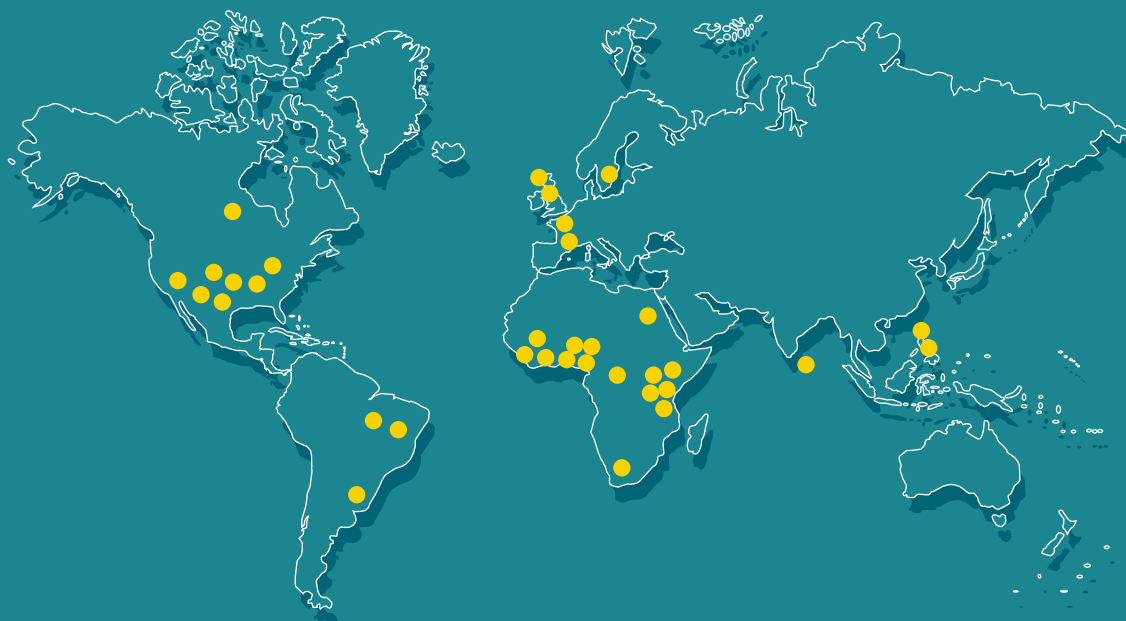


Tropical Disease Research



▶ Results Portfolio

A collection of Final Reports
from projects selected by
independent scientific experts



Chagas disease

► For the first time the efficacy of specific drug treatment during the early chronic phase of Chagas disease in children has been demonstrated. This has major implications as it illustrates the need to devise a clinical management and treatment policy for *Trypanosoma cruzi*-infected children aged 7-12 years in areas where Chagas disease is endemic, including those areas that are undergoing vector elimination.

► This work represented the continuation of a double-blind, randomized clinical trial to test the efficacy of benznidazole in seropositive children. Carried out in a rural area of the State of Goiás, Brazil, between 1991-95, the trial showed that a 60-day (7.5 mg/kg daily) course of benznidazole treatment of early chronic *T. cruzi* infection was effective in producing negative seroconversion of specific antibodies in 55.8% of cases. It was also shown that after five years of follow-up, treated children had significantly less risk of developing electrocardiographic lesions, which are indicative of chronic chagasic cardiomyopathy.

In the rural area where the study was undertaken the progression of Chagas disease is accelerated, thus reinforcing the need for systematic treatment of infected children.

Experts from Argentina, Bolivia, Brazil, Chile, Colombia, Honduras and Venezuela discussed these results at a meeting at the Oswaldo Cruz Institute in Rio de Janeiro in April 1998. The group recommended development of a public health policy in all endemic countries for the treatment of infected children (aged 7-12 years) in the early chronic phase of Chagas disease, to avoid the evolution to irreversible cardiac lesions. This is particularly important as, up to now,

no medical treatment has been applied or recommended for this age group. The proposal was later endorsed by the XIII International Congress of Cardiology.

The work was carried out in parallel with a similar project (Project No. 900342) in Argentina, which produced identical results. ■

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Sosa Estani, S. et al. (1998) Efficacy of chemotherapy with benznidazole in children in the indeterminate phase of Chagas disease. *American Journal of Tropical Medicine and Hygiene*, 59(6): (in press)

PROJECT No. 960295

Monitoring Chagas disease cardiopathy among children in a high risk area

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Quality of Care

▶ A participatory methodology consisting of a series of workshops was used to sensitize health workers to the quality of service they provide, and help them identify how it could be improved. The process, welcomed by health staff, improved provider-client relations, facility level functioning and staff relations, and had some impact at system level. Commitment to change at system level enhanced the positive impact. Results indicate that greater returns could have been realised for health systems, had managers embraced the enthusiasm and thrust for change generated and supported it more fully.

Quality of health care is linked with the context - social, economic, cultural and structural - in which the health services and those seeking care are located. These contexts, and their interactions, influence the recognition of illness, health seeking behaviour, and responses of health providers and health services. *Health Workers for Change* (HWFC) consists of a series of 6 workshops designed to explore this matrix and help health workers improve the quality of care they provide, especially in relation to female clients. The intervention, developed by the Women's Health Project in South Africa, was also tested in Uganda, Zambia, Mozambique and Senegal. The workshops help health workers examine their jobs, the way they do them, gender issues and other factors influencing their interaction with colleagues and clients, and what was required to improve the situation. The intervention proved highly acceptable and relevant within widely different social and cultural contexts.

A multi-centre study to test the impact of HWFC was undertaken in 7 rural and urban sites in Nigeria, Ghana, Tanzania, Kenya and Argentina. Comprehensive post-intervention assessments showed a decline in the time spent by patients after the intervention in 5 sites, and clients reported that they felt their time in the health services was better spent. Clients identified comprehensive positive changes,

including a more polite and respectful attitude on the part of providers, improved explanations, prompt attention, availability of drugs and not having to pay bribes. Positive changes for facility level staff included improved team work, implementing action plans staff had proposed, improved frequency of staff meetings, taking initiative for solving problems, and more rational drug ordering. At system level, some positive changes occurred, but generally lack of responsiveness of the system to the momentum created by the intervention was identified at all sites as a major constraint.

Health Workers for Change helped to: identify fundamental problems in the health system and address problematic issues; improve provider-client relations and promote problem solving and initiative on the part of health workers; work synergistically with other concurrent health sector reform initiatives; support decentralisation; promote gender mainstreaming.

