Pasteur Institute, Tehran: An electrophoresis gel of the ‘cpa’ gene identified in Leishmania parasites. Photo: WHO/TDR/Crump
New basic knowledge
The TDR activities which generate new knowledge (area B of the TDR strategy) are diverse. They include research in biological disciplines such as genomics, proteomics, immunology, pathogenesis and molecular entomology on the one hand, and social science disciplines such as economics, health policy research, sociology, and anthropology on the other.

Bioinformatics or computational biology is needed to help us understand how information contained in the genome is transformed into biological reality. A multi-disciplinary international network for bioinformatics applied to pathogen genome research was created during the biennium, through which TDR is strengthening institutions and training researchers from disease endemic countries (DECs), helping them to participate in this important area of modern biological research. In 2001, a two-pronged approach was developed by TDR to facilitate training and application of bioinformatics and genomics in DECs. For the first step, an international training-of-trainers workshop on bioinformatics applied to genome studies, held at Fundation Oswaldo Cruz (FIOCRUZ) research institute in Brazil, was supported. The objective was to develop the network and, in addition, prepare the participants to facilitate similar teaching courses in their regions, promoting South-South and South-North interactions in bioinformatics.

TDR also awarded support to four institutions in DECs selected from 18 responses to a call for applications for regional training centres in bioinformatics. The four centres – South African National Bioinformatics Institute (SANBI), South Africa; University of Sao Paulo (USP), Brazil; Mahidol University, Thailand; International Centre for Genetic Engineering and Biotechnology (ICGEB), India – have taken on the responsibilities of organizing annual training workshops for young investigators from Africa, Latin America and Asia respectively. The centres are also developing and facilitating regional networks for applications of bioinformatics and applied genomics to tropical diseases.

The availability of genome sequences for a number of the TDR pathogens has generated a large amount of information available on public domain databases, providing an unprecedented opportunity to use whole genome-based methodologies, computational biology and functional genomics to identify new drug targets and diagnostic reagents. For this, TDR established a Working Group on Applied Genomics for Drugs and Diagnostics under its Pathogenesis and Applied Genomics Committee.

Research on entomology continues with the focus on developing a mosquito incapable of transmitting malaria or dengue. The need for a careful approach to future studies involving potential modified vectors is emphasized, and the aim is to organize a scientific group to facilitate discussions between all parties interested and involved in transgenesis and its potential applications, ethics, and related policy issues.

Since social, political and economic inequalities are central to the persistence and spread of the TDR diseases, and the performance of health systems in protecting vulnerable populations from the impact of these diseases often falls far short of potential, TDR emphasizes the importance of the basic social sciences for identifying opportunities to improve the prevention and control of diseases of poverty. The aim is to contribute to a better understanding

TDR-supported regional training centres in bioinformatics:

Africa
SANBI, Cape Town, South Africa
http://www.sanbi.ac.za/tdrcourse/

Latin America
USP, Sao Paulo, Brazil
http://ich.ime.usp.br/tdr/

Asia
ICGEB, New Delhi, India
http://www.icgeb.res.in/~whotdr/
Mahidol University, Bangkok, Thailand
http://www.sc.mahidol.ac.th/scbc/cbag/index.htm
of the implications of globalization and changing social, political and civil structures for health equity and the persistence, emergence and resurgence of TDR diseases, reflecting growing interest in the complex relationship between poverty and health.

The TDR target diseases form a spectrum stretching from those which are neglected and lack adequate tools for their control to those where tools have been available for many years and the scene is set to reach elimination of the disease as a threat to public health. Depending on the category, the approaches applied are naturally very different and so are the outcomes. While we are still groping in the dark to find targets for vaccines, diagnostics and drugs regarding some of the infections under study, the control of others has become more a question of guiding scientists towards the social, economic and behavioural aspects. Research support has been widely dispersed over the ten diseases and various topics, and this overview of activities of the last biennium concentrates on topics where most progress has been made.

