Tropical Disease Research

Progress 1995–96

Thirteenth Programme Report

UNDP/World Bank/WHO Special Programme for Research & Training in Tropical Diseases (TDR)

Tropical Disease Research
1995-96
UNDP/World Bank/WHO

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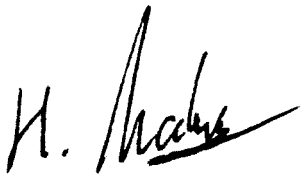
Foreword

It is estimated that approximately 500 million people, most of them poor, suffer from tropical diseases. This exposes them to many kinds of suffering, including deformity, blindness, brain damage and premature death. Tropical diseases are a serious impediment to individual and national development. They impair intellectual and physical growth, make fertile land unuseable, and burden economies with huge costs for treatment and control.

The Special Programme for Research and Training in Tropical Diseases (TDR) is jointly sponsored by WHO, UNDP and the World Bank. Its task is to control eight particular tropical diseases. It does this by supporting research on, and developing, new tools to prevent, diagnose and treat its target diseases. It also supports the training of scientists from developing countries and contributes to improving research facilities in developing countries. TDR coordinates multicentre and multicountry studies effected through networks created among research groups, institutions, ministries and industry. This process involves minimal investment in infrastructure and produces effective, decisive results. In this way, new products are developed very cost-effectively.

Substantial progress has been made on four of TDR's target diseases (lymphatic filariasis, onchocerciasis, Chagas disease and leprosy), although some research remains to be done if the current success is to be followed through to elimination of the diseases as public health problems. In contrast, new tools are urgently needed to control schistosomiasis, sleeping sickness, leishmaniasis and malaria. Malaria, in particular, remains a considerable challenge. The fight against tropical diseases can be won, but it needs the wholehearted support of the entire international community.



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Note

This report is compiled disease by disease, and TDR is organized along the lines of Strategic Research, Applied Field Research, Product Research and Development and Research Capability Strengthening. To facilitate reading therefore, each chapter has been colour coded according to research area.

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Photographs: Page 2 Poster: B.K.Tyagi. Page 26 Top WHO/TDR/Crump; Page 26 Bottom (L) WHO/TDR/Crump; Page 26 Bottom (R) WHO/TDR/CDC/Gethaney. Page 32 Top left WHO/TDR/Edwards; Page 32 Top right WHO/TDR/Crump; Page 32 Bottom WHO/TDR/Chandran. Page 38 WHO/TDR/Edwards. Page 41 WHO/TDR/Crump. Page 45 WHO/TDR/Martel. Page 53 WHO/TDR/Crump. Page 63 WHO/TDR/Crump.
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CHAPTER 1

The reorganization of TDR:

There was a good response from the scientific community in developing countries to TDR's poster competition. This winning entry illustrates that TDR is helping produce solutions to disease problems across the tropical world. This is TDR's gift as we move into the 21st century.

Achievements and new challenges

The reorganization of TDR began in early 1994. Three years later is an appropriate time to review this reorganization from an 'internal' perspective. Has it worked? Has the programme become more efficient? Has it become more effective? Is it serving health/science better than before? What lessons can we draw?

A review of TDR from another perspective is also under way. The Third External Review of TDR, for which this programme report provides input and background, is due to be completed by June 1998, and it will look at the reorganization from an external perspective. (Two earlier external evaluations took place in 1981-82 and 1986-87.)

Why did it happen? The old structure and rationale for change

Until 1994, TDR was largely organized by disease and operated through a series of steering committees. These committees covered all aspects of research within a disease, from basic research to operational research (Fig. 1); although malaria and leprosy were exceptions, each having several steering committees:

malaria:

- immunology
- chemotherapy
- applied field research

leprosy:

- immunology
- chemotherapy.

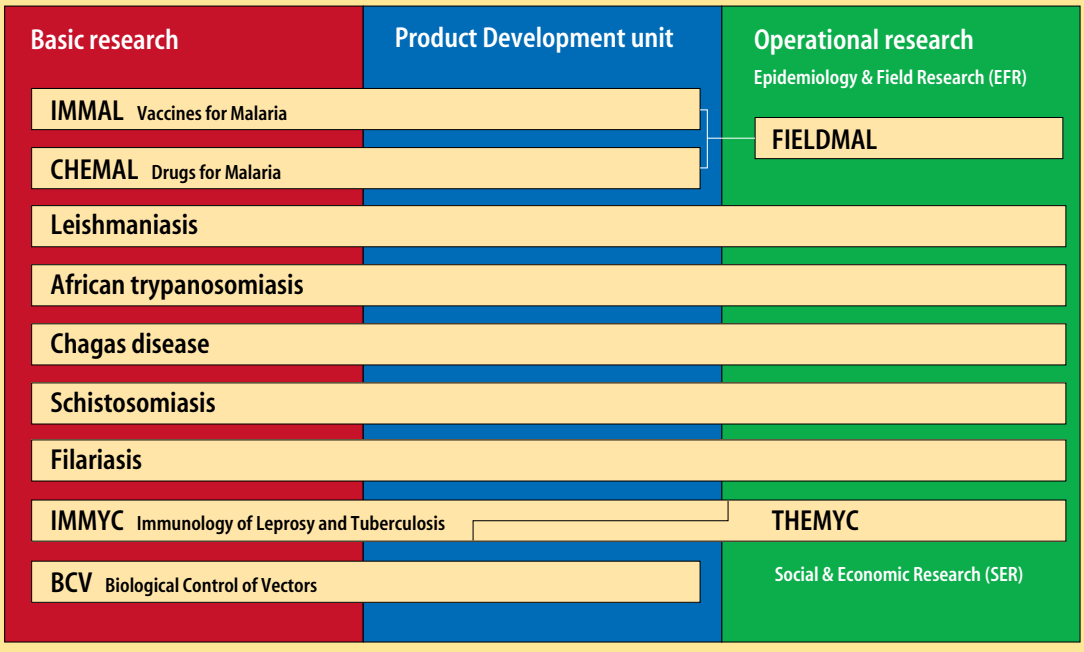
In addition, there were two discipline-specific groups:

- social sciences
- epidemiology.

The strength of this organization was that each activity was very circumscribed and by and large reflected what was happening in the scientific community. It was easy to manage. Projects were investigator-initiated and resources were allocated on the basis of relevance and merit. However, there were several limitations:

i. Being in a situation of no growth, the time came when we could no longer cover all aspects of research and had to become selective. The structure

Fig. 1 TDR R&D structure pre-1994



and operating mechanisms did not lend themselves to more focus, nor could high-priority tasks be backed with sufficient resources.

ii. There was not enough focus or prioritization and we realized that, if the outomes of the research were to be used for disease control and policy development, they needed to be based on multicentre, multicountry studies.

iii. It was difficult to achieve a 'critical mass' of expertise in any area such as basic research, product development and applied field research.

iv. Projects were analysed according to both relevance and merit. This meant that support went to projects that were neither good basic research nor good product development; and product development (especially in the field of diagnostics) tended to end when the incentive for the scientist ended – i.e. with a publication rather than a disease control tool.

v. Managerially, the programme was rigid. There was one person in each disease component and cross-fertilization was difficult.

Because of the recognition among TDR staff that needs in product development were not being met, a product development unit was introduced in 1990 to overview product development and manage a special product development initiative. However, its relationship with the steering committees remained blurred and the intentions were not achieved.

Limitations in applied field research were also recognized. In particular, projects had become largely intervention related; the necessary multidisciplinary composition was not being achieved and it was felt, by the Social and Economics Research steering committee, that the social sciences should permeate all areas of applied field research.

In basic research, we strove to get first-rate molecular biologists into each of the steering committees in the late 1980s. But by the 1990s, the 'game' had changed, and the potential of science had become more limitless. There were quantitative leaps, and 'big' science won ground. TDR could not exploit this by staying in a disease-by-disease mode.

In short, why did it happen? Because TDR staff recognized that it needed to happen and wanted it to happen.

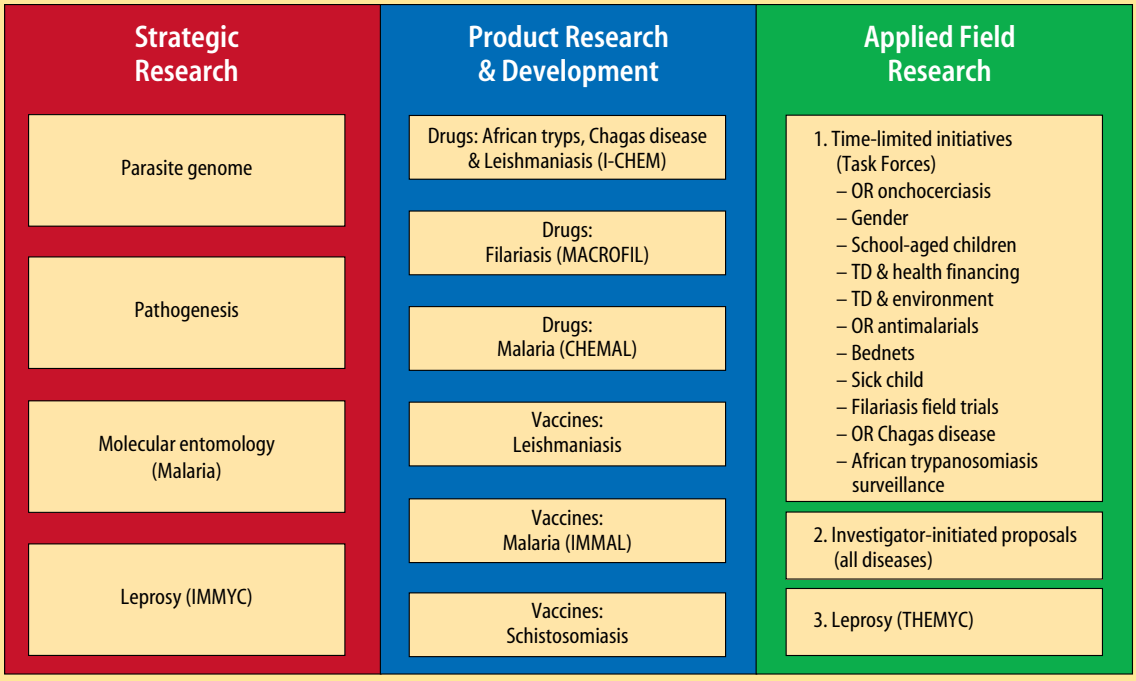
The new overall structure

These considerations and an assessment of TDR's comparative advantage (Box 1)[1] led to the following new TDR structure and management:

i. Division of TDR's R&D into three areas:

- Strategic Research,
- Product Research and Development,
- Applied Field Research.

Fig. 2 TDR R&D structure post-1994



The fig. shows the principal changes which took place in 1994. However, some changes took place before 1994, e.g. the establishment of MACROFIL and I-CHEM in 1991. The shown changes in Strategic Research were arrived at after the first meeting of the Strategic Research Steering Committee in September 1994.

Box 1. TDR's comparative advantage[1]

TDR's strengths give it certain advantages over other actors on the development scene:

- *Unlike national research councils,* TDR has an international research network and a global vantage point from which to identify unmet research needs and to plan and carry through international research projects or programmes that uniquely fill those needs.
- *Unlike bilateral funding agencies,* TDR mounts projects that combine research and research training that involve, simultaneously, individual scientists, research groups, institutions and disease control programmes, and that cover several countries or regions.
- *Unlike private foundations and agencies,* TDR has the international backing and base, and the organizational and financial staying power to see large-scale projects through to completion.
- *Unlike private industry,* TDR can follow-up on product development leads that may require a heavy investment in time, work and manpower without a strong guarantee of a commercially acceptable return on the investment.

Box 2. Definitions of TDR areas of research before the reorganization (1992)[1]

- **Strategic research** employs the latest tools and advances in basic science to explore basic disease mechanisms, such as host-parasite relations and parasite biology, in order to achieve its *strategic* goal of producing radically new solutions that could strengthen disease control.
- **Product research and development** selects from the leads and prototype tools stemming from strategic research those with the greatest potential and takes them through the development process up to and beyond the Phase III (large-scale clinical trials) stage.
- **Applied field research** seeks to identify the health problems and needs of communities and disease control programmes with a view to determining the best, most cost-effective ways of reaching affected communities with solutions most likely to be accepted and to have the strongest impact on disease.

Each of these areas covers selected elements of all TDR diseases (Fig. 2).

Definitions of the terms were set out in a background document and are reproduced in Box 2.[1]

ii. In order to increase the flexibility and manœuvrability of the programme, each programme manager is assigned different tasks, often within different areas.

iii. Research topics are selected to meet certain targets and outcomes. This requires proactive, rather than investigator-initiated, management.

The changes that took place are described below.

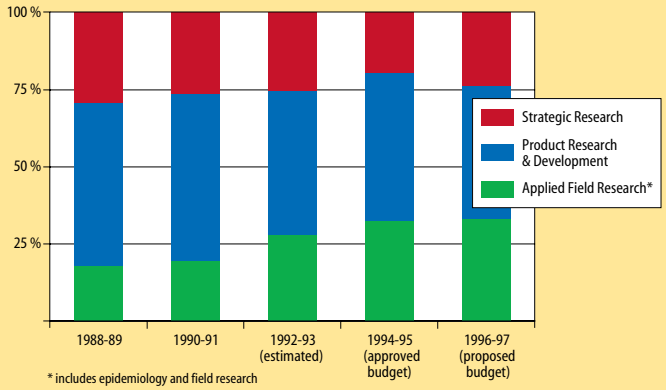
Strategic Research

In order to focus TDR's research, we defined our involvement at the more basic end as 'strategic' research. While the purpose of basic research is to generate new knowledge, we defined strategic research as basic research that would develop new product development opportunities (Box 2).

The three components chosen during 1994 for this area were:

- **Parasite genome**. For each parasite, except malaria, a genome project was launched (malaria was excluded because an international project, initiated by the Wellcome Trust, was already ongoing). The tasks were to identify the strain of parasite to be used, develop gene libraries, and map and sequence the genomes. Genome research represents a paradigm shift in biology. By pioneering this approach for the parasites, I believe TDR has fully exploited its comparative advantage by launching a focused, forward looking, international project. The investment has been remarkably small – only US$ 100 000 – 200 000 per year per genome project. A quantum leap has been made in DNA sequencing and a basis made for high-throughput sequencing for several parasites. Discussions about the post-genome agenda have already started, about how to use genome information for product development.
- **Molecular entomology**. This component is also highly focused (on malaria) and directive. It has a very practical long-term objective: the development of mosquitos that are resistant to the malaria parasite and the permeation of this resistance through wild mosquito populations. Progress has been remarkable, and particularly rapid in regard to unravelling the molecular basis of the parasite-vector relationship. The component was an initiative of the MacArthur Foundation, with which TDR joined forces; although the Foundation is now

Fig. 3 Trend in TDR's distribution of resources for R&D



phasing out its support (a characteristic of foundations is their lack of staying power, whatever the promise and outcome).

- **Pathogenesis**. This is the third area in strategic research. It is very different from the two above in that it is fully investigator-initiated. Because the competition has been overwhelming, the grant approval rate has initially been less than 20%.

In general, investigator-initiated strategic research was considered to be the area where TDR's comparative advantage was the lowest – it is in this area that many research councils invest substantially, according to scientific merit. When TDR was reorganized therefore, we cut back on overall funding in this area (from 25% to 18% – Fig. 3). However, because the genome and molecular entomology components turned out to represent pioneering approaches, and the pathogenesis component turned out to be very competitive as far as funding is concerned, the Scientific and Technical Advisory Committee (STAC) has given financial priority to strategic research through increased funding (to over 23% for the current biennium – Fig. 3).

When Strategic Research was first planned, there were concerns that the committee for pathogenesis would lack a critical mass for each of the diseases. But we have not found this to be a handicap. Rather, the similarities between diseases are such that cross-fertilization has occurred, and this has been emphasized enthusiastically by the committee. Our general experience after the reorganization is that the composition of committees depends more on discipline and area of expertise than on disease-specific expertise. Many scientists are quite familiar with a range of tropical diseases.

Strategic Research was planned on the basis of there being one committee to cover all three areas, each area being represented by a subgroup of that committee. But this did not materialize. Rather, the committee for each area has become increasingly independent. However, so far they have met jointly for one day to report on their respective activities and hold a seminar focusing on new technological developments (for genome research, combinatorial chemistry, etc.).

A characteristic feature of the reorganization in Strategic Research was that we took on the management of emerging areas of research with existing staff, even though they may have lacked experience in, for example, genome research. This was possible because of our long tradition of letting committees of experts make decisions about funding and, to some extent, direction of research – through the 'steering' committee mechanism, which allows for new areas of research being taken up without recruitment of new staff. Indeed, it would be very difficult to change the composition of staff at the pace required to meet the emerging needs in TDR's programme. Moreover, the qualifications of people prepared to serve on a TDR committee are often considerably higher than those that can be recruited into full-time positions.

Product Research and Development

Already in the late 1980s we had begun to intensify and improve our product development activities, but we needed to become more professional. First, in 1990, we established a product development unit, for which a staff member was recruited from industry. The purpose of the unit was to manage a selected number of products. However, it never completely fulfilled its expectations, and the reorganization of TDR brought with it a new opportunity – to merge the unit with other selected components into an area that would that have product R&D as its sole focus. Thus, the product development unit was disestablished and the new Product R&D area took up the responsibility for taking specific product 'leads' through from discovery to preclinical and clinical development (Box 2), and ultimately to registration.

Since product development is expensive, we had to prioritize our activities. Considering that the eight TDR diseases were each in need of drugs, vaccines, diagnostics and tools for vector control (excluding leprosy), this represented 31 potential lines of product development – and we had been supporting projects in most of these areas. Therefore, R&D relating to vector control was phased out because we had only one promising vector control tool in development *(Bacillus sphaericus* for filariasis, which was moving into field research). R&D relating to diagnostics was also phased out, because of the time and effort it takes to develop diagnostics (almost as much as for drugs and vaccines) and because field resources are so scarce that only rarely are diagnostic laboratory tests actually affordable.

Fig. 4 Schistosomiasis steering-committee supported activities

1993	Drug Development	Vaccines Diagnostics	Pathogenesis	Control related	Total
Strategic Research	2 Projects US$ 93 842	9 Projects US$ 345 948	3 Projects US$ 89 146	5 Projects US$ 99 097	19 Projects US$ 534 191
Product Research		5 Projects US$ 127 181		2 Projects US$ 23 700	7 Projects US$ 150 881
Product Development		1 Project US$ 14 200			1 Project US$ 14 200
Applied Field Research				3 Projects US$ 85 580	3 Projects US$ 85 580
Total 1993	US$ 93 842	US$ 487 329	US$ 89 146	US$ 208 377	US$ 784 852
1994					
Strategic Research					
Product Research		8 Projects US$ 369 057			8 Projects US$ 369 057
Product Development		3 Projects US$ 152 943			3 Projects US$ 152 943
Applied Field Research					
Total 1994		US$ 522 000			US$ 522 000

Fig. 5 Leishmaniasis steering-committee supported activities

1993	Drug Development	Vaccines Diagnostics	Pathogenesis	Control related	Total
Strategic Research	2 Projects US$ 37 700	1 Project US$ 20 000	7 Projects US$ 206 724	CTD/EMR/TDR US$ 46 763	US$ 311 187
Product Research		10 Projects US$ 248 351			10 Projects US$ 248 351
Product Development	4 Projects US$ 114 950	9 Projects US$ 221 000			13 Projects US$ 335 950
Applied Field Research			(HIV-Leish) US$ 13 065	US$ 66 280	US$ 79 345
Total 1993	US$ 152 650	US$ 489 351	US$ 219 789	US$ 113 043	US$ 974 833
1994					
Strategic Research					
Product Research		8 Projects US$ 262 852			8 Projects US$ 262 852
Product Development		11 Projects US$ 264 268			11 Projects US$ 264 268
Applied Field Research					
Total 1994		US$ 527 120			US$ 527 120

Vaccine and drug R&D was finally restricted to 9 of 16 options based on the following criteria:[1]

i. need, in relation to disease burden

ii. potential for impact on disease

iii. scientific opportunity and feasibility

iv. expected time needed for development

v. TDR's special advantage in achieving the goal

vi. cost of development

The nine options became clustered in components:

Drugs:

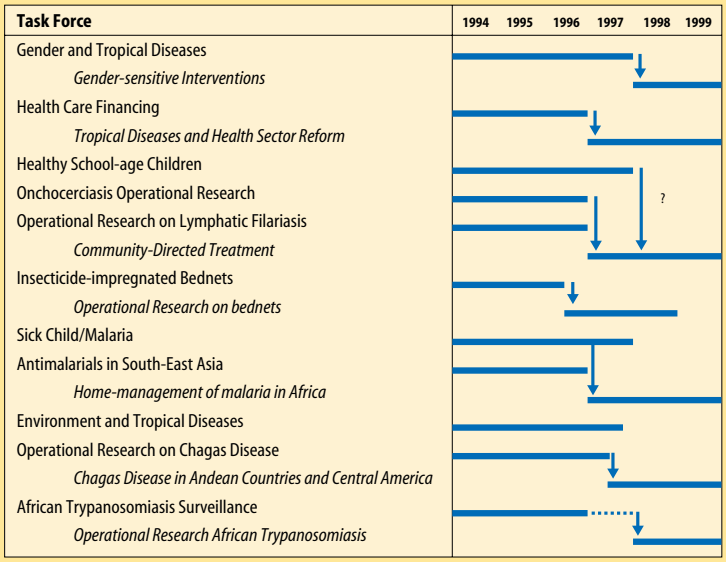
- malaria,
- macrofilaricides for onchocerciasis and lymphatic filariasis,
- leishmaniasis, African trypanosomiasis and Chagas disease.