A Spanish version of HWCG is being incorporated into technical cooperation initiatives with national and local health systems and services by the Pan-American Health Organization. ■

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Ouma, W., Agyepong, A., Zakari, M., Fonn, S., Vlassoff, C. *The impact of Health Workers for Change in seven settings: a useful management and health system development tool.*
Health Policy and Planning (In press)

**PROJECT No. 961008,
960754**

*Health Workers for Change:
An intervention to improve
quality of care*

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Malaria

► Researchers in Sudan have demonstrated a correlation between outbreaks of malaria and increased numbers of parasite clones in the blood, indicating that clinical malaria is associated with the presence of novel parasite genotypes. Mixed infections were detected among blood samples taken from individuals, while people harbouring parasites during the dry season proved not to be at increased risk in the following malaria season. The results have wide-ranging implications for our understanding of how malaria parasites interact with the immune system and for vaccine research.

► This project used Polymerase Chain Reaction (PCR), rosette formation (agglutination) assays and other cutting-edge techniques, to characterize *Plasmodium falciparum*-infected erythrocytes *in vitro* to evaluate associations between virulence, parasite epitopes, and host immune responses. It was carried out in an area of seasonal and unstable malaria transmission in eastern Sudan, where malaria can be absent for up to two years and where *P. falciparum* is the predominant species.

Study populations consisting of cohorts of 32-64 individuals, seven years old and upwards, provided blood samples before, during, and after the time of transmission. Agglutination assays were carried out with cultured parasitized erythrocytes originally provided by cohort members during a period of acute malaria. Agglutination of parasitized cells turned out to mainly depend on the time of sampling relative to the transmission season. More than 50% of all samples obtained before the transmission season failed to agglutinate any of the lines of cultured parasites, while all the post-transmission season samples agglutinated at least one of the parasite lines and around 75% were capable of agglutinating two or more lines.

Increased agglutination capacity was not correlated to malaria attacks dur-

ing the transmission season. This supported the notion that stimulation of agglutination antibodies can occur, even in the absence of overt disease, and demonstrating, for the first time, substantial seasonal fluctuations in individual responses to infection. Although, levels of agglutinating antibodies have a tendency to decrease between transmission seasons, the scientifically more far-reaching alternative was corroborated, i.e. a shift with respect to antigen epitopes recognized by the host. Mixed infections were detected among clinical isolates and clinical malaria was shown to be associated with infection with novel genotypes not previously detected in that particular person. Individuals harbouring parasites during the dry season were shown not to be at increased risk when transmission returned. Clear association of malaria attacks with the appearance of novel genotypes strongly suggests that the malaria parasite is capable of antigenic variation. ■

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Giha, H.A. et al. (1998) *Seasonal variation in agglutination of P. falciparum-infected erythrocytes. American Journal of Tropical Medicine & Hygiene, 58: 399-340*

Roper, C. et al. (1998) *Seasonal changes in the P. falciparum population in individuals and their relationship to clinical malaria: a longitudinal study in a Sudanese village. Parasitology, 116: 501-510*

PROJECT No. 960448

Role of antigenic variation in maintaining asymptomatic malaria infections through the Sudanese dry season.

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Malaria

► To be fully effective, bednets need to be treated with insecticide once or twice a year. A kit designed to allow families to safely and effectively dip their own bednets at home - irrespective of their degree of literacy - has now been produced following research work in Tanzania. Previously, communal 'dipping days' or treatment centres that had to be visited by net-users, failed to produce a high rate of re-treatment. The success of the project has driven major manufacturers to commence production of formulations and kits suited for home treatment.

► Mosquito nets need to be re-treated with insecticide every 6-12 months. Neither 'dipping days', where insecticide is delivered to a community and all nets are dipped at the same time, nor a dipping service at centres where nets are taken to be dipped by trained staff, achieve high re-treatment rates - especially when the insecticide is sold rather than given away.

This study developed a 'dip-it-yourself' kit that allows people to dip their nets at home and that could be sold locally. This has many advantages: it saves work for the insecticide supplier, gives autonomy to net users, and allows dipping to be carried out as part of the domestic washing routine. The work was carried out in Tanzania using groups of experienced net users. In comparing ways of packaging doses of insecticide, users expressed a preference for locally produced plastic-metal foil sachets.

The hardest task was to devise a set of instructions for safe and effective use that could be understood even in semi-literate communities. Points of difficulty and possible confusion in instructions were identified through observations of naive users and focus groups. Instructions combined both words and pictures and lack of literacy was not identified as a major problem. Net sizes were best defined in terms of the width

of the bed that a net would fit, and amount of water for treatment in numbers of soda bottles. Users handled the kit safely and effectively, avoiding wastage and conforming to instructions for hand-washing and safe disposal of all materials after use.

The instructions were thoroughly tested in both urban and rural communities, and have since been adopted by two social marketing projects in Tanzania. A similar methodology has been used to test instructions in Rwanda.

At the outset, the idea of a dip-it-yourself kit as a novel form of commercial domestic insecticide for use in developing countries was regarded with scepticism by most people, including public health authorities and major chemical manufacturers. It is now widely recognised as the way in which most nets will be treated in the future, and major pesticide producers are now developing formulations and packaging adapted to this purpose. ■

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Miller J.E.M., Jones C.O.H., Lines J, et al. (1998) *A new strategy for treating nets: Part 2. The effect of net users perceptions and practices on insecticide dosage*. *Tropical Medicine and International Health*, (in press).

PROJECT No. 950625
Testing and further development
of a kit for home impregnation
of mosquito nets

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Malaria

► Researchers in Benin and Côte d'Ivoire have shown that personal protection provided by pyrethroid insecticide treated bednets (ITNs) is good, even in areas where *Anopheles gambiae*, the major malaria vector, has developed strong pyrethroid resistance. The practical implications are important. ITNs are likely to be effective for malaria prevention even in areas where pyrethroid resistance is present. In addition, the use of ITNs is unlikely to induce resistance in areas where malaria vectors are free of resistance genes.

► The use of bednets impregnated with insecticide (ITNs) is an important component of the global strategy proposed by WHO to prevent people being infected by vector mosquitoes. Recently, *Anopheles gambiae* s.s., the major malaria vector in Africa, was found to be pyrethroid resistant in some West African countries. Efficacy of the pyrethroid insecticide, permethrin, was dramatically reduced under laboratory conditions when tested on mosquitoes that had developed resistance. Serious concern was therefore raised about the efficacy of ITNs in resistance areas. Field trials supported by TDR, using experimental huts fitted with permethrin or deltamethrin impregnated nets, were recently carried out independently in Côte d'Ivoire and Benin.

