges or salivary glands. The results suggest that (1) MEL contains a factor(s)

which can regulate the pleomorphism of *T.b. brucei* BSFs as seen earlier with BHb, (2) the monomorphism expressed by the MEL-treated BSFs is not host-dependent, (3) the MEL-treatment may eliminate BSFs, which are able to transform to stumpy forms, as well as to PCFs, from the pBSF populations, and (4) the *in vitro* system developed in this study may facilitate further studies on the mechanism underlying this stage-specific differentiation of *T. brucei*.

TR2.22 *In vitro* regulation of the virulence of *Trypanosoma brucei*

Scientists: T. Yanagi, H. Hirumi

Degrees in the virulence of *Trypanosoma brucei brucei* GuTat 3.1 were regulated *in vitro* by maintaining originally pleomorphic bloodstream forms (pBSFs) under different culture conditions. This was done in three steps, using moderately virulent pBSFs which rose to successive parasitaemic waves in Balb/c mice and killed the hosts within three weeks to three months. Step I: the original pBSF population was maintained at 37°C using RPMI 1640 medium containing bovine haemoglobin for one month as described earlier (ILRAD, *Ann. Sci. Rep.*, TR.2.9, 1988). The pBSFs maintained under such conditions changed to monomorphic(m) BSFs and became highly virulent, killing Balb/c mice within two weeks when inoculated with 10^4 mBSFs/animal: this constituted a highly virulent group. Step II: the mBSFs were then maintained at 27°C in the presence of feeder layers derived from either (a) adult mouse brain cells (AMBC) or (b) foetal bovine spleen cells (FBSC) for more than six months. The majority of the mBSFs maintained in the presence of AMBC-feeder layers penetrated the intercellular spaces and retained the same level of high virulence. On the contrary, the mBSFs maintained with FBSC-feeder layers continued to proliferate without penetrating the intercellular spaces and showed various degrees of lower virulences. Step III: the mBSF populations with different degrees of virulence were divided into an additional three groups: a moderately virulent group which developed successive parasitaemic waves and killed the hosts within three weeks to three months by inoculating with 10^5 mBSFs/animal (equivalent to the original virulence); a less virulent group which also developed parasitaemic waves, killing the hosts four months after inoculation with 10^6 mBSFs/animal, and an "attenuated" group which was avirulent in host animals inoculated with 10^7 mBSFs/animal. The mBSFs in the latter group consistently established infection during the initial three days, appearing in the peripheral blood of various host animals, including Balb/c, C3H/He, C57Bl/6 and Swiss mice, as well as in SD rats and rabbits, but thereafter disappeared. No relapse was developed in all the forty Balb/c mice for more than twelve months. The results indicated that (1) host cell-parasite interaction may play an important role in developing the various degrees of virulence, (2) mBSFs are not always highly virulent, and (3) the remission of parasitaemia can occur without the transformation of long, slender BSFs to stumpy forms, at least at a detectable level.

TR2.23 Characterisation of *in vitro*-induced avirulent bloodstream forms of *Trypanosoma brucei*

Scientists: T. Yanagi, H. Hirumi, S.K. Moloo, N.B. Murphy, M. Kishima, M.K. Shaw

A preliminary characterisation of the avirulent bloodstream forms (A-BSFs) of *Trypanosoma brucei brucei* GuTat 3.1, which were induced *in vitro* in a previous study (TR2.22), was carried out. The A-BSFs were morphologically similar to long, slender BSFs and were covered with an electron-opaque surface coat. When mice were inoculated intraperitoneally with 10^7 A-BSFs/animal, they consistently appeared in the peripheral blood of the hosts during the initial three days and disappeared thereafter, whereas procyclic forms (PCFs) were not seen in the blood during the same period, even when the animals were inoculated with up to 10^8 PCFs/animal. Of fifty-four Balb/c mice inoculated with a total of 1 - 2 x 10^7 A-BSFs at 2 - 4 month intervals, only seven mice (13%) were immune to tsetse challenge with GuTat 3.1.

An examination of chromosome-sized DNA molecules of A-BSFs and virulent(v)-BSFs by means of orthogonal field-alternation gel electrophoresis revealed differences in the DNA band profiles between the two populations. The v-BSF populations consistently contained an approximately 500 kb chromosome band, whereas the A-BSF populations lost the 500 kb band but contained a 200 kb band not present in v-BSF. Two-dimensional polyacrylamide gel electrophoresis and autoradiographic analyses of ^{35}S-methionine labelled proteins demonstrated at least seven qualitative and five quantitative changes in minor proteins between the two populations. The results suggest that the *in vitro*-induced A-BSFs are not applicable to the immunisation of host animals against infective tsetse challenge. They nevertheless may be suitable for studying the mechanisms underlying the determination of virulence in these parasites.

TR3.1 Characterisation of bovine B-cell markers for the Ist Bovine Leukocyte Antigen Workshop held in Hannover, 1989

Scientists: J. Naessens, D.J.L. Williams
Research Associate: N.D. MacHugh

A panel of twenty monoclonal antibodies (MAbs), produced by six laboratories throughout the world, was analysed and divided into clusters. The following tests were performed with the panel:

(1) immunofluorescence analysis with cells from blood (PBM) and other lymphoid organs and on *in vitro* cultured cell lines and clones; (2) immunoprecipitations with radioiodinated PBM from an animal with a high percentage (50%) of B cells; (3) two-colour immunofluorescence on PBM; (4) ELISA tests using purified bovine immunoglobulins; (5) immunohistology. The data were presented at the Ist Workshop on Bovine Leukocyte Antigens held in Hannover.

Of the twenty MAbs tested, three detected immunoglobulins, one detected class II, and three were negative in all the tests. Three MAb clusters were defined at the workshop: (1) Cluster WC3, composed of 4 antibodies which detect a 145 kDa antigen. The antigen is present on mature B cells and may be similar to human CD21. (2) Cluster WC4, composed of 2 MAbs, detects a 90 kDa antigen which is weakly expressed on all B cells. (3) Cluster WC5 is also composed of 2 MAbs which detect a 46 kDa antigen on B cells but which is also present on activated T cells.

Other MAbs were analysed which could not be assigned to a cluster. Two antibodies recognised a 200 kDa antigen which was expressed weakly on B-cells, but strongly on dendritic reticulum cells. The antibodies did not inhibit one another, and not enough data were collected to assign them the status of a cluster. One other MAb detected an antigen which was strongly expressed on B cells and is different from all the others.

TR3.2 Reactivity of monoclonal antibodies with buffalo (*Syncerus caffer*) and waterbuck (*Kobus defassa*) peripheral blood cells

Scientist: J. Naessens

A panel of 144 monoclonal antibodies (MAbs) from the Ist Bovine Leukocyte Antigen Workshop was tested on peripheral blood cells from three buffalo and three waterbuck, to identify MAbs which cross-reacted between the three species.

Many of the MAbs which reacted with bovine cells did not react with either buffalo or waterbuck cells. In contrast, some of the antibodies raised against sheep antigens, which did not react with bovine cells, reacted very well with cells from the wild bovids. It is striking that in some clusters almost all the MAbs cross-reacted (CD2, CD8), while in others almost no cross-reacting MAbs could be found (CD4, CD5). Further, one cluster (CD6) occurs in buffalo but not waterback.

From each cluster two to three positive antibodies were selected and further tested by two-colour immunofluorescence on buffalo PBM to make sure that they recognised

similar populations as in bovine PBM. All those tested reacted as expected. The CD4 and CD8 subpopulations were distinct, did not overlap and were contained in the CD2 T-cell population. We observed small percentages of $CD8^+$ $CD2^-$, $CD2^+$ $CD6^-$ and $CD2^+$ $CD5^-$ cells. Two anti-B-cell MAbs (ILA65 and an anti-Ig which was not included in the workshop) did not overlap with the T-and "null" cell populations. However, two anti-MHC class II antibodies (not on the workshop list) reacted with all buffalo B, "null" and most T cells.

Some of the antibodies were used to precipitate antigen from buffalo PBM, and the molecular weights were identical to those obtained for cattle PBM.

From these results it is clear that we do not need new monoclonal antibodies to compare lymphocyte subpopulations between cattle and buffalo, and possibly waterbuck.

TR3.3 Cellular interactions involved in antigen-specific *in vitro* responses of bovine lymphocytes

Student: V. Lutje
Supervisor: S.J. Black

In vitro antigen-specific responses of bovine lymphocytes were analysed using an ovalbumin (OA)-specific system. Antigen-specific proliferation of primed PBL was shown to be dependent on the presence of CD4 positive cells, recognized by MAb ILA12; their presence could not be replaced by the inclusion of TCGF in the culture medium. It was possible to further dissect the response using MAb ILA47, which recognises a subset of bovine T-cells; the cell population proliferating to OA appeared to be ILA12 positive, ILA47 negative. OA-induced proliferative responses were also influenced by CD8 positive cells (recognized by MAb ILA51), and by "null" cells, recognised by MAb ILA29. Proliferative responses of primed PBL were enhanced after the depletion of these cell subsets.

Antigen presentation by macrophages and B cells was also analysed. When macrophages were added to populations of MHC class II-negative cells (MAb J11-negative cells), a reconstitution of the proliferative response was observed. When resting B cells, recognised by MAbs ILA30, ILA59 or CC51, were used as antigen-presenting cells, no proliferation was observed. When PWM-activated B cells were used as antigen-presenting cells, high specific proliferative responses were constantly observed, probably because of the persistence of the mitogen on the surface of the B cells. LPS-activated B cells, on the other hand, appeared to be able to mediate antigen presentation.

TR3.4 Analysis of proliferative and antibody responses induced by pokeweed mitogen in bovine leukocyte populations

Student: V. Lutje
Supervisor: S.J. Black

Pokeweed mitogen (PWM), a polyclonal activator of human and mouse lymphocytes, was used to induce proliferative and antibody responses of bovine lymphocytes. The cellular interactions involved in the responses were analysed by selectively depleting PBL of distinct

cell subsets, identified by specific MAbs, by FACS sorting. Proliferation was measured by ^{125}IUDR incorporation after three to five days in culture. Immunoglobulin (Ig) production was detected by a sandwich ELISA using culture supernatants. Both proliferative and Ig responses were reduced, but not abolished, by the depletion of CD4 positive cells, identified by MAb ILA12; the readdition of ILA12-positive cells induced a partial reconstitution of the response.

The opposite effect was observed after depletion of CD8 positive cells, recognised by MAb ILA51. Cell proliferation was also reduced after the depletion of macrophages, recognised by MAb ILA46. However, both proliferative and Ig responses could be induced in populations of purified B-cells, obtained by FACS sorting with specific MAbs ILA30, ILA59 and CC51.