Vaccines:

- malaria,
- leishmaniasis,
- schistosomiasis.

This prioritization process allowed us to put more resources behind the development of a more limited range of products (Figs. 4, 5). The limitation allowed us to reduce the size of the committees and ensure that they were composed of expertise directly relevant to product development (e.g. medicinal chemistry, toxicology, pharmacokinetics, clinical trials). The reorganization also made the commonalities between the different drug development groups more apparent, and common systems for the handling of compounds, screening and data were established.

Fig. 6 Evolution of AFR Task Forces



Whereas earlier TDR had tended to fund a lot of projects at the interface of strategic research and product research and development, the reorganization led to the development of a wedge between these two areas. This has had some advantages in that all areas of product development have moved into development 'mode'. On the other hand, product discovery research needs to be strengthened, and plans to do this are under way.

Applied Field Research

The area of Applied Field Research (AFR) was to cover post-registration research up to operational research, and to find generic solutions to operational disease control problems. Because site-specific research in disease control was the responsibility of different disease control programmes (Control of Tropical Diseases – CTD, Leprosy – LEP, both of WHO; the Onchocerciasis Control Programme in West Africa – OCP, and the African Progammme for Onchocerciasis Control – APOC), and in order to secure good links with disease control, the area of AFR is jointly managed by Director CTD and TDR, and is physically located within the premises of CTD.

The area of AFR then became subdivided into a series of components which address specific problems and are time limited to three years. These components are called task forces, the composition and evolution of which are shown in Fig. 6. The intention was that each task force would be composed of researchers who actually coordinate and carry out the work. A task force manager is responsible for one or usually two task forces and the activities of each task force are based on well articulated workplans with precise time frames.

The whole area of AFR is overseen by a steering committee composed of disease-specific experts and experts in relevant disciplines of the social sciences; the Socioeconomic Research (SER) steering committee having been merged with the AFR steering committee. In addition to overseeing the task forces, a responsibility of the AFR Steering Committee is to solicit and review investigator-initiated proposals.

During the reorganization of AFR, there were concerns that TDR was moving away from a peer review steering committee approach. Some donors felt uncomfortable about the 'looser' task force approach. In my view however, the change to the proactive task force approach has worked well in the

majority of cases, although some task force managers were slow to change from the reactive steering committee approach. Moreover, it is a continuing challenge to ensure that chosen research topics are translated into large-scale, multicountry studies on which firm policy decisions can be based. In spite of the freedom of operation, most task forces have adopted appropriate mechanisms for project design, development and review and, depending on needs, have become multidisciplinary in composition. The AFR Steering Committee has been very conscientious in reviewing the task forces, but, at the same time, the performance of the task forces has raised the issue of frequency of review. This has been accentuated by the overlapping review pattern of AFR and STAC, which puts further demands on task force managers. Because of this, we have taken the following steps:

i. Some STAC members are now invited to attend the AFR Steering Committee meeting and report back to STAC. This avoids overlap.

ii. The frequency of AFR review of task forces is under consideration.

iii. As part of its mandate, the External Review will evaluate all operations of TDR including the area of AFR.

To my mind, one considerable advantage of the task force approach has been the time limitation, which has given TDR and its review bodies an opportunity to look afresh at particular task force areas on a regular basis. As a consequence, after the first three years, considerable changes are now proposed. For instance, we plan to reduce the number of task forces from eleven to six or seven (Fig. 6), because we have found that the burden of managing more than one task force can slow progress down.

The process of change

Time is required when a programme with many partners to consult, like TDR, needs to introduce change; and we started our staff retreat in January 1992 with the aim of introducing change on 1 January 1994, the beginning of a new budget biennium.

The change was very much staff driven. Some staff expressed concern about losing the disease orientation, and some were sensitive that change implied criticism of the past. But, by the end of 1992, most staff firmly backed the modifications. The scientific community was more sceptical and felt threatened by the change: many thought of the lost opportunity for scientific support in their particular area. This assumption was correct but, being of a culture accustomed to change (the need to acquire new skills, etc.) and competition, many scientists faced the challenge by adapting some of their research to meet the emerging TDR needs.

Another fear was that TDR priorities would become the global priorities as adopted by other funding agencies. This was also a real threat, which could have a negative impact; because TDR's comparative advantage lies in its difference from other agencies (Box 1). Fortunately, as far as we can tell, this fear has not arisen to the degree anticipated.

Views about the approaching changes were quite diverse among Joint Coordinating Board (JCB) members, developing countries and donors, especially at the first consultation. Some were very positive but did not think the changes went far enough; others were especially concerned about the changes in the functioning of steering committees. Some donors emphasized that they could only justify applied field research; others expressed concern about reductions in investigator-initiated basic research; others thought that product development was the core function of TDR. And regional concerns were expressed about regional diseases. To some extent, these divergent views perhaps reflected a lack of clear information; although divergent views like this are not uncommon in the field of international health! However, for whatever reason, when the proposal was later considered in a reworked form, based on comments received, the reorganization of TDR was unanimously approved by the JCB at its 1993 session. I believe that one important factor in this was the time element – which allowed the external community also to consider and digest the proposed changes for about one year.

Implications of R&D reorganization for Research Capability Strengthening

We also considered TDR's Research Capability Strengthening (RCS) activities in connection with the R&D changes, and developed various options for integrating RCS activities with R&D activities. A closer integration of the two components had long been a concern of JCB. STAC considered the various options for structural integration, and chose to postpone it until we had gained experience with the new structure. However, RCS activities were streamlined; and RCS and AFR steering committee meetings began to be held back-to-back to facilitate overlap.

Subsequently, and as a result of the clear delineation of Strategic Research (SR), Product Research & Development (PRD) and AFR, close collaboration between R&D and RCS developed across the range of activities. For example, in Strategic Research, support has been given

Fig. 7 TDR contributions 1977-96

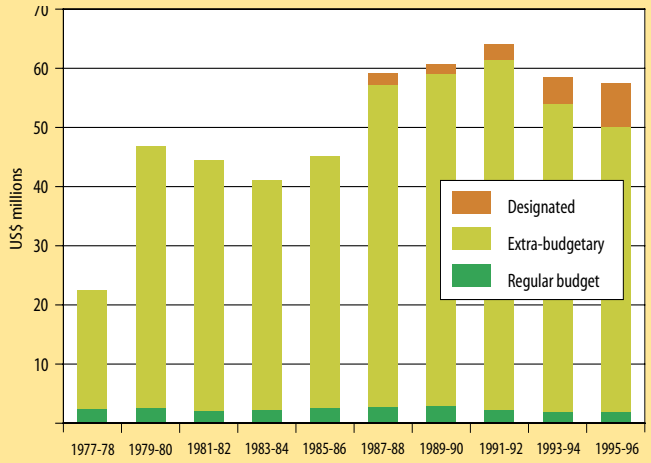
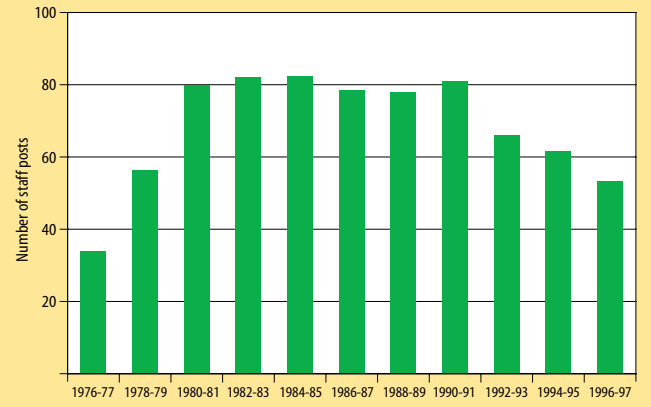


Fig. 8 TDR personnel 1976-97



for training in genome research and molecular entomology. In pathogenesis, RCS has supported for one year those proposals that scored just below the threshold for the Pathogenesis Committee (which is determined by the funds available to it), allowing scientists the chance to re-compete the next year. As a result, the proportion of grants from the Pathogenesis Committee going to scientists in developing endemic countries has steadily increased and had reached 40% at the last round. In addition, grants for the transfer of high-throughput DNA sequencing technology have been provided by RCS.

In the area of PRD, training workshops for clinical trials monitors were supported by RCS in the early 1990s. Later, and as a result of specific deliberations at the JCB, a new initiative was launched with a focus on the transfer of technology required for product discovery and development (drugs and vaccines). These grants are linked to the transfer of specific drug or vaccine candidates for malaria. A major aim of the effort is to facilitate countries' own goals to acquire full-fledged R&D capability for pharmaceuticals early in the 21st century. The costs are largely covered by the countries themselves and therefore represent very significant contributions to the development of products for the control of malaria.

But it is in the area of Applied Field Research that the closest collaboration has developed. In this area, RCS has supported a number of workshops for field research methodology development and the task forces have been instrumental in identifying trainees for RCS, particularly in countries where research is very limited, such as Laos.

Maintaining manœuvrability under financial constraints

As shown in Fig. 7, TDRs financial contributions have shown a downward trend since 1991-92. The reorganization took place against a background of anticipated resource constraints; it was intended to help TDR remain flexible and able to respond to new challenges. I have outlined above how the reorganization allowed us to take up new initiatives such as genome research and task forces. Four additional factors can be emphasized:

1. A consistent and stringent personnel policy relating to retirement at 60, to keeping posts vacant to the extent possible, and to farming out tasks (often to previous TDR staff), has allowed us to reduce TDR posts from 81 in 1991 to 53 in 1996 (Fig. 8). In the next biennium we propose to keep to this level, and will keep as many posts as possible vacant until after the External Review. This will give us the flexibility to take up new directions. The curtailment of staff has also led to curtailment of staff costs, estimated at 23.3% for 1996-97, close to the target of 20% which both STAC and JCB urged should be implemented with caution. Consequently the proportion going to operations in 1997 is estimated to be 68.1%, close to the target of 70%. The ability to move operational funds around enhances flexibility.

2. In the new structure of TDR, most staff agreed to carry out more than one task, often in different areas of the programme. Having a foot in two camps means that a staff member's input no longer stands and falls with a single component. This again enhances flexibility.

3. Having projects which are time-limited is important. It is a common feature of research management. Direct funding from programme components to principal investigators at country level also helps. Keeping the operation flat in terms of management structure is a key strategy.

Fig. 9 Network development: Location of centres involved in onchocerciasis operational research studies

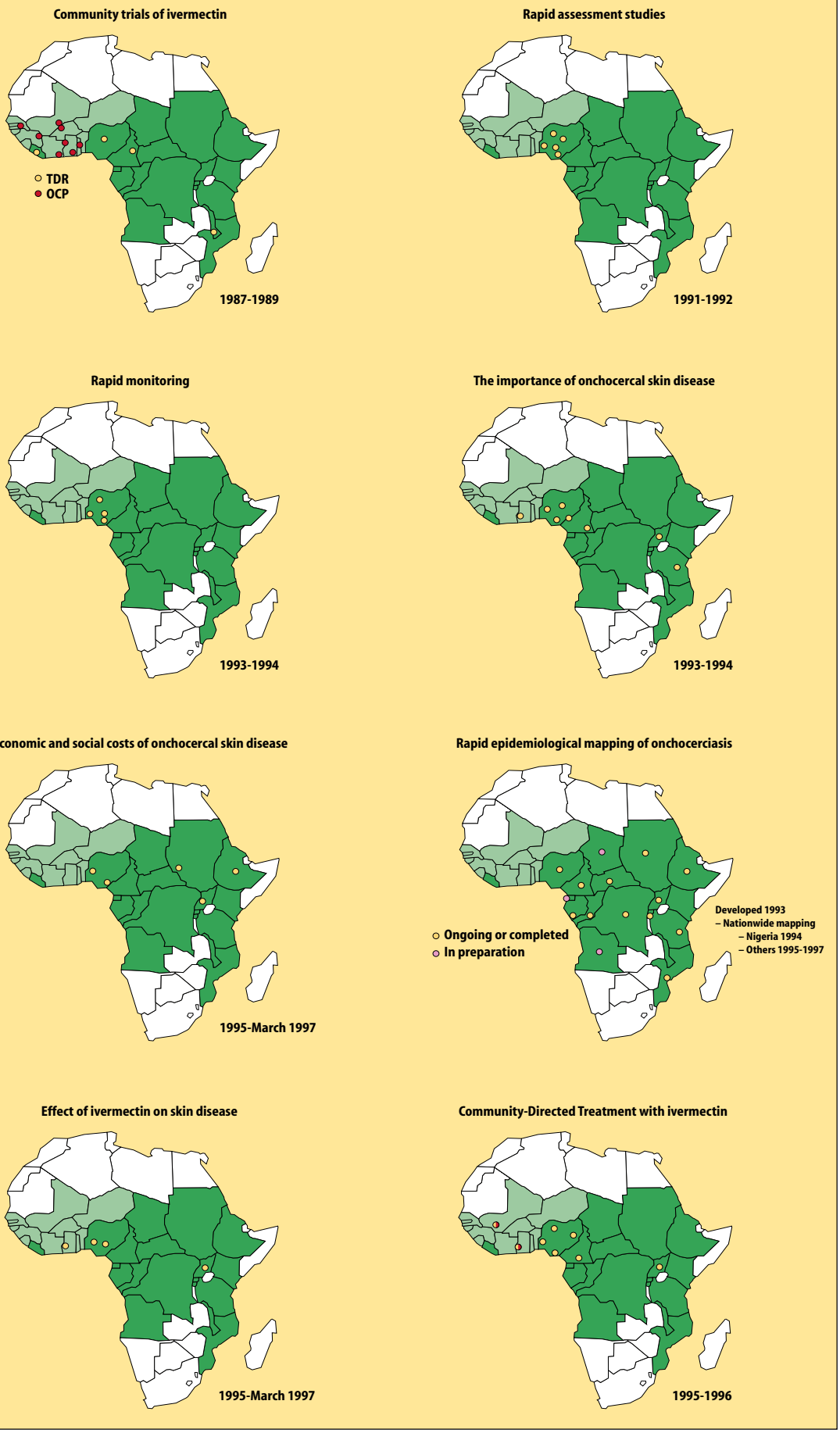
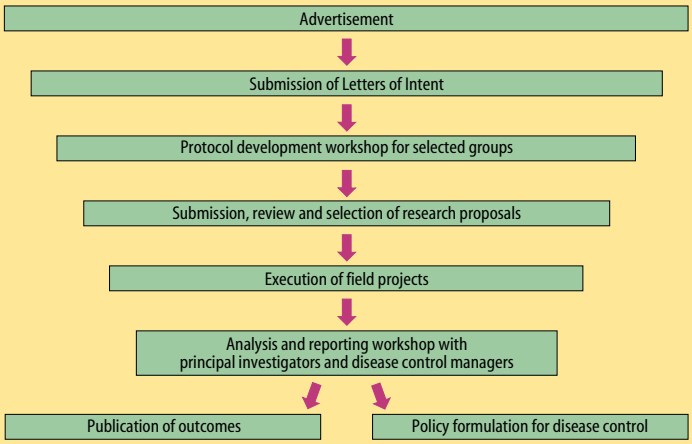


Fig. 10 TDR approach to Applied Field Research



4. A continuing challenge is to design studies in such a way that they not only solve problems but also provide a sufficient basis from which to tackle the next problem and a solid basis for control policy. This is illustrated by the ivermectin networks shown in Fig. 9.

From network to network: driving ivermectin towards disease control

When, in 1987, it became clear that ivermectin would become available free of charge, our concern was only the question of safety and we did not give much consideration to other aspects of ivermectin distribution. However, step by step we recognized the need for rapid assessment of onchocerciasis at the village level, and subsequently for rapid epidemiological mapping, a fundamental requirement before ivermectin could be distributed. Then the discovery of the true impact of onchocercal skin disease was made, which required confirmation in different settings. This led to the need for testing ivermectin on skin disease and, finally, to the issues relating to the supply and distribution of ivermectin in the community.

These issues have been dealt with by a series of investigations, each a multicentre trial, initially starting in Nigeria but later becoming multicountry studies (Fig. 9). As outlined in Fig. 10, they have each followed the 'TDR approach' to this type of research. It is interesting to note that, of 44 investigations, only 13 centres have carried out more than one trial; and most trials have been highly diverse in terms of discipline (comprising economists, anthropologists, psychologists, pathologists, epidemiologists, parasitologists and dermatologists). Despite the multidisciplinary requirements, through our approach and advertisements, we have always been able to find African teams able to carry out the research.

In research management, discussions about the role of networks often crop up. Our experience in TDR suggests that networks are formed and disappear as problems are solved. Surely we would not have achieved our tasks as speedily, and at such a low cost, had we selected only a fixed number of centres to carry out our applied field research; nor would over 150 scientists from 17 countries have gained from participating in this research. Since we found all the scientists we needed for our multidisciplinary activities in Africa, I sometimes wonder if its not more a question of capacity 'utilization' than of capacity 'building'.

TDR outcomes and health impact, 1995-96

Outcomes can be measured from a managerial as well as product perspective. My predecessor, Professor A.O. Lucas, once showed TDR as a stepwise process: projects, publications (scientific publications in the peer reviewed press), products, and packages. This reflected a flow of events typical of TDR, and also, to some extent, the evolution of the programme. In the beginning, the programme was evaluated very much by the growth of projects and subsequently by the growth of publications supported through TDR grants. In the biennial report for 1985-86 we introduced a products table, and, in 1991-92, a targets table, with time frames against which future activities would be measured. The latter is clearly a managerial type of outcome (the termination of a product development activity is a successful managerial outcome). TDR has never used meetings or other process indicators as targets. Both the product and target tables have been updated in this report (pages 16-25).

When considering potential for health impact, the three most important outcomes of TDR activities during the biennium have been:

i. The establishment of APOC, building on the applied field research carried out by TDR since 1987 and relating to the use of ivermectin for onchocerciasis.

ii. The results of the mega field trials of insecticide-impregnated bednets and materials, showing that they can save the lives of one in four children in sub-Saharan Africa.

iii. The development of a comprehensive new strategy by CTD for the control of lymphatic filariasis based on TDR-supported field trials. These trials have shown that a single dose once or twice a year of ivermectin, DEC, or particularly a combination of the two, has a profound effect on microfilaraemia and on transmission of the disease. Other studies have shown that simple treatment (with soap and water) can ease, and prevent worsening, of lymphoedema.

Box 3. Third External Review

Objectives

The proposed activities of the Third External Review and evaluation of the Special Programme are as follows:

Fundamental basis

- To review the continuing need for the Special Programme, bearing in mind the contexts of changes in disease patterns and changes in the international scientific environment.

Research progress and links to control

- To examine progress and effect/impact under the strategy for TDR Towards The Year 2000: by the consolidated areas of Strategic Research, Product Research and Development, Applied Field Research (including research on cross-cutting issues), and Research Capability Strengthening (including the functions and activities of the Research Strengthening Group); and by disease.
- To examine links between TDR's research activities and the operational control of tropical diseases and to what extent TDR's Strategic Research priorities are influenced by control issues.
- To review the methods currently used to measure performance and progress in achieving positive outcomes and to make recommendations for improving Programme evaluation.

Future directions

- To review the future portfolio of TDR's target diseases, the Programme's future directions and its priorities into the next millenium.
- To consider any implications for TDR's priorities resulting from the report and recommendations of the Ad Hoc Committee on Health Research Relating to Future Intervention Options.
- To examine future resource needs, opportunities and implications for tropical disease research; and to propose alternative financing requirements for the next ten years by Programme component, based on two scenarios: (i) all future needs and (ii) highest priority needs as determined by the External Review Committee.

Collaboration

- Taking into account the stage of development of each programme, to examine the effectiveness of linkages between TDR and relevant programmes inside WHO, especially the Division of Control of Tropical Diseases, the Global Tuberculosis Programme, and the Division of Emerging and Other Communicable Diseases Surveillance and Control; between TDR and other tropical disease programmes outside WHO; and between TDR and the pharmaceutical industry.
- To examine a global partnership for tropical disease research with other major actors in this field.

Organizational structure

- To examine the outcome of the Programme's new organizational structure and its ability to carry out the strategy for TDR Towards The Year 2000, including whether it reflects the most efficient use of the reduced funds available to the Programme.
- To examine the balance of TDR's resources allocated to the areas of Strategic Research, Product Research and Development, Applied Field Research and Research Capability Strengthening.
- To examine the prioritization of the task forces under the Applied Field Research component.
- To examine the financial management, administrative structure and governance of the Programme.