**AFRICAN TRYPANOSOMIASIS**

A start has been made to characterize the *T. brucei* genome project reference stock with respect to growth and differentiation in vitro, comparing the profiles of the bloodstream form to the procyclic form based on a map of protein expression during differentiation. While, due to post-transcriptional processing of transcripts, more changes were expected at the protein level than at the mRNA level in gene expression, little variation was in fact observed despite considerable differences in metabolism. This work is currently being followed up through the network of the European *T. brucei* functional genomics consortium. In another development related to research aimed at finding new drug targets in *T. brucei*, the existence of polyphosphates in the parasite, the size and distribution of these compounds, and their sub-cellular localization and variation under normal growth and stress conditions, have been documented.

With respect to social, economic and behavioural research on this disease, research on the cost-benefit of control is encouraged including the effect on health systems, and community participation in the control of re-emergence and epidemics. This line of research aims at producing important information regarding the impact of inequity of access to health services, and of environmental and behavioural risk factors for infection, particularly in refugee populations.
The assessment, monitoring, and evaluation of behaviours and community-based interventions related to dengue need to be addressed and better understood. These activities include identifying and building partnerships among donors, the public sector, NGOs, and the private and commercial sectors. In 2001-2002, TDR contributed to the development of a package of guidelines aimed at aiding those involved in dengue control and research to better assess, monitor, and evaluate behaviours related to preventing and controlling dengue disease and epidemics. Based on WHO’s behaviourally focused social mobilization and communications planning framework COMBI (communication-for-behavioural-impact), the guidelines demonstrate how to plan and manage a combination of interventions including water container management, improved treatment-seeking behaviour, partnership building, and policy/legislative strengthening. The guidelines are illustrated with examples from over a dozen countries. A trial edition of the guidelines (not for distribution) was completed in October 2002 and then field-tested with eight national dengue teams attending workshops in Laos and Nicaragua. They will become available in 2003.
The network-based systematic analysis of gene function in *Leishmania* has been very successful. The transformation efficiency of the genome reference strain has been improved and so has the polymerase chain reaction (PCR)-mediated transfection approach originally developed in yeast. Moreover, the application of fluorescent activated cell sorting (FACS) to the selection of transformants is deemed valuable for future progress.

Proteophosphoglycan (PPG) is an important virulence factor in the sandfly vector; it blocks feeding and thereby promotes multiple probing and regurgitation of the parasites into the mammalian host. After cloning the ppg-1 gene in *L. major*, it quickly became apparent that there is a complex family of genes and proteins of large size, repetitive nature and complex post-translational modifications. Based on continuous electrophoresis it was possible to demonstrate that at least one sub-population of PPG is expressed by the amastigote but not the promastigote. Moreover, PPG has been shown to be localized on the parasite surface, in the endosomes, and in the multi-vesicular tubule/lysosome, where it is responsible for binding specifically to macrophages, so enhancing parasite infection of these cells. BALB/c mice infected with PPG-suppressed promastigotes did not produce lesions over a period of several months, while those infected with control parasites produced the expected pattern of disease. Promastigote lipophosphoglycan is the major molecule involved in acid sequence homology to the unrelated membrane glycoprotein PSA-2 (promastigote surface Ag 2 complex) and this homology is restricted to a region of leucine-rich repeats present in the amino terminal of PPG. Amastigotes have also been shown to be capable of infecting and surviving in a newly identified dendritic cell population, the mouse equivalent of the human plasmacytoid dendritic cell that is abundant in the spleen, lymph nodes and thymus.

Parasites like *Leishmania* cannot synthesize purines *de novo* but instead depend on a salvage pathway. Based on the rationale that this pathway is sufficiently different from that in humans, a structural investigation of enzymes involved in purine metabolism of *Leishmania* has been undertaken. The crystal structures for adenylosuccinate lyase, adenine phosphoribosyltransferase (APRT) and hypoxanthine guanine phosphoribosyltransferase at around 2Å have been cloned, expressed and crystallized. The search continues through screening for lead compounds in the form of inhibitors of APRT.
An important milestone towards controlling transmission of malaria was achieved in 2002 through completion of the *Anopheles gambiae* genome project. The whole genome sequence of this mosquito, the main vector of malaria in sub-Saharan Africa, has now been assembled and is being annotated. In addition, a peptide consisting of 12 amino acids and known as SM1, which binds to the mosquito midgut and salivary tissues, has been described. This peptide is capable of blocking invasion of salivary glands and midgut epithelium, implying that it recognizes a common ligand. Constructs containing this peptide were introduced into the germline of *An. stephensi* resulting in mosquitos that were severely impaired in their ability to transmit *P. berghei*.