The presence of ITNs strongly deterred entry of malaria vector mosquitoes into a room, whether or not they were resistant. Surprisingly, resistant mosquitoes were killed in the field almost as efficiently as susceptible mosquitoes. Since resistant mosquitoes also partly lose their response to the irritant effect of pyrethroids, they spend longer time than susceptible ones in contact with treated netting and thus pick up a higher quantity of insecticide. It was also expected that resistant mosquitoes should have a great advantage in blood feeding. However, it was found that this was not the

case since treatment of bednets dramatically reduced blood feeding, independently of the resistance level.

The practical implications of these studies are of major importance. Since, in the experimental huts, resistant mosquitoes had little or no apparent advantage in the presence of insecticide, when Kdr (the gene associated with pyrethroid resistance) is not already present, it is unlikely to be selected by large scale use of ITNs. In areas where this resistance is already present, even at high level, ITNs are likely to still be effective, at least as a personal protection tool. A much larger scale field trial is now planned in order to confirm these very encouraging findings. ■

**PROJECT No. 960431,
960387**

*Impact de la résistance
d'*Anopheles gambiae* à la
perméthrine sur l'efficacité des
moustiquaires imprégnées*

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Malaria

▶ A previously unknown factor required for the development of the malaria parasite in the mosquito has now been identified. It is xanthurenic acid - which has been demonstrated to be crucial for the induction of gametogenesis in *Plasmodium*. As gametogenesis is the first obligatory step in the development of the malaria parasite in the mosquito, this discovery could form the basis of rational new approaches to interrupt malaria transmission.

▶ *Plasmodium* is transmitted to the mosquito from vertebrate hosts as gametocytes. These blood-living stages are responsible for the sexual events of the parasite life cycle. Within minutes of ingestion into the bloodmeal, the gametocytes form gametes, which then fuse to form zygotes. It has been known for over 50 years that the pH and temperature of the bloodmeal, in association with unknown 'factors' from the mosquito, play key roles in regulating the induction of gametogenesis. In the 1970s, it was shown that a mosquito extract could stimulate gametogenesis in vitro. However, the identity of the active factor(s) remained unknown.

Two collaborating groups, using micro-scale chemical derivatization and mass spectrometry, have now conclusively identified the gametocyte-activating factor as xanthurenic acid (XA), a by-product of the tryptophan metabolic pathway that forms insect eye pigments. Studies on the insect-derived molecule were subsequently supported by parallel studies using synthetic, pure XA.

This project has also demonstrated the complex interdependency of three gametogenesis-regulating factors in vitro. Gametogenesis can be induced in vitro by simultaneous exposure to two stimuli: a drop in temperature, and either a rise in pH or the addition of

XA. In vivo, the temperature shift was known to be essential, but not sufficient for gametogenesis. Research has now also ruled out the involvement of pH change. It therefore seems likely that XA is the natural trigger.

These studies were carried out with the rodent parasite *P. berghei* and the mosquito *Anopheles stephensi*. Ongoing studies with mosquito lines genetically deficient in XA metabolism suggest that no single combination of temperature, pH and XA adequately explains all aspects of gametogenesis regulation in all parasite/vector combinations. It is possible that other, as yet unknown, factors may be involved.

Nevertheless, the demonstration that a single synthetic molecule can regulate the sequential passage from one vital stage of the life cycle to the next could lead to the development of novel strategies to block malaria transmission – either by designing new drugs which interfere with XA activity, or selecting mosquitoes in which gametogenesis is blocked. ■

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Billker, O. et al (1998). Identification of xanthurenic acid as the putative inducer of malaria development in the mosquito. *Nature* 392, 289-292.

PROJECT No. 950559
Mosquito factors regulating gametogenesis in Plasmodium

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Filariasis

▶ The concerted efforts of researchers in 5 countries have made impressive progress in the identification and mapping of genes of the human filarial parasite *Brugia malayi*. At the start of the project, only 50 *Brugia* genes had been identified. Currently, over 6000 different genes have been discovered - more than one-third of the total number thought to be present. Mapping studies are well under way, and functional genomic programmes have already been initiated.

▶ The overall goal of the Filarial Genome Project is to clone, sequence, identify and map genes expressed during all developmental stages of filarial parasites. *Brugia malayi* was chosen to be studied, due to the availability of all stages of the parasite life cycle. In 1995, the project began identifying genes by sequencing ESTs (expressed sequencing tags) from cDNA libraries derived from various life-cycle stages. Well over 16 500 ESTs have been sequenced, resulting in the discovery of over 6000 new genes. About half of these are unique to *Brugia*, and may be extremely useful in developing new drugs, vaccines or diagnostic tests. This efficient gene discovery rate is due partly to the construction of new cDNA libraries each year from additional life-cycle stages and many of the transition stages. Currently, 11 cDNA libraries are available, which have been distributed to over 100 research labs worldwide. The development of "subtracted" libraries (cDNA libraries from which abundant clones have been removed) has also ensured that the rate of new gene discovery remains high.

Excellent progress has also been made on chromosome mapping studies. By the end of 1998, 1000 ESTs will have been mapped to gridded genomic libraries. The training and involvement of scientists from laboratories in countries where filarial diseases are en-

demic has added dramatically to the success of the project. In addition to the participants from Egypt and India, a new laboratory from Indonesia was recently added to the network.

The Filarial Genome Project Resource Centre has been set up to store and distribute all filarial DNA libraries and clones. These have been sent out to a substantial number of investigators around the world. All data are available on the Web site <http://helios.bto.ed.ac.uk/mbx/fgn/filgen.html>

Given the rapid progress of this project, due primarily to the close interaction of the participating laboratories, functional genomics programmes investigating functions of discovered genes have already been initiated. ■

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**PROJECT No. 950549,
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Filarial Genome Project

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African Trypanosomiasis

▶ Eflornithine has significant potential for treatment of the gambiense form of African trypanosomiasis, although it is expensive. This 4-nation study showed that, for relapsing cases (patients who had previously been treated with melarsoprol but who had relapsed) a 7-day course of eflornithine was as effective as the usual 14-day treatment. This should allow considerable savings for health systems in endemic countries. The 14-day regime remained effective for new cases in all countries, except Uganda, where efficacy was significantly lower.