Future directions

By the time this report is published, the Third External Review will have begun. The terms of reference are outlined in Box 3. The review will cover fundamental questions like the need for TDR's continued existence, its disease portfolio and its longer term evolution. Instead of dealing with these issues here, I will simply indicate some considerations for the next biennium.

Strategic Research

TDR must be fully conversant with the emerging 'post-genome' agenda, that is, with the application of genome information to practical outcomes in pathogenesis and product development. To this end, close collaboration between genome, pathogenesis and molecular entomology must remain; and we need new skills on the committees.

Product Research and Development

As outlined above and as a consequence of the new structure, high on the agenda will be: strengthening discovery of new drugs and vaccines through the development of separate committees for these activities; better linkage to strategic research through cross-membership; and collaboration with industry to

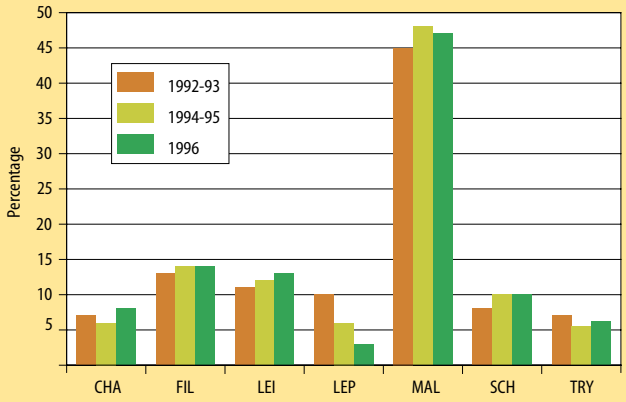
incorporate TDR parasite targets in high-throughput screening. Development activities will be further prioritized around key products, for which collaboration with advanced developing countries will be strengthened.

Applied Field Research

In this area, the number of task forces will be reduced, partly through elimination and partly through consolidation. The main focus will be to move new tools towards control (e.g. operational research relating to the application of insecticide-impregnated bednets and materials; application and assessment of impact of the Healthy Women Counselling Guide); to complete the activities of the task forces on Healthy School-age Children and Tropical Diseases and the Environment; and to switch current activities into related areas (such as moving Chagas disease operational research from the Southern Cone countries to the Andean and Central American countries, and expanding onchocerciasis operational research to lymphatic filariasis). During 1997, some projects will be completed which could form the basis for expanded activities (e.g. the impact of malaria chemoprophylaxis on anaemia in infants in high transmission areas; home practices in relation to the management of severe illness). Other projects are being initiated, such as: female genital schistosomiasis and its possible impact on HIV transmission; the social consequences of male genital lymphatic filariasis; and new approaches to rapid epidemiological mapping of malaria. For the moment, these will remain parts of other task forces, but they could evolve to larger-scale projects depending on progress and outcomes.

Malaria is a top priority in most countries of sub-Saharan Africa. All our studies show that improving the case management of malaria relates, to a large extent, to the health system. A major challenge is to see how our tools can be better adapted to the system; and how the system can be modified to be more effective against malaria. Some of the issues are: development of drug packaging systems at district level to improve dosing and compliance; better collaboration between public and private sectors to improve case management of malaria; and improved supervision of drug vendors by district pharmacists. These issues are being addressed in close collaboration with other programmes dedicated to health policy, drugs, etc., and with health sector reform initiatives.

Fig. 11. Disease spending



Priorities by disease

Close to 50% of TDR's resources are allocated to malaria. This has been the case in recent years, and we do not foresee a dramatic shift in the next biennium (Fig. 11).

Research Capability Strengthening

The priority for RCS during the next biennium will be to implement the new strategy (see chapter 3). In this regard, the activities in Least Developed Countries will be the biggest challenge: to keep them sufficiently concentrated and to build around identified individuals with exceptional talent and commitment. In this area we also need to build on lessons learned from our 20 years of RCS experience; twinning with stronger research groups and incorporating research and training arrangements where appropriate.

In conclusion

To return to the questions presented at the beginning:

Has it worked?

There are many pointers to the success of the reorganization; although we await the conclusions of the Third External Review.

Has the Programme become more efficient in project management?

The overall data do not suggest that this is so. We use about the same percentage of resources on staff, operational support and operations as before, and the number of individual projects has remained the same. However, it is too early to identify clear trends. In a sense, the earlier passive, investigator-initiated approach was more efficient than the current proactive, more demanding approach, although it lacked focus and product perspective. But whereas the earlier approach may have been as efficient, it seemingly was not as productive.

Has the Programme become more effective in moving products and tools towards policy decisions and disease control?

This was a major goal of the reorganization; and the reorganization has indeed helped us to move products along the pipeline more quickly. For instance, many components in the area of product R&D have come up with a new series of product candidates (e.g. I-CHEM and CHEMAL), while others have accelerated the development of specific products (e.g. vaccines for leishmaniasis and schistosomiasis).

In the area of AFR, the task forces have completed their tasks on time, with major implications for disease control policy and action.

As a consequence, it is likely that we are serving health better.

Are we serving the science of tropical diseases better?

At this stage, it is difficult to answer this question. Initiatives such as the genome and molecular entomology projects have clearly enhanced the science of tropical diseases and brought new first-rate scientists into the field. But what have we missed discovering in the areas we no longer support? Will we retain our position among funding agencies as having stimulated the most publications in the field of malaria, and as having stimulated the most publications per million dollars invested – as found in an independent review?[2]

Are we better able to respond to new challenges, even in a no-growth situation?

We are more flexible; and surely better able to respond.

What are the implications for others?

The UN system is widely perceived as being rigid and difficult to change. The change in TDR has been substantial; and we have shown that significant change is possible. This change started from the inside. Had it been forced from outside, the change would likely have been cosmetic rather than productive.

Have all the intentions of the reorganization been met?

Some intentions remain, as yet, unmet – we are, after all, a bureaucracy. But we are still changing; and are confident about living with change and striving to improve the world's health in the face of declining resources.

My sincere thanks to all those who went through the process and made it happen.

Tore Godal

References

1. *TDR Towards The Year 2000: strategic considerations.* Geneva, World Health Organization, 1992, TDR/PTR-SCI/92.3 Rev.4.

2. *Malaria Research: an audit of international activity*. London, The Wellcome Trust, Unit for Policy Research in Science and Medicine Report no. 7, 1996.

Products table

This table indicates potential manufactured products for disease diagnosis, treatment or control to which TDR has contributed. The table does not include techniques, unless they represent manufacturable items.

Column A indicates potential products which have been undergoing laboratory evaluation for safety and biological efficacy as a prerequisite for clinical and field trials.

Column B indicates potential products which have been undergoing evaluation in humans to show safety and efficacy, and diagnostics and vector control tools which have been under field trial.

Products listed in column C have been used in disease diagnosis, treatment or control since the date indicated.

Bracketed years indicate developments which took place outside TDR.

Product	A – Initiation of preclinical/ pharmaceutical development	B – Initiation of clinical or field trials	C – In disease control use since
MALARIA			
DRUGS			
Artemether – injectable for severe malaria	[1980s] 1989	1992	[1991]
Arteether – injectable for severe malaria	[1984] 1991	1991	–
Artesunate – rectal for prevention of severe malaria and death	[1992] 1997	1996	–
Chlorproguanil/dapsone – oral for uncomplicated malaria	1996	–	–
Mefloquine and mefloquine combinations	[1977]	1979	1984
Pyronaridine – oral for uncomplicated malaria	[1980s] 1995	1996	–
VACCINES			
Plasmodium falciparum asexual blood-stage vaccine	1977	1992	–
P. falciparum sporozoite vaccine	–	1986	–
P. vivax sporozoite vaccine	1989	–	–
P. falciparum transmission-blocking vaccine	1989	1995	–
DIAGNOSTICS			
DNA & RNA probes to monitor drug resistance	1989	–	–
Microtest kit for measuring *P. falciparum* sensitivity to antimalarial drugs	–	–	1985
DNA probes for detection of *P. falciparum* and *P. vivax* in blood	–	1987	–
VECTOR CONTROL			
Diagnostic monoclonal antibody-based (Zavala) test for species-specific detection of sporozoites in mosquitos	–	1986	1989
Insecticide-impregnated bednets	–	1991	1994
OTHER			
Portable incubator	–	–	1986

SCHISTOSOMIASIS			
DRUGS			
Praziquantel drug combinations for multidrug therapy	1990	1991	1994
DIAGNOSTICS			
Morbidity assessment by ultrasonography *(S. haematobium)*	–	1992	1997
Morbidity assessment by ultrasonography *(S. mansoni, S. japonicum)*	–	1992	–
Diagnostic urine filtration technique	–	1980	1983
Immunodiagnostic assays	1992	–	–

Product	A – Initiation of preclinical/ pharmaceutical development	B – Initiation of clinical or field trials	C – In disease control use since
FILARIASIS			
DRUGS			
Ivermectin for onchocerciasis	[1978]	1982	1987
Ivermectin for lymphatic filariasis	–	1988	–
Amocarzine (CGP 6140) for lymphatic filariasis	–	1996	–
UMF 078 for onchocerciasis/lymphatic filariasis	1989	–	–
DIAGNOSTICS			
DNA probes for *Brugia malayi* infective larvae	–	–	1989
DNA probes for *Onchocerca volvulus*	–	1989	1991
Monoclonal antibody for *Brugia malayi*	–	–	1989
Antigen assays for routine serodiagnosis of onchocerciasis and lymphatic filariasis	1989	1992	–
DNA probes for detection of microfilaria in blood in onchocerciasis and lymphatic filariasis	–	1989	–
LEISHMANIASIS			
DRUGS			
New regimen for antimony compounds	–	1979	1984
Allopurinol therapy	–	1989	–
Gamma-interferon + antimony compounds	1984	1988	–
Amidazoles	1982	1989	–
Paromomycin i.m. (aminosidine)	[1950]	1994	–
Paromomycin ointment (aminosidine)	[1950]	1994	–
Antitubulin compounds	1989	–	–
Lipid associated amphotericin B	[1988]	1992	1994
Lysophospholipids	1994	–	–
VACCINES			
Killed leishmania vaccine, New World	[1960]	1992	–
Killed leishmania vaccine, Old World	[1960]	1991	–
DIAGNOSTICS			
Direct agglutination test (DAT)	1983	1992	1994
Dot-ELISA	–	1992	–
Standard leishmania skin test antigen	–	1991	1994
CHAGAS DISEASE			
DIAGNOSTICS			
Agglutination test for blood bank screening	–	–	1989
Serodiagnostic test using synthetic peptides	–	1992	1994
VECTOR CONTROL			
Fumigant canister	–	–	1990
Insecticidal paints	–	–	1990
Triatomine detection box	–	–	1990
Crystal violet/sodium ascorbate to kill parasites in infected blood in blood banks	–	1989	1994

Product	A – Initiation of preclinical/ pharmaceutical development	B – Initiation of clinical or field trials	C – In disease control use since
AFRICAN TRYPANOSOMIASIS			
DRUGS			
Eflornithine	1978	1986	1990
DIAGNOSTICS			
Card Indirect Agglutination Test for Trypanosomiasis (CIATT)	–	1993	–
Card Agglutination Test for Trypanosomiasis (CATT)	1978	1980	1983
Miniature anion-exchange centrifugation technique	1979	1981	1984
VECTOR CONTROL			
Insecticide-impregnated screens	1984	1989	1989
Monoconical insecticide-impregnated traps	1984	1989	1989
Pyramidal tsetse fly trap	1984	1991	1991
LEPROSY			
DRUGS			
Clarithromycin	1991	1993	–
Combined drug regimens for PB leprosy - WHO/MDT	–	–	1982
Combined drug regimens for MB leprosy - WHO/MDT	–	–	1982
Ofloxacin	1985	1991	–
Minocycline	1991	1994	–
Ofloxacin/rifampicin combination	–	1991	1997
Ofloxacin/rifampicin/minocycline combination, single dose	–	1994	1997
VACCINES			
Heat-killed *Mycobacterium leprae* vaccine	–	1983	–
DIAGNOSTICS			
PCR to detect small numbers (<100) *M. leprae* organisms	1991	–	–
Native/recombinant and synthetic antigens for diagnostics	1989	–	–
BIOLOGICAL CONTROL OF VECTORS[a]			
Bacillus brevis	1989	–	–
Bacillus sphaericus	1983	1990	1993
Bacillus thuringiensis H-14	1976	1980	1982
Clostridium bifermentans	1990	–	–
Competitive snails	1978	1985	–
Lagenidium giganteum	1982	1986	–
Larvivorous fish	1975	1975	1975
Novel vector control organism by genetic manipulation	1985	–	–

[a]Due to financial constraints, TDR no longer supports work in this area

Targets table

TARGET	ACTIVITIES / RESULTS (reason for delay or termination)	STATUS	TARGET YEAR original/new
MALARIA			
APPLIED FIELD RESEARCH			
Diagnosis and treatment for the malaria component of the Sick Child initiative	Development of strategy for highly endemic areas Development of strategy for malaria in low endemicity areas	completed continuing	1994/1995 1996/1997
Strategy for control of African urban malaria	No activities (resource constraints)	discontinued	1994
Development of appropriate diagnostic tools and detection of antimalarials in urine	Commercial dipstick assay evaluation/one test available (Resource constraints)	completed discontinued	1996 1995
Community-based strategies for treatment of malaria	Studies in South-East Asia Studies on care-seeking and therapy for sick children in Africa Effect of chemoprophylaxis and/or iron supplementation on malaria and anaemia in first year of life	completed 7 projects ongoing on track	1995 1997 1997
Methods to reduce mortality in pregnant women	Two full treatments during pregnancy (2 studies completed – conclusions awaited)	completed	1995/1996
Rapid response to outbreaks of severe and complicated malaria	Evaluation of surveillance methods (lack of technical progress)	discontinued	1997
Overcoming factors that prevent women from seeking therapy	Healthy Women Counselling Guide	on track	1997
Rapid assessment methods	Development and evaluation of methods in South-East Asia	new	1998
PRODUCT RESEARCH AND DEVELOPMENT			
Insecticide-treated bednets for preventing childhood mortality	Four efficacy trials in Africa Technology promotion and implementation Definition of areas for bednets use Cost-effectiveness cf. other interventions	completed on track on track completed	1995 1999 1999 1996
Anti-TNF monoclonals in childhood cerebral malaria	Phase III trials Final analysis – no significant benefit from anti-TNF	completed completed	1994 1994/1995
Artemether in childhood cerebral malaria	Efficacy trials Registered for use in developing endemic countries; approved for use in France on named patient basis	completed completed	1994 1994/1995
Drugs for severe malaria	Clinical development of injectable arteether Registration in country of origin	ongoing planned	1995/1997 1998
Evaluation of artemisinin-type compounds	Analysis of clinical and animal toxicology data	on track	1998
Peripheral therapy to prevent evolution to severe malaria	Development of artesunate rectal caps Registration in USA and Europe	on track planned	1997 1997
Drug discovery mechanisms	Database for compound acquisition and screening	on track	1998
Partnerships in drug discovery	Technology transfer to Thailand and Malaysia	on track	1999
New drug leads	Selection for development of one lead from 2nd generation peroxidic drugs, phospholipid or proteinase inhibitors	on track	1998
Inexpensive drugs to replace chloroquine	Evaluation of pyronaridine and short half-life antifolate drugs Development of pyronaridine up to registration including technology transfer to Malaysia	completed planned	1995/1996 2000
Transmission-blocking vaccine	Preclinical development Phase I trials Investigation of novel adjuvants Evaluation of optimum antigen-adjuvant combination Phase II trials	completed completed on track on track on track	1994 1996 1998 1998 1999
SPf66 synthetic Colombian vaccine	Phase III trial in Tanzanian children Phase III trials in The Gambia and Thailand – variable results Repeat study in Tanzania in high-risk group infants	completed completed on track	1994 1995/1996 1998
RTS,S pre-erythrocytic vaccine (WRAIR/SKB)	Monitor Phase I/II trial in The Gambia	on track	1998
Cost-effectiveness of vaccine in areas of high mortality	Can be cost-effective even at relatively low levels of effectiveness	completed	1996

TARGET	ACTIVITIES / RESULTS (reason for delay or termination)	STATUS	TARGET YEAR original/new
MALARIA			
STRATEGIC RESEARCH			
Systems for genetic manipulation of the parasite	Development of a stable transfection system for *Plasmodium*	completed	>1997/1995[a]
Identification of factors responsible for disease and protective immunity	Research on cerebral malaria and mechanisms for erythrocyte adhesion	on track	>1998
Clarification of the basis for drug resistance	Studies on antifolate resistance in *P. falciparum*	on track	>1998
Genetic basis for parasite development in the mosquito	Midgut, haemocoel and salivary gland stages of *Plasmodium* under investigation	on track	>2000
Transgenic mosquitos resistant to malaria	Investigation of genetic mechanisms of mosquito germline transformation	on track	>2000
Genetically engineered biological larvicides	Transformation of a few aquatic organisms achieved (resource constraints)	terminated	1996
SCHISTOSOMIASIS			
APPLIED FIELD RESEARCH			
Introduction of praziquantel for control	Identification of most efficient means Feasibility of combining drugs for children	completed completed	1995 1995
Rapid identification in highly endemic communities	Trials for rapid identification of urinary schistosomiasis Field testing of manual for urinary schistosomiasis	completed completed	1995 1996
Risk factors and preventive measures for female genital schistosomiasis (FGS)	Guidelines for diagnosis of FGS Strategies to increase women's understanding of FGS Projects on prevalence of FGS Assessment of risk of FGS for HIV Impact of praziquantel treatment on FGS	completed ongoing 2 completed new new	1995/1996 1999 1999 1999 1999
Provision of treatment to children who do not attend school	Comparison of schistosomiasis in children who do and do not attend school Cost-effectiveness of provision of treatment through the school system to out-of-school children	new new	1997 1998
PRODUCT RESEARCH AND DEVELOPMENT			
Co-administration of albendazole and praziquantel in schoolchildren	Feasibility studies Development of a manual for use in control programmes (resource constraints)	completed delayed	1994 1997
Identification of protective antigens	Preclinical screening service for *S. mansoni* candidate antigens in mice	completed	1995
Preclinical development of *S. mansoni* candidate vaccine antigens	6 candidate antigens subjected to trial for human correlate immune responses (technical constraints)	delayed	1995/1996
Clinical trials of *S. mansoni* antigens	Initiation of trials of one candidate (technical constraints)	delayed	1996/1998
Preclinical development of *S. japonicum* antigens	3 candidate antigens in farm animal trials	new	1997
Production of *S. mansoni* vaccine antigens	Initiation of scale-up and large-scale trials	planned	1999
Monoclonal antibodies for detecting circulating antigens in diagnosis	Trials of selected antibodies Trials of new diagnostic methods (resource constraints)	completed terminated	1994 1996
STRATEGIC RESEARCH			
Genetic maps	EST and random DNA sequencing for creation of low-resolution maps	on track	>1999
Molecular biology of host-parasite association	Research on granuloma formation and its treatment	new	>1997
Development of parasite cell lines for drug screening and vaccine research	Technical constraints in achieving immortality of schistosome cells	delayed	>1998

[a]completed ahead of schedule

TARGET	ACTIVITIES / RESULTS (reason for delay or termination)	STATUS	TARGET YEAR original/new
FILARIASIS			
APPLIED FIELD RESEARCH – LYMPHATIC FILARIASIS			
Assessment of the social and economic costs of acute adenolymphangitis (ADL) and chronic disease	ADL shown to cause 3-4 full days of incapacitation per episode in certain communities Chronic disease affects output of certain groups of workers	completed completed	1995 1996
Clarification of geographical distribution and identification of high-risk communities	Rapid assessment of endemicity Rapid epidemiological mapping	ongoing new	1995/1997 1999
Appropriate treatment regimens for use in control programmes	Single-dose treatment with DEC or ivermectin/basis of new global control strategy DEC + ivermectin treatment more effective than ivermectin alone Effect of DEC + ivermectin on transmission	completed completed ongoing	1994 1996 1996/1998
Improved control strategies	Combining vector control with mass treatment DNA probes for vector infection Modelling of control strategies (resource constraints)	completed completed delayed	1997 1996 1996/1998
Improved distribution of ivermectin and/or DEC	Development of Community-Directed Treatment Operationalization of DEC-fortified salt	new new	1999 1998
Imaging methods for diagnosis of lymphatic filariasis	Use of imaging methods in practice	completed	1996
APPLIED FIELD RESEARCH – ONCHOCERCIASIS			
Increased knowledge of onchocercal skin disease	Assessment of sociopsychological burden of skin disease Economic impact of skin disease	completed on track	1995 1997
Clarification of geographical distribution and identification of high-risk communities	Rapid assessment of endemicity level/in control use since 1994 Rapid epidemiological mapping and development of a GIS/in control use since 1996	completed on track	1994 1997
Optimal treatment regimens	Impact of ivermectin on skin disease Impact of 7 years of annual treatment on ocular disease	on track completed	1997 1996
Improved distribution of ivermectin	Identification of cost-effective means in Nigeria Community-Directed Treatment with ivermectin/ now APOC strategy Integrated approaches to Community-Directed Treatment Rapid monitoring of large-scale ivermectin treatment	completed completed new validation ongoing	1994 1996 1999 1995/1997
Elimination of the disease	Identification of most efficient method in Latin America Feasibility of vector eradication in isolated foci in Africa	partly completed completed	1995 1996
PRODUCT RESEARCH AND DEVELOPMENT			
Immunodiagnostic assay for lymphatic filariasis	Trials completed Field evaluation of diagnostic kits Workshop on relative value of the assay in rapid assessment	completed completed planned	1994 1995 1997
Vector control using *Bacillus sphaericus*	Assessment of efficacy Cost-effectiveness studies	completed completed	1995/1994[a] 1996
Immunodiagnostic assay for detection of *Onchocerca* re-invasion	Production Evaluation (inferior to the DEC patch test)	completed completed	1995 1996
New macrofilaricides	Amocarzine in onchocerciasis/not macrofilaricidal Amocarzine in lymphatic filariasis, Phase II study UMF 078 – preclinical studies – clinical studies 2 new compounds in early preclinical studies for identification as projects	completed planned on track to be initiated on track	1996 1997 1997 1998 1997
Ivermectin resistance in nematodes	Determination of mechanisms of resistance Cloning of the genes involved in resistance	good progress on track	1997 1997
Detection of ivermectin resistance in onchocerciasis	Development of molecular probes Development of field assays	ongoing delayed	>1998 >1998