In all the culture supernatants tested, only IgM was detected. From these results, it appears that PWM activation of bovine B cells is relatively more independent of the influence of T cells and macrophages than in other species. However, CD4-positive and CD4-negative cells also have a modulatory effect on the response. The phenotype of the proliferating cells will be examined in more detail by double-fluorescence stains with activation markers and T- and B-cell markers.

TR3.5 T-cell responses during infection with *Trypanosoma congolense* IL1180 in Boran cattle

Scientist: J.N. Flynn

The responses of peripheral blood mononuclear cells (PBMC) to trypanosome antigens and to the T-cell mitogen Concanavalin A (Con A), were assessed in a group of four Boran cattle during primary challenge with *Trypanosoma congolense* IITat 3.1. On days seven to twenty one post-infection (p.i.) there was a marked drop in the proliferative responses of T-cells when cultured *in vitro* with Con A. This effect was most marked fourteen days p.i. The mitogenic responsiveness then returned to levels approaching those pre-infection. In three out of the four infected cattle, this immunosuppressive phase was followed by antigen-specific proliferative response. This response was noted in peripheral blood mononuclear cells (PBM) collected from twenty-two to thirty-six days p.i. and was most noticeable at day twenty eight. The antigen specific response was transient and resulted in low-level, yet significant, proliferation *in vitro*. A further ten Boran cattle were infected with *T. congolense* IITat 3.1 and eight were killed at weekly intervals p.i. to compare responses of cells from other lymphoid tissues with those of the peripheral blood. Splenic mononuclear cells exhibited depressed mitogenic responses similar to those seen in the blood. However, this suppressive effect was most noticeable in mononuclear cells prepared from lymph nodes draining the bite site and also the contralateral lymph nodes. They exhibited a profound depression of their mitogenic responses which continued until thirty-five days p.i.

Splenic antigen-specific proliferative responses paralleled those seen in the blood, although they appeared to lag seven days behind. An antigen-specific response was not observed in lymph node tissue at any time.

TR3.6 Analysis of activation of bovine macrophages/monocytes in *Trypanosoma congolense*-infected cattle

Scientist: M. Sileghem

The aim of the project is to analyse the role of macrophages and monocytes in the differential resistance of Boran and N'Dama cattle to *Trypanosoma congolense* infection. Since most of the assays to measure macrophage activation are lacking in cattle, our first goal was to develop assay systems to quantitate different aspects of macrophage activation.

We developed bio-assays to quantitate secretion of different monokines by bovine monocytes and macrophages. The spontaneous secretion of IL1 was greatly increased in a group of *T. congolense* -infected Boran, two to three weeks post-infection. The kinetics of IL1 secretion were similar in peripheral blood monocytes and splenic macrophages but the quantity secreted was higher in the monocyte fraction. Other monokines such as transforming growth factor (TGF) do not appear to be induced by *T. congolense* infection, suggesting that the increased IL1 production was not attributable to an overall, non-specific stimulation of monokine production. Assays to titrate tumour necrosis factor (TNF) and interleukin 6 (IL6) have been developed, but attempts to quantitate these factors in the bovine system have not, so far, been successful.

Other assay systems were developed to measure the phagocytic and microbicidal potential of bovine monocytes and granulocytes using two-colour cytofluorimetric analysis. Peripheral blood monocytes were labelled with specific monoclonal antibodies conjugated to phycoerythrin and the phagocytic cells detected following uptake of fluorescein-labelled bacteria. Thus analysis of the phagocytic cell population could be undertaken without prior purification, a factor which offers many advantages.

These assays will be used to determine if breed susceptibility to *T. congolense* infection is associated with differences in activation of the mononuclear phagocytic system.

TR3.7 *In vitro* analysis of erythrophagocytosis in Boran calves following *Trypanosoma congolense* infection

Student: V.O. Taiwo
Supervisors: L.L Logan-Henfrey, D.J.L. Williams

A standard chromium 51 (Cr^{51}) release assay was used to assess erythrocyte phagocytosis and cytotoxicity by monocytes purified from peripheral blood (PBM) and macrophages (MQs) from the spleen and sternal bone marrow (BM) from eight Boran calves infected with *T. congolense* IITat 3.1 and from two controls. PBM and MQs from the spleen and BM were cultured for 24 or 96 hours respectively, before use. Cr^{51}-labelled erythrocytes, from one control calf, were incubated with the MQs at an effector cell:target cell ratio of 1:20 for four and eighteen hours in medium alone or in medium containing 1:120 dilution of post-infection serum taken from a Boran calf previously infected with *T. congolense* IL13-E3. The amount of radioactivity released into the supernatant was used to assess cytotoxicity whilst phagocytosis was determined by the amount of radioactivity taken up by MQs after unphagocytosed erythrocytes in the cell pellet were lysed by warm Tris-ammonium chloride.

MQs were incubated with unlabelled erythrocytes, harvested on slides and stained with Diff-quik for light microscopic examination.

In vitro erythrophagocytosis by PBM, spleen and BM MQs increased fourteen days post-infection (d.p.i.) in an antibody-independent manner. In some instances, the presence of post-infection serum resulted in an increased rate of erythrophagocytosis. From thirty five d.p.i. onwards, both phagocytosis and cytotoxicity increased by four-fold above control animals.

Morphologically, MQs became progressively enlarged, had extensively vacuolated cytoplasms, frilled cell membranes and pseudopodia. After four or eighteen-hour cultures, MQs usually contained two to four erythrocytes in vacuoles, often in close proximity to the nucleus. From 21 d.p.i. onwards, MQs taken from the Ficoll interface contained red cells. Lymphocytes, neutrophils and eosinophils, which occasionally contaminated the MQ cultures, were also phagocytosed.

Our results suggest that erythrophagocytosis occurred as a result of an activated mononuclear phagocytic system rather than as a result of the removal of erythrocytes which had been damaged by parasite antigens.

TR3.8 Analysis of lymphocyte populations in *Trypanosoma congolense* - infected Boran cattle

Scientists: J. Naessens, D.J.L. Williams

We measured changes in T and B lymphocyte subpopulations in different lymphoid organs of *T. congolense* -infected cattle. We used monoclonal antibodies against bovine CD4, CD8 and CD2 to measure T-cell subsets, and MAbs against bovine CD5 (Ly-1), CD11b (Mac-1), immunoglobulin (Ig) and WC3 to measure B-cell subsets.

A small percentage (15 - 25%) of peripheral blood B cells from normal animals expressed CD5, and a higher proportion (15 - 35%) expressed CD11b. Using three-colour fluorescence analysis, we showed that the $CD11b^+$ B cells included the $CD5^+$ B cells. These two subpopulations were also found in spleen (0 - 8%), but were undetectable in lymph nodes, Peyer's patches and tonsil.

Following infection with *T. congolense*, the percentage of $CD5^+$ and $CD11b^+$ B cells remained constant until day fourteen, then increased rapidly to reach 60% or 80%, respectively, by day twenty-one. This increase occurred about one week after the rise in parasitaemia. A similar rise in $CD5^+$ and $CD11b^+$ B cells was observed in the spleen but not in other lymphoid organs.

There was no change in the CD4:CD8 ratio in lymph nodes and blood of cattle during infection, but an increase in $CD8^+$ T cells was observed in the spleen. This increase was not observed when using an anti-CD8 MAb which was specific for the heterodimer form of CD8. This implies that some spleen cells, including some $CD4^+$ T cells, must have acquired one CD8 polypeptide chain, which was expressed as a homodimer on the cell surface.

TR3.9 Specificity of immunoglobulins produced by CD5$^+$ B cells in cattle during trypanosome infections

Research Associate: J. Newson
Scientists: J. Naessens, D.J.L. Williams

An increase in the numbers of CD5$^+$ B cells in peripheral blood and spleen occurs during *Trypanosoma congolense* infections of cattle. Between fourteen and twenty days after infection, levels of CD5$^+$ B cells rise from 5 - 30% to 40 - 70%. In the mouse, these cells have been associated with increases in IgM levels and autoantibody production. Since autoantibodies directed against erythrocytes and platelets have been reported in trypanosome infections of man, cattle and mice, we investigated the function of these cells to determine whether they could be contributing to the pathology of trypanosomiasis.

In mice, bromelain-treated red blood cells (RBC), which are uniquely recognised by CD5$^+$ B cells, have been used as targets for autoantibodies in haemolytic plaque assays. It was found that spleen and lymph node cells from *T. congolense* -infected cattle produced antibodies against bromelain-treated RBC, and occasionally against normal RBC. However, since polyclonal activation of spleen and lymph node cells by pokeweed mitogen also induced antibodies against bromelain-treated RBC, the response by infected cattle might be due to polyclonal activation.

Spleen and lymph node culture supernatants and cell lysates from cattle at different stages of infection were examined for antibody which bound to intact RBC, RBC ghosts and platelets and for rheumatoid factor. Activity against these was minor and sporadic, as found in *T. vivax* infections in cattle, and we conclude that autoantibodies are unlikely to be a major factor in the anaemia of trypanosomiasis. Moreover, the majority of B cells producing IgM and those producing antibody against bromelated RBC were from the CD5$^-$ population. A role for CD5$^+$ B cells in trypanosome infections has therefore still to be defined.

TR3.10 An assay to detect antibodies specific for conformational epitopes on the surface coat of *Trypanosoma congolense*

Scientist: D.J.L. Williams
Technician: M. Opollo

To determine whether antibodies specific for conformational epitopes of the variable surface glycoprotein (VSG) coat of trypanosomes are involved in controlling trypanosome infections in cattle, it is essential to have a reliable assay in which these conformational epitopes are conserved. Our studies have revealed that the glutaraldehyde or formalin fixation techniques usually used in assays with *Trypanosoma brucei brucei* resulted in the loss of the conformational epitopes of *T. congolense*, irrespective of the method used to purify trypanosomes from blood. Thus the only assay available in which the conformational epitopes of *T. congolense* are conserved appears to be the complement lysis assay (CLA), in which live trypanosomes are used. However, the CLA is tedious and limited in that complement fixing antibodies alone are detected and in order to determine which antibody class mediates killing, immunoglobulin separations of each test sample are required.

Therefore we have devised an assay which retains the specificity of the CLA but incorporates the advantages of a conventional ELISA. Briefly, living trypanosomes were incubated initially on ice, with 1% normal rabbit sera, washed, and then with dilutions of heat-inactivated test sera, taken from *T. congolense*-infected cattle. Trypanosomes were washed thoroughly, transferred to an ELISA plate and fixed overnight with 1% formalin. The ELISA plates were then probed with alkaline phosphatase-labelled monoclonal antibodies, specific for bovine immunoglobulins, to detect antibody which had bound to the living trypanosomes.