▶ A multicentre, randomized comparative study of the 14-day versus 7-day treatment of late-stage *T. brucei gambiense* African trypanosomiasis (sleeping sickness) with eflornithine was initiated in 1993. The rationale was that if the 7-day regimen was as equally effective as the 14-day regimen, treatment costs could be reduced by half. 320 patients from four countries (Côte d'Ivoire, Congo, Democratic Republic of Congo [DRC - formerly Zaire] and Uganda) were recruited for treatment from July 1993 to February 1996. Two categories of patients were involved: relapsing patients, those who had been previously treated with melarsoprol and then relapsed; and new cases, patients who had not been previously treated. The 2-year follow-up of patients ended in April 1998.

The following conclusions and recommendations were made:

For relapsing cases, the 7-day regimen is highly effective and could be recommended as an acceptable alternative to the 14-day regimen and may be used by national control programmes and hospitals, provided that patients are closely followed up.

For new cases, in Côte d'Ivoire, DRC and Congo, the 14-day regimen was highly effective and continues to be recommended. In Uganda, the 14-day

regimen was considerably less effective than in the other three countries. This observation may be related to the low sensitivity to eflornithine shown by *T. b. gambiense* strains in north-western Uganda.

The adoption of the short regimen has important implications in the clinical management of the disease and in diminishing hospital costs, which are reduced on average by half, from US\$ 884 to US\$ 392, in endemic areas. ■

**PROJECT No. 960720,
960721, 960722**

Multicentre comparative study of two treatment durations with eflornithine for late-stage T. b. gambiense sleeping sickness

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Health Sector Reform

► Research in the Philippines to examine the impact of decentralization on health administration and services focused on the National Tuberculosis Control Programme (NTP). Case studies in 8 municipalities, representing different geographical and economic situations, found negative results of devolution to Local Government Units (LGU) in all regions studied. The findings identified elements that need to be put in place at the local level prior to devolving disease control programmes from the central system to the periphery.

Five major conclusions could be drawn from this project:

1. Compared to pre-devolution, the level of compliance with NTP guidelines deteriorated. Cases diagnosed by X-ray increased after devolution, indicating avoidance of the policy to use sputum microscopy as the diagnostic tool. Patients were required to pay, so many did not have sputum microscopy or did not return for treatment. Patients were advised to collect drugs monthly rather than weekly. Fair to poor compliance with recording and reporting of TB statistics at the municipal level was also observed.
2. Accomplishment of NTP targets in terms of the number of patients treated, worsened in all municipalities, especially during the early post-devolution years.
3. Frequency of supervision and training declined after devolution, as most LGUs suspended or drastically reduced such activities.
4. Health became increasingly politicised. In all municipalities, the mayor hired more health staff, in most cases without prior knowledge of the Municipal Health Officer. Hiring political allies was deemed to be a privilege of the mayor. This led to situations in which 60-80% of the total municipal health budget went on personnel, leaving insufficient funds for other services. No funds were budgeted locally for TB other than emergency pur-

chases of anti-TB drugs. In all resource-rich municipalities and even in some which were resource-poor, funds were spent on renovating and improving health facilities rather than on services after devolution. This was a political decision to assure electoral mileage for the mayor.

5. With devolution, Municipal Health Officers were required to become managers of a much wider range of activities than before, including multi-programme monitoring, for which none of them had received any management training. Nor had they any experience in the political skills that had become an integral part of the health system.

Discussions with staff from all levels of the health system followed the research results, and the "Model NTP in the Devolved System" was developed, with important implications for all priority health problems;

Reciprocity: for every output provided by the LGU there will be a corresponding assistance package from the central Department of Health (DOH). Regular feedback between the DOH and LGUs will occur alongside pre-defined evaluation mechanisms.

Direction: NTP objectives, policies and guidelines will be set by the DOH.

Logistics: support provided by the DOH for drugs, supplies, and staff training, will be phased out as the LGUs reach political and administrative maturity and fiscal autonomy. ■

PROJECT No. 960698

Effect of devolution on the Philippines National Tuberculosis Control Programme: Cases studies

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Schistosomiasis

► The long-term effect on infection and morbidity of praziquantel treatment and re-treatment of urinary schistosomiasis was evaluated in Mali. The rate of re-infection and of urinary morbidity were significantly higher in school-aged children than in older age groups. It was concluded that, although repeated treatment did not significantly reduce the rate of infection, it has a major impact on morbidity as assessed by ultrasound examination of the urinary system. The results of the study provided the scientific basis for recommending regular treatment, every 2 years, as a morbidity control strategy.

► Supported by a Re-entry grant, this investigation was designed to evaluate the long-term impact of praziquantel treatment on urinary schistosomiasis in high to moderate endemic areas in Mali, and to relate the findings to the age at which patients are treated, in order to contribute to improvement of the treatment policy for urinary schistosomiasis. The research work involved clinical, parasitological and ultrasound examination. Surveillance of a cohort of 648 individuals from 3 villages was carried out from February 1991 to December 1995. Physical examination focused on the presence of haematuria, dysuria and pain, and on ultrasound investigation of bladder abnormalities (polyps, masses, wall thickening, wall irregularity and presence of calcification), dilated ureter and renal pelvis.

Results demonstrated that the rate of reinfection and of urinary tract morbidity were significantly higher in school-aged children than in older age groups. Before treatment, peak prevalence of infection and morbidity (85%) occurred in children aged 7-14 years. Three and a half years after treatment, prevalence of infection had returned to the initial level. However, the rate of urinary tract lesions was 50% lower than before treatment - but significantly higher than in older age groups. At 12-15 months after treatment, some 80% of urinary tract lesions are re-

versed with praziquantel, with no significant difference between adults and children as regards the rate of reversion.

It was not clear whether repeated treatment guaranteed a significant reduction in the rate of reinfection, but it can have a real impact on morbidity due to urinary schistosomiasis. In the areas studied, re-treating children under 17 years old at least every two years is an effective morbidity control strategy. This work demonstrated that such re-treatment campaigns can be targeted specifically on children. The research work supported by this Re-entry grant has made an important impact on the National Schistosomiasis Control Programme in Mali. ■

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PROJECT No. 940897

Long-term effect of praziquantel: modelling reinfection/evolution of morbidity due to urinary schistosomiasis

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Malaria

► In collaboration with international partners, the Research Institute for Tropical Medicine (RITM) in Manila has contributed greatly to national capacity and sustainability for vaccine research, development and production in the Philippines. RITM has become the focal institution for construction of a new Biological Production Service (BPS), and for establishing Rules and Regulations for the Bureau of Food and Drugs. RITM has also become the central facility for, and assumed the lead role in, a proposed Centers for Disease Control (CDC) where all national disease control programmes will be housed.