[a]completed ahead of schedule

TARGET	ACTIVITIES / RESULTS (reason for delay or termination)	STATUS	TARGET YEAR original/new
FILARIASIS			
STRATEGIC RESEARCH			
Genetic map of *Brugia malayi*	Species- and stage-specific libraries created EST and random DNA sequencing for low-resolution mapping	completed proceeding fast	1997 >1999
Cell lines for drug screening and vaccine research	(Resource constraints)	delayed	>2000
Vaccine research	Studies on microfilarial chitinase for vaccination	completed	1996
Pathogenic mechanisms in onchocerciasis	Mapping of pathogenic epitopes in *Onchocerca volvulus*	on track	>1997
LEISHMANIASIS			
APPLIED FIELD RESEARCH			
Women's understanding of the disease	Studies in Morocco	completed	1996
Children's compliance with treatment	Study in Tunisia	completed	1995
Women's treatment for cutaneous leishmaniasis	Studies in Tunisia, Colombia and Costa Rica	completed	1995/1996
PRODUCT RESEARCH AND DEVELOPMENT			
Vaccine for cutaneous leishmaniasis	Phase III trials with single injection of candidate vaccine Phase III trials with multiple injections	in progress due to start	1996 1997
Vaccine for visceral leishmaniasis	Phase III trial in Sudan Langur monkey model for visceral leishmaniasis	ongoing on track	1996/1998 1997
'Cocktail' vaccine	Use of recombinant molecules and novel adjuvants	on track	2005
Genetically modified parasites for vaccines	Development of a genetically attenuated strain of *L. major* for use as vaccine	completed	1997
Amphotericin B/lipid complexes	One product registered in one country in 1994 Additional Phase III dose-finding trials	completed completed	1997 1996
New drugs for: cutaneous leishmaniasis	Topical aminosidine for *L. major* infection Use of allopurinol alone/of no use	completed completed	1996 1996
visceral leishmaniasis	Submission of dossier for registration of injectable aminosidine	ongoing	1998
mucocutaneous leishmaniasis	Pilot clinical trial of an oral alkyl lysophospholipid Trial of injectable aminosidine in combination with antimony	new ongoing	1997 1997
Direct agglutination test for visceral leishmaniasis	Final evaluation of simple diagnostic kit	near completion	1997
STRATEGIC RESEARCH			
Genetic maps	Sequencing and identification of *L. major* genes	fast progress	>1999
Identification of pathogenic genes	Localization of genes used in stage differentiation of the parasite in the human host	ongoing	>1999
Knowledge of cellular differentiation associated with the life cycle	Identification of genes regulating promastigote cellular differentiation	on track	>1999
Clinical studies on the pathology of *Leishmania*	Clarification of how *L. braziliensis* spreads in the human host	completed	1996

TARGET	ACTIVITIES / RESULTS (reason for delay or termination)	STATUS	TARGET YEAR original/new
CHAGAS DISEASE			
APPLIED FIELD RESEARCH			
Understanding the distribution and genetic structure of triatomine populations	Non-domiciliated triatomines in Central America and Andean countries	new	1999
Combinations of vector control measures	Southern Cone countries Central American and Andean countries	completed on track	1996 1998
Improved housing and health education in rural and semi-urban areas	Central American and Andean countries	ongoing	1994/1998
Improved surveillance of infants and women during pregnancy	Identification of measures to apply in Bolivia and Argentina (2 projects)	completed	1994/1996
Prevention of transmission by blood transfusion	Identification of effective strategy in Andean and Central American countries	ongoing	1995/1998
PRODUCT RESEARCH AND DEVELOPMENT			
Diagnostic kits for screening of blood products	Introduction of diagnostic kits for screening Epidemiological validation	completed completed	1993 1996
Simple PCR-based test for screening blood products	(Technical constraints)	terminated	1996
Preclinical and/or clinical development of new drugs	An inhibitor of sterol biosynthesis effective in experimental infections Multicentre trial (Brazil, Argentina) of benznidazole in chronic Chagas disease	on track completed	1998 1996
STRATEGIC RESEARCH			
Genetic maps	Gene identification and sequencing for creation of low-resolution maps	on track	>1999
Understanding of mechanisms of tissue damage	Genes important for parasite virulence and viability identified	on track	>1998
Characterization of cellular machinery and signalling	Research on protein folding and its production in the parasite cell	on track	>1998

TARGET	ACTIVITIES / RESULTS (reason for delay or termination)	STATUS	TARGET YEAR original/new
AFRICAN TRYPANOSOMIASIS			
APPLIED FIELD RESEARCH			
Community-based surveillance	Selection and evaluation of strategies (initial studies not conclusive; follow-up research required) Community involvement in diagnosis (CATT) and tsetse control	ongoing on track	1995/1999 1997
Geographic Information System	Continued by CTD	ongoing	-
Determination of gender differences in detection and control of sleeping sickness	Preliminary results indicate that gender differences exist, particularly in treatment-seeking behaviour	on track	1997
Determination of effect of sleeping sickness on children	Growth and academic performance of children with late-stage disease who received melarsoprol treatment was significantly affected	completed	1996
PRODUCT RESEARCH AND DEVELOPMENT			
Field trials of monoclonal antibody-based diagnostic tests (CIATT)	Analysis of studies Pilot study for integration in surveillance	completed to start	1995/1997 1997
Improved treatment with eflornithine	14- vs 7-day treatment / follow-up for 12 months so far shows no significant difference in relapse rate between the two doses	on track	1997
Cheaper production of eflornithine	Testing of eflornithine produced by a new route Change method of crystallization to improve purity profile	completed follow-up study	1995/1997 1997
Clinical trials of new drugs	A diamidine compound undergoing preclinical studies (technical constraints due to toxicity)	delayed	1997
STRATEGIC RESEARCH			
Genetic maps	Gene identification and sequencing for creation of low resolution maps of a *T. brucei* strain	on track	>1999
Understanding of mechanisms of parasite-associated brain damage	Research on brain nuclei involved in sleep/wake cycles	on track	>1998
Identification of common non-variant surface antigens	(Resource constraints)	terminated	>1997
Characterization of the trypanolytic factor (TLF) and its role	TLF1 characterized; further work on TLF2	on track	>1997
Elucidation of parasite growth and signalling	Studies on the lymphocyte triggering factor (TLTF) of *T. brucei* Studies on the role of tumour necrosis factor (TNF) in the pathogenesis of trypanosomiasis	on track on track	>1997 >1997

TARGET	ACTIVITIES / RESULTS (reason for delay or termination)	STATUS	TARGET YEAR original/new
LEPROSY			
APPLIED FIELD RESEARCH			
Community awareness of, and involvement in, control of leprosy	Studies completed and manual developed in India	2 studies completed	1995; 1996
Reduced risk of infection in women	Information on pregnancy risks incorporated into Indian manual (above)	completed	1997
Shorter treatment regimens	Studies in animal model and clinical trials Follow-up of patients in multicentre trial	completed on track	1996 2004
Minimum treatment for patients with single lesion, paucibacillary leprosy	Combination of the 3 most potent drugs as a single dose	completed	1997
STRATEGIC RESEARCH			
Immunoepidemiological surveillance tools	Evaluation of peptide-based skin tests in humans Simplification of polymerase chain reaction technology	on track completed	1999 1996
Vaccine trials (TDR input: provision of *M. leprae* for vaccine production)	Trials in Malawi Trials in India	completed on track	1996 1999
M. leprae genome sequence for pathogenesis studies	(Financial constraints)	delayed	1996/1999
Knowledge about mechanisms of nerve damage	(Lack of technical progress)	delayed	1997/1999

CHAPTER 2

How does TDR work?

AFR – Bednets reduce mortality in African children

PRD – A leishmaniasis vaccine could be universally adaptable

SR – Identification of mosquito eye colour genes has promoted genetic transformation work

Following a review of TDR and its ability to meet its mandate most effectively during the next decade, the Scientific and Technical Advisory Committee (STAC) recommended in March 1993 that the structure of TDR be changed in order to achieve the right balance between its efforts in basic ('strategic') research, product development and field research. One of the major motivating factors was the recognition of a growing demand for applied field research, and the need to develop a flexible structure which could develop rapid solutions to field problems.

The trans-disease approach

STAC recommended that TDR's disease-specific steering committees be phased out and replaced by three main components covering Strategic Research (SR), Product Research and Development (PRD) and Applied Field Research (AFR). Disease-specific issues would still continue to be addressed under the new structure.

STAC recommended, then and in subsequent meetings, that:

- SR be concerned with furthering the understanding of host-parasite/parasite-vector and parasite biology in order to develop leads toward more effective disease control tools, and should incorporate three steering committees: Parasite Genome (GENOME), Pathogenesis (PATHO), and Molecular Entomology (BCV);
- PRD be responsible for developing the leads identified through to registration, and should incorporate six steering committees: Drugs for Malaria (CHEMAL); Drugs: Macrofilaricides (MACROFIL); Drugs for African Trypanosomiasis, Chagas Disease and Leishmaniasis (I-CHEM); Vaccines for Malaria (IMMAL); Vaccines for Leishmaniasis (LEISH); and Vaccines for Schistosomiasis (SCH);
- AFR be concerned with applied field research for improved disease control through building on the activities of the previous Social and Economic Research (SER), Field Research in Malaria (FIELDMAL) and the Epidemiology and Field Research (EFR) components of TDR, and through operational research on lymphatic filariasis, onchocerciasis, Chagas disease and African trypanosomiasis.

The recommendations of STAC were accepted by the Joint Coordinating Board (JCB) of TDR during its session in June 1993 and the new strategy and structure of TDR came formally into effect on 1 January 1994.

Rationale

Application of disease control tools at the most peripheral level of health services requires integration. Unless such integration starts at the central level in WHO/HQ, constraints and tensions are created in the health system. This calls for unified approaches (or packages) at all levels of the health system.

A trans-disease approach has allowed TDR to:

- address issues that are common to many health problems, including tropical diseases, and hence to develop cost-effective and comprehensive solutions (e.g. GENOME, PATHO, MACROFIL, I-CHEM, task forces on Sick Child, Gender, Healthy School-aged Children, Environment, Health Financing);
- develop partnerships and complementary relationships with other programmes within WHO, other donors and research organizations working in tropical diseases and related fields;
- respond more rapidly to both new opportunities in science and new demands from the field, and to build quickly upon new findings as they emerge;
- become increasingly proactive in identifying research targets of critical importance to tropical disease control, and in focusing research on these targets;
- incorporate a multidisciplinary approach to research questions, including for example, social and economic sciences, epidemiology, clinical science and parasitology;
- incorporate research strengthening and training into projects by the development of common protocols for multicountry studies in disease endemic countries by Developing Disease Endemic Country (DEC) researchers;
- promote operational research to facilitate the implementation and subsequent improvement of disease control tools and strategies.

Challenges of the trans-disease approach

The focus on trans-disease issues such as gender, school-aged children, environment, and health care financing has the advantage of incorporating a wide number of issues, including tropical diseases but also broader health and development issues, under one umbrella. However, there are risks in such a targeted approach, mainly risks of omission. It is not possible, for example, to focus on all health or even tropical disease issues of relevance to gender or school-aged children in one task force and priorities have to be set. Similarly, research on tropical diseases tends to attract highly specialized scientists who are not accustomed to working in multidisciplinary teams and who may find it difficult to assess the disciplinary needs of a cross-disease project or to identify appropriate collaborators with the necessary skills. TDR therefore must assist researchers in setting up teams and in continually assuring, through RCS and training workshops, that a sufficient number of researchers are trained in the many disciplines required for the growing diversity of research approaches to the control of tropical diseases.

Priority setting

TDR's research is focused on clearly defined research targets which have been identified in a thorough prioritization process based on the following criteria:

- Global need, according to disease burden and the effectiveness of existing control tools to reduce that burden.
- Potential impact on disease control of reaching the research target, taking into account the cost-effectiveness, affordability, acceptability, and expected useful life of the resulting product or solution.
- Scientific opportunity for, and feasibility of, the required research.
- Expected time needed to reach the target.
- TDR's specific ability, compared with other institutions, to reach the target.
- Cost of the work required to reach the target.

It is important to note that TDR's priorities are specifically those of TDR, and correspond to its mandate, special capabilities, and budgetary limitations. While TDR efforts have been reduced in certain areas, such as in strategic research, the need for these to remain priorities of other organizations working in similar areas is recognized. Moreover, TDR priorities, being based on global need, are different from regional and national disease priorities. Therefore, in TDR considerable emphasis is put on partnerships with other organizations supporting related research in order to avoid duplication and make the best use of limited resources.

TDR's new structure, as described in Chapter 1 and above, is based on three main lines of research: strategic research, product research and development, and applied field research.

Setting and reaching research targets in each TDR area

Strategic Research: building on scientific advances to develop future intervention tools

Building on opportunities created by recent scientific advances, Strategic Research (SR) aims to find new ways for disease control. As the development from scientific breakthrough to field applicable intervention tool takes considerable time, SR has a long-term perspective and targets which may take more than a decade to reach. For example, the target to develop, using recent advances in molecular entomology, field applicable methods to ensure that *Anopheles gambiae* will not transmit malaria is set in a time frame of at least 15-20 years. Another main target, that of analysing and mapping the genomes of the TDR parasites with the aim of more efficient product development (vaccines, diagnostics, drugs), will also take 15-20 years to reach. The third SR target, that of pathogenesis, is broader and more vague, but is basic to all other activities. Pathogenesis is the only TDR activity which has a target without a clear time frame.

Mode of operation: TDR research priorities and targets are announced regularly. In SR, all research supported by the Pathogenesis Committee is investigator-initiated.

Product Research and Development: from product lead to registered intervention tool

Priorities for Product Research and Development (PRD) are more cost-effective drugs for TDR diseases, and vaccines for malaria, schistosomiasis and leishmaniasis. Drug development is approached through multiple mechanisms which represent a hybrid between public and private sector approaches. In some areas, diseases caused by similar parasites are combined for more efficient development of products (lymphatic filariasis + onchocerciasis; African trypanosomiasis + Chagas disease + leishmaniasis).

PRD has two types of target: the identification of leads, and the development of leads through to registered products. Each steering committee has a workplan which, for each identified lead product, specifies in detail the steps to be followed in the development process to reach the target of registration within a stipulated time frame. The usual time frame is 5-15 years.

Mode of operation: In PRD, discovery research is a mixture of external investigator-initiated projects (drugs and vaccines) and in-house directed screening activities (drugs only); the latter focus on the whole parasite but increasingly at the target level. Product development projects arise increasingly from research supported by other public sector agencies and as spin-offs from the private sector. They are driven by professional managers and expert advisors with detailed knowledge of the product development process.

Applied Field Research: providing the technical foundation for more effective disease control policies

Applied Field Research (AFR) is aimed at developing generic solutions to practical problems of disease control in endemic countries, thus providing the technical basis for more effective disease control strategies. Products are taken into AFR after their development to registration has been completed through PRD. Some research is prompted by the need to solve generic operational problems following the introduction of a new intervention tool (e.g. insecticide-impregnated bednets for malaria control or ivermectin for onchocerciasis control), but other research is aimed at achieving more effective control with existing tools (e.g. schistosomiasis control with praziquantel, but through schools) or at developing radically new cross-disease approaches to community-based disease control strategies (e.g. the Healthy Women Counselling Guide or Community-Directed Treatment with ivermectin).

Mode of operation: Most research is undertaken through task forces consisting of independent scientists with the required expertise and direct links to disease control programmes or general health services. The task forces address priority problems in tropical disease control and try to develop generic solutions within three years. Each task force has a workplan with defined targets, time frame and budget. An important characteristic of the task forces is their direct links to disease control programmes and/or health services, which ensure a proper focus for research activities and direct feedback of research findings into control activities. Progress of the task forces is reviewed annually by the AFR Steering Committee. Investigator-initiated proposals on AFR topics not covered by the task forces are also funded, but there is only limited funding for such research and the process is extremely competitive.

Integration of research targeting with capability strengthening

Research Capability Strengthening (RCS) is aimed at building the capacity within developing disease endemic countries (DECs) to carry out research needed for developing intervention tools. Scientific merit and relevance to the workplans of the different TDR steering committees/task forces (SCs/TFs) are the major criteria for support. Thus the programme aims at close integration between its R&D and RCS areas.

Mode of operation: SCs/TFs define what research is needed in developing countries and RCS develops the capacity in target epidemic foci to carry out the defined research – through training of individuals and groups, building up of research groups, setting up of linkages and communications – with a view to establishing a sustainable research environment.

Mechanisms for responding to priority issues

Investigator-initiated research

With the reorganization, more support has been devoted to other mechanisms, often with clear objectives and shorter time frames and addressed from a multidisciplinary, and/or cross-disease approach. Nonetheless, in all programme areas funds are available for investigator-initiated research in order to ensure that new ideas and creativity continue to stimulate the research.

Goal-oriented task force initiatives

In order to reach their targets within the allotted time, task forces, particularly in AFR, have a proactive mode of working. They launch specific research initiatives in which researchers from endemic countries are invited to participate. Combining peer review and scientific rigour with assistance to investigators where required, most task forces are in fact doing both R&D and research capability strengthening. As with the AFR task forces, TDR's PRD task forces also have the task of recognizing and setting priorities and assuring that these are activated.

Research studies

A special AFR research model which can have a significant impact on disease control policies is the multidisciplinary study. This approach is generally based upon a core protocol developed with researchers from endemic countries and a range of disciplines.

Small grants

Small grants have been used as a mechanism to stimulate research on tropical diseases in areas where more information is needed or to encourage new researchers to enter the field. A small grants programme in Latin America for social and economic research on tropical diseases has been supported by TDR since 1989, and most of the projects are now completed. The activity has been highly successful in generating research on all TDR target diseases endemic to the region. The project completion rate has been 100% and many publications in scientific journals have emerged. The programme has also been successful in encouraging junior researchers to work in the field of social sciences research on tropical diseases and their control, and many of the results are now being used for disease control, especially in the area of health education materials.

For example, researchers working on the knowledge, attitudes and practices of women in rural Ecuador concerning cutaneous leishmaniasis have collaborated with the Ministry of Public Health in the development of a practical guide to leishmaniasis and its treatment for the use of health care workers in endemic areas. Findings from the study have been useful for providing practical examples and illustrations from the field of people's experience of the disease.

One of the reasons for the success of the programme lies in the commitment of the coordinator, Dr Roberto Briceño-Leon, and the steering committee of experts consisting of social scientists and disease control personnel from five Latin American countries who have nurtured the process through direct interaction with researchers, through training and project development workshops, and through correspondence.

A similar small grants programme was initiated in 1992, in collaboration with the WHO Regional Office for the Eastern Mediterranean (EMRO) and the WHO Control of Tropical Diseases Programme (CTD). The

Box 1. What became of SER?

The Social and Economic Research (SER) component and steering committee was dissolved as a separate entity in 1994 and was integrated with the Applied Field Research (AFR) area. Many of its features remained in the new structure, including the carry-over, for a full year, of the previous chairperson, Dr Burton Singer, and several members. Social and economic researchers remain key participants in the AFR secretariat, steering committee and task forces, and are key participants in the multidisciplinary teams supported by AFR.

Several SER activities have continued beyond the lifetime of SER itself, including small grants programmes in Latin America and Africa (now completing their activities) and the production of five SER reports based on final reports received after 1994. These include SER Project Reports 14, 15 and 16, and *Methods for social research in tropical diseases* 2 and 3.

The malaria manual, No. 2 of the Social Research Methods series, produced by Dr Irene Agyepong and colleagues, presents well-validated guidelines for the rapid assessment of social, economic and cultural aspects of malaria. It represents an important contribution to describing tools for collecting information on issues relating to community-based malaria control and their adaptation to distinct socioecological settings.