Preliminary results suggest that this assay is specific for conformational epitopes of *T. congolense* and should prove useful in measuring the quantity and isotype of variant-specific antibody in *T. congolense*-infected cattle.

TR3.11 Interaction between live trypanosomes and specific antibodies to exposed VSG epitopes

Student: D.C.W. Russo
Supervisor: D.J.L. Williams

Clones of *Trypanosoma brucei*, *T. vivax* and *T. congolense* were tested for their ability to internalise surface bound IgG and IgM monoclonal antibodies and polyvalent antisera via the flagellar pocket. All the clones tested were found to be capable of antibody internalisation. $F(ab)_1$ monomers directed toward exposed VSG epitopes of *T.b. brucei* S427 clone 22 were also cleared from the cell surface via the flagellar pocket. Trypanosomes continued to clear antibody from their surface under conditions of complete inhibition of protein synthesis or in the presence of cytoskeleton inhibitors.

Internalisation of antibodies was rapid since trypanosomes incubated on ice with limiting dilutions of antibody (0.5 µg Ig /5 x 10^6 cells in one ml culture), when resuspended in antibody-free medium at 37°C, cleared VSG-bound antibody in less than 8 minutes. However, this clearance was delayed by up to 90 minutes if the amount of antibody used was increased to 50 µg/ml.

Following internalisation through the flagellar pocket, electron microscope studies detected anti-VSG antibody intracellularly in tubules and vesicles, some of which were associated with one face of the Golgi apparatus. $F(ab)_1$ monomers were detected in the flageller pocket after modulation from the cell surface but were not detected intracellularly.

Giemsa stains of *T.b. brucei* S427 clone 22 which had been cultured in the presence of VSG-specific IgG, revealed that antibodies blocked cell division in telophase, the number of cells bearing two nuclei and two flagella increased from 5 - 10% to 80% of the total population after eight hours incubation. This block was irreversible if trypanosomes were incubated with the VSG-specific IgG for more than 48 hours.

TR3.12 Analysis of antibody responses in different lymphoid organs taken from *Trypanosoma congolense* -infected Boran calves

Scientists: D.J.L. Williams, J. Naessens
Research Associate: J. Newson
Technicians: M. Opollo, F. McOdimba, J. Maloba

Eight 6-month-old Boran calves were infected with *T. congolense* IINaT 3.1. One animal was slaughtered every seven days and mononuclear cells purified from the left prefemoral (draining the infection site) and right prescapular lymph nodes, the spleen and the peripheral blood. 10^6 cells from each organ were cultured in one ml of complete medium and the supernatants (SN) harvested after 5 days. Also, 10^8 cells from each organ were lysed in one ml of lysis buffer and the SN harvested.

Culture SN, lysates and sera from each animal were analysed for presence of immunoglobulin (Ig) and parasite-specific antibody. Parasite-specific antibody was detected using whole trypanosome lysate extract in an ELISA.

The results showed a massive IgM response in the spleen which occurred as early as day fourteen post infection, i.e., during exponential growth of the first wave of parasitaemia. Most of the antibody produced was not specific for the whole trypanosome lysate extract. Therefore the IgM must be either specific for the variable surface glycoprotein (VSG) coat of the trypanosome, or produced in response to a polyclonal activation of splenic B cells.

Activation of lymph node cells was less marked and occurred after day twenty one.

Low levels of parasite-specific IgG antibody were detected in the serum on day fourteen in half the animals tested. All the animals tested had high antibody titres by day twenty-one, titres ($\log_{10}$) = 3.

We are currently investigating the VSG-specific response.

TR3.13 Humoral immune responses in N'Dama and Boran cattle following a secondary challenge with *Trypanosoma congolense*

Scientist: D.J.L. Williams
Research Associates: J. Newson, J. Scott
Technicians: M. Opollo, H. Gathuo

A group of N'Dama (trypanotolerant) and a group of (susceptible) Boran cattle were given a secondary challenge with *Glossina morsitans centralis* infected with *Trypanosoma congolense*, clone IL13-E3, 32 months after the primary challenge. A group of age and sex-matched Borans which had had no previous infection was included as the infection control.

The N'Dama experienced a mild infection with low parasitaemia and no anaemia. The rechallenged Boran group had high parasitaemia and chronic anaemia but three out

of five eventually self-cured. The primary infected Borans had high parasitaemia, severe anaemia and all required drug treatment by day 56 to prevent death.

Total IgM levels increased from around 1 mg/ml^{-1} prior to infection to over 12 mg/ml^{-1} in some individuals, with a more marked increase in the N'Dama compared to the Boran groups. Total IgG_1 levels increased in all three groups. No increases in the IgG_2 levels were detected.

A complement lysis assay was used to detect variable surface glycoprotein (VSG)-specific antibodies against trypanosome populations taken at different times throughout the infection. The rechallenged N'Dama group produced a more efficient VSG-specific antibody response than the primary or secondary challenged Boran groups. In the N'Dama, the majority of the antibodies which lysed the populations of parasites tested were of the IgG class, suggesting either that the response in N'Dama switched from IgM to IgG earlier than in the Boran or, that in the primary infection, a memory response was generated to the trypanosome populations which we tested.

TR3.14. Non-VSG antigens recognized by *Trypanosoma congolense*-Infected N'Dama and Boran cattle

Scientists: E. Authié, D.J.L Williams
Technicians: N. Saigar, D. Muteti

Animals infected with African trypanosomes produce antibodies against both variable surface antigens and non-variable common antigens. A more efficient immunological response to the VSG antigens may account for the better control of parasitaemia in resistant cattle. Recognition of non-VSG antigens common to trypanosomes of different variable surface antigenic types might contribute to host defence against the effects of infection. A study was therefore undertaken to identify the antigens of *Trypanosoma congolense* which give rise to antibody formation in cattle, and to determine whether the recognition of particular antigens correlates with the course of infection. A Western blot analysis of the immune response of five N'Dama (*Bos taurus*) and five Boran (*Bos indicus*) was performed during an experimental reinfection with *T. congolense*, clone IL13-E3. The various antigens of *T. congolense* were mainly recognized by IgG1. IgG1 from the sera of infected N'Dama showed a wider range of reactivities than that observed in the Boran. Three major antigens of *T. congolense* were identified: Two proteins of relative molecular mass 69 kDa and 23 kDa elicited a strong antibody response 0 - 4 days after the detection of parasitaemia. The IgM and IgG2 anti-69 kDa responses were not different in the two groups of cattle, but the IgG1 response was higher in the N'Dama. A 33 kDa protein was recognized by IgG1 already present prior to reinfection in the sera of all the N'Dama, and in sera of the two Boran which were clinically least affected. Antibodies against this antigen were not detected in the sera of the two Boran which required treatment. All the N'Dama, but none of the Boran, had recognised this protein during the first experimental infection with *T. congolense* IlTat 3.1, in 1985. The ability to mount an IgG1 response to the 69 kDa and 33 kDa proteins of *T. congolense* may therefore be related to resistance to disease. Work is in progress to characterise these proteins and to determine their possible role in host-parasite interactions.

TR3.15 The immunological basis of resistance to trypanosomiasis in mice

Research Associate: J. Newson
Scientist: S.J. Black

By six days after infection of susceptible C3H/He and resistant C57Bl/6 mice with *Trypanosoma brucei brucei* GuTat 3.1, splenic plasma cell responses of both strains of mice were similar in terms of plasma cell number, intracellular Ig, Ig secretion, Ig class and Ig specificity for exposed variant surface glycoprotein (VSG) epitopes on the infecting organisms, despite higher parasitaemia in the C3H/He mice. By seven days after infection, however, although splenic plasma cells from both strains of mice had amplified their Ig responses, those from C57Bl/6 mice (which cleared parasites from their bloodstream by six to seven days after infection) contained and secreted 3 - 5 times more Ig specific for exposed VSG epitopes than those from C3H/He mice which did not clear parasites from their bloodstream. The main amplification of the VSG-specific response in C57Bl/6 mice occurred after, and not before, parasite clearance.

Trypanosomes *in vitro* were shown to absorb large amounts of VSG-specific antibody produced by splenic plasma cells. This probably accounts for the undetectable serum VSG-specific levels found in C3H/He mice, whose parasitaemia is 2.5 - 10 times higher than that of C57Bl/6 mice. However, removal of trypanosomes from spleen cell cultures from seven-day-infected C3H/He mice did not result in any change in amount of VSG-specific antibodies detected. It seems that between six and seven days after infection, the C3H/He mice selectively lose the capacity to support development of plasma cells specific for exposed VSG epitopes on the infecting organisms and that this is a consequence rather than a cause of differences in the peak levels of first wave parasitaemia.

TR3.16 Trypanotolerance in buffalo: *In vivo* and *In vitro* observations.

Scientists: J.G. Grootenhuis*, S.J. Black, D. Reduth**, R.O. Olubayo*

(*Veterinary Research Laboratory, Wildlife Disease Section, Kabete, Kenya)
(**Swiss Tropical Institute, Basel, Switzerland)

Previous observations showed that *Trypanosoma congolense* infection in buffalo as compared to infection in Boran cattle is characterised by reduced skin reactions at the tsetse bite site, prolonged prepatent period (twenty days in buffalo versus twelve days in Boran), no decrease in PCV, 100 - 1000 fold reduced parasitaemia and a detectable neutralising antibody response prior to onset of parasitaemia. Intravenous infections with *T. congolense* IINat 3.1 in buffalo revealed that the parasite growth control is not initiated at the skin level, but is due to systemic factors.

In vitro experiments comparing growth of *T. brucei* in sera from cattle and buffalo indicated the presence of a toxic factor for trypanosomes in buffalo serum. A protein molecule with a molecular weight of 185 kDa was purified from buffalo sera. This protein killed *T. brucei, T. vivax* and *T. congolense in vitro*.

Future studies will aim at studying the kinetics of this molecule *in vivo* and its contribution to trypanotolerance shown by buffalo.

TR4.1 BoLA and trypanotolerance

Scientists: S.J. Kemp, A.J. Teale. In collaboration with the International Livestock Centre for Africa
Technician: J. Mwakaya

The highly polymorphic bovine lymphocyte antigen (BoLA) system might be expected to provide a means of marking genes which contribute to the performance of cattle under trypanosome challenge. A prerequisite for such a study is groups of cattle on which there is extensive disease and performance data. The ILCA/ILRAD trypanotolerance network is able to provide this.

In 1988, a study was made of N'Dama cattle from Zaire. Disease and productivity information on sixty four breeding cows over a period of forty months was analysed. This rather small data set revealed some apparently powerful associations between productivity and reactivity with three BoLA-typing reagents.