This Partnership Grant was designed to help support wide-ranging initiatives in malaria transmission blocking vaccines research and development, and pave the way for future clinical and field trials. Strengthening the capabilities of the Philippines National Control Authority and the Bureau of Food and Drugs (BFAD), in order to regulate and assure the safety, efficacy and quality of vaccines and other biological products, was seen as a prerequisite for success. This proved most timely in view of advances in the country related to vaccine research and production. In addition, vaccine-specific expertise and experience was required within BFAD with respect to vaccine product evaluation, evaluation of clinical performance, and current Good Manufacturing Practice (cGMP) inspection.

Under the grant, a Task Force on Vaccines and Biologicals was created to review BFAD guidelines for developmental vaccines, upgrade local regulatory standards, and strengthen cGMP procedures, including quality assurance and quality control. A series of four seminar-workshops, with national and international participants, were undertaken. They resulted in updated standards for product registration and manufacturer licensing; specific, clearly defined requirements and evaluation processes for vaccines

and biological products; identification of additional cGMP requirements and special consideration for local manufacturers of vaccines and biologicals. Specific guidelines for developmental vaccine approval processes and requirements for clinical trials were also established.

RITM, BPS and BFAD, as national institutions, all benefit from the outcome of these past and ongoing pursuits to promote the application of biotechnology to tropical disease research. The Filipino population also derives significant benefits and protection from the newly established safety standards and guidelines for vaccines and other biological products.

The grant also provided support for a sociobehavioural component on the implementation and sustainability of a Community Volunteers (CV) programme in an area of low malaria endemicity. The programme, based on early case detection and treatment coupled with health education, was undertaken during 1994-96 in collaboration with the University of Queensland, Australia. Malaria incidence over the period fell significantly. In 1997, the CV programme was turned over to the Local Government Unit and it has now been replicated in other areas of the country. The project was awarded the Philippines' Outstanding Health Research Award in 1998. ■

PROJECT No. 930817
Development and evaluation of new methods for controlling malaria transmission in low endemic areas: groundwork for vaccine production and regulation

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Schistosomiasis

► Studies in Brazil have shown that interleukin-10 (IL-10) - one of a set of immunoregulatory cytokines - plays a major role in controlling pathology in individuals infected with *Schistosoma mansoni*, while resistance to (re)infection is linked with high levels of another cytokine, gamma interferon (IFN- γ), and specific immunoglobulins of the antibody class E (IgE). These results contrast with results obtained in the mouse model and suggest that mice and humans deal with this infection differently. The project has significantly promoted understanding of the immune response and pathogenesis in schistosomiasis.

Immune reactions induced by *S. mansoni* infection result in either asymptomatic infection, serious morbidity or protection. The outcome is determined by a balance between different immune responses - modulated by certain cytokines - which are directed both against larval and adult stages of the parasite, as well as parasite eggs trapped in the tissues. Following the initial acute phase of the disease, the great majority of people infected with *S. mansoni* develop a chronic, largely asymptomatic form of the disease (intestinal disease), while some individuals progress to severe hepatosplenic schistosomiasis which can sometimes lead to death. The nature of the cytokine-modulated immune responses, associated with the typical granulomatous reaction to parasite eggs, determines progression from the acute stage to chronic forms.

A Career Development Grant was awarded to promote research in this area. Cytokine responses were analysed in three groups of patients from an endemic area of Brazil: those with acute disease; those with intestinal disease, and those with the hepatosplenic form. By 'blocking' IL-10 activity in cultures of peripheral blood mononuclear cells (PBMCs) taken from patients belonging to the intestinal group, significant increases in cellular proliferation, including granulomatous cells, were observed - a result contrasting with PBMCs taken from the other

groups. This implies that IL-10 down-regulates immunopathology in the majority of patients, i.e. those with intestinal schistosomiasis, but exerts little influence on other disease forms.

To understand more about the mechanisms of protective immunity, responses were also characterized in individuals identified as naturally resistant to (re)infection. It was shown that PBMCs from naturally resistant individuals secreted high levels of IFN- γ when cultured *in vitro* with adult schistosome antigens, and that specific anti-parasite IgE was elevated in this group compared with susceptible controls. These results suggest that in humans, both cell-mediated (Th1-type) and humoral (Th2-type) responses are needed in the elimination of *S. mansoni* infection. This contrasts with the situation in the mouse model, where Th1-type responses are associated with protection and Th2-type responses are associated with pathology, and demonstrates that human studies, though logistically much more difficult, are absolutely needed. ■

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Brazilian Journal of Medical and Biological Research, 31: 171-177

PROJECT No. 920479

Cytokine regulation of immune response and development of resistance to Schistosoma mansoni.

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Malaria

▶ Two aspartic proteinases, plasmepsin I and plasmepsin II, found in the malaria parasite *Plasmodium falciparum* have been identified as potential targets against which to develop new drugs. These enzymes play a key role in the degradation of haemoglobin within the digestive vacuole of the parasite. This research resulted in the first crystal structure determination of a malarial enzyme and provided initial insights into how new antimalarial drugs could be appropriately designed for these targets. In addition, it engaged new specialists in drug R&D to work on malaria.

Malarial parasites develop in human erythrocytes and, during occupation of these cells, the parasites degrade up to 80% of the cell's haemoglobin. This provides essential nutrients for parasite growth and creates growing space for the parasite within the cell. Two aspartic proteinases - plasmepsins I and II - have already been identified as having a pivotal role in the degradation of haemoglobin, which occurs within a lysosomal digestive vacuole of the parasite.

This study investigated the haemoglobin degradation process and assessed the potential of these enzymes as targets for the rational development of new antimalarial drugs. This approach was supported by the discovery and development of new aspartic proteinase inhibitors for the treatment of HIV.

Key results included:

- Demonstration that both plasmepsins are involved in the initiation of haemoglobin degradation, cleaving the native haemoglobin molecule and exposing it for completion of proteolysis.
- Production of recombinant plasmepsin II for kinetic analysis and crystallisation studies.
- Crystallisation and structural determination of plasmepsin II (the first reported crystal structure of any malarial enzyme) which demonstrated key structural differences between the

malarial enzyme and other human enzymes of this class. This opened up the possibility of rationally designing new classes of antimalarial drug.