SER Project Report 14 is a report by the Kikwawila Study Group on a TDR workshop on Qualitative Research Methods held in Ifakara, Tanzania, in April, 1994. At the time of planning the workshop the fieldwork was seen essentially as a training exercise and utilization of the results was not foreseen. However, the results of the fieldwork were so interesting that TDR disseminated them as an SER Project Report so that they could be shared with researchers, health planners and policy-makers concerned with health-seeking behaviour and its determinants.

SER Project Report 15, by the Red Urine Study Group, presents the results of a multicountry study involving eight African countries, of a successful low-cost method of identifying communities with high levels of schistosomiasis based on the perceptions of school teachers and children. A companion document – the *Guide for the rapid identification of communities with a high prevalence of urinary schistosomiasis* (SER Methods series No. 3) provides concise step-by-step instructions for using this methodology.

SER Project Report 16, by Dr Pimpawun Boonmongkon of the Faculty of Social Science and Humanities, Mahidol University, reports on an anthropological study of leprosy, the 'disease of social loathing', in rural North-East Thailand. The study found that the Buddhist faith both reinforced and mitigated the effects of leprosy stigmatization. It makes practical recommendations for changing health workers' attitudes and reinforcing community support for leprosy patients.

Box 2. Gender and Tropical Diseases Award

An award for the best paper in the area of gender differences in the impact of tropical diseases was initiated by SER to stimulate research in this area, where relatively little was known, at a very low cost. The award, referred to as the Gender and Tropical Diseases Award, is sponsored by Canada's International Development Research Centre (IDRC), for an amount of C$5000, and is administered by TDR. The first competition, in 1991, invited researchers to submit papers on any topic relating to women and tropical diseases. The award stimulated nearly 50 entries, and the ten best papers were published by IDRC in a Manuscript Report, entitled *Women and tropical diseases*. This competition highlighted many gaps in research pertaining to both males and females, their contrasting roles and how these affect, and are affected by, tropical diseases. As a result of the broader focus on both men and women and their sociocultural context, subsequent competitions have focused on 'gender' as opposed to 'sex'. Titles have included *Gender and tropical diseases: Facing the challenge*; *The female client and the health care provider*; and *Gender, health and technology*.

The papers are judged independently by a panel of representatives from IDRC and the Gender and Tropical Diseases Task Force (TDR), who are unaware of the authors' identities. Each paper is then scored according to criteria including relevance to gender issues, scientific merit, originality and knowledge of the field. In 1995/96 no prize was awarded on the topic of *Gender, health and technology* because none of the submissions adequately addressed the theme. While several papers made interesting contributions on gender and tropical diseases, the technological aspects were neglected. The award for 1997 is for the best paper on *Gender issues in the operationalization of bednets use*.

goal was to encourage researchers in the Eastern Mediterranean region to develop field research projects with a focused research question, where the result could quickly lead to a disease control activity. The grants programme first focused on leishmaniasis, followed by schistosomiasis and malaria, but in the past two years applications have been accepted on any of the TDR target diseases endemic in the region. The grant applications are reviewed by an expert committee representing the WHO partners and outside experts, and are judged on a fully competitive basis. Applications have been received from all countries in the region. In the first four years of the programme, over 300 applications were reviewed and approximately 35 applicants were successful in obtaining research funds, many of whom have completed the work satisfactorily.

In francophone West Africa, a small grants training and research network was established in 1993 to build around a continuum of activities and support. This process, grouping five French-speaking countries (Benin, Burkina Faso, Guinea Republic, Mali and Niger), involves a protocol development workshop for pairs of researchers from the same country working on the same subject, low-level funding of the best protocols, and execution of the research with support from experienced scientists. The process ends with a final workshop for data analysis and writing up of results for final publication and/or application in control programmes in the researchers respective countries. The programme has, so far, focused on malaria and schistosomiasis. The hub of activity is the Department d'Epidémiologie des Affections Parasitaires of Mali medical school in Bamako, and the Institut National des Recherches en Santé Publique in Bamako, where the first series of workshops was held. Both institutions have successfully carried out projects under two of TDR's institutional strengthening grants with additional peer support from the US-based National Institutes of Health (NIH) and the University of Rome, Italy, and they have the best research infrastructure for tropical disease research among the partners as well as a rapidly increasing core of PhD trainees completing their training abroad.

This cycle can be repeated with other groups of young researchers, and it can allow the phasing out of member countries that have achieved progress to allow other countries to enter the network. The process has, in addition, proved invaluable in identifying young persons with potential for research (for example among those who are successful in planning and executing the small grants) from the five countries of the network. These promising researchers have been encouraged to apply for formal degree (doctorate or masters) training through TDR.

Awards

The mechanism of awards has been used to a limited extent by TDR in order to encourage research in new areas and to collect existing information, that has not been widely disseminated, on a particular subject. TDR has used this mechanism to stimulate journalists in developing countries to write about a subject of interest, for a poster competition, and to foster research on gender and tropical diseases (Box 2).

CHAPTER 3

Research Capability Strengthening:

'Training through research'

'Learning by doing'

Updating the strategy

'*To strengthen – through support to institutions and through training in biomedical and social sciences – the capability of Developing Disease Endemic Countries to carry out research required to improve the control of TDR target diseases, and to participate in the global effort to develop new methods of prevention, diagnosis and treatment of these diseases.*'

Three interrelated factors led to a need to review and reassess TDR's capacity building activities and the Research Capability Strengthening (RCS) component. Firstly, the extraordinary growth during this past decade of scientific knowledge and techniques with application to tropical disease research and the growing ability of scientists in Developing Disease Endemic Countries (DECs) to participate in the generation and application of this knowledge. However, there exists a group of Least Developed Countries (LDCs) that remain underserved and which require special attention. Secondly, global financial pressures have limited the funds available for all forms of development assistance. But at the same time, the demand for assistance is increasing. Thirdly, in partial response to the previous two factors, TDR was restructured in 1994 to reflect changes and advances in tropical disease research. However, at that time, TDR's RCS component was left untouched. With TDR's new operational structure and RCS's revised strategy, approved by the Joint Coordinating Board in June 1996, an update on TDR's RCS strategy is presented below, with special attention given to the relationship between RCS and research and development (R&D) activities in TDR.

Bilateral policies for RCS

At the same time as reviewing its own RCS programme, in 1996 TDR commissioned the Danish Bilharziasis Laboratory (DBL) to review the policies and programmes of Belgium, Canada, Denmark, France, Germany, Norway, Sweden, the Netherlands and the United Kingdom, and their responsible agencies for overseas aid, for research capability strengthening. The objective of the review was to provide TDR with an overview of selected TDR-related donor activities in the DECs, with emphasis on the LDCs, so that TDR might better understand the bilateral and multilateral priorities for capacity building. The review showed that research capability strengthening, or capacity building, is receiving increased attention for development cooperation in a wide range of AID/ODA organizations and countries. It was noted, however, that few countries had an explicit policy for such activities, although all implicitly supported the concept through various mechanisms. Consistently similar to TDR's approach, was recognition of the need for human resource development, institutional strengthening and networking activities. All three aspects were seen as fundamental to promoting self-sustainability following the end of financial support. Overall, the representatives of the countries interviewed for the review expressed satisfaction at the approach adopted by TDR and recommended it continue with its strategy.

A differentiated approach for capacity building: LDCs and ADCs

In 1992, TDR adopted an explicit differentiated approach to capacity building that grouped Developing Disease Endemic Countries (DECs) based on their scientific maturity. These were the (United Nations defined) Least Developed Countries (LDCs), the (TDR defined) Advanced Developing Countries (ADCs),[a] and the remainder (see Figure 1). This allowed for development of country-specific strategies and approaches for LDCs and ADCs respectively. The LDCs often have a weak research infrastructure and investment targeted to individual training and to strengthening potential research groups. TDR programmes for ADCs on the other hand, are targeted to the strongest research groups, on a highly competitive basis, to encourage them to assume a full role in priority areas of R&D on tropical diseases. In the DECs where scientific maturity falls between the two ends of the spectrum, a case-by-case approach is used to determine the most appropriate mechanism for TDR support, according to the country's needs and TDR's priorities. It should be noted that the distinction between ADCs and LDCs is not a rigid dichotomy. There are some LDCs that successfully compete for a full range of TDR funding and there are some ADCs that require special attention. However, overall, the research conducted in leading DEC institutions in the past decade has dramatically shifted from northern-dominated to southern-driven, and increasingly includes product development activities such as chemotherapy, vaccine trials and the development of diagnostic tests.

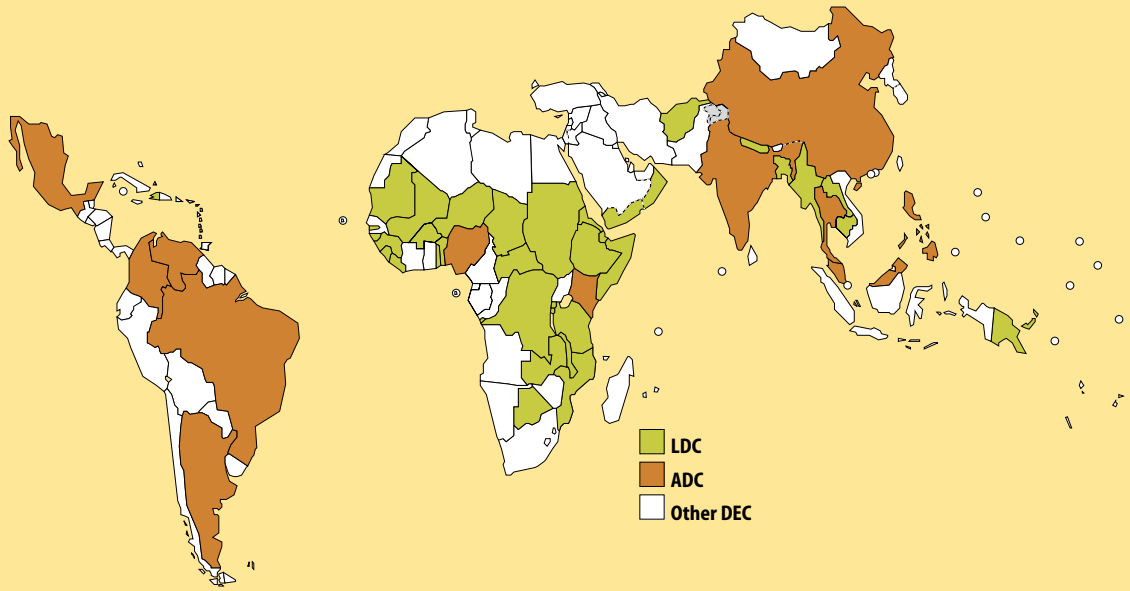
Priorities for Research Capability Strengthening

LDCs and ADCs

The LDCs are generally countries exhibiting the heaviest burden of TDR diseases and, at the same time, the weakest economies. They are often expected to solve their own disease control problems with inadequate resources, their research and control programmes operating under conditions of financial and political uncertainty. The constraints and limitations in LDCs include: small numbers of experienced researchers who often work in scientific isolation; a research environment with insufficient resources – financial, material, bibliographic; and lack of collaborative research linkages, all of which are exacerbated by the lack of national training opportunities and facilities, especially at the doctoral level.

The results of these deficiencies in LDCs, pertaining to TDR-related research, are poorly-designed research proposals submitted to TDR and other funding agencies, and insufficient personnel and equipment necessary to

Figure 1. Advanced Developing Countries (ADCs) and Least Developed Countries (LDCs)



[a] *An Advanced Developing Country is a country which, within its own region, receives a significant and consistent proportion of the region's TDR R&D funding. There is also a logical pattern in RCS funding where, over time, institutions and principal investigators have successfully competed for higher level RCS grants. Three external indicators are also used as markers of ADCs – the Human Development Index score (1987), real GDP per capita (1985), and scientists and technicians per 1000 population (1970-87). These five indicators show the presence of a research infrastructure and a national cadre of scientists able to sustain research activities and withstand a change in membership.*

undertake the proposed research. As a consequence, it is important for LDCs that TDR increase its emphasis on strengthening research capabilities through training, and develop and promote mechanisms to sustain evolving research capacities. These objectives support TDR's drive for research capability strengthening. But within the context of LDCs and the constrained financial and personnel resources in TDR, the RCS priorities need to be adapted. This means a more proactive approach for soliciting applications for grants and for monitoring of the grants, and better use of the Research Strengthening Group members or members of other TDR steering committees and task forces. The number of grant formats will not be expanded but the current flexibility within the formats will be maintained. TDR will emphasize the acquisition of basic skills lacking in LDCs. Support for strategic research in LDCs will have to be critically evaluated, tailor-made and limited to building research groups around exceptional talent.

Table 1. RCS priorities

	LDCs	ADCs
Applied Field Research	High	Low
Product Research & Development	High	High
Strategic Research	Low	High

For the ADCs, TDR's priorities are to support the acquisition of new skills and technologies in emerging disciplines, for instance, molecular entomology, high-throughput DNA sequencing and combinatorial chemistry. Through partnership with TDR, some ADCs can invest in new technologies that might lead to income-generating endeavours and hence become sustainable. In addition, TDR will continue to support current priority areas, including health economics and health education. For all countries, TDR support will continue to be linked to disease control priorities when possible.

In terms of an approach within the country groups (ADCs vs LDCs), TDR's priorities, in general, are described in Table 1. However, the unique characteristics of a country may provide opportunities that necessitate a deviation from this general principle.

Integration with TDR's Research and Development programme areas

TDR now links its RCS activities with its three Research and Development (R&D) programme areas – Strategic Research, Product Research and Development, and Applied Field Research – to a greater extent. As far as possible, every attempt is made to accomplish capacity building within the priorities and workplans of the various steering committees and task forces. TDR's R&D priorities are focused on the development of tools needed for the control of the target diseases, and research capability strengthening in the DECs further enhances the chance of reaching the ultimate goal – control of tropical diseases. The following three objectives for determining priority RCS activities pertain:

- Within Strategic Research, the RCS objective is to identify and support exceptionally well-qualified persons with the potential to be competitive in emerging, high-technology areas at the international level.
- Within Product Research and Development, the RCS objective is to promote capacity for product discovery, for example by combinatorial chemistry and screening of products, and process development for the manufacture of drugs and vaccines; and to strengthen the ability of DECs for early (Phase I, Phase II) clinical studies, as well as pivotal Phase III clinical trials.
- Within Applied Field Research, the RCS objective is to increasingly link activities to identified national priorities, awarding an explicit value to national disease control programmes and those needs identified as essential national health research. This linkage will involve the integration of DEC researchers trained by TDR into disease control programmes. Training and capacity building will be provided through AFR-funded projects, training of scientists in the AFR disciplines, and selective small grants programmes to encourage young scientists to enter the field of tropical disease research. RCS grants in this area will favour LDC researchers.

The integration of RCS activities with TDR's redefined priorities is under way. In collaboration with Strategic Research, RCS has provided funds for DEC scientists to receive training within the genome area, and a new round of RCS competitive grants were awarded for high-throughput DNA sequencing. RCS adopted a fast-track approach to training DEC researchers in the field of strategic research relevant to molecular

entomology by supporting numerous training courses and scientists, and awarded two partnership grants to DEC scientists to pursue this area of research (Box 2). In collaboration with Product Research and Development, three partnership grants were awarded to DEC scientists for developing Good Manufacturing Practice (GMP) facilities and human resources for vaccine scale-up and production, and a new round of RCS competitive grants were awarded for combinatorial chemistry. The links with Applied Field Research are firmly established following the recommendations made by the Joint Coordinating Board in previous years.

Box 2. Strengthening research capacity in molecular entomology

With the rapid advances in molecular biology in the past decade, a global initiative has emerged with the goal of controlling malaria through the genetic manipulation of its anopheline vectors. In this context, RCS in 1991, in collaboration with the then Steering Committee on the Biological Control of Vectors (BCV), invited leading scientists in the fields of molecular biology, biochemistry and genetics to launch a new initiative for malaria control. From the beginning it was understood that success would be dependent upon the participation of scientists from developing disease endemic countries (DECs). As a consequence, RCS quickly embarked on supporting DEC scientists to upgrade their skills and train new scientists in the relevant fields of strategic research related to molecular entomology. From 1992, TDR has supported courses on the biology of disease vectors, funded regional workshops on the molecular biology of malaria vectors, and funded numerous scientists for advanced training in ongoing molecular entomology projects and at universities for formal degree training. Partially as a result of these efforts, DEC scientists are now playing an active role in many molecular entomology projects worldwide.

Strategies and mechanisms for Research Capability Strengthening

If good science is the foundation of RCS grants, then successful training is the cornerstone, and the fundamental RCS strategy remains 'training through research' or 'learning by doing'. TDR-funded training, in different formats, has become the predominant mechanism for capacity building. Even within the now-replaced Institution Strengthening Grants and the Re-entry Grants, training has assumed an ever-increasing and important role. For example, Partnership Grants provide considerable opportunities for training, and previous reviews have shown that training is an important dividend of this grant format.

The fundamental mechanism is still the Research Training Grant. With a better developed research infrastructure in the ADCs, research training grants focus on local training of individual applicants from an existing research environment. For the LDCs, TDR focuses on individual applicants (for local and overseas training) and on developing research groups. In these contexts, four strategies have been introduced based on existing grant formats (Fig. 2). These are training, post-training, research group development, and research sustainability.

Training grants

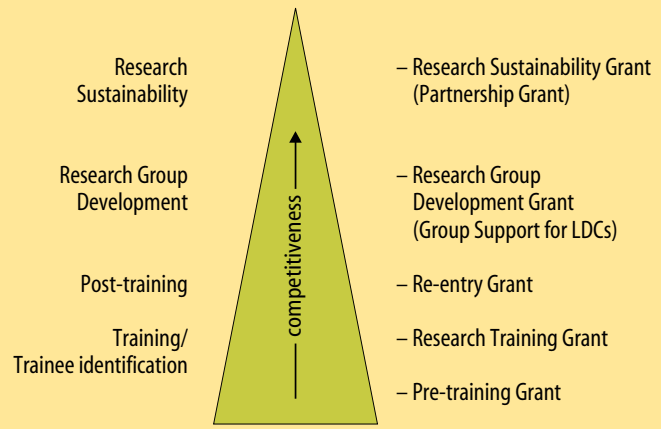
Trainee identification grants

The pyramid concept illustrated in Figure 2 implies a larger base and in this respect TDR promotes small Pre-training Grants to sensitize as large a group as possible to research, at limited cost. This serves as a trainee identification process – a screening mechanism to help identify persons with a potential flair for research who might then apply for formal degree training through TDR. One possible means is through research training networks, first initiated in 1994 in francophone West Africa. The concept is to link a small group of countries, or institutions within a country, where young individuals have an expressed interest in research, around an institution with a comparative advantage in the network. The other members of the network can draw upon the expertise of this institution operating in the same or a similar economic and professional environment.

The francophone West Africa training and research network was built around the concept of a continuum of activities and support. This process involves a protocol development workshop (for pairs of researchers from the same country working on the same subject), low-level funding of the best protocols, execution of the research work with support from experienced scientists, and a final workshop for data analysis and writing up of results for possible publication and/or application in control programmes. This cycle can be repeated with other groups of young researchers, or it can allow the gradual phasing out of member countries that have achieved progress to allow other countries to enter the network. Phasing out members of the network would be dependent on their ability to compete for increasingly competitive grants.

Other possible mechanisms for trainee identification include regional or country-specific small grant programmes that provide funds, on a competitive basis, to young researchers, either to support thesis research or to support research projects immediately following

Fig. 2 TDR Pyramid of Research Capability Strengthening



completion of a graduate training programme. These grant programmes are implemented in cooperation with the WHO Regional Offices, the WHO Representatives' offices and/or the involved national authorities, and reflect local and regional requirements. In the past several years, TDR has supported such programmes in China and Sudan, and with the Regional Offices for the Americas (AMRO) and the Eastern Mediterranean (EMRO).

Formal Research Training Grants

TDR emphasizes PhD training as this prepares the trainee for independent research and a leadership role. MSc training and short-term training are also supported. MSc training is a practical step in which young researchers, already possessing a professional degree in areas such as medicine or pharmacy, can acquire research competence. Short-term, non-degree training allows researchers to acquire specialized techniques, and can be undertaken within TDR R&D projects. Whenever possible, TDR encourages local training or training in other DECs. Training in the North is limited to short attachments, as part of a local PhD, to allow acquisition of skills not available in the DECs, or when it is clearly the most appropriate training location.

Training within TDR R&D projects

This primarily includes non-degree training for acquisition of special skills and techniques, usually not exceeding a period of one year, and short-term group learning activities. It also includes short attachments with R&D projects as part of formal degree training. Integrating RCS activities within the TDR R&D components is challenging, given the differing expectations and particularly the R&D focus on timeliness and output. In an attempt to overcome this challenge, RCS funds are made available to the R&D steering committees to provide short-term training in support of research projects. The selection of projects and persons is undertaken in consultation with the RCS secretariat and the Research Strengthening Group.