These results were considered to be of sufficient potential importance to require further investigation. A second study in an independent population was therefore conducted in the N'Dama herd of OGAPROV near Franceville in Eastern Gabon. Approximately 1,000 animals were sampled. So far, only the data relating to 130 of these have been examined.

In this group, reactivity with one of the sera which showed significance in Zaire was too rare to examine, another showed a significant but reversed effect and one (EDA013) continued to show association with trypanosensitivity.

Closer examination of EDA013 reactivity revealed serological differences between the Zaire and Gabon populations. It therefore seems likely that the gene product detected in Gabon is not the same as that detected by EDA013 in Zaire. This raises interesting questions about the mechanism of the association. If this preliminary evidence for an association is supported by further data, then this would suggest a direct involvement of BoLA in trypanosensitivity.

These preliminary results are promising and will be pursued. However, it must be remembered that there appear to be multiple genes involved in trypanotolerance and MHC or MHC-linked genes cannot be the sole factors. However, they may make a useful contribution to an approach based on a range of markers spread throughout the genome.

TR4.2 Further studies of polymorphism of the bovine CD4 T lymphocyte antigen

Scientists: W.I. Morrison, C.J. Howard*
Research Associate: C.A. Hinson
Technician: E. Awino

(*AFRC Institute for Animal Health, Compton Laboratory, Compton, UK)

The detection by monoclonal antibodies (MAbs) of polymorphic determinants on leukocyte differentiation antigens limits the use of such antibodies as phenotyping reagents. However the polymorphic determinants provide useful markers for studies of the genetics of resist-

ance to disease. During the course of studies carried out to characterise MAbs specific for the bovine CD4 antigen it was noted that, of six MAbs tested, two (CC26 and ILA18) reacted with cells from only 87% of cattle. Studies of half- and full-sibling families produced from bulls and dams of defined phenotypes, indicated that the two MAbs recognise a distinct allelic form of bovine CD4 and that the alleles are co-dominantly expressed. The frequency of the allele detected by these MAbs was similar in Boran, Friesian and N'Dama cattle. In the last year, biochemical studies have revealed a second level of polymorphism, namely in the relative molecular mass of the CD4 antigen.

Two allelic forms of CD4 with relative molecular masses of 52/57 kDa and 57/64 kDa have been detected by immunoprecipitation. All of the $CC26^+$ alleles examined were of the 52/57 kDa form. The 57/64 kDa form has not been detected in Friesian cattle, but in Boran cattle represented about 80% of the $CC26^-$ alleles examined. The CD4 antigens of N'Dama cattle have not yet been examined biochemically. These initial observations indicate that the high molecular weight form of the bovine CD4 antigen may be restricted to *Bos indicus* cattle and thus may be a useful marker in genetic studies of trypanotolerance.

TR.4.3 Embryo transfer in Boran (*Bos indicus*) cattle and N'Dama cattle

Scientists: T. Jordt, A.J. Teale, H. Hirumi, K. Hirumi
Student: E.Lorenzini

The studies on the relationship between embryo production and dosage of follicle stimulating hormone continued. Boran cows were superovulated with 25.2 mg Folltropin (Vetrepharm, Canada) or 1.3 IU Ovagen (Immuno-chemical Products Ltd, New Zealand).

The average embryo production per superovulation (AEPS) was 2.9 when superovulating with 25.5mg Folltropin and 3.7 (preliminary data) when using 1.3 IU Ovagen. Both results are better than the result obtained when superovulating with FSH-P (28mg, 32mg & 36mg) or Folltropin (19.5mg & 22.4mg), AEPS: 2.2.

The bovine genome (N'dama cattle)

Four N'Dama bulls and twelve boran cows were selected for this project, with each bull being allocated four cows. The objective is to produce large families from each bull-cow combination; the cows will therefore be superovulated four times yearly. The first calves are due to be born in January 1990.

Embryo transfer in N'dama cattle

The first calves from the second generation of N'Dama cattle (total twenty-six calves), which were produced by embryo transfer, reached maturity in 1989. After having confirmed that the two heifers, born in September 1987, had regular oestrus-cycles and also that their reproductive tracts were suitable for non-surgical embryo-collection, they were superovulated with 1.1 IU Ovagen, but only unfertilized ova were recovered.

In vitro culture of early embryos

In vitro culture of early embryos, which were removed from the oviducts of superovulated Boran cows forty-eight to fifty-two hours after being artificially inseminated, were studied.

Epithelial cell monolayers were made from surgically removed oviducts from Boran cows. The monolayers were grown and maintained in either B-2 Menezo medium (supplemented with 0.25% lactalbumin hydrolysate and 10μg/ml serotonin) or M-199, both supplemented with 7.5% fetal bovine serum and 1% antibiotic. They were cultured in a humidified atmosphere of 2.0% CO_2 in air at 37°C. Both media supported the cells equally well.

B-2 Menezo (supplemented as above), M-199 and Dulbeccos Modified Eagle medium, all supplemented with 7.5% fetal bovine serum and 1% antibiotic, were used to culture early embryos on the ephthelial monolayers. Forty-nine embryos/ova (1 - 4 cells) were recovered from the oviducts. Twenty seven embryos showed at least one cleavage of cell/s. Six of these, all cultured in M-199, passed the twelve cell block stage. Four developed on fresh oviduct epithelial cells monolayers, whereas two developed on frozen-thawed epithelial cell monolayers. Two embryos were transferred into recipients after four days in culture.

TR4.4 BoLA-PC: Software for the storage and analysis of bovine tissue-typing data

Scientists: S.J. Kemp, A.J. Teale, G. Gettinby*

(*Department of Mathematics, University of Strathclyde, Glasgow, UK)

A working set of computer programmes for the IBM-PC for the manipulation of serological tissue-typing data has been in place in ILRAD since 1984. The programmes have now been extensively rewritten in collaboration with the University of Strathclyde and have been released to a number of BoLA labs around the world under the name "BoLA-PC". The improvements to the software over previous versions are predominantly in the area of the "user interface". The menu system has been improved and provided with comprehensive but unobtrusive help facilities.

In ILRAD, approximately 3100 cattle have been tested against some 190 BoLA-typing reagents since 1983. This is equivalent to 589,000 cell/antibody reactions and all of these data, together with additional information on the individual animals, are stored in the form of BoLA-PC data files. This is believed to be the most comprehensive set of tissue-typing data on African cattle in existence.

The software is being used to provide information related to two areas. The first of these is work on the cellular immunity to *T. parva*. A group of cattle and cell-lines of known BoLA-phenotype has been built-up over the last few years and this information is being used by a number of ILRAD projects. The second area in which the BoLA-PC programmes are being used is in the search for associations between BoLA and trypanotolerance.

The latter of these two projects has tested the capabilities of the software over the past two years. Since 1987 1,223 West African cattle have been typed in connection with this study. BoLA-PC has been used to receive the data from an automated typing system,

to incorporate disease and productivity data and to perform relative risk and gene frequency analysis.

The future development of BoLA-PC depends on the trends in research techniques; it seems likely that the importance of serology in this field will decline. However, the advances in microcomputer hardware and software, particularly the advent of parallel processing, offer the possibility of dramatic improvements in performance in the critical areas relating to cluster analysis.

TR4.5 Haemopoietic and lymphoid changes in Boran cattle infected with *Trypanosoma congolense*

Scientist: L.L. Logan-Henfrey, J. Naessens, M. Shaw, D.J.L. Williams, S.K. Moloo
Student: V. Taiwo
Research Associates: J. Magondu, N. MacHugh, J. Scott
Technicians: F. McOdimba, J. Kamau, M. Opollo, P. Theuri, J. Maloba

Even though anaemia is a hallmark of bovine trypanosomiasis, few publications on the disease have included observations on bone marrow. Consequently, very little is known about the bone marrow response during acute trypanosomiasis and even less is known of what happens during chronic infection In an earlier report, we infected eleven Ayrshire-Guernsey calves with a haemorrhagic isolate of *Trypanosoma vivax* to study haemopoietic response during infection. The acute disease had early onset of elevated parasitaemia ($5x10^6$/ml), followed by rapid onset of severe anaemia, marked pancytopenia, sever thrombocytopenia, and an inadequate haemopoietic response characterised by few reticulocytes in the blood, only mild erythroid hyperplasia, myeloid suppression, and marked hyperplasia of the lymphoid and monocytic cells. There was widespread macrophage phagocytosis of erythroid and granulocytic cells and platelets. In order to confirm whether these pathological findings represented a common pathway of suppressing marrow cell production during acute trypanosomiasis infections in other breeds of cattle and with other species of trypanosomes, the present study was done. Eight Boran calves were infected with *Trypanosoma congolense* (IL 1180) and their haemopoietic and lymphoid response was studied by weekly examination of bone marrow and lymphoid organs during a forty-six day infection. Differential counts were made from sternal marrow smears collected by aspiration biopsy. A panel of monoclonal antibodies recognising bovine leukocyte antigens was used to stain marrow cells and a fluorescence activated cell sorter (FACS) was used to analyse marrow cells. Animals examined between days twenty-one and forty-nine were severely anaemic and leukopaenic. Leukopaenia was due to neutropaenia, as both lymphocytes and monocyte levels in blood were within the normal range. The M:E ratio in bone marrow dropped due to a mild erythroid response and a granulocytic lineage cell suppression. There was a two fold-rise in numbers of lymphocytes and a six-fold rise in monocytes in the marrow by differential counts. The increased numbers of lymphocytes was not detected by FACS analysis. As two-thirds of the lymphocytes seen were large or blast forms, there may have been a failure to detect these cells by FACS due to their immaturity and lack of epitopes for the monoclonal markers used.

TR4.6 Role of the chancre in induction of immunity to tsetse-transmitted *Trypanosoma (Nannomonas) congolense* in goats

Student: V.O. Taiwo
Supervisors: V.M. Nantulya, B.O. Ikede*
Scientist: S.K. Moloo,

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Local skin reactions (chancres) developed in goats at the sites of deposition, by tsetse flies, of metacyclic forms of *Trypanosoma congolense*. The chancres developed much faster and were more pronounced when ten infected tsetse were allowed to feed on a spot as compared to only one fly per spot. The initial host cellular reaction in the chancre was predominantly polymorphonuclear followed at the peak of development of the chancre by a predominantly lymphoblastic and plasmacytic reaction. Trypanosomes were found in varying stages of division as well as degeneration in chancre biopsies taken at various days post-infection. The majority of the trypanosomes recovered from the chancre tissue fluid were found to bear the same variable surface glycoprotein (VSG) epitopes as the corresponding metacyclic trypomastigotes for as long as thirteen days post-infection (p.i.), as revealed by direct immunofluorescence using anti-metacyclic VSG hyperimmune sera and monoclonal antibodies. immunisation of goats with metacyclic trypanosomes, by exposure to infected tsetse bites followed by treatment of the infected goats on day thirteen p.i., gave rise to the development of complete protection to homologous tsetse-transmitted challenge, while immunisation by intravenous inoculation of the metacyclics did not induce such protection. Chancre formation would thus appear to be vital for the induction of comprehensive immune recognition of the metacyclic variable antigen (MVAT) repertoire deposited in the skin by infected tsetse and hence development of protective immunity.