- Identification of a series of compounds, in collaboration with Pharmacoepia Inc., that are specific against the enzyme and the malarial parasite in culture, demonstrating the validity of these enzymes as drug targets.
- Further insights into the haemoglobin degradation process; how the plasmepsins are targeted to the food vacuole, and how they are activated from their proenzyme form. This offers the possibility of discovering further antimalarial drug targets and novel chemotherapeutic approaches.

This work established a basis for the discovery and development of new antimalarial drugs that target the parasite food vacuole. Through focusing on aspects of drug discovery, the project also resulted in collaborations that introduced new scientists with specific drug R&D expertise to the malaria field. ■

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PROJECT No. 950195
Plasmodium falciparum aspartic protease inhibitor development

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Leprosy

► Identifying the molecular basis of *Mycobacterium leprae* neurotropism is a high point in recent leprosy research. This study has shown that the G domain of the $\alpha 2$ chain of endoneurial laminin is crucial in the invasion of peripheral nerves by *M. leprae*. Furthermore, α -dystroglycan has been identified as the laminin $\alpha 2$ -G receptor on the Schwann cell and a candidate protein receptor on the surface of *M. leprae*. This work is a profound contribution to our understanding of the pathogenesis of leprosy and may have important implications for the design of interventions to control leprosy-induced nerve damage.

► *M. leprae*, the organism that causes leprosy, needs invasion of peripheral nerve cells for bacterial survival, replication and establishment of infection. *M. leprae* causes significant damage to peripheral nerves, leaving patients with disabilities and deformities. Although antibiotic therapy is an effective cure, it does not reverse nerve function loss.

Pathogenic bacteria exploit host cell receptors to serve as initial targets for interaction with a specific cell type. This attachment occurs by direct binding between bacterial "adhesins" and host cell receptors, or indirectly by absorbing a bridging ligand of host cell origin onto the bacterial surface. In leprosy, the initial and crucial step in the disease process is *M. leprae*'s attachment to the basal lamina that surrounds the Schwann cell-axon unit. In the endoneurium of the peripheral nerve, this basal lamina is comprised of laminin, type-IV collagen, entactin/nidogen and heparin sulphate proteoglycan. Using a combination of antibodies directed against different isoforms of laminin, recombinant fragments of the laminin $\alpha 2$ chain and *dy/dy* mice, the nerves of which are devoid of laminin $\alpha 2$, it was shown that it is the C-terminal portion, the so-called G domain, of the laminin $\alpha 2$ chain that mediates *M. leprae* adherence to Schwann cells *in vitro* and *in situ*. Furthermore, that α -dystroglycan,

a laminin receptor of the dystrophin-glycoprotein complex, is a cell receptor for the binding of G domain-coated *M. leprae* to Schwann cells. This binding results in aggregation of cell surface α -dystroglycan and colocalization of bacteria with these aggregates. Identifying laminin $\alpha 2$ as the bridging molecule and α -dystroglycan as the receptor on the nerve, leaves the question, how is *M. leprae* binding to laminin? Using binding of radiolabelled laminin to *M. leprae*, the investigators identified a single cell wall protein of 21kDa, which appears to be the major adhesin of *M. leprae* for the interaction with peripheral nerve.

This study represents a major breakthrough in our understanding of the molecular mechanisms that govern the interaction between *M. leprae* and the peripheral nerve. Despite indications that parallel (e.g. non-laminin-mediated) mechanisms may also be at work, these findings represent a crucial step towards developing strategies to prevent *M. leprae*-induced nerve damage. ■

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Cell, 88:811-821

PROJECT No. 960147
Molecular mechanism of Mycobacterium leprae invasion of Schwann cells of the peripheral nerve.

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Malaria

▶ A new cell type - Ross cells - has been discovered in the midgut epithelium of mosquitos. During their development in the mosquito, *Plasmodium* parasite ookinetes were found to preferentially invade Ross cells when penetrating the mosquito midgut wall to form oocysts. It has also been shown that monoclonal antibodies recognizing midgut surface antigens inhibit oocyst formation. Since crossing of the midgut is essential for completion of the parasite cycle in the mosquito, this discovery may lead to new strategies for blocking malaria transmission.

▶ When crossing the midgut epithelium in *Aedes aegypti* mosquitoes, *Plasmodium* ookinetes have been found to invade a unique class of cells named 'Ross cells'. These cells have distinct features such as reduced basophilicity, smaller and more sparse microvilli, and abundant expression of a vesicular ATPase. As Ross cells constitute only 3-5% of epithelial cells, these results strongly suggest that Ross cell invasion involves specific cell surface ligands.

In an attempt to characterize cell surface molecules, two sets of monoclonal antibodies (MAB) were prepared against *Ae. aegypti* midgut epithelium. One set was made against purified plasma membranes and another against total midgut homogenates. Three of the MAB significantly reduced the number of oocysts formed (up to 90%), when incorporated into an infective blood meal. These antibodies recognized antigens localized specifically on the brush border and cross-reacted with *Anopheles gambiae*, *An. freeborni* and *An. stephensi* antigens. On Western blots, they produced a complex pattern of multiple bands.

Electron microscopy suggested that ookinetes interact with 3 types of structures on the midgut surface: a fibrous network which appears to be distinct from the peritrophic matrix; microvilli; and vesicular structures of unknown origin that are found inter-

spersed between the microvillated cells of the midgut. The interactions are specific, require carbohydrates, and the parasites undergo extensive morphological changes.

These results show that midgut epithelium invasion occurs in at least two steps: the parasite attaches to the epithelium via a carbohydrate-containing ligand, then invades a specific cell type (Ross cell), presumably through interaction with a specific ligand.

In addition, the gene encoding the vesicular ATPase β -subunit was cloned and an antibody against the recombinant protein was produced. The anti-vesicular ATPase antibody will allow further study of the specificity of Ross cell invasion. This work may help develop new ways of interfering with malaria transmission. ■

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PROJECT No. 950476
Identification of mosquito midgut receptors for Plasmodium ookinetes

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Chagas disease

► Routine detection and clinical management of congenital Chagas disease has been improved via the development of new molecular biology tools. Comparison of several diagnostic techniques found that the polymerase chain reaction (PCR) had a sensitivity comparable to conventional serology. Furthermore, the Shed Acute Phase Antigen (SAPA) protein, when used in an ELISA system, allowed for unequivocal diagnosis of congenital Chagas disease, identifying those infants infected by *Trypanosoma cruzi* but that were not detected by conventional serology.