Post-training grants

Re-entry Grants

There is a great need to continue supporting young scientists following their training and on return to their home country. In this context, the Re-entry Grants are seen as a means to maintain the skills acquired during training and also to initiate research activities in the home institution. The objective of TDR's Re-entry Grant is to develop the competence and independence of young scientists who have recently completed a period of postgraduate training by financially supporting a research project upon their return to the home institution. Such grants also strengthen the institution's research capacities. The Re-entry Grant is viewed as a bridge between training and successful competition for R&D funds from TDR and/or other national and international sources. In this context, it is important that Re-entry Grants (as training grants) reflect the priorities of TDR's steering committees and task forces as well as the specific research needs of disease control programmes in the applicant's country. As with all RCS grants, preference is given to applications that provide opportunities for training junior researchers in the home institution, thereby producing a multiplier effect.

Re-entry Grants are awarded on a yearly basis, with the possibility of one renewal period, subject to satisfactory progress. Eligibility requires completion of a PhD or a 2-year Master's programme within the applicant's own country, or a minimum of nine months academic training abroad during the 12 months prior to the meeting of the Research Strengthening Group. Preference is given to applicants who are former TDR trainees, LDC scientists and scientists whose research is related to TDR's R&D activities. TDR has assumed a more proactive role in developing and following up on research projects.

Research Group Development Grants

Subsequent to post-training grants for individuals who have demonstrated their potential for research, TDR further supports their growth through Research Group Development Grants. In both the LDCs and ADCs, TDR identifies exceptional individuals and strengthens their capacity to initiate research. In the LDCs, TDR support may also be required to help develop an institutional research environment. This

helps to bring together scientists who have demonstrated a basic level of scientific maturity. In both the ADCs and LDCs, principal investigators are expected to create a functional research team and provide training opportunities. Young researchers working within this grant structure may themselves apply for TDR support. This grant format replaces both the Career Development Grant and the Institution Strengthening Grant.

Research Sustainability Grants

These grants represent the top of the pyramid and involve only those research groups that have demonstrated a high level of scientific competence and maturity. The pinnacle of RCS institutional support, formerly called the Partnership Grant, was established in 1988 in cooperation with the Rockefeller Foundation. This grant linked DEC institutions with one or more northern partners. The premise was to pool the resources and strengths of both South and North to develop research proposals that could make a significant contribution to disease control and scientific literature in general. Research training and technology transfer were also requisites for this grant. These grants proved to be an outstanding success and additional rounds of the grants have been awarded. The partnerships are primarily South-North with the southern partner being the driving force. The approach represents a proven means to sustain research activities at a high scientific level.

While the first two rounds of Partnership Grants were open to investigator-initiated proposals in disciplines related to the TDR target diseases, the new Research Sustainability Grants awarded in 1995 and 1996 were in response to a focused Call for Applications. The three grants awarded in 1995 were for vaccine scale-up and production; the four grants awarded in 1996 were for combinatorial chemistry and high-throughput DNA sequencing.

TDR also works with bilateral donors to help sustain research capacity in the DECs. In many countries where TDR provides support, there are ongoing parallel bilateral projects. There may be opportunities for TDR to play a role by co-sponsoring research activities and providing technical expertise and assistance for training. This type of cooperation requires sustained effort and negotiation between the bilateral donors, TDR and the recipient countries.

Fiocruz, Rio de Janeiro: one of the institutes that have benefitted from RCS activities.

Conclusions

The training and research capability strengthening strategies presented are measured steps that help DEC researchers, particularly those from LDCs, to gradually improve their research capacities, eventually reaching a level that allows them to sustain their own research agendas. Aside from the common characteristics shared by LDCs, specific conditions and requirements are considered in relation to RCS activities. This ensures that opportunities to build on the comparative advantage of each LDC are not lost, and that RCS activities meet specific needs. The best scientists and research groups are assisted and made more able to compete for TDR R&D grants and other international funding. As such, they are ready to compete better at an international level.

CHAPTER 4

Malaria

Applied Field Research

- ✓ there is growing evidence that people delay longer before seeking treatment after the introduction of user charges
- ✓ some behaviours associated with the introduction of revolving drug funds may be detrimental to the control of malaria
- ✓ self-medication is the preferred option for treatment of malaria, using drugs purchased from private, often unlicensed, sellers
- ✓ insecticide-impregnated mosquito nets have been shown to significantly reduce child mortality in a variety of settings and to be a potentially very cost-effective way of improving health
- ✓ in an integrated approach, TDR is helping define and evaluate potential interventions, including iron supplementation and malaria chemoprophylaxis, for infants in highly endemic areas
- ✓ blister packaging, appropriate information, and quality control of drugs can help stave off the spread of drug resistance
- ✓ 17-fold increases in malaria resulting from development projects have been documented, as has the potential for increased malaria transmission in tree crop plantations
- ✓ women's first choice for their illnesses is home remedies and traditional medicines

Product Research and Development

- ✓ newer technologies, such as combinatorial chemistry and high-throughput screening, are complementing classical approaches in the search for new drugs
- ✓ some synthetic trioxanes now in preclinical development have *in vitro* activities several thousand times greater than that of artemisinin
- ✓ artemether for intramuscular use has been registered in more than 30 countries for the treatment of severe malaria
- ✓ artesunate suppositories offer considerable promise, especially for the treatment of childhood malaria
- ✓ a vaccine able to reduce overall childhood mortality by 30% or more, with a duration of immunity of three years or more, would represent a very cost-effective intervention strategy for use in Africa
- ✓ three types of malaria vaccine are now in clinical trials: asexual blood-stage vaccines, transmission- blocking vaccines, and pre-erythrocytic vaccines

Strategic Research

- ✓ a novel class of polypeptides has been shown to contribute to the 'stickiness' of malaria-infected cells for uninfected red cells and endothelium
- ✓ susceptibility to severe and complicated malaria is correlated to two alleles associated with high levels of tumour necrosis factor (TNF-α) secretion
- ✓ research into genetic modification of mosquitos to disrupt development of the parasite in the vector is advancing along three main avenues

Malaria remains one of the leading causes of morbidity and mortality in the tropics. An estimated 300-500 million cases of malaria each year result in about one million deaths, mainly children under five in Africa. Malaria has been estimated to represent 2.3% of the overall global disease burden and 9% in Africa,[1] ranking third among major infectious disease threats, after pneumococcal acute respiratory infections (3.5%) and tuberculosis (2.8%). Pregnant women and children under five continue to constitute one of the most important risk groups. The disease is often linked to the movement of refugees and populations seeking work, and to environmental change, including forestry, mining and water development projects. In Africa alone, the estimated annual direct and indirect costs of malaria were US$ 800 million in 1987, and were expected to exceed US$ 1800 million by 1995. In 1990, malaria was responsible for the loss of 31.7 million Disability Adjusted Life Years (DALYs).[1]

Malaria is caused by protozoan parasites of the genus *Plasmodium*; of which four species infect man. The most serious infections are caused by *P. falciparum*, which, amongst other things, causes cerebral malaria, anaemia and kidney failure. The other species which infect man are *P. vivax*, *P. malariae*, and *P. ovale*. The parasites are transmitted by mosquitos of the genus *Anopheles*. During its life cycle, the malaria parasite passes through a number of distinct stages in its human and mosquito hosts (see Fig.2).

The current WHO Global Malaria Control Strategy aims to reduce malaria mortality by the year 2000 by at least 20% compared to 1995 in at least 75% of affected countries. This strategy recognizes that varied transmission and operational drug-resistance patterns worldwide lead to different epidemiological patterns of malaria

illness, for which certain risks are particularly important and certain approaches to control more likely to succeed than others. Since malaria represents a moving target, control programmes need to identify and monitor epidemiological patterns.

To control malaria, better ways must be found to apply existing control methods; improved tools must be developed; and solutions identified to circumvent and combat emerging problems, which include social/political unrest, the widespread resistance of the malaria parasite to existing drugs,[2,3] and the potential change in distribution and incidence of malaria due to anthropogenic climate change.

Disease control and trends

Malaria control was previously based largely on mosquito eradication and vector control. Unfortunately, experience has shown that mosquito control is often not cost-effective in areas where the disease is most severe and where interruption of transmission cannot be sustained. In this respect, the use of insecticide-impregnated bednets, which TDR has been supporting, represents an exception.

The current WHO Global Malaria Control Strategy emphasizes early diagnosis, treatment with effective antimalarials, and the selective use of preventive measures, including vector control, where they can be sustained.

The emergence of drug resistance continues to be a serious global problem. Chloroquine was until recently the drug of choice in Africa, but resistance has now spread to all major malaria endemic regions. In South-East Asia, in areas of unmanaged economic opportunism, especially on the borders of Thailand with Myanmar, Laos

Fig. 1. Malaria distribution and problem areas

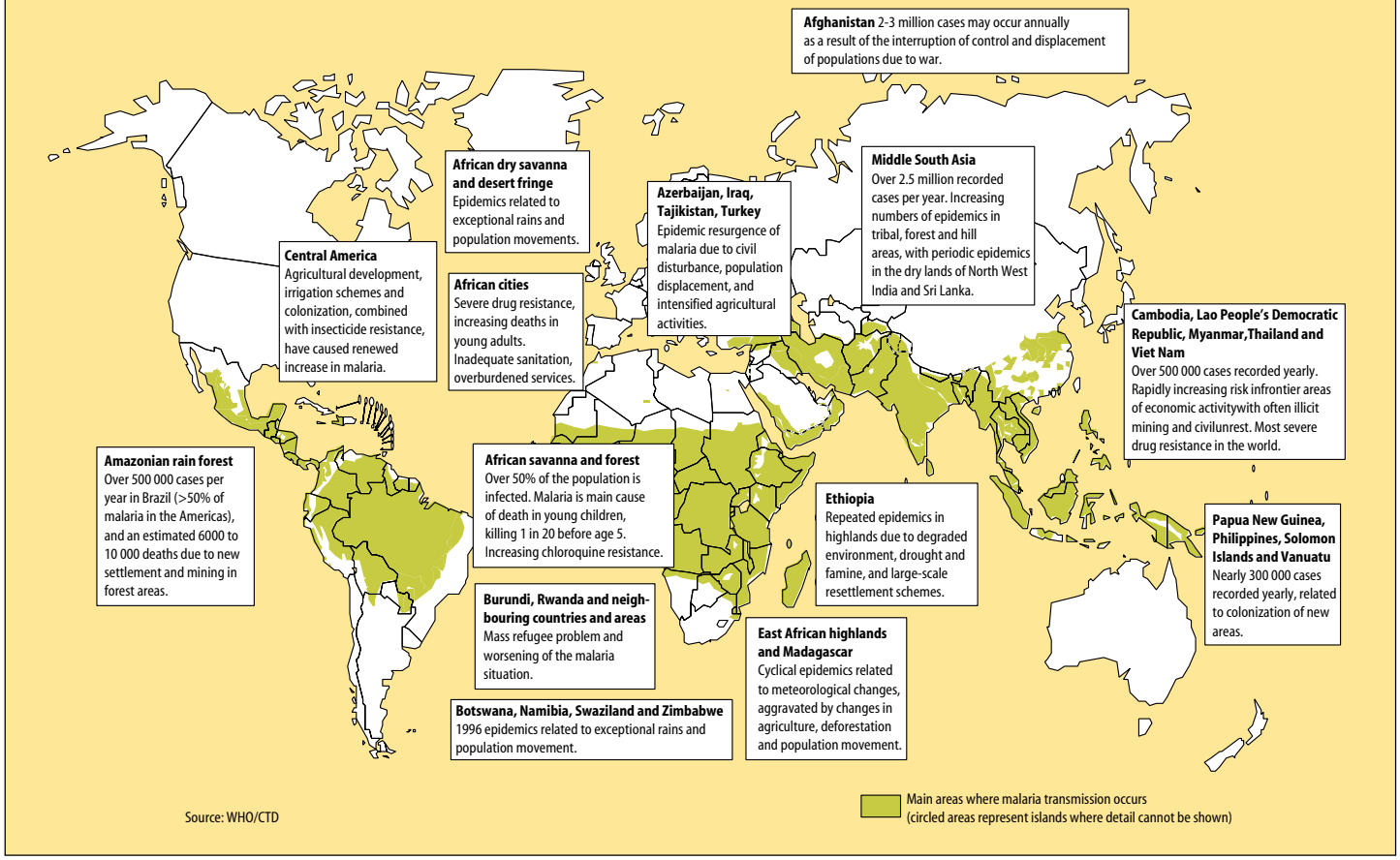


Table 1. Estimated cost-effectiveness of malaria control interventions in Africa[a]

Intervention	Total cost (US$)	Total DALYs[b] gained	Cost/DALY (US$)
Current treatment practice [c]	210 000–297 000	20 882	10.1–14.2
Impregnated bednets[d]	142 900	10 400–20 800	7–14
Hypothetical vaccine [e] (30% reduction all-cause childhood mortality) (a) EPI delivery; duration of immunity 1-5 years	7800–229 000	21 123	0.4–11
(b) No EPI delivery; duration of immunity 1 year	497 000	21 123	24

[a] Calculations based on a cohort of 10 000 live births, followed over expected lifetime
[b] As calculated here, DALYs are identical to the number of discounted years of life gained
[c] G. Azene and D. Evans (1997; in preparation)
[d] See Reference 4 for more detailed information
[e] See Reference 1 for more detailed information

and Cambodia, malaria cases no longer respond to sulfadoxine/pyrimethamine or mefloquine, and a decreased sensitivity to quinine has been reported. New drugs are being developed, but resistance to some of these is also being reported at an alarming rate. Improvements in malaria control face immense challenges in many regions of the world where there is a lack of basic health care infrastructure.

TDR objectives in malaria research include the development and evaluation of novel, cost-effective control tools, such as new and affordable drugs, improved diagnostic methods, and an effective vaccine, which would help curb the emergence of drug resistance. There is still an urgent need to improve early diagnosis, treatment and case management in order to reduce the avoidable mortality from *Plasmodium falciparum* malaria. Interestingly, a cost-effectiveness analysis of the current pattern of treatment against malaria infection in sub-Saharan Africa compares favourably with estimates for reduction in overall childhood mortality by the use of newer intervention tools such as impregnated bednets or a hypothetical vaccine applied through the Expanded Programme on Immunization (Table I). It should be noted that any intervention costing between US$ 1-20 per life saved represents a very good investment of scarce resources.

Applied Field Research

Health sector reforms and malaria

The impact of user charges on demand

TDR studies on the impact of user fees have confirmed that an increase of fees (or their introduction) leads to a fall in the overall demand for care for all diseases, including malaria. However, there is some evidence that malaria symptoms may be regarded as being sufficiently serious to warrant some protection. For example, in 11 health centres in Uganda overall attendances had fallen by 21% approximately two years after the introduction of fees compared with the same period beforehand, while attendances diagnosed as malaria had fallen by only 13%. This pattern was not identical in all health centres, and in one centre malaria attendances actually rose in the face of an overall decline in demand.

The possibility that attendance for malaria treatment is less affected by the introduction of fees than attendance for other diseases needs further study, but any falls in attendance are undesirable for children with symptoms of severe malaria.

There is also growing evidence that people may delay longer before seeking care. In rural Ghana a study found that mothers used self medication as the first line of treatment for just under 60% of infants suffering from malaria-like symptoms and 66% of children. This figure rose to 69% for adult males. Qualitative research suggested that people felt, after the introduction of user charges at health facilities, that they now waited longer before seeking care; and this perception was shared by health care providers who felt they were now seeing more serious cases. This has yet to be verified more widely but would not be desirable in the case of children with fever or convulsions.

Supplier behaviour

A study in Ghana suggested that supplier behaviour may be partly determined by the type of financing mechanism, and that some behaviours associated with the introduction of revolving drug funds may be detrimental to the control of malaria. The study compared the way patients were treated for malaria in four facilities which operated revolving drug funds with those in four facilities which still offered free outpatient care. It found that over 76% of those who had to pay for drugs obtained injectable chloroquine compared to only 30% of patients who received free care. Many explanations are possible, but this difference could be due to both supply and demand factors associated with the introduction of fees. On the supply side, the mark-ups on chloroquine injections are relatively high compared to chloroquine tablets, so that injections provide higher surpluses for revolving funds. On the demand side, injections are culturally preferred in many parts of Africa, and patients might demand them more strongly when they have to pay for treatment. This result has led the Task Force on *Health Care Financing and Tropical Diseases* to focus more on the impact of financing changes on provider behaviour.

Decentralization

Many health sector reforms taking place all over the world feature decentralization to different degrees, where sub-national levels of government are required to plan, execute and sometimes finance health care services and activities. In many cases, programmes such as malaria control, which were previously funded and managed from the central level, have been integrated into primary health care. TDR sponsored a series of studies by district medical teams in countries where forms of decentralization have occurred – in Ghana, Nigeria, Zambia and Uganda.

The first stages of the studies were aimed at describing the current patterns of treatment and control of malaria in districts and at identifying problems. The fact that the majority of fever cases are not treated in the public sector, and that self medication is the preferred option using drugs purchased from private, often unlicensed, sellers, has been consistently highlighted in the studies. Convulsions in children are not perceived to be malaria but caused by supernatural forces, and traditional healers are preferred for treatment. Underdosing and the use of multiple drugs, many of which are unnecessary, are common in both the public and private sectors.

The second phase of the studies is focused on developing and testing interventions to resolve some of the problems and on improving malaria control. Only interventions which are believed to be sustainable in the long term by district health teams using available resources are being tested. A number of the interventions involve interactions with private chemical sellers to develop ways of ensuring that patients receive appropriate drugs in appropriate quantities. Other interventions involve health facilities and are aimed at encouraging patients to comply with dosing recommendations and at improving the quality of malaria services offered in the public sector. Results of the studies will be available in late 1997.

Insecticide-treated bednets and curtains

On the basis of results from a TDR-funded trial conducted in the Gambia in 1990-1991 to evaluate the impact of insecticide-treated nets on child mortality, which showed a reduction of 63% in mortality from all causes in children (1-5 years of age) sleeping under the treated nets compared with a control group, the Gambian government initiated a country-wide programme of net-impregnation in villages with a primary health care clinic. More recently, large-scale studies including an evaluation of the Gambian national bednet impregnation programme[4] and intervention studies carried out in Kenya,[5] Ghana[6] and Burkina Faso have confirmed this important impact on all-cause child mortality in differing epidemiological situations, with observed reductions of between 16% and 33% in children under five years of age. In the Kenya study, hospital admissions for severe and complicated malaria were also reduced by 44% in the group receiving treated nets compared with the controls.

The results of these trials suggest that approximately 500 000 African children might be saved each year from malaria-related mortality if the nets, treated with a biodegradable pyrethroid insecticide, were widely and properly used. The findings have emphasized the underestimated contribution of malaria to child mortality in Africa and the potentially large benefits of malaria-preventive interventions. An international meeting held in the WHO Regional Office for Africa in Brazzaville, Congo, in March 1996, reviewed the results of the trials and called for a 'phased and continuously monitored introduction of treated nets' in Africa.

After success in Africa, could impregnated bednets become the main means of malaria control in Asia?

Now that there is a substantial body of evidence for the efficacy of treated nets in Africa, TDR's focus will be on operational research related to the cost-effectiveness and social and economic factors affecting the feasibility of routine utilization and re-treatment of nets, and on issues involved in the sustainability of this intervention, all of which are addressed in an International Development Research Centre/WHO publication.[7] Priorities for research include the selection and evaluation of strategies for product development to enhance the technology of treating and re-treating the nets, and a search for

alternative methods of implementation and promotion that will maximize the impact and sustainability of nets in malaria control. Evaluation of the long-term impact of nets on age-specific mortality will be carried out through extended follow-up of mortality patterns in the Kenya and Burkina Faso trial sites.

Studies comparing the efficacy of treated nets with indoor residual spraying of insecticide will soon be completed in trial sites in China, India and Myanmar to determine, based on an analysis of cost-effectiveness, whether or not treated bednets could replace residual spraying as the primary means of malaria control in certain epidemiological settings in Asia.

Sustainability of impregnated mosquito nets

Now that impregnated mosquito nets have been shown to significantly reduce child mortality in a variety of settings and to be a potentially very cost-effective way of improving health, questions arise of how best to finance and deliver them, and encourage their appropriate use. Two studies on health financing have focused on delivery methods and sustainability.

The first study sought to define a way of assessing the extent of net use in a country before delivery strategies are developed. The indications were that school questionnaires may be the most accurate way of mapping out current patterns of use. In addition, surveys at bus terminals may provide useful initial mapping of distribution in countries where people travel extensively. School-based surveys may also be a means of monitoring changes in use after an intervention.