TR4.7 Pituitary-Adrenal Axis in Trypanosomiasis

Student: G. Abebe
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Previous work showed significant differences between the mean concentration of both ACTH and cortisol in preinfection and infected Boran cattle when the pituitary was stimulated with exogenous bovine Corticotropin Releasing Factor (bCRF) After i/v injection of bCRF the mean peak ACTH concentration for preinfection animals was 306.6 pg/ml where as the mean peak ACTH concentration for infected animals was 83.0 pg/ml. Similarly, after injection of bCRF, the mean peak plasma cortisol concentration was 9.3 µg/100 ml in preinfection animals, as compared with mean peak plasma cortisol concentration of 4.8 µg/100 ml in infected animals.

The low concentrations of ACTH and cortisol in response to injection of bCRF in infected animals could be due to either increased turnover of hormones in the plasma, or a less responsive pituitary, or in the case of cortisol, a less responsive adrenal gland. To test this, porcine ACTH was administered to a group of Boran cattle before and during infection. Intravenous injection of pACTH appeared to have a similar effect on the rise of

plasma cortisol concentration in both infected and uninfected animals. Thirty minutes after injection of pACTH the mean peak cortisol concentration for preinfection and infected animals was 9.5 µg/100 ml and 9.9 µg/100 ml respectively.

Our experiments have shown that, (1) in cattle infected with trypanosomes, the pituitary gland is less responsive to the stimulatory effect of bCRF than uninfected animals, (2) trypanosomiasis in cattle does not impair the ability of the adrenal glands to respond to exogenous ACTH. At present the evidence suggests that during trypanosomiasis the low responsiveness of the pituitary-adrenal axis to exogenous bCRF could be due to an impaired pituitary response.

EPIDEMIOLOGY AND SOCIO-ECONOMICS

ESE.1 The definition and characterisation of the impact of livestock disease and its control

Scientists: B.D. Perry, B.E. Grandin, A.W. Mukhebi, J. Delehanty, R. Kruska*
Student: P. Lessard

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This programme is studying the impact of livestock diseases and the implications of their control in Africa. The theme behind the specific research projects is that there is a strong need to better define the current constraints imposed on agricultural systems in Africa by livestock diseases, and to predict the likely consequences of reducing or even removing these constraints. A variety of well-researched methods are available to control many livestock diseases prevalent in Africa, including tick-borne diseases and trypanosomiasis to which ILRAD is currently paying particular attention. This research programme is attempting to define the consequences of widespread application of these and any new (immunological) technologies in epidemiological, socioeconomic and environmental terms. In addition, it aims to characterise the circumstances which will permit the successful widespread application of these methods in the field, and which will avoid deleterious consequences.

This continues to focus on the effect of improved control of theileriosis and other tick-borne diseases in eleven countries of eastern, central and southern Africa. However, much of the methodology being developed will be appropriate for application in other areas and to other livestock diseases. The principal obstacles to these assessments, particularly with vector-borne diseases where conditions for vector survival and development vary considerably in the continent are the marked and independent heterogeneity in disease epidemiology, farming systems and socioeconomic status within the continent. This means that while very site-specific studies are necessary in order to attain the required level of detail, extrapolation of the results will only be valid in other areas that share the same or similar sets of circumstances, in terms of epidemiology, farming system, socioeconomic status and environment. The research being carried out thus has three main focuses.

1. Baseline data continue to be assembled and analysed on livestock production, disease incidence and epidemiology and the socioeconomics of livestock production and disease control for the eleven study countries. (ESE 2).

2. Detailed assessments at specific, district level, sites are being carried out on disease incidence and epidemiology, farming systems and farm economics, and the sociological and ecological framework in which livestock production occurs. These will be followed by an evaluation of the impact of a disease control intervention on defined multidisciplinary parameters. This research is being carried out with a focus of East Coast fever control in two districts of Kenya, Kilifi and Uasin Gishu. (ESE 3, ESE 4, ESE 6).

3. The third focus of the research is on identifying the key parameters in disease epidemiology, farming systems and socioeconomic status which can be used to characterise the impact of disease control, and to develop methods of extrapolation, based on this characterisation, to wider areas of Africa. This approach involves studying the use of geographic information systems (GIS) in developing extrapolation methods. So far, the only subject studied in this way has been theileriosis epidemiology, with a continental level collation of databases on the subject and their manipulation in a GIS. It is anticipated that

this work will proceed in several directions. Firstly, theileriosis epidemiology studies also are being done at a country level (Zimbabwe, ESE 8) and at a district level (Kenya, ESE 5). Studies of disease incidence and epidemiology will be developed at the same three levels of resolution (continent, country, district or equivalent) for other tick-borne diseases, and eventually it is hoped for other livestock diseases. Concurrently, it is hoped to develop and expand the subject components of GIS studies at these three levels of resolution to include farming systems and socioeconomics. Such studies have been already initiated in Uasin Gishu and Zimbabwe.

In addition to these three areas, preparation is underway for a study of the potential environmental ramifications in Africa of improved control of major livestock diseases. Broad categories of possible impact have been identified, which include among other things changes in livestock numbers and income levels (both an increase and a decrease), and a data review and collation process will be conducted over the next year, leading to formal hypothesis generation.

ESE.2 Macro socioeconomic statistics of countries affected by East Coast fever

Scientists: A.W. Mukhebi, R. Kruska*

(*United Nations Environment Programme, Nairobi, Kenya)

East Coast fever (ECF) is a major disease of cattle and has been reported in eleven countries in Eastern, Central and Southern Africa. These countries are Kenya, Uganda, Sudan, Tanzania, Rwanda, Burundi, Zaire, Zambia, Malawai, Mozambique and Zimbabwe. Intensive research activities aimed at developing improved methods for the control of ECF are being conducted by several institutions, including ILRAD ,in several countries. Assessment of the impact of such improved ECF control methods at macro (national) levels will require country level, in addition to micro (farm/production system) level socio-economic data. In addition, these data will be used for reviews of the role of livestock and the importance of livestock diseases as a constraint in the economies of the target countries.

In this study, country level socio-economic data continue to be systematically compiled from secondary sources. These sources include latest available country reports and publications by international organisations and institutions such as the World Bank, the Food and Agriculture Organisation of the United Nations, the International Livestock Centre for Africa, aid missions, donor agencies, other international research centres, government ministries and universities.

The data are entered and stored in a micro-computer spreadsheet by source, year, unit of measurement and country. They are organised under five headings: demography, economic indicators, general livestock and cattle. They are being digitised in a Geographic Information System (GIS) for display of information on the economics of livestock in the target countries.

Demographic data contain twenty-two items on human population, population distribution in rural and urban areas and labour force. Economic indicators consist of thirteen items pertaining to gross national products, gross domestic product, inflation rates, life expectancy and physical quality of life index. The general agriculture section consists

thirty-three items on land, land use, crop production, consumption and trade. The general livestock and cattle data include 191 items on domestic animal populations, production, consumption, trade, health expenditures and staffing. These data are continuously updated and expanded as new data become available.

ESE. 3 The impact of theileriosis control in Kilifi, Kiambu and Uasin Gishu districts of Kenya

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Following approval by the Department of Veterinary Services, Kenya, to conduct pilot immunisation using the *infection and treatment* method in smallholder farms in Kilifi district, initial studies to define the agricultural systems, the role of livestock in agriculture, the prevalence of disease and the importance of tick-borne diseases in particular have been initiated in collaboration with the Kenya Agricultural Research Institute (KARI) and the International Livestock Centre for Africa (ILCA). These studies form part of a broader research project coordinated by KARI and ILCA on constraints to smallholder dairy development in the sub-humid coastal zone.

In Uasin Gishu district, similar studies to define agricultural systems and disease epidemiology have been initiated.

In Kilifi district, Kaloleni division was selected as the study area. The division spans three agroecological zones, coconut/cassava (CL3), coconut/cashew (CL4) and semi-arid coastal lowlands (CL5). Study design has been based on the following sequence:

1. Farm-level socioeconomic research to describe the major farming systems in the zone.
2. Livestock census study
3. Study on the prevalence of tick-borne diseases, trypanosomiasis and other disease and nutritional constraints to cattle production.
4. Farm economics study, on a sample of farms representative of the three agro-ecological zones, cattle types and cattle grazing systems, and administered by personal interview questionnaire.
5. Ethnoveterinary studies of farmers' understanding of disease and disease control.

In Uasin Gishu district, an equivalent of stage 1 above has been run, using a different methodology. Broad characterisation of farming systems using potential key variables for subsequent stratification of studies was carried out by an interview survey of key informants throughout the district.The main features of Kaloleni division are small farm size (mean 3.7 ha), with 84% of respondents forming less than 5 ha, and a small proportion of farmers owning cattle (about 8%). About 13% of cattle owning farms have grade cattle, and 79% have zebu, the remainder keeping both grade and zebu. A total of 770 grade cattle are kept in 141 herds. The zebu cattle population is almost 37,000, and the mean zebu herd size

32. There are distinct differences by location and agroecological zone, with a large number of the zebu population in the semi-arid zones. Interestingly, only 13% of the grade cattle are managed on zero-grazing, with 52% being kept on free range grazing. All grade cattle have been blood sampled, and serological assasys for *T. parva, T. mutans, T. taurotragi, B. bigemina, B. bovis, A. marginale* are being run. Stratification for a stratified random sampling of the zebu population for serum sampling is currently underway. Preliminary results of the farm enterprise and ethnoveterinary studies are reported under ESE 4 and ESE 6. Results of of preliminary farming systems characterisation of Uasin Gishu are reported under ESE 5.

Results so far confirm the heterogeneity in farming systems, the role of livestock, and tick-borne disease epidemiology between the two study areas, and also within Uasin Gishu.

ESE.4 Farm-level financial and economic effects of current and alternative tick and tick-borne disease control strategies in Kilifi and Uasin Gishu districts of Kenya

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This study is part of the wider study on the impact of theileriosis control in Kaloleni division of Kilifi district and Uasin Gishu district of Kenya. It aims to identify and quantify the0major types of crop and livestock farm enterprises, their input-output and profitability relationships, the proportion of animal health costs in the livestock enterprise budgets, the proportion of tick and tick-borne disease (TBD) control costs in the animal health cost component, and the contribution of livestock to the household economy total farm income, under current and alternative tick and East Coast fever (ECF) control strategies. The information generated will highlight financial and economic effects of tick and TBD and demonstrate justification (or lack of it) for alternative ECF control strategies under varying farm level production circumstances.