Germline transformation of the major African malaria vector, *Anopheles gambiae*, was achieved using the piggyBac transposable element marked with enhanced green fluorescent protein (EGFP) injected into mosquito embryos. Two G1 generation male mosquitos expressing EGFP were identified among 34,143 larvae screened. Particularly high levels of expression were observed uniformly in salivary glands and, in most individuals, in the anterior stomach. An improvement in the injection technique at the end of the studies resulted in increased G0 hatching, transient expression and EGFP-expression rates among G1 progeny. These results represent an important step towards the genetic transformation making *An. gambiae* incapable of transmitting malaria.

In further work on the mosquito genome, the members of two important gene families expected to play central roles in olfactory signal transduction in *An. gambiae* have been isolated and characterized. These are odorant receptors (AgOns) and sensory arrestins which are critical for desensitization pathways. Sequence information of five AgOn genes was obtained. Furthermore, AgOns 1-5 are all specifically expressed only in the olfactory apparatus of *An. gambiae*. AgOn1 displays female specific expression while AgOns 2-5 are detected in both male and female. AgOn1 expression is down-regulated directly after a bloodmeal.

A study of effective population size of *An. gambiae* has found that, contrary to initial expectations, the size was in the thousands and not correlated with drastic shifts in seasonal...
abundance, supporting the hypothesis of a low-density deme (locally interbreeding group) with extensive migration between demes. This finding has important implications for the spread of insecticide resistance and of genetically modified mosquitos.

Different *P. falciparum* dihydrofolate reductase-thymidylate synthase (DHFR-TS) recombinants have been expressed in vitro to allow for development of a rapid drug screening assay. Substantial progress has been made with regard to purifying the bi-functional DHFR-TS enzyme as well as improving the expression of DHFR with the goal of subsequent crystallization and structural studies. Although minor contaminants remain, the purified enzyme does form crystals in the presence of WR99210 (an experimental antifolate) and these are now being exploited to obtain structural information. The experiments have also shown that a full-length junctional sequence in addition to the enzyme sequence is required to express a catalytically active enzyme.

Another line of research, aimed at identifying both hepatocyte proteins that interact with the *P. berghei* thrombospondin-related anonymous protein (TRAP) and also the sporozoite proteins that interact with the C-terminus of TRAP, has produced glutathione S-transferase-fusion of the TRAP A-domain, present in integrins and other adhesive proteins, and generated antibodies against the expressed protein.

Further, a project designed to investigate the role of chemokines (soluble molecules that alter the behaviour of white blood cells) and chemokine receptors in the development of cerebral malaria has delivered new information on the up- and down-regulation of a variety of immuno-modulatory genes in murine non-cerebral malaria (NCM). Expression-profiling of brain material comparing murine NCM brain to normal mouse brain has been carried out and results confirmed by quantitative reverse-transcriptase PCR.

A two-year prospective study of costs and performance of the malaria control programme in two counties with endemic *P. vivax* malaria in Henan Province, China, showed great variations in case management. Of 12,325 suspected malaria cases, only 131 (1%) received excellent care while a further 4,414 (36%) received mediocre case management. However, as many as 7,780 (63%) cases were dealt with in a clearly inadequate manner. The researchers concluded that efforts should be made to improve the efficiency of case management if malaria eradication is to be achieved, and that good management requires continued government investment in malaria control. Case management would improve if village doctors began anti-malaria treatment more promptly and continued treatment longer. This can actually be achieved without increased government investment since other aspects of case management, such as rapid patient access to care and choice of drug, are already operating well. Decreased government spending would increase the costs for the suspected cases and their families and delay treatment. Such a development would increase the risk of recrudescence transmission and ultimate breakdown of control, as has occurred in the endemic countries of South Asia but so far not in China.