The second study focused on the coverage achieved by a mail order delivery programme. A scheme already existed in Namibia and TDR funded a trial in Zimbabwe. People place orders for nets by mail or telephone, nominating the closest post office to their residence for delivery. Payment is obtained on delivery to the postal outlet. Coverage was low among poor people in rural communities but a considerable number of nets were sold in urban areas and to teachers. Health centre staff were not receptive to this form of marketing and did not seem to use nets widely. This suggests that, not surprisingly, mail order involving full cost recovery is unlikely to have a significant impact on malaria incidence among the poor. However, it may be a useful adjunct to other types of delivery mechanism in that mail orders to the more affluent sections of society could generate profits which could be used to subsidize delivery to poorer communities. The other interesting implication is that 'demonstration' effects may be better targeted at school teachers than at health centre staff.

The UNICEF/WHO initiative for integrated management of childhood illness

Twelve million children die each year before they reach their fifth birthday, many during the first year of life. Seven in ten of these child deaths are due to malaria, diarrhoea, pneumonia, measles or malnutrition – and often to a combination of these conditions. Due to the considerable overlap in signs and symptoms of several of these diseases, for example in the case of malaria and pneumonia, a single diagnosis for a childhood illness is often not appropriate. A focus on an apparent single problem may lead to an associated, and potentially life-threatening, condition being overlooked.

As part of a UNICEF/WHO initiative for *Integrated Management of Childhood Illness*, under the direction of WHO's division of Child Health and Development (CHD), TDR formed a task force focusing on the malaria component of management of childhood illness. Following contributions to development of the 'fever' component of the integrated approach, and in conjunction with the malaria unit of the WHO division of Control of Tropical Diseases – CTD/MAL – field research has focused on clinical diagnosis of malaria in areas where most fevers are not due to malaria and where microscopy is not available for parasite confirmation. The ability to exclude malaria when a fever is due to other causes results in economies of drug use and more appropriate treatment for the true cause of disease. In addition, appropriate treatment for malaria in these areas should be associated with reduction of severe and complicated disease episodes, and in many cases, mortality.

Iron deficiency, the main cause of nutritional anaemia worldwide, is especially common in developing countries. In areas where malaria is endemic, the incidence and prevalence of anaemia increase, and severe life-threatening anaemia, often accompanied by malaria parasitaemia, is one of the most common causes of hospital admission and death among infants and children. Effective, appropriate public health interventions addressing anaemia should be incorporated into the integrated approach to management of childhood illness. TDR is funding studies to define and evaluate potential interventions, including iron supplementation and malaria chemoprophylaxis, in infants in the most highly endemic areas of Africa.

TDR has also supported research to adapt this integrated approach for use in parts of South-East Asia where the pattern of etiological agents associated with 'fever' is different from that observed in Africa, principally in the inclusion of dengue, dengue haemorrhagic fever and typhoid. Development and validation of treatment modalities for management of severe and

complicated malaria in inpatient facilities and at the periphery are also being supported by TDR in Malawi.

In addition to efforts which are directed towards better clinical management of malaria in the health facility, TDR has made concerted efforts to improve the home management of sick children since this form of care – both preventive and curative – continues to dominate, and can save lives. The short-term objective being implemented jointly with WHO/CHD is to conduct community-based intervention trials which are carefully designed to improve case management of sick children. The medium- to long-term objective is to evaluate the role and benefit of improved home management in preventing severe forms of, and death from, *P. falciparum* malaria.

Three research strategies are being pursued simultaneously:

i. community trials to improve early maternal recognition of danger signs and symptoms – e.g. change in mental status – and rapid action in case management;

ii. community trials to upgrade the potential for early, effective treatment of children at risk at traditional places of consultation;

iii. technical improvements in antimalarial products: paediatric formulations, compliance-friendly packaging and marketing.

Research already funded by TDR includes:

- Interventions to improve recognition of early signs of complicated malaria.
- Improving compliance with multidose drug regimens at the periphery.
- Optimizing private sector diagnosis and quality of care for mild malaria.
- Communications interventions to improve advice to patients and caretakers.

Improving the use of antimalarials in South-East Asia

Because of regional concerns regarding the rapid and accelerating spread of multidrug resistance to antimalarials and a desire to prolong the effective life of qinghaosu compounds (derived from artemisinin – a potent antimalarial extracted from the plant *Artemisia annua*), TDR has funded research to examine the viability of improving the quality of drugs and compliance with multidose antimalarials within mainland South-East Asia.[8]

The results have been encouraging and have demonstrated:

- that blister packaging improves compliance with the full antimalarial regimen – particularly in complex or combined courses;
- the value of appropriate information and good communication for encouraging purchase and ingestion of a full antimalarial regimen;
- the importance of quality control in countries which produce and market antimalarials over-the-counter;
- a method for accurately measuring compliance with short-course therapy, through the use of a pharmacological marker.

In China, for example, blister packaging of drugs for malaria produced 97% compliance compared with 83% in the control group to whom drugs were handed out in simple envelopes (which are often lost or disintegrate in the rainy season associated with malaria). Compliance was improved specifically in relation to an 8-day course of primaquine. In Cambodia, where a variety of dangerous and injectable drugs are commonly used at a cost to the patient which exceeds the full treatment cost of quinine/tetracycline (Q/T), community-based communication methods have shown improved advice given by pharmacies and shops on compliance with the full regimen. Although there was no significant increase in the proportion of patients purchasing Q/T, there was a highly significant rise in the number of patients purchasing and ingesting the full dose, and not purchasing unnecessary or dangerous drugs.

In Myanmar, a community-based trial to test the viability of a subsidized system for providing mefloquine with artesunate for the treatment of uncomplicated malaria at the periphery showed that a voucher subsidizing mefloquine was ineffective in increasing uptake of the drug for treatment of uncomplicated malaria. This was largely because the voucher was redeemable only at one location, and hence only 5% of all patients took up the option. In a follow-up community trial, the viability of an unsubsidized but combined package of a 5-day course of artesunate with mefloquine (included on the second day) was explored. A control group of patients received an identical package which contained a placebo in place of mefloquine. Cure rates at 7 and 28 days, and compliance to day 3 and day 5, measured through detection of a pharmacological marker, were measured. In total, 360 patients were followed to day 28. Cure rates were significantly higher in the intervention (mefloquine and artesunate) group ($p = 0.01$). Ninety-nine per cent of patients purchased the combination package, thus supporting the marketing of drug combinations to achieve higher cure rates and increase the longevity of multidose therapies.

Malaria and the environment

With vector-borne diseases, a change in risk of a disease can be the unintended result of economic activities or agricultural policies that change land use, such as the creation of dams, irrigation schemes, commercial tree cropping and deforestation. The impact on illness and death may be very serious if non-immune people are involved in new or intensified transmission.

Land-use change is invariably financed following appraisal of the economic benefit and cost of the development activity. Health outcomes are often not taken into account in this appraisal for two reasons: the lack of convincing quantitative information on how much the risk of disease is uniquely attributable to the land-use change; and the difficulties of valuing health benefits and costs in financial terms. To resolve this problem, the Task Force for *Tropical Diseases and the Environment* has made strides in attempting to quantify the health benefits and costs of tropical diseases, and in valuing the health outcomes in economic terms. Armed with epidemiological data on project impact as well as estimates of the health costs and benefits, the Task Force has influenced the manner in which project appraisal takes place in major multilateral lending institutions, and thereby has helped the move towards the inclusion of minimization of health risk in the design of development projects.

Results/products available from projects funded in the first year of operations include:

- Documentation of 17-fold increases in malaria resulting from development projects which have compelling and high agricultural or water benefits in semi-arid areas of Africa.
- Documentation of the potential for increased malaria transmission and disease incidence in South-East Asian commercial tree crop plantations (rubber, teak, fruit orchards) and of the re-emergence of drug-resistant malaria in these newly created habitats (Box 1).
- The *World Bank environmental sourcebook update on health*[9] – a procedural guide for multilateral funding institutions in their appraisal of development activities which modify the environment. This was a co-development project.
- Guidelines for the preparation and implementation of future loans at the interface of environmental and land-use change and health. These are being prepared on the basis of case studies, from the loan portfolio of multilateral funding institutions, which illustrate the impact of projects on tropical diseases. The guidelines provide detailed advice for project staff on where improvements can be made in development project design and/or implementation.

Box 1. Malaria risk in commercial tree plantations in South-East Asia

In South-East Asia, from Bangladesh to Viet Nam, the mosquito which is most important in transmitting malaria, *Anopheles dirus,* is extremely sensitive to light and thrives in closed-canopy forests – a habitat now reduced by extensive deforestation, with a resultant reduction in the incidence of malaria. However, TDR research indicates that *A. dirus* has adapted well to new commercial tree plantations – orchards, rubber or teak plantations. Man-biting rates by *A. dirus* are higher in these new plantations than in its natural forest habitat, and there is a corresponding effect upon disease incidence.

Plantation practices which affect malaria incidence include:

- **The use of coconut shells for the collection of latex in rubber plantations. When discarded on the ground, the coconut shells provide good breeding sites for *A. dirus*.**
- **Rubber tapping in the early morning, the time that latex must be collected if it is to be of best quality. The hours before dawn coincide with the peak biting time of the vector.**
- **The hiring of workers on a daily basis in smallholder rubber plantations in Thailand, which means that the costs of malaria have to be found from outside, in contrast to the situation in Viet Nam, where plantations are state-owned and the costs of malaria covered by the employer.**
- **Harvesting in orchards at a time which coincides with the peak transmission season for malaria.**

This information provides several lessons:

- **If a project is likely to increase transmission, then the characteristics of any population movement provoked by the project are crucial.**
- **If a high rate of return on financial investment in commercial tree crops occurs, despite adverse health consequences, then measures should be incorporated into the design and implementation of the project to minimize the negative impacts on health.**
- **Solutions – changes in plantation practices, supplemented by malaria control measures – are required from the agricultural sector in order to minimize the increased malaria incidence that occurs in commercial plantations.**

Gender studies on malaria

Background research for the Healthy Women Counselling Guide (HWCG) was conducted in three African countries (Kenya, Nigeria and Sierra Leone) in 1994. During the studies, women's health in the context of the community was assessed, and women's health needs from their own perspective, as well as from that of men, health providers (informal and formal), local leaders and community groups, were examined. The results of this research have provided the basis for further work towards the development of the Guide.

The studies have highlighted how cultural expectations often demand stoic acceptance of endemic diseases such as malaria, and the low status of women in their communities – a woman may need her husband's permission before she seeks medical care. In most cases, women choose first to use home remedies and traditional medicines for their illnesses. If these fail and symptoms persist, they will seek low-cost treatment in the form of over-the-counter drugs. Women consider this type of treatment to be the most effective for malaria.

Communication strategies, including radio programmes and illustrated material on various aspects of health, were developed in the first half of 1996 in the three countries where background research for the HWCG had been undertaken.

Radio as a medium for communication in Africa was selected because it can create awareness rapidly, reach a wide audience, overcome literacy problems and be cost-effective. Developing radio programmes in participation with community members enhances comprehension and encourages the community to take appropriate health-related actions. As radio is more effective when combined with other media and interpersonal approaches, illustrated pictorial material was developed to support and enhance the radio programmes. Taped radio programmes can be used in listening groups – to overcome difficulties of access to a radio and provide a forum for discussion and problem-solving.

Multidisciplinary teams, made up of a social scientist, medical doctor, communication expert and an illustrator, carried out pilot projects in the three countries. Each team highlighted the specific health issues identified by the communities in the areas where the interventions were to be implemented. In Kenya, malaria was identified as a key health issue. Gender issues pertaining to health topics were also identified. The teams developed radio programmes and illustrated materials in consultation with community members. All programmes and materials were pre-tested with groups of women and men in the communities and changes made as necessary.

In Kenya, focus group discussions were held in Chiakariga with Tharaka men and women to:

- identify communication structures at the family and community level;
- identify the roles of family and community members during a woman's illness;
- map out the health resources in the community e.g. distances travelled to health facilities and shops selling over-the-counter medication;
- simulate messages about malaria and gender in a story relevant to the Tharaka community, testing various dramatic situations and other formats, including dialogue, role plays, question and answer methods, and illustrations;
- produce radio segments and illustrated material.

A sample of the story outline for a radio programme is given in Box 2.

Box 2. 'Mucoore (my friend) : Small insects, big trouble!'

- Kagendo is sick with malaria. Her husband, M'Makembo, spends his time drinking 'marua' with his friends and doesn't care about his family or wife. Kagendo has a friend, a 'mucoore' (trusted woman friend), called Karimi who comforts her and takes action during times of illness. They also share information on topics like pregnancy and malaria. Kagendo is lying on a mat outside her hut. Her children are playing nearby. She gets up and starts preparing the mid-day meal and then goes back to sleep on the mat. She sends her daughter Makena to keep the cooking fire going. Her mucoore comes to visit, finds her sick and questions her on her illness and what medications she has taken. Kagendo explains that she had taken left-over drugs from her son, Gitonga's, last illness. She has also taken mukununi, mutongu herbs and a local brew made from honey. Her mucoore asks her what her husband has done since she got sick. She responds by saying that he has sold a cow but has not used the money to treat her illness. Various gender issues are raised in their discussion, such as the neglect of the wife by the husband, the work burden on women, lack of financial resources among women and the problem of alcoholism in the community.
- The brother-in-law comes, expresses surprise that the brother left his wife sick, and asks about the symptoms of her illness and the medication she has taken. He then quickly sends Gitonga to buy malaria drugs at Mr Nyaga's shop. He then goes to look for his brother. The two mucoores continue discussing.
- At Nyaga's shop, Gitonga explains that his mother is sick with malaria and that he has been sent to buy chloroquine tablets. Mr Nyaga asks him about the symptoms of his mother's illness and then sells the medicine. He tells Gitonga that his mother should take four tablets at once, two after six hours, and two every morning for three days. Gitonga returns with the tablets and passes on the information. The mucoore tells Kagendo that she will come back in the evening to personally give her the right dosage and ensure that she takes the correct dosage for the remaining days.
- The drunken husband enters with the brother-in-law and is surprised to find his wife's mucoore in the house. He says he is not sure whether the wife is sick or just pretending, since she has been doing so from the time he married her. At this point, the brother calls him aside and tells him that his wife is seriously sick with malaria and that she is not malingering.
- On the fifth day, after completing the treatment, Kagendo's condition has become worse. She is now vomiting, can't walk and feels drowsy. The husband is now worried and realises his wife is not pretending. He sends for his brother. On arrival, he tells him (his brother) that his wife's illness has become worse and he has sent Gitonga for Nyaga's vehicle to carry her to the hospital. His brother makes an 'itarati' (the traditional Tharaka stretcher) to carry Kagendo to the hospital instead of waiting for the vehicle. He sends Makena to call his wife's mucoore. M'Makembo tells the mucoore that his wife is now very sick and he fears for her life. The mucoore accompanies them to the hospital after organizing her household and leaving a message for her husband about where she has gone.
- Kagendo is placed on the itarati and the safari to hospital begins with the mucoore, husband and brother-in-law. At the health facility, the doctor examines her, asks for the symptoms, the medication she has taken and tells them that she has to be admitted. He reassures them that Kagendo will be fine. He also explains that the reason why the drugs were not effective is because of parasite resistance to chloroquine tablets. So even though Kagendo took the correct drugs and correct dosage, she still became sick. The story ends with an interview in which a member of the community asks the doctor questions on malaria.

Product Research and Development

Malaria drug development

The discovery of antimalarial drugs and their subsequent preclinical and clinical development is the concern of the TDR *Steering Committee on Drugs for Malaria* (CHEMAL). Emphasis is placed on influencing university and government research in the area of drug discovery, on fostering industrial partnerships in the area of drug development, and on establishing research and development (R&D) capability in malaria endemic countries.

Drug discovery

The discovery and characterization of drug targets which are unique to the malaria parasite, and validation of these targets, is a top priority for TDR.[10] Potential targets must constitute essential features of the parasite life cycle, be parasite specific and/or be sufficiently different from analogous structures or processes in the host to make possible selectivity between host and parasite. Emphasis is placed on testing compounds for inhibition of specific targets and for activity on the intact parasite.

Priority areas for discovery include:

- the *Plasmodium* digestive vacuole, which has multiple targets (e.g. haemoglobin degradation, haem polymerization);
- nucleic acid metabolism, particularly those parts of folate metabolism which relate to this;
- phospholipid metabolism.

In order to identify additional targets, there is a need for more information on the basic metabolic and biochemical processes of the malaria parasite. In addition, it must be recognized that few potential targets have been validated to date. Therefore, efforts to develop lead compounds for inhibiting putative targets are also a high priority. A programme for compound acquisition or synthesis and screening is in place in which newer technologies, such as combinatorial chemistry and high-throughput screening, complement classical approaches, thus increasing the number and diversity of compounds tested.

Preclinical development

Selection of compounds for preclinical development is made on the basis of ease of synthesis, stability/solubility, comparative efficiency, and patentability/proprietary status. High priority is given to the pre-development of selected compounds, a management-intense and costly activity which is vital for 'transitioning' compounds to a stage of development that could be of interest to the pharmaceutical industry.

Three priority projects have so far led to a significant number of leads:

Phosphatidyl choline inhibitors

Blood stage malaria parasites require large amounts of phospholipids (PL) which are synthesised from free fatty acids imported from plasma. The main PL in *P. falciparum* is phosphatidylcholine (PC), for which choline is a precursor and choline transport a limiting step in the synthetic pathway. The project focuses on blocking choline transport. During the past ten years, over 350 compounds have been synthesized and structure-activity relationships developed.

First-generation leads include a quaternary ammonium compound, but most recent bioisosters show better absorption and lower toxicity. The lead compounds are active against mature trophozoites, in which PL biosynthesis is most intense. There is no cross resistance with known antimalarials and, in monkey models, some compounds have been shown to clear very high parasitaemias. Preliminary toxicology studies have indicated that the compounds are not mutagenic.

Whilst some basic studies are still being conducted (such as characterization of molecular targets and resistance mechanisms), the main thrust is now on selecting candidates for further development, and a number of active leads have been identified. The problem of proprietary status of these compounds has been solved and a potential industrial partner identified.

Second generation endoperoxides

This project is focused on generating more stable derivatives of artemisinin and on generating synthetic trioxanes and tetroxanes.

With respect to artemisinin derivatives, most attention is focused on chemical changes (e.g. removal of the oxygen atom at C-10 in the lactone ring) which lead to compounds that are broken down more slowly in the body than are the parent compounds, bypassing the intermediate dihydroartemisinin (which is common to the currently used artemisinin derivatives, artemether and artesunate, although not to artemisinin itself). Evidence that derivatives not possessing oxygen at C-10 are less neurotoxic than standard artemisinins is also emerging (the neurological lesions caused by standard artemisinin derivatives, as seen in animal studies, are a prime concern). A very interesting sub-family of

derivatives, which possess a particular substitution pattern at C-9 and have enhanced activities *in vitro* against both chloroquine-resistant and chloroquine-sensitive strains of *P. falciparum,* has also been pinpointed.

In the area of synthetic trioxanes and tetroxanes there are exciting developments. The *in vitro* activities of some synthetic trioxanes are several thousand times greater than that of artemisinin. The compounds are easily prepared via non-photochemical routes, and either yield enantiomerically-pure compounds or, in some cases, are achiral. This is important from the drug regulatory and manufacturing points of view. Tetroxanes also have advantages. They are very easily prepared in a minimum number of steps from cheap, accessible starting compounds. Clearly these compounds warrant collaboration with the pharmaceutical industry.

Proteinase inhibitors

The roles of proteinases in essential cellular functions and pathogenesis are being increasingly recognized. As a result, inhibitors of proteinases are being screened for activity against such diseases as HIV and hypertension. Degradation of haemoglobin provides a principal source of amino acids for protein synthesis in malaria parasites. Three proteinases in the *Plasmodium* digestive vacuole play a major role in this degradation: two aspartic proteinases (plasmepsin I and II, similar to human cathepsin D) and one cysteine proteinase (falcipain, analogous to human cathepsin L). Together the three proteases account for virtually all the haemoglobin-degrading capability of the digestive vacuole. Preliminary data with inhibitors indicate that all three proteinases may be novel chemotherapeutic targets. TDR funds four projects on malaria proteinase inhibitors, but there is a need to test larger numbers and a greater variety of compounds. In this respect, agreements are being made for drug companies to include cysteine proteinase and plasmepsin II in their screening systems (plasmepsin I has only recently become available for high-throughput screening). Of the potential inhibitors so far synthesized and tested, some have high *in vitro* activity against the target enzyme and a few are also active against the whole parasite.

CHEMAL is now looking for sources of compounds which inhibit cathepsin D or L, are of likely oral bioavailability and do not inhibit the analogous mammalian enzymes.

Clinical studies

Pyronaridine

CHEMAL sees pyronaridine as a potential replacement for chloroquine in the treatment of uncomplicated malaria. However, available preclinical and clinical data do not meet international standards for marketing authorization while the current price (approximately US$ 3-4 per course of treatment) makes the drug far too expensive for extensive use. There is therefore a need for more data on the chemistry and pharmacy of pyronaridine, for further preclinical and clinical studies, and for the price of treatment to be substantially reduced (to below US$ 1). Critical review has shown that synthesis of the compound can be shortened, so reducing the cost, while Phase I studies have shown that dosing regimes can be optimized by using a more suitable formulation (capsules instead of tablets). The cost issue will be constantly monitored.