In collaboration with researchers from the Kenya Agricultural Research Institute (KARI) data are collected from cross-sectional field studies of representative farm samples to prepare farm enterprise budgets for the major crop and livestock enterprises. Enterprise land, labour and capital inputs and enterprise products are identified, quantified and valued to derive enterprise returns and farm income. Following immunisation, selected enterprise input-output relationships will be monitored for several years to assess immunisation effects on enterprise costs and returns and total farm incomes. Issues of resource ownership and allocation between on-farm and off-farm activities, division of products between household consumption and sale, input-output prices and markets and perceived farmer objectives and constraints are investigated.

In Kilifi district the major crop and livestock enterprise types in Kaloleni division were identified from a collaborative KARI/ILCA/ILRAD farm survey and a KARI/ILCA cattle census. The major cash crops grown were coconuts (90% of farms), cashewnuts (51%), citrus fruits (27%), mangoes (18%) and oil crops (9%). The subsistence crops were maize (92% of farms), cassava (50%), peas (27%), rice (21%), bananas (14%) and beans (10%).

The cattle census covered 1563 herds, of which 88.9% were zebu cattle, 4.7% grade cattle and 6.4% mixed zebu-grade cattle.

A cross-sectional farm enterprise budget study is underway on a sample of farms.

In Uasin Gishu district a reconnaisance survey identifying major factors influencing variations in the farming systems and hence ECF risk has been completed. The district is being stratified by these factors for the purpose of selecting a representative farm sample for a cross-sectional farm enterprise budget study.

ESE.5 Socioeconomic and environmental characterisation in Uasin Gishu district, Kenya

Scientists: J. Delehanty, A. Mukhebi, B. Grandin

In preparation for field trials of theileriosis immunisation by infection and treatment in Uasin Gishu District, a project is underway to describe farming systems there, in order to portray both spatially and across the socioeconomic spectrum the variability in cattle management conditions and strategies likely to impinge on theileriosis risk and its effective control. The project is proceeding in two steps: (1) collection and analysis (quantitative and cartographic) of general data specific to each of Uasin Gishu's approximately 650 Land Registration (L.R) units; and (2) collection and analysis of more detailed, farm-level data obtained from a stratified sample of the first set.

Preliminary results confirm initial assumptions of extreme heterogeneity in farming systems and cattle management practices in Uasin Gishu District. Three variables illustrate this diversity, and suggest the importance of socioeconomic characterisation to the effective implementation of a disease control intervention: (1) Farm size. In twenty percent of the L.R units in Uasin Gishu District the modal farm size is ten acres or less, while in fourteen percent it is 500 acres or more. This is an unusually broad range. Cattle management practices vary substantially between small and large farms, and it will be important to tailor interventions carefully to account for Uasin Gishu's extreme diversity in this regard. (2) Main cattle type. In 47 percent of the L.R. units in Uasin Gishu District exotic cattle dominate, in 37 percent exotic-local crosses dominate, and in 6 percent local cattle dominate. (In 10 percent of the L.R. units information on this subject is thus far unavailable or unreliable). Geographically specific information of this sort is key to the planning of a strategic control program. (3) Land tenure. In only twelve percent of Uasin Gishu's L.R. units do most farmers have title deed to their land. Where farmers do not have title deed, tenure ranges from very secure, to reasonably secure, to tenuous. Tenure patterns matter because the willingness and ability of farmers to invest in livestock technologies that follow logically on an improved disease control package often is a function of the actual or perceived security of their land claim. These examples illustrate the kinds of information sought in this ongoing project, and some of the uses to which that information will be put.

ESE.6 Delivery, adoption, and sustainability of animal health interventions on the farm: Ethnoveterinary studies in Kilifi district, Kenya

Scientists: J. Delehanty, B. Grandin

A sustainable intervention is one that accounts for and adjusts to preexisting practice. Before a new animal disease control method is introduced in any locale, it is important to learn to what farmers there already know or believe about animal diseases, particularly the ones targeted.

In preparation for field trials in Kaloleni Division of the infection and treatment method of theileriosis immunisation, detailed ethnoveterinary information is being gathered from all farmers who presently keep cattle. A lexicon of cattle disease or syndrome names is being assembled using each of the eight languages or dialects spoken there. For each disease or syndrome named, local conceptions of cause, aetiology, prevention, and cure are described. Particular attention is given to locally defined or described maladies that seem to be vector-borne. A major intent is to depict farmers' own understanding of the rudiments and the relative importance of theileriosis, so that the forthcoming intervention might be tailored to meet local concepts.

Among our preliminary findings are the following: (1) Most cattle keepers in Kaloleni Division are able to identify anywhere from two to seven cattle diseases, or assemblages of clinical signs which they recognise as diseases. (2) Diseases and clinical signs are commonly confounded. Many of the separate syndromes identified by farmers may be the product of a single causative organism, but are viewed as distinct by the farmer because clinical signs vary with disease stage, age of animal, or season. In addition, given the confusion between signs and diseases, any one syndrome may go by several names, even within an ethno-linguistic group. (3) The number of farmers who recognise disease names in either English or Kiswahili is minimal. This suggests that farmers in this area may benefit from being shown the advantages of a disease control intervention in their working language, because they cannot be counted on to translate foreign terms over to their working tongue through the morass of confused sign and disease categories. (4) In general, cattle-keepers in Kaloleni Division appear not to draw a strong connection between ticks, tick bites, and the assemblage of clinical signs we would label theileriosis. Many dip their cattle, and know they do so in order to control ticks, but there is a general unwillingness to draw explicit links between this activity and the control of those signs that we associate with theileriosis. This apparent unwillingness is almost certainly a function of the brevity and sparsity of a cattle-keeping tradition among most of the peoples of Kaloleni.

ESE.7 Development of geographic information systems for studying the epidemiology of cattle diseases caused by *Theileria parva*

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It is considered that one of the major obstacles to defining the impact of a disease control measure and the sets of circumstances in which its application is most warranted, is to identify the key differences in epidemiology of the disease in question throughout its distribution. This is particularly challenging when selecting tick-borne diseases as the target of study, as not only do the circumstances defining the disease epidemiology vary markedly in Africa, even within small regions of a country, but so do (independently) the livestock production systems in which the diseases occur.

This study focused on the development of methods for translating epidemiological information on theileriosis into a format that will assist in the design of theileriosis control strategies on a geographic basis. and provide an assessment of ECF risk to livestock in Africa. Geographic information systems (GIS) provide methods of portraying epidemiological parameters pertaining to theileriosis and analysing the complex relationships between them and the disease on a geographic basis.

In a collaborative project with UNEP, the risk assessment has initially been determined at a continental level from a set of parameters for which geographical information is available. These include distributions of cattle, cattle types, buffalo, tsetse fly (*Glossina spp.*) and protected areas. They also include the known and potential distribution of the tick (*Rhipicephalus appendiculatus*) and ECF (clinical disease and/or *T. parva* antibodies). The potential distribution for both the tick and the disease is based on zones of suitability for survival and abundance of *R. appendiculatus*. These zones of suitability are calculated by the climate matching model CLIMEX from an interpolated climate data base and the satellite derived Normalized Difference Vegetation Index (NDVI).

Results provide a systematic geographic representation of available data on a series of determinants of theileriosis epidemiology. In addition, the study has developed methods forbetter defining the environmental parameters which influence tick-borne disease distribution and prevalence in Africa. The ecoclimatic index (EI) calculated by CLIMEX from the interpolated climate database shows a good correlation with *R. appendiculatus* distribution and abundance in eastern Africa. In central Africa, distribution of suitable EI values provides a good indicator of tick distribution, but not of its abundance. This is probably due to the unfed adult ticks entering a day length-induced diapause for a period of three to six months thus avoiding the effects of heat stress during the period of its greatest intensity. Diapause does not appear to occur in the tropical areas of the tick's distribution. The CLIMEX model is least sensitive in South Africa probably due principally to cold stress during the southern winter and a combination of dryness and cold and dryness and host stress in specific regions of South Africa.

The principal climatic indices exerting stress on *R. appendiculatus* over wide areas are heat and dryness. Cold stress restricts distribution in tropical Africa in limited mountain zones only, but becomes a major influence in the temperate south of the continent. Wet

stress appear to have no significant effect on distribution. In eastern Africa, heat and dryness are the dominant stresses. Further south in Zambia, Zimbabwe, Malawi and Mozambique, heat stress plays a limited role, and is confined to the lowland river valley and coastal areas. In these countries, the apparently high dry stress does not reflect the high tick burdens which can be seen during the single wet season. It is during this season that adult instars are predominantly active. The more dessication-sensitive stages (eggs and larvae) thus occur in the season of extreme favourability, whereas the hardier nymphs and adults survive through the dry season. The annual EI calculated on monthly average climatic data thus underestimates climatic suitability in central Africa.

In southern Africa, although heat stress in significant in the Kalahari desert and the Limpopo valley, it is dry and cold stress which exert the greatest influence. The Drakensburg mountains form a cold physical barrier to northerly extensions of the tick's distribution, beyond which the distribution is also limited by dry stress. The next step is to use the databases reported here in integrated analysis to determine disease-risk zones, of value in defining control strategies and research priorities. It is anticipated that by the further supplementation of these data bases to improve parameter quality and to increase the number of determinants studied, such GIS systems will become invaluable tools in aiding decisions on disease control.

ESE.8 Economic assessment of current and alternative tick and tick-borne disease control strategies in Zimbabwe

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The depletion of vegetation cover by overgrazing in most of Zimbabwe's communal areas has created environmental conditions unsuitable for the existence of R. Appendiculatus and considerably reduced the risk of Theileriosis in these areas. Despite this very low risk of Theileriosis in the communal areas, the Department of Veterinary Services (DVS) continues a policy of costly intensive, twice weekly, cattle dipping to control ticks and tick-borne diseases. The DVS is spending over 50% of its annual budget on providing dipping services, most of which is in scarce foreign exchange for the importation of acaricides. However, the DVS is considering alternative control methods for efficacy and cost-effectiveness to justify a revision of its dipping policy for a more efficient allocation of its scarce human, material and foreign exchange resources.

This study was carried out under the auspices of the DVS as part of its collaborative work with the International Laboratory for Research on Animal Diseases (ILRAD).