Examination of several different techniques for the diagnosis of *T. cruzi* infection was undertaken to help establish the prevalence of *T. cruzi* in newborns and infants of seropositive mothers (congenital Chagas disease). Serological diagnosis took place in two departments in Paraguay (Cordillera and Paraguari) before being expanded to the whole country.

From 1996-98, 27,626 pregnant women were tested for *T. cruzi* antibodies. In Cordillera, 15.5% were positive and in Paraguari, 12%, illustrating the public health importance of *T. cruzi* infection in women. The prevalence of congenital Chagas infection in these departments was 7%. Serologically positive women were referred to health centres for clinical evaluation and follow-up, and confirmed cases were treated with benznidazole.

Of the techniques evaluated, use of Shed Acute Phase Antigen (SAPA) in an ELISA system proved the most significant. Testing this system in babies aged 3-8 months indicated its importance and accuracy, allowing for identification of congenital cases among those that gave false negative and false positive by conventional serology. Detection of anti-SAPA antibodies for routine confirmation of congenital infection demonstrated the power of this marker for the unequivocal diagnosis of congenital

cases. In comparison, direct microscopic observation showed low sensitivity, missing 77 of 104 infected newborn babies. The PCR technique proved comparable to conventional serology using ELISA and immune fluorescence techniques, missing 7 and 2 infected babies respectively. However, PCR, although useful, is costly (US\$ 4 per test) when compared with traditional serology (US\$ 0.04). These observations showed that conventional serology is still useful for detecting congenital infection.

It was also found that *T. cruzi* IgG antibodies of maternal origin occurred in non-infected children for much longer periods - up to 7 months - than previously thought, suggesting that congenital infection can be confirmed only when IgG specific antibodies of non-maternal origin are found at least 8 months after delivery. ■

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Diagnostic techniques applied for the detection of congenital Chagas disease in Paraguay, Journal of Clinical Microbiology. (In press)

PROJECT No. 950279

Assessment of a locally sustainable system for Chagas disease diagnosis in two endemic regions of Paraguay

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African Trypanosomiasis

► New results have provided major insights into how signals released by interactions between trypanosomes and the human immune system can cause the severe nervous dysfunction characteristic of sleeping sickness. During *Trypanosoma brucei* infection, CD8+ cells secrete IFN- γ which has been shown to suppress the immune response and enhance parasite proliferation. In addition, the trypanosome-derived lymphocyte triggering factor (TLTF) has been isolated and characterized, and an animal model of African trypanosomiasis has been developed, which can reproduce phenomena seen in humans.

This research, conducted over several years, has led to a wide range of discoveries of great significance in providing a better understanding of the pathogenesis of African trypanosomiasis. The demonstration that CD8+ cells secrete IFN- γ in trypanosomiasis and the elucidation of the effects of this particular cytokine on various parasite strains is a new and important finding. Besides its suppressive effects on the humoral immune system, IFN- γ has been shown to enhance the proliferation of *Trypanosoma brucei*, both *in vivo* and *in vitro*. Further achievements have included the characterization and isolation of the activator, trypanosome-derived lymphocyte triggering factor (TLTF); the identification of molecules released during infection that activate newly detected IFN- γ receptors in the spinal cord, which in turn, elicit the pain-related behaviour typical of African trypanosomiasis; and the revelation that the suprachiasmatic nucleus of the anterior hypothalamus in the brain - the pacemaker for circadian rhythms - is an important target for pathogenesis.

A rat model of chronic African trypanosomiasis has also been established that has demonstrated increased pain sensitivity responses and slow-wave sleep fragmentation, similar to that observed in human trypanosomiasis. This model has also

been shown to reproduce the changes in melatonin secretion, and binding to its receptor in the suprachiasmatic nucleus, which has been described in human patients. This project has also produced evidence that correlates cytokine-regulating factors in the brain with the induction of pro-inflammatory cytokines and neurodegeneration. In addition, a marked mRNA expression for TNF- α has been observed, in parallel with a highly selective degeneration of certain nerve fibres, without evidence of nerve cell degeneration or death. ■

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Journal of Comparative Neurology (in press)

PROJECT No. 960557,
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*Signalling molecules in
Trypanosoma brucei nervous
system interactions*

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Malaria

► Research in Sri Lanka has found that genetic regulation of the expression of TNF- α and TNF- β cytokines governs the clinical picture of *Plasmodium falciparum* malaria infection. Correlations between allelic types and TNF- α levels in patient plasma suggests a causal link between the presence of the TNF- α 2 allele and strain variations of parasites in the induction of high levels of TNF- α . It was also found that the adherence of infected red blood cells to certain cell receptors is also implicated - under the influence of other genes. Increased binding of parasites to the receptor, ICAM-1, may also be associated with severity of disease.

Studies of the distribution of the human TNF- α cytokine genotype have found marked differences in malaria in individuals who are homozygotes (TNF- α 1/TNF- α 1) compared with those who are heterozygotes (TNF- α 1/TNF- α 2). Severe malaria is found significantly more frequently in heterozygotes. Indeed, only two homozygote individuals were found to have severe malaria among the several hundred patients studied.

Investigation of associations between TNF- α and TNF- β alleles further revealed that TNF- α 2/TNF- β 2 (homozygote state) was higher in individuals with severe, complicated malaria, whereas TNF- α 1/TNF- β 2 (heterozygote state) was commonly found in those with uncomplicated disease.

Conversely, the TNF- α 2/TNF- β 1 combination was significantly higher in the uncomplicated malaria group, consistent with previous findings. Elucidation of the role of these alleles, previously shown to be linked to the levels of TNF- α secretion, is not straightforward, but the results suggest that both TNF- α and TNF- β play significant roles in regulating immunopathologic pathways involved in severe and uncomplicated malaria disease.

Patients with severe malaria were also observed to exhibit higher adherence of infected red blood cells, due to an

increased expression of the ligand ICAM-1, while the adherence to cells expressing the CD36 marker did not differ significantly. Plasma from patients with cerebral malaria resulted in a greater expression of ICAM-1 than did plasma from patients with uncomplicated malaria or with multiple organ failure. There was no difference in the regulation of CD36 expression between these groups, suggesting that the binding of infected red cells to ICAM-1, but not CD36, plays an important role in the pathogenesis of severe, complicated malaria. Results also showed that binding of ICAM-1 is specifically involved in the pathogenesis of cerebral malaria and that the causal pathogenic mechanism may be different from that causing multiple organ failure.

Follow-up work also found a clear correlation between higher plasma cytokine levels of not only TNF- α , but also, of signal molecules such as IL-6 and IL-10 and severe, complicated disease. ■

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Clinical and Experimental Immunology, 115: 350-355.