The unravelling of the schistosome genome has progressed smoothly, leading to rapidly increasing volumes of data. Sequencing techniques and PCR-based approaches have been applied to the coding regions of the mitochondrial genomes of all five species of schistosomes capable of infecting humans. Little intra-species variation has been found, but there is clear inter-species variation. These observations were made within the nad4L gene, believed to be involved in transcription regulation and in respiration, and the findings could help identify new drug targets.

Further genome-related research has resulted in the production of complementary DNA (cDNA) libraries representing different developmental stages of two species, and these are useful as templates. A consortium of five laboratories generated over 14 000 ESTs for S. mansoni and 1850 for S. japonicum, representing various schistosome developmental stages. Annotation and characterization of these sequences showed that over half – 7269 for S. mansoni and 1134 for S. japonicum – are unique. Another research group working to produce a low-resolution map for chromosome 3 of S. mansoni has constructed three contigs using a bacterial artificial chromosome (BAC) library which was localized to the chromosome by using the fluorescence in situ hybridization (FISH) technique. ESTs were then used to fingerprint the BACs, resulting in 14 791 end sequences.

Results from a closely related area of research have demonstrated, for the first time and using the S. mansoni heat shock transcription factor (SmHSF) as a model, that structural diversity modulates the DNA-binding activity of SmHSF at different stages of the parasite life cycle, resulting in patterns of alternative splicing. Binding and inhibition studies revealed that the DNA binding domain (DBD) fragment and the full length SmHSF molecule differ in DNA sequence recognition, suggesting that elements inside and outside of the DBD contribute to the binding specificity of SmHSF.

Recent results from the field of immunology and pathogenesis of schistosomiasis indicate that different clinical presentations, e.g. acute schistosomiasis or intestinal disease, could be due to individual variations in immunological response to the infection. In addition, the antigens released from the egg have been shown to contain at least 22 different components divided into six major families. It was demonstrated that macrophages respond to the majority of these released egg antigens with interleukin-6 (IL-6) production, resulting in the stimulation of a Th1 response which, after the first week of infection, changes to a Th2 response.
There have been significant advances in understanding the role of antibodies to *Trypanosoma cruzi* P protein ribosomal proteins in the pathology of chronic Chagas heart disease. Fine-mapping of these epitopes has elucidated the stimulus giving rise to the antibody response, which contributes to the development of cardiac symptoms. The antibodies have been shown to react with receptors coupled to the second extracellular loops of the heart membrane G protein that resembles the hydrophilic C-terminal epitopes of the P protein. The specificities of the functional antibody demonstrated are potential novel markers for ventricular arrhythmia and sinus-node dysfunction in Chagas disease.

Experiments designed to study the influence of parasite and host genetic variation on the tissue distribution of *T. cruzi* have confirmed that there is a strong influence of host genetic background as well as differences with regard to the genetic configuration of the infecting clone. Experiments using mixed infections with different *T. cruzi* clones with varying degrees of genetic distance, as revealed by micro-satellite patterns, showed a selection for two of the strains in all tissues analysed, except in the heart of the animals where there was a predilection for one specific strain. In addition, in single or double infections using two different cloned *T. cruzi* strains in five different mouse strains, some with the same and some
with different haplotypes, a clear haploid-dependence on tissue tropism was found. However, preliminary efforts to genetically type T. cruzi parasites isolated in different tissues of Chagasic patients indicate great profile-diversity among the T. cruzi populations analysed.

In addition, substantial progress towards the major goal of obtaining structural and functional information of trypanosomal trans-sialidases has been achieved. The optimal conditions for protein expression and purification of the recombinant forms of Trypanosoma rangeli sialidase and T. cruzi trans-sialidase (TcTS) have been established and the high-resolution structures of both enzymes elucidated, alone and in complex with various ligands. The structural analysis, complemented with a detailed mutagenesis and kinetic study of the two enzymes, allowed a general mechanism to explain the molecular mode of action of trans-sialidase to be proposed. Furthermore, the crystallographic structures of unliganded, substrate-bound and inhibitor-bound forms of TcTS provided a framework for the rational design of specific inhibitors with potential therapeutic applications. In parallel collaborative studies, the trans-sialidase from T. brucei has also been identified and characterized. Structural studies of the T. cruzi and T. brucei trans-sialidases in complex with potential inhibitor compounds and transition-state intermediate analogues are under way.