Resources (facilities, equipment, acaricides, personnel, etc.) expended under current and alternative tick and tick-borne disease control strategies with particular emphasis on the communal areas were identified, quantified and costed. The control methods being considered are intensive dipping (weekly dipping during the summer, fortnightly the rest of the year), reduced dipping (fortnightly in the summer and monthly the rest of the year) and a combination of strategic (weekly dipping for three summer months only supplemented by natural or artificially acquired herd immunity to tick-borne diseases) and minimal dipping (a

dipping equivalent of four acaricide immersions annually). For each control method, target populations and assumptions of likely consequences are being defined.

The DVS budget for the 1988/89 financial year was Z$ 32.5 mil (about US$ 16.3 million). Of this, Z$ 18.5 million or 56.9% was the cost of dipping a communal herd of 4 million cattle in 42 immersions (or Z$ 4.62 per head). In a reduced dipping strategy, it is considered that the number of immersions per annum could be reduced to twenty two without increasing the risk of ECF. This option would release an estimated Z$ 6.5 million for alternative re-allocation within the DVS budget, and reduce the cost of dipping by 35.3% from the current Z$ 4.62 to Z$ 2.99 per head of cattle per year. It is considered that such a strategy would still require excessive acaricide use, and should form part of a transitional phase to a combination of strategic and minimal dipping in the communal areas.

ESE.9 The environmental impact of improved livestock disease control in Africa

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Preparation is underway for a study of the potential environmental ramifications in Africa of dramatically improved control of major livestock diseases. The work is expected to focus geographically on African transect to include several countries comprising a variety of ecological circumstances in which the same disease control measure or measures would likely occur. A central assumption will be widespread effective control of vector-borne cattle diseases under the differing circumstances prevailing in the transect. Broad categories of possible impact have been identified, and a major literature review and data collection exercise will be conducted over the coming year to verify, elaborate, reject, or add to preliminary conjectures, leading in the end to formal hypothesis generation. A preliminary report is anticipated in late 1990, but the modelling of various scenarios in a Geographic Information Systems framework will be an ongoing activity of the Unit into the foreseeable future.

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STAFF PRESENTATIONS AT INTERNATIONAL MEETINGS

C.L. Baldwin

2nd International Veterinary Immunology Symposium, July 1989, Hannover, Federal Republic of Germany. Paper presented: *Cloning and sequencing of cDNA coding for the bovine homologues of CD3 gamma, delta and epsilon.* C.L. Baldwin, K.P. Iams and H. Clevers.

7th International Congress of Immunology, August 1989, Berlin, Federal Republic of Germany. Paper presented: *Theileria-specific antigens recognised by immune lymphocytes.* C.L. Baldwin, D.J. Grab, K.P. Iams and W.C. Brown.

A. Bensaid

2nd International Veterinary Immunology Symposium, July 1989, Hannover, Federal Republic of Germany. Paper presented: *Nucleotide sequence of bovine alpha and beta T-cell receptor cDNA clones.*

7th International Congress of Immunology, August 1989, Berlin, Federal Republic of Germany. Paper presented: *Long-range mapping of the bovine MHC.*

E. Jay Bienen

Annual meeting of the American Society of Tropical Medicine and Hygiene, December 1989, Honolulu, USA. Paper presented: *Metabolic preadaptation of metacyclic African trypanosomes for the mammalian bloodstream.* J. Kiaira, W.R. Fish and E.J. Bienen.

T.T. Dolan

8th International Congress of Protozoology, July 1989, Tsukuba, Japan. Paper presented: *Serum factors and haemophages in recovery of cattle from* ***Theileria parva*** *infection.* T.T. Dolan, P.R. Spooner and L.K. Juma.

FAO/IAEA Seminar - Improving the Health and Reproductive Efficiency of Livestock through Radioimmunoassay and Related Techniques, September 1989, Harare, Zimbabwe. Paper presented: *The effectiveness and use of control measures against East Coast fever.*

J.J. Doyle

Seminar on Agricultural Biotechnology - Opportunities for International Development, May 1989, Canberra, Australia. Paper presented: *Biotechnology and Animal Health.*

Y. Eshita

8th International Congress of Protozoology July 1989, Tsukuba, Japan. Paper presented: *Identification of metacyclic-specific surface antigens expressed in a* ***Trypanosoma congolense*** *cDNA library.* Y. Eshita, P.A.O. Majiwa, H. Hirumi and W.R. Fish.

B.M. Goddeeris

2nd International Veterinary Immunology Symposium, July 1989, Hannover, Federal Republic of Germany. Paper presented: *The influence of the restricting MHC phenotype on*

*the parasite strain specificity of **Theileria parva** - specific cytotoxic T cells.* B.M. Goddeeris and W.I. Morrison.

7th International Congress of Immunology, August 1989, Berlin, Federal Republic of Germany. Paper presented: *Strain specificity of bovine **Theileria parva** - specific cytotoxic T cells is determined by class I MHC phenotype.* B.M. Goddeeris and W.I. Morrison.

H. Hirumi

8th International Congress of Protozoology, July 1989, Tsukuba, Japan. Paper presented: ***In vitro** cultivation of **Trypanosoma brucei** bloodstream forms in a low serum containing medium without feeder layer cells.* H. Hirumi and K. Hirumi.

7th Japan and German Cooperative Symposium on Protozoan Diseases, July 1989, Tokushima, Japan. Paper presented: ***In vitro** propagation of **Trypanosoma congolense** bloodstream forms in a feeder layer-free system.* H. Hirumi and K. Hirumi.

24th Joint Conference on Parasitic Disease, The United States-Japan Cooperative Medical Science Program, August 1989, Madison, USA. Paper presented: *Recent progress in cultivating African trypanosomes.*

B.O. Ikede

20th Meeting of the International Scientific Council for Trypanosomiasis Research and Control, April 1989, Mombasa, Kenya. Paper presented: *Sequential immunohistology of lymphoid organs in cattle infected with a haemorrhagic strain of **Trypanosoma vivax**.* B.O. Ikede, L.L. Logan, W.I. Morrison, and F. McOdimba.

S.J. Kemp

2nd International Veterinary Immunology Symposium, July 1989, Hannover, Federal Republic of Germany. Paper presented: *Lymphocyte Antigens as Markers of Trypanotolerance.*

L. Logan-Henfrey

20th Meeting of the International Scientific Council for Trypanosomiasis Research and Control, April 1989, Mombasa, Kenya. Paper presented: *Haemopoiesis in Ayrshire-Guernesey calves infected with Galana stock of **Trypanosoma vivax**.* L.L. Logan, V.O. Anosa and M.K. Shaw.

25th Trypanosomiasis Seminar of the British Society for Parasitology, September 1989, Glasgow, UK. Paper presented: *The pathology and functions of the bone marrow in **Trypanosoma vivax** infection of ruminants.* V.O. Anosa, L.L. Logan-Henfrey, I.O. Igbokwead and M.K. Shaw.

J.D. Lonsdale-Eccles

Annual Meeting of the American Society of Cell Biology and American Society for Biochemistry and Molecular Biology, February 1989, San Francisco, USA. Abstracts presented:

1. *Endocytosis and peptidolytic activity in **Trypanosoma brucei**.* Y. Verjee, J.D. Lonsdale-Eccles and P. Webster.
2. *Serine esterases of **Trypanosoma brucei**.*

3. *Identification of 10nm "intermediate-sized" filaments in* ***Trypanosoma brucei brucei.*** W.O. Endege, P. Webster, J.D. Lonsdale-Eccles and O.K. ole-MoiYoi.

N.D. MacHugh

2nd International Veterinary Immunology Symposium, July 1989, Hannover, Federal Republic of Germany. Paper presented: *Selection of murine L cells expressing the bovine CD8 antigen following transfection of genomic DNA.* N.D. MacHugh, C.J. Howard, P.G. Toye, A. Bensaid, K.R. Parsons, B.V. Jones and W.I. Morrison.

D.J. McKeever

2nd International Veterinary Immunology Symposium, July 1989, Hannover, Federal Republic of Germany. Paper presented: *Bovine T-cell clones specific for a variable glycoprotein of* ***Trypanosoma brucei.*** D.J. McKeever, B.M. Goddeeris and V. Nene.

7th International Congress of Immunology, August 1989, Berlin, Federal Republic of Germany. Paper presented: *Phenotypic and functional differences between bovine blood monocytes and afferent lymph veiled cells.* D.J. McKeever, N.D. MacHugh, B.M. Goddeeris and W.I. Morrison.

W.I. Morrison

2nd International Veterinary Immunology Symposium July 1989, Hannover, Federal Republic of Germany. Papers presented:

1. *The effects of pathogenic organisms on the functional integrity of the lymphoid system.*
2. *A monoclonal antibody specific for a clonotypic epitope on the bovine T cell receptor.* W.I. Morrison, B.M. Goddeeris, J. Sandio and H. Clevers.
3. *Transfection of bovine Class I MHC genes into mouse L cells: evidence for two Class I MHC loci.* P.G. Toye, N.D. MacHugh, A. Bensaid, S. Alberti, A.J. Teale and W.I. Morrison.
4. *Summary of the first international workshop on leukocyte differentiation antigens in cattle, sheep and goats.* C.J. Howard and W.I. Morrison
5. *An overview of the use of current molecular biological techniques for defining CD antigens in domestic animals.* H. Clevers and W.I. Morrison.

7th International Congress of Immunology, August 1989, Berlin, Federal Republic of Germany. Paper presented: *Monoclonal antibodies identify polymorphisms of the bovine CD4 differentiation antigen.* W.I. Morrison and C.J. Howard.

S.P. Morzaria

8th International Congress of Protozoology, July 1989, Tsukuba, Japan. Paper presented: *Cloning and molecular characterization of* ***Theileria parva.*** S.P. Morzaria, T.T. Dolan, P. Spooner, R.P. Bishop and A. Norval.

N. Murphy

8th International Congress on Protozoology, July 1989, Tsukuba Japan. Paper presented: *The development of molecular biology in African trypanosomes through studies on VSG genes.*

J. Naessens

1st International Workshop on Bovine and Ovine Leukocyte Antigens, July 198, Hannover, Federal Republic of Germany. Papers presented: *Further studies on workshop monoclonals reacting with B cells.* J. Naessens and D.J.L. Williams.

2nd International Veterinary Immunology Symposium, July 1989,Hannover, Federal Republic of Germany. Paper presented: *Bovine lymphocyte activation markers.*

7th International Congress of Immunology, August 1989, Berlin, Federal Republic of Germany. Paper presented: *$CD5^+$ and $CD11b^+$ B cells in trypanosome-infected cattle.* J. Naessens and D.J.L. Williams

25th Trypanosomiasis Seminar of the British Society for Parasitology, September 1989, Glasgow, UK. Paper presented: *CD5 positive B-cells in cattle infected with **T. congolense**.*

V. Nene

8th International Congress of Protozoology July 1989, Tsukuba, Japan. Paper presented: *Molecular approaches to the development of a vaccine against East Coast fever.* V. Nene, K.P. Iams and A.J. Musoke.