**PROJECT No. 940434,
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Pathogenesis of severe and complicated Plasmodium falciparum malaria

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Malaria

► An *in vivo* model for the maintenance of *Plasmodium falciparum* parasites in human red blood cells has been developed. This breakthrough is of great practical value. Using this new mouse model infected by either drug-sensitive or multi-drug resistant parasites, treatment with a wide range of antimalarial drugs showed that the course of parasite clearance and the morphological effects on the parasite parallels that seen in infected humans, and hence validates the use of the model for novel drug development.

► Malaria research has been given a major boost by the development of a new Severely Compromised Immune Deficient (SCID) mouse strain that may also have important applications in other research fields. This new *in vivo* model, allowing maintenance of *Plasmodium falciparum* parasites in human red blood cells, is a significant breakthrough because it emulates the situation in infected humans. Results using the model showed that infected mice, when treated with antimalarials such as chloroquine, quinine, mefloquine, artemisinin, proguanil, or inhibitors of phospholipid metabolism, exhibited parasite clearance and morphological effects on parasites similar to that observed in humans. The new model is particularly useful in view of both the shortage of primates susceptible to *P. falciparum* and its appearance at a time when the synergistic effect of existing drugs needs to be systematically studied to counter the challenge of rapidly-spreading, multi-drug resistance. Previously, finding treatment regimens which allowed sustained, stable, low numbers of macrophages to be maintained was a major problem. While animals can live without B and T lymphocytes, they cannot survive without macrophages. Yet for parasites to grow, the number of macrophages must be decreased by about 80%. This can usually be achieved quite easily, but *P. falciparum*

is a potent macrophage lineage activator. Despite conflicting treatment effects, a balance has been achieved which, without destroying 100% of the host macrophages, prevents the parasite from recruiting monocytes and inducing macrophage production.

In contrast to *in vitro* studies, the long-lasting parasitemia obtained in SCID mice permits the sequential study of various components of the immune system. Furthermore, improved regulation of physiological parameters provided by *in vivo* systems allows more reliable conditions of study. The model was already been used in several ways. Repeated inoculations of clinically active total IgG from African immune adults in the model had no effect upon the course of parasitemia. Similarly, repeated grafting of lymphocytes or monocytes had no parasitological consequences, whereas the combination of both resulted in parasite killing *in vivo*. The model was also used to evaluate vaccine candidates and, in at least one case, the repeated injection of human monocytes followed by specific antibodies, reproducibly induced parasite clearance at the same speed as that observed with chloroquine. ■

References:

Badell, E. et. al.
Human malaria in humanised mice: a new tool to study defense mechanisms (submitted)

PROJECT No. 960617

A *Plasmodium falciparum* SCID mouse model applied to the study of immune mechanisms against asexual blood stages

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African Trypanosomiasis

▶ A vervet monkey model of human African trypanosomiasis, developed in the 1980s with TDR support, has been used to evaluate a promising candidate for drug development. Results in this monkey model showed that CGP40215, a diamidine compound which had shown promise against *Trypanosoma brucei rhodesiense* infection in a mouse model, was not suitable for further development.

▶ CGP40215, a diamidine compound synthesized by Novartis (formerly Ciba Geigy), inhibits the synthesis of polyamines by preventing the hydroxylation of ornithine to form putrescine. This step is catalysed by the enzyme *S*-adenosylmethionine decarboxylase (SAM-DC). CGP40215 was originally synthesized as an anti-cancer agent, but it was later found to have activity against several trypanosome species, both *in vitro*, and *in vivo*, in the mouse model of African trypanosomiasis. This study in Kenya:

- 1) investigated whether the compound was hypotensive,
- 2) determined its efficacy against early-stage *T. brucei rhodesiense* infections, and
- 3) measured its pharmacokinetics in blood and cerebrospinal fluid (CSF) in the vervet monkey model of human African trypanosomiasis.

The latter was undertaken using a sensitive bioassay method developed at the Swiss Tropical Institute in Basel. Results showed that the compound was not hypotensive in vervet monkeys up to a dosage of 8mg/kg (intravenous injection), but at higher dosages it was markedly hypotensive. It had no effect on blood clotting time and was not curative for early-stage *T. b. rhodesiense* infection in the monkey. The half-life of the drug was found to be 3-4 hours. No drug was detected in the CSF, even after increasing the

dosage to 4 mg/kg given twice daily for 8 days, indicating that the compound did not cross the blood/brain barrier. Based on these results, CGP40215 was not considered suitable as a drug development candidate for African trypanosomiasis.

There is a basic need for animal models in the evaluation of promising compounds in drug development. During the early 1980s, TDR sponsored the development of the vervet monkey model of human African trypanosomiasis caused by *T. b. rhodesiense*, and contributed to the establishment of primate facilities at the Kenyan Trypanosomiasis Research Institute (KETRI). These facilities have been used both for the evaluation of lead compounds in the drive to find new drugs against African trypanosomiasis, and to study the pathogenesis of the disease. The Kenyan Government currently maintains the primate facilities at KETRI for specific studies on promising compounds such as CGP40215. ■

References:

Brun, R., et. al.
CGP40215: efficacy against *T. brucei rhodesiense* infection and pharmacokinetics in vervet monkeys. (in preparation)

PROJECT No. 980785

CGP40215: pharmacokinetics, efficacy against early stage *T. b. rhodesiense* infections and effect on blood pressure in vervet monkeys

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▶ The Special Programme for Research and Training in Tropical Diseases (TDR) is an independent global programme of scientific collaboration. Established in 1975 and co-sponsored by the United Nations Development Programme (UNDP), the World Bank and the World Health Organization (WHO), it aims to help combat 10 major tropical diseases: African trypanosomiasis, Chagas disease, Dengue, Leishmaniasis, Leprosy, Lymphatic filariasis, Malaria, Onchocerciasis, Schistosomiasis and Tuberculosis.

Objectives

- i) Research & Development: to develop safe, acceptable and affordable methods of prevention, diagnosis, treatment and control of target diseases.
- ii) Training & Strengthening: to strengthen the capability of developing disease-endemic countries to undertake the research required to realize new disease control technologies.

TDR does not work alone: it selects, guides, funds, encourages and develops research done by others. In this way, TDR acts as a 'global facilitator' of both research and training. Great emphasis is placed on forming partnerships with a variety of organizations around the world, including research institutions, industry and non-governmental organizations.



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