**LEPROSY**

Five studies on development of new diagnostic tools for early infection with leprosy and on pathogenesis of nerve damage are ongoing. One study has identified an M. leprae component that activates monocytes via toll-like receptor 2 (TLR2). Data have been accumulated on the role of cytokines in modulating TLR2 expression, and activation and expression profiling has been initiated to investigate the consequences of activation. Alpha-defensin has been shown to be upregulated in lipopeptide-activated monocytes and in transfectants to inhibit growth of mycobacteria.

In the social sciences, a study in Ethiopia will explore the influence of socioeconomic and cultural factors, and the resultant gender differentials, in male and female leprosy patients. The study is currently undergoing ethical approval hence no data are yet available.

**LYMPHATIC FILARIOSES**

In the past few decades, self-help organizations have become increasingly important for dealing with illness and disability in Western industrial nations, and evaluation studies report positive impacts of support group participation on members’ abilities to cope with health problems. However, support groups have received little attention in developing countries. An experimental study of support groups for women with lymphoedema and elephantiasis caused by lymphatic filariasis was conducted in Haiti. Five groups in urban and rural communities were evaluated over two years to determine the factors which
influence participation and assess the impact of the intervention on illness management and outcomes. Analysis of the data collected demonstrated that participation in support groups brings benefits in quality of life, understanding of the disease, home care practices, and illness symptoms. The support groups were sustainable, despite scarcity of resources, and thus offer a low-cost intervention for people living with chronic disease and disability in resource-poor settings.

**ONCHOCERCIASIS**

Several possible prenylation substrates in *O. volvulus* have been explored; the b protein farnesyltransferase (bPFT) has been expressed and purified, but functional activity cannot be studied until aPFT is also available. Full-length clones are not yet available for this gene but it is hoped they will appear in the genome sequencing project. Inverse PCR or genomic library screening could also be employed to clone the genomic locus.

**Health sector reform and social, economic and behavioural research**

On a worldwide scale, parasitic infections and other diseases disproportionately affect populations living in poverty. The last decade has witnessed a series of government-level reforms aimed at improving equity of access to care and thus the general health status of all citizens. The current expanded TDR support of research into health sector reform in the developing world grew out of a set of ideas developed and initiated in the mid-1990s, and was, in large part, sponsored by the Norwegian government. TDR takes an active interest in this area since it feels that health management in general has a strong impact on its overall approach to improving the lot of those at risk for, and very often infected by, the common infectious endemic diseases of the developing world.\(^\text{[10]}\)

Health legislation alone is seldom capable of creating true community participation in settings with a limited history of democratic participation, and there is little correspondence between official policy and reality when distinctions between public and private health care disappear. Research supported by TDR with regard to the impact of broader reforms in the overall organization of Zambia’s health care system has provided valuable insights regarding the forces preventing governments from taking the actions necessary to reform the hospital system. Similarly, serious implementation problems emerged in Colombia despite radical overhaul of the entire system of health financing for the poor including substantial increases in resources. In India, research on hospital accreditation in Mumbai reveals what seems to be a real willingness to participate in a voluntary accreditation system but large gaps in access to health care remain between rich and poor. Several interesting findings are reported from China:


12 Learning from experience: research on health sector reform in the developing world. *Health Policy and Planning*, 2001, 16 (Suppl. 2).
Increasing costs and breakdown of the insurance system have widened the gaps in health access between rural and urban, and high and low income, groups.

Economic and social barriers leave the growing migrant population without adequate prenatal care, leading to poorer pregnancy outcome compared to permanent residents.

Public hospitals function essentially as private enterprises, making it impossible for many people to afford medical care.

A study from South Africa reveals a surprising neglect of sexually transmitted disease (STD) treatment, and that doctors tend to prescribe less expensive and less convenient treatment regimens to poor patients. A similar issue in the different world of rural health centres is reported from Uganda. Like in China, the central government has urged fee exemptions to protect the poor but has not provided funding to pay for lost revenue, which has stymied implementation of the initiative at local level. In Laos, it is reported that user fees and bureaucracy in public facilities have led to growth of the private health care sector where now both the rich and the poor get most of their care, the rich mainly from private clinics and the poor from pharmacies selling drugs without examination. However, with regard to the impact of specific changes in health care financing on expenditures and service utilization, several new results have emerged:

- A successful pharmaceutical cost-containment policy for hospitals in Shanghai.
- A new policy for hospital financing in Indonesia.
- A shift of relative health care utilization in Zambia, from hospitals towards health centres.