J. Newson

25th Trypanosomiasis Seminar of the British Society for Parasitology, September 1989, Glasgow, UK. Paper presented: *Antibody responses of spleen cells from resistant and susceptible mice infected with **T. brucei brucei** GuTat 3.1.*

O.K. ole-MoiYoi

Human Genome Organisation (HUGO) meeting, June 1989, Moscow, USSR. Paper presented: *Points of view from, and potential areas of participation by, developing countries.*

A. Peregrine

20th Meeting of the International Scientific Council for Trypanosomiasis Research and Control, April 1989, Mombasa, Kenya. Paper presented: ***Trypanosoma congolense:** clonal variation in expression of resistance to isometamidium and diminazene.* A.S. Peregrine, G. Knowles, A.I. Fasogbon, J.R. Scott, and S.K. Moloo.

B.D. Perry

Consultation on Information Storage and Retreival in Vector Biology - World Health Organisation, November 1989, Geneva, Switzerland. Paper presented: *The use of geographic information systems for monitoring tick-borne diseases of cattle in Africa.* B.D. Perry, R.A.I. Norval, P. Lessard, T.T. Dolan and K. Kundert.

I.A. Sutherland

20th Meeting of the International Scientific Council for Trypanosomiasis Research and Control, April 1989, Mombasa, Kenya. Paper presented: *Studies on the prophylactic and therapeutic activities of isometamidium chloride (Samorin) in Boran cattle against a tsetse*

transmitted drug-resistant clone of ***T. congolense.*** I.A. Sutherland, S.K. Moloo, P.H. Holmes, and A.S. Peregrine.

25th Trypanosomiasis Seminar of the British Society for Parasitology, September 1989, Glasgow, UK. Paper presented: *In vitro uptake of isometamidium by* ***T. congolense.*** I.A. Sutherland, A.S. Peregrine, J.D. Lonsdale-Eccles, P.H. Holmes and G.M. Urquhart.

A. J. Teale

6th International Conference of Institutions of Tropical Veterinary Medicine, September 1989, Wageningen, The Netherlands. Paper presented: *Potential approaches in the study of the genetic control of trypanotolerance in cattle.*

Biotechnology 1989 - International Conference on the Application of Biotechnology to Livestock in Developing Countries, September 1989, Edinburgh, UK. Paper presented: *Biotechnologies applied to the diagnosis of disease, with special reference to trypanosomiasis and theileriosis of livestock.*

25th Trypanosomiasis Seminar of the British Society for Parasitology, September 1989, Glasgow, UK. Paper presented: *Genetics of Trypanotolerance.*

D.J.L. Williams

20th Meeting of the International Scientific Council for Trypanosomiasis Research and Control, April 1989, Mombasa, Kenya. Paper presented: *Analysis of peripheral blood leukocyte populations in N'Dama and Boran cattle following* ***Glossina morsitans*** *centralis transmitted* ***Trypanosoma congolense*** *infection.* D.J.L. Williams, J. Naessens, S.K. Moloo and J.R. Scott.

2nd International Veterinary Immunology Symposium, July 1989, Hannover, Federal Republic of Germany. Paper presented: *Humoral immune responses in trypanotolerant and susceptible breeds of cattle following secondary challenge with* ***Trypanosoma congolense.*** D.J.L. Williams, J. Newson and J. Naessens.

T. Yanagi

58th Annual Meeting of the Japanese Society of Parasitology, April 1989, Tokyo, Japan. Paper presented: *The approach to the attenuation of* ***Trypanosoma brucei brucei.*** T. Yanagi, H. Hirumi, S.K. Moloo, and K. Hirumi.

31st Annual Conference of the Japanese Society of Tropical Medicine and Hygiene, November 1989, Nagoya, Japan, Paper presented: *In vitro induction of monomorphic bloodstream forms from pleomorphic bloodstream forms of* ***Trypanosoma brucei brucei.*** T. Yanagi, H. Hirumi, and S.K. Moloo.

VISITING SCIENTISTS

Visiting Scientists - Short-term 1989

Name	Project	Dates
Ms. J. Atwood Iowa State University USA	Hemoprotozoal diseases diagnostic techniques	3/1/89 - 3/2/89
Mr. C. Harms Iowa State University USA.	Hemoprotozoal diseases diagnostic techniques	3/1/89 - 3/2/89
Ms. S. Dunlap Boston, Massachusetts USA.	Characterisation of bovine T-cell receptor genes	28/1/89 - 25/7/89
Dr. M. Eguchi TARC, Ibaraki Japan	Immunochemicall characterisation-*T. parva*	17/2/89 - 15/4/89
Dr. Dalgliesh University of Glasgow Scotland, UK	Immunology techniques	18/2/89 - 25/2/89
Dr. H. Dawkins Regional Vet. Lab. Victoria, Australia	ELISA for *T. evansi*	7/3/89 - 30/3/89
Dr. D. Reduth STI, Basel Switzerland	Trypanosome growth factors	18/3/89 - 6/5/89
Dr. T. Baltz University of Bordeaux France	Trypanosome biochemistry	14/4/89 - 17/4/89
Dr. J-C. Maillard C.R.T.A., Burkina Faso	Bovine immmunogenetics	5/4/89 - 22/4/89
Dr. B. Allsopp Molteno Institute Cambridge, UK	*Theileria* genes	17/4/89 - 15/ 6/89
Prof. P. Holmes University of Glasgow Scotland, UK	Trypanosome chemotherapy	25/1/89 - 30/1/89 29/6/89 - 3/7/89
Ms. A. Flanagan University of Glasgow Scotland, UK	MAbs to polymorphic determinants on bovine CD4 molecules	4/7/89 - 15/8/89

Dr. D. Rock Inst. of Agriculture & Natural Resources Nebraska, USA	Transfer of monoclonal antibody hybridoma cell lines to Plum Island, USA	14/7/89 - 24/7/89
Dr. E. Khomba National Institute of Med. Research Tanzania	Evaluation of ELISA for sleeping sickness	15/7/89 - 21/7/89
Mr. T. Collins Johns Hopkins University Baltimore USA	Biochemistry of trypanosomes	17/7/89 - 18/8/89
Mr. J. Kiaira Department of Biochem. University of Nairobi Kenya	Metabolism in metacyclics trypanosomes	17/7/89 - 31/10/89
Dr. S. C. Gouk M.A.F. Hamilton, New Zealand	ELISA technology	19/7/89 - 22/7/89
Profesor J. E. Womack Texas A & M University USA	Bovine immunogenetics	7/8/89 - 12/8/89
Dr. J. F. Turrens South Alabama University USA	Trypanosome enzyme fumarate reductase	14/8/89 - 19/8/89
Professor Y. O. Aliu Ahmadu Bello University Nigeria	Trypanocide pharmacokinetics	21/8/89 - 21/12/89
Dr. R. S. Silayo Sokoine University Tanzania	Trypanocide pharmacokinetics	21/8/89 - 21/12/89
Dr. H. Clevers Utrecht University The Netherlands	Molecular biology of bovine immune genes	24/8/89 - 3/9/89
Dr. R. O. Williams ITG, Karlesruhe West Germany	*Theileria* genes	3/9/89 - 17/9/89

Dr. D. Zilberstein Technion - Israel Inst. of Technology Haifa, Israel	Differential expression of glucose transporters in *T. brucei*	3/9/89 - 30/9/89
Dr. Lucy Mutharia University of Nairobi Kenya	Trypanosome neuraminidase and 2-D gel assays	8/5/89 - 9/6/89 7/8/89 - 14/10/89
Dr. K. Gale CSIRO,Indoorpilly Australia	*Babesia* and *Theileria* antigens	22/9//89 - 27/10/89
Dr. P. K. Thornton Edinburgh School of Agriculture, Scotland, UK	Economic modelling in agriculture	23/9/89 - 27/9/89
Mr. B. Byrom Strathclyde University, Glasgow, Scotland, UK	ECF epidemiology models	25/9/89 - 14/10/89
Dr. G. Gettinby Strathclyde University Glasgow, Scotland, UK	Consultant in biostatistics and modelling	27/3/89 - 14/4/89 29/9/89 - 7/10/89
Professor N. Citri The Hebrew University Jerusalem, Israel	Biochemistry/diagnostics	1/10/89 - 31/3/89
Dr. P. Leegwater Utrecht University The Netherlands	DNA polymerase of *T. brucei*	1/10/89 - 31/12/90
Dr. T. Shotake Nagoya City University Japan	Bovine immunogenetics	1/10/89 - 15/11/89
Mr. R. Suzuki Nagoya City University Japan	Genetic and biochemical characterisation of N'dama	1/10/89 - 30/9/91
Dr. G. L. Tilney Pennsylvania University USA.	Parasite-cell relationships in *Theileria parva*	6/10/89 - 8/2/90
Dr. R. Brun Swiss Tropical Inst., Basel, Switzerland	Collaborative programmes on trypanosomiasis	11/10/89 - 22/10/89

Mr. J. Gerhards ITG, Karlesruhe University West Germany	Expression of *Theileria* cDNAciones in mammalian cells	21/ 8/89 - 1/10/89 12/10/89 - 11/11/89
Mrs. E. Gault University of Glasgow Scotland, UK	Evaluation of Samorin ELISA	15/10/89 - 14/11/89
Dr. D. D.Whitelaw University of Glasgow Scotland, UK	Evaluation of Samorin ELISA	15/10/89 - 14/11/89
Prof. V. O. Anosa Ibadan University Nigeria	*T. vivax*-induced anaemia	16/10/89 - 14/11/89
Dr. M. Hutchinson Australian National University, Canberra Australia	Collaborative research on GIS	18/10/89 - 23/10/89
Ms. J. McMahon Australian National University, Canberra Australia	Collaborative research on GIS	18/10/89 - 23/10/89
Dr. G. Medley Parasite Epidemiology Imperial College London, UK	Consultant in disease epidemiology models	28/10/89 - 18/11/89
Dr. F. Kooy Utrecht University The Netherlands	Diploidy in metacyclic African trypanosomes	16/11/89 - 31/1/90
Prof. H. Nix Australian National University Canberra, Australia	Predictive models of *Theileria* control	22/11/89 - 26/11/89
Dr. J. A. Kornblatt Concordia University Montreal, Canada	Trypanosome alternative oxidase	30/12/89 - 31/7/90
Dr. M. J. Kornblatt Concordia University Montreal, Canada	Trypanosome lysosomes	30/12/89 - 31/7/90
Dr. S. Inumaru National Institute of Animal Health Ibaraki, Japan	Immuno-characterisation of *Theileria*	12/1/90 - 3/3/90