Social, economic, political, behavioural, and health system factors all affect, and are affected by, disease patterns and disease control efforts. Social research to elucidate these factors is key to effective infectious disease control programmes, product development, and implementation. The TDR Steering Committee on Strategic Social, Economic and Behavioural (SEB) promotes and manages social research and training on a number of critical issues related to tropical disease control and international public health, particularly focusing on:

- Inequality of access to health care.
- Policy processes.
- Gender-sensitive interventions.
- Conflict and infectious diseases.
- Ethics of social research and biotechnology.

SEB seeks to bring a broad array of social science methodologies to bear on the chief causes of morbidity and mortality among the world’s poorest. Only by embracing a true trans-disciplinary “bio-social” approach can we intervene successfully. For example, when irrigation schemes or dam construction alter the epidemiology of schistosomiasis or filariasis, the views of health workers as well as of policy-makers should be taken into account. When recurrent drug stockouts characterize a tuberculosis control programme, patient attitudes and practices may be of less relevance to the emergence of drug resistance than fluctuating drug prices, tariffs, and poor drug quality. When poor blood-screening practices lead to an increased prevalence of Chagas disease, the anthropology of blood banking and blood-bankers is called for. The emergence of new strains of infectious agents resistant to our therapeutic arsenal could be thought of as classic biosocial problems. There is simply no way to understand the dynamics of emerging drug resistance without an understanding of both microbial and human behaviour.13

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There is much evidence that armed conflict and infectious diseases are intimately linked together, so the interface of the TDR diseases with conflict and other crises is an important new SEB priority. The first activity in this field was a workshop in Manila in April 2002 which was attended by 25 social scientists, epidemiologists and health systems researchers from 12 different countries, including some which are currently unstable as a result of current or previous conflict. The aim of the workshop was to develop a framework for examining how communities and health systems cope with instability, insecurity and infectious diseases, with a view to identifying and supporting resilience. It has been suggested that 65% of infectious disease epidemics occur in unstable countries, while direct war-related mortality ranks in the top 12 most common causes of death worldwide and is projected to become the 8th most common cause of death by 2020. Displaced people typically experience high mortality, especially in the period immediately following their displacement; infectious diseases and nutritional disadvantage are key factors. In conflict settings, the quantity and quality of health care available is usually greatly reduced; vector control programmes, outreach services, training, referral, and drugs distribution are all typically impeded. In Ethiopia and Mozambique, epidemics of malaria were associated with deterioration in disease control activities and, in Nicaragua, an increased risk of malaria was associated with war-related population and troop movements, inability to carry out timely disease control activities, and shortages of health personnel. In central Africa, work presented at the SEB workshop highlighted the linkages between the spread of the Ebola virus and military movements. Paradoxically, however, conflict situations can also have positive impacts on societies and health systems, as has been demonstrated in Nicaragua, Mozambique, Viet Nam, Eritrea and Tigray. In the popular conflict against the Ethiopian ruling class, the Derg, community-based political movements in Eritrea and Tigray engaged strongly in building community structures for participation and decision-making, facilitated the development of multi-sectoral health promotion strategies, and identified innovative community-financing systems. Outputs of the workshop included:

- Country profiles focusing on conflict with regard to infectious diseases, health systems and community responses.
- A cohort of enthused research activists who are keen to explore, understand and promote improved policy responses to infectious diseases and conflict.
- A decision to commission a review of how infectious diseases and conflict interact and how communities and health systems respond.
- An agenda for research on conflict and health systems.

TDR, including SEB, is interested in distributing research results to a wider public, and grantees are encouraged to publish in international peer-reviewed journals. The outcome of TDR scientific writing workshops has been publication in three TDR-sponsored special issues of a high-quality scientific journal (see: Technical Information). In addition, three SEB publication series were established during the biennium (see: Technical Information).