Current negotiations with a potential industrial partner include developing a workplan to cover all stages of drug development and the transfer of research and development technology to a developing country.

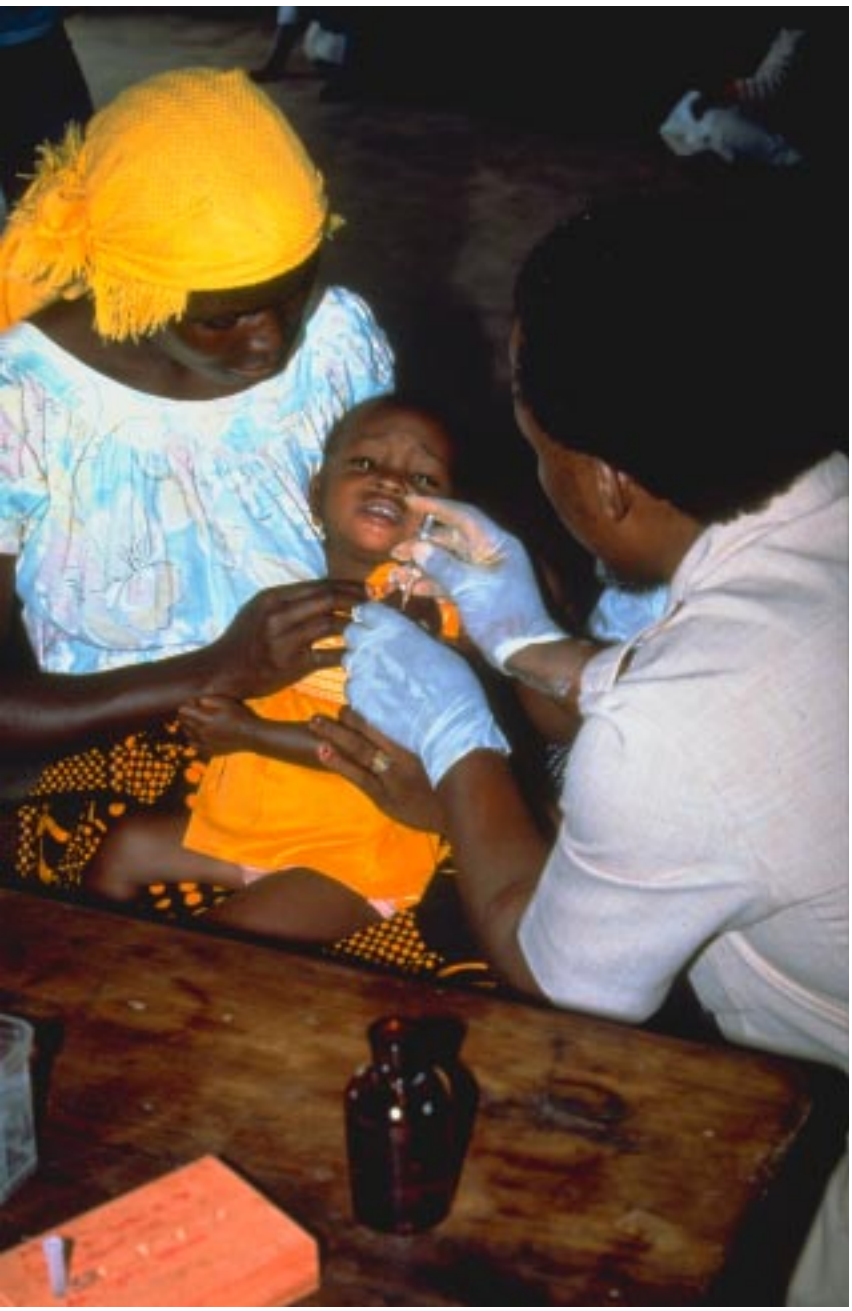
Chlorproguanil/dapsone

TDR supported the screening of this combination which led to a Phase II pilot study in Kenya. This study indicated that 3-day treatment with the combination may be a valid alternative to treatment with sulphadoxine/pyrimethamine. A Phase I study on dose selection followed, the results of which are being analysed, while an agreement with a potential commercial partner is under discussion.

Artemisinin and derivatives

Artemisinin drugs, first discovered and developed for use in China from the herbal compound known as Qinghaosu, have been a TDR priority since the early 1980s. A number of derivatives have proved particularly effective in the treatment of severe and multidrug-resistant falciparum malaria. Although most information on qinghaosu compounds concerns the treatment of adults, there are sufficient data to conclude that children tolerate the drugs very well and that the therapeutic response is similar to that observed in adults.[11] Hundreds of thousands of persons have received antimalarial treatment with artemisinin or its derivatives and demand for it is high because of its efficacy, lack of apparent toxicity, rapid action and low cost. However, inappropriate drug use could lead to the emergence of resistant parasites and untoward toxicity. Potential for neurotoxicity has been reported in animal models, although the significance of this with respect to use in humans remains to be elucidated. Artemisinin and its derivatives are marketed in oral, parenteral and rectal formulations.

Artemether for intramuscular injection. Through TDR's efforts, and under a collaborative agreement with Rhone-Poulenc Rorer Doma, where the necessary safety and efficacy studies were completed, artemether has



SPf66 vaccine trial: Drugs, vaccines and methods of reducing human/vector contact will be needed in the fight against malaria.

been registered for intramuscular (i.m.) use for the treatment of severe malaria in more than 30 malaria endemic countries. A preferential price for the public sector was obtained in exchange for data from TDR-funded non-clinical and clinical studies.

Arteether for intramuscular injection. Phase I clinical pharmacology studies of this drug (with partial funding from the Netherlands Ministry of Development Cooperation, in collaboration with Artecef, B.V., Holland, and the Walter Reed Army Institute of Research, USA) showed the injection to be well tolerated. Subsequent Phase II studies in adult, non-severe, multidrug-resistant falciparum malaria patients in Thailand were also completed and showed the drug to be well tolerated and effective in 5-day treatment regimens.

Both an adult i.m. formulation and a paediatric formulation have been developed and Phase III clinical trials in more than 200 severe falciparum malaria patients, including three trials in children and two trials in adults, have begun in Africa and Asia. It is anticipated that these trials will finish by mid-1997. This will be followed by regulatory filings in the Netherlands and in malaria endemic countries.

Artesunate suppositories. Case fatality rates from severe falciparum malaria vary from 10-40% depending on the facilities available and the time of starting treatment. Death from cerebral malaria usually occurs within 96 hours of admission[12] and therefore in cerebral malaria the aim of treatment is to achieve therapeutic concentrations of antimalarial as soon as possible. Parenteral drugs cannot be given where facilities for injections and/or infusions are not available. Certain patients, often because of vomiting, cannot take medicine by mouth, and in malaria endemic areas with high mortality from the disease, treating these *non per os* (NPO) patients as early as possible with effective therapy, such as artesunate suppositories, may offer considerable promise. The benefits could be quantified in lives saved, avoidance of severe disease and reduction of acute case load (and associated costs) at peripheral hospitals. Furthermore, because of their safety and ease of administration in children, suppository formulations of artemisinin derivatives are potentially valuable in childhood malaria. They can be administered by minimally trained personnel and are unlikely to be abused.

The impact of artesunate suppositories in terms of efficacy and mortality will be seen in the treatment of suspected severe malaria in peripheral settings where facilities for parenteral drugs are not available or safe. However, before field-based studies can be conducted to assess the effect of prompt treatment on the morbidity and mortality of severe and complicated malaria, additional data on artesunate suppositories are required: What is a safe and effective initial dose? How often should one suppository formulation be administered? Do the pharmacokinetics and metabolism differ between healthy volunteers and malaria patients? Do the pharmacokinetics and metabolism differ with disease status and age?

In preparation for field trials, the *Artesunate Suppository* Task Force, in collaboration with Mepha Pharmaceuticals Research at Aesch-Basle in Switzerland, has completed Phase I tolerability and pharmacokinetic studies in healthy volunteers. Detailed pharmacokinetic cross-over studies have also been completed in Thai adults and African children. The results of the trials are extremely promising, particularly in African children, and data from them have been used to design and implement large, population-based pharmacokinetic studies in high-risk malaria patients in Asia, Africa and Latin America. Preparations are also under way for Phase III field-based studies to be implemented in 1997-1998.

In addition, efforts are being made to develop standardized methodologies for measuring the pharmacokinetic and pharmacodynamic properties of the compounds and to complement the existing dossier on the drug with pharmaceutical and pre-clinical data meeting international standards. Preparations are being made to prepare a dossier for registration of the drug in Europe for its limited use in moderately severe malaria or severe malaria where no other drug or method of administration can be used for treatment in children and adults.

Malaria vaccine development

The development of an effective malaria vaccine represents one of the most important approaches to providing a cost-effective intervention for addition to currently available malaria control strategies. Over the last decade there has been considerable progress in the understanding of immune mechanisms involved in conferring protection to malaria and the identification of vaccine candidates and their genes. However, due to the complexity and cost of malaria vaccine development, and the relative lack of commercial interest, few vaccine candidates have so far progressed to clinical trials. This is now changing and several new vaccines have recently entered phase I/II trials. Most of these candidates represent unmodified parasite antigens in traditional adjuvants such as alum. Moreover, work over the last 15 years which focused on the identification of vaccine candidate antigens is now being augmented by new adjuvants soon to be available for human use, and new approaches such as DNA vaccines and structural modification of antigens, to circumvent some of the strategies the parasite uses to avoid the host's immune response.[13] Thus malaria vaccine development is entering a crucial period with unprecedented opportunities.

Cost-effectiveness

Recently, TDR has established some estimates of the relative cost-effectiveness of a hypothetical vaccine against falciparum malaria as compared to currently available interventions for use in high risk groups in Africa, especially young children.[1] A malaria vaccine which could be implemented through existing Expanded Programme of Immunization activities (similar to the hepatitis B vaccine) and which was able to reduce overall childhood mortality by 30% or more, with a duration of immunity of 3 years or more, would represent a very cost-effective intervention strategy (Table I). However, as no single intervention tool is likely to represent a panacea for malaria, an effective vaccine would be applied together with other appropriate cost-effective methods (e.g. impregnated bednets) in an integrated, sustainable approach. One major challenge will be the identification and/or strengthening of appropriate delivery systems for a malaria vaccine, depending on the epidemiological and operational situation(s) of the country/region in question.

Candidate vaccines

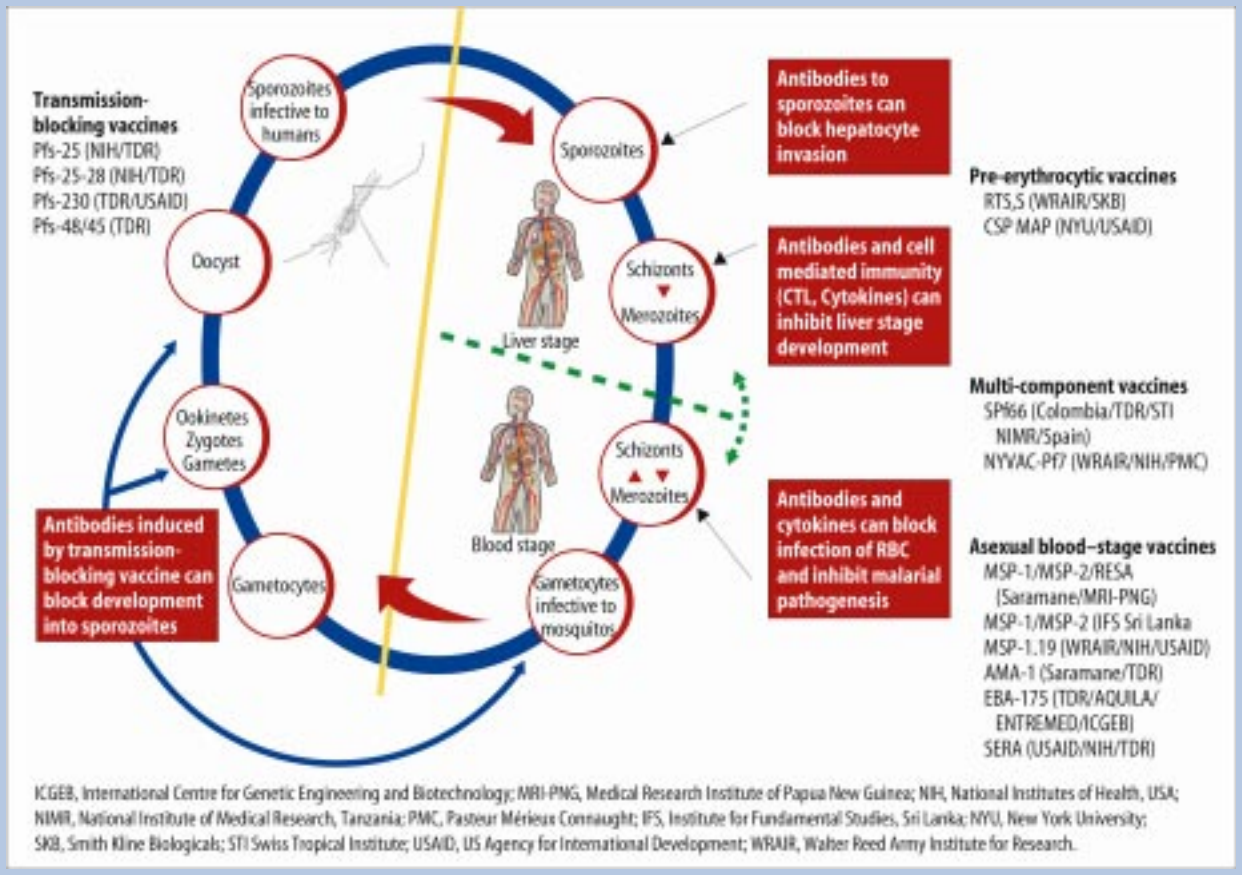
There has been considerable progress in the identification of vaccine candidate antigens and their genes but, due to the complexity and cost of vaccine development and the relative lack of commercial interest, only recently have they started to enter clinical trials. Basically, there are three main types of vaccine – asexual blood-stage vaccines, transmission-blocking vaccines and pre-erythrocytic vaccines (Fig.2).

Functional *in vitro* assays for predicting protection have been difficult to identify and validate, and only exist for transmission-blocking vaccines. Even the membrane-feeding assay used for measuring transmission-blocking activity remains to be validated in the field. It should be noted that vaccines directed to the asexual blood-stage antigens may constitute 'anti-disease' vaccines and that the objective of such vaccines is to reduce severe morbidity and mortality from malaria, but not necessarily to provide sterile immunity in the vaccinated individual. Indeed, it is extraordinarily rare for sterile immunity ever to be developed in nature.

Asexual blood-stage vaccines

In 1993, a TDR-sponsored task force evaluated some 20 asexual blood-stage candidate *P. falciparum* antigens and prepared a strategy for their development, leading to clinical testing and field trials. Merozoite Surface Protein-1 (MSP-1) was determined to be a leading candidate antigen, and several recent clinical trials in

Fig.2. Vaccine targets in the malaria parasite life cycle



ICGEB, International Centre for Genetic Engineering and Biotechnology; MRI-PNG, Medical Research Institute of Papua New Guinea; NIH, National Institutes of Health, USA; NIMR, National Institute of Medical Research, Tanzania; PMC, Pasteur Mérieux Connaught; IFS, Institute for Fundamental Studies, Sri Lanka; NYU, New York University; SKB, Smith Kline Biologicals; STI Swiss Tropical Institute; USAID, US Agency for International Development; WRAIR, Walter Reed Army Institute for Research.

humans have involved vaccines with portions of this antigen, including the multicomponent asexual blood-stage vaccine candidate SPf66 developed in Colombia. Several additional leading blood-stage candidates, including AMA-1, EBA-175 and SERA, are in various stages of preclinical development and should progress to clinical testing over the next 1-4 years. Some vaccines currently being evaluated are:

i. SPf66, a synthetic cocktail peptide vaccine against *P. falciparum* malaria, which was demonstrated to give partial protection in *Aotus* monkeys and subsequently in humans. This is the first multi-component blood-stage vaccine to undergo extensive field trials, in South America, Africa and South-East Asia. Results to date demonstrate that the peptide vaccine as formulated with alum is safe and capable of giving partial protection against malaria attacks under conditions of high as well as low malaria transmission.[14] However, results from a study in The Gambia failed to demonstrate protection against seasonal malaria when tested in infants aged 6-11 months at their first injection.[15] Results from a recent study in children 2-15 years old in Thailand, using vaccine produced in California, also gave no protection.[16] A recently conducted 'meta type' analysis of malaria vaccine efficacy trial results by the Cochrane Collaboration[17] showed that in six trials of SPf66 conducted in various age groups and epidemiological zones, the vaccine had a combined estimate of efficacy of 23% (95% C.I. 12%-32%) in reducing incidence of the first attack of clinical *P. falciparum* malaria. A second Phase III trial in Tanzania, in the high-risk group of infants under the age of one year, is currently under way with TDR providing an independent trial monitor. In spite of mixed results, the many field trials conducted to date with SPf66 have had a major impact on the thinking and design of vaccine trials in naturally exposed populations.[18]

ii. A mixture of three recombinant asexual blood-stage *P. falciparum* antigens, corresponding to parts of MSP-1, MSP-2 and RESA. A human Phase I trial of this vaccine has been completed in Australia by the SARAMANE/Hoffmann-La Roche group, using an oil-based adjuvant, Montanide ISA 720. The study was designed to test the effect of vaccination with a mixture of the three antigens versus vaccination with

the three antigens at separate sites. A subsequent Phase I study in Papua New Guinea (PNG) has demonstrated safety and immunogenicity in immune adults of an endemic area, and a proposed Phase I/II study in 6-12 year olds will be initiated in mid-1997 in PNG. It should be noted that the epidemiological pattern(s) of malaria in PNG resemble those observed in many areas of Africa, with high disease impact in young children and pregnant women.

iii. A yeast-derived clinical grade MSP-1.19 C-terminal antigen, which has been produced as part of a USAID/WRAIR/NIH collaboration. Human Phase I trials with this vaccine began in mid-1996 in Texas. The 19 kDa C-terminal processing fragment of *P. falciparum,* MSP-1, which has two cysteine-rich epidermal growth factor-like domains, is a conserved region of the molecule. It is thought to play a crucial role in red cell invasion and has been shown to play a role in the induction of protective immunity in murine malaria models. In addition, the target of invasion-inhibiting antibodies against the *P. falciparum* MSP-1 molecule has been identified as the first of the two EGF-like domains. TDR is also supporting the development of an *in vitro* assay designed to monitor the processing events for the MSP-1 C-terminal, which occur during invasion and may provide a correlate marker for attempts to inhibit that invasion by vaccination.

iv. NYVAC-Pf7, an engineered, attenuated vaccinia virus, multistage, multicomponent *P. falciparum* vaccine which includes a transmission-blocking vaccine candidate Pfs-25 (see below), together with six additional leading candidate antigens (three pre-erythrocytic proteins – CS, SSP2/TRAP and LSA-1; and three asexual blood stage antigens – MSP-1, AMA-1 and SERA). This recombinant vaccine, developed by Pasteur Mérieux Connaught/WRAIR/NIH, entered human Phase I/IIa safety, immunogenicity and efficacy studies in 1995. The engineered, attenuated virus vaccine approach, if successful, would provide a particularly cost-effective means of delivering multiple antigens in one vaccine formulation designed to elicit both humoral and cellular immune responses. Indeed, the concept of priming with an attenuated viral vaccine, followed by a booster injection with a recombinant antigen – the 'prime boost' principle – has considerable support in the HIV vaccine development field. Natural boosting may be very relevant in the case of malaria.

Transmission-blocking vaccines

An effective transmission-blocking vaccine would help deal with the emergence of drug-resistant parasites or potential escape variants selected by partially effective pre-erythrocytic stage or asexual blood-stage vaccines. It may also be effective at eliminating transmission in areas of low endemicity. However, it is considered unlikely that a transmission-blocking vaccine would be used on its own in areas of high endemicity, but rather would be used in combination with effective pre-erythrocyte and asexual blood-stage antigens. Studies have been designed to validate the membrane feeding assay as a tool for use in the field to monitor the development of transmission-blocking activity in humans.

Pfs-25, the leading *P. falciparum* transmission-blocking vaccine candidate under development by TDR and NIH, recently entered Phase I trials for safety and immunogenicity as an alum formulation in the USA. Preliminary results suggest that the vaccine is safe and induces antibodies, but that the antibodies lack functional transmission-blocking activity in the *in vitro* membrane feeding assay. Additional pre-clinical studies are testing new, more potent adjuvants and antigen combinations. In addition, a second Phase I trial is currently ongoing in the USA designed to test the 'prime boost' concept for generating functional transmission-blocking immunity, whereby volunteers previously vaccinated with NYVAC-Pf7 are boosted with one dose of recombinant Pfs-25. Several other candidate antigens are also under investigation by TDR, including Pfs-25-28, Pfs-230 and Pfs-48/45.

Pre-erythrocytic vaccines

Pre-erythrocytic malaria vaccine development has been driven by the observation that solid immunity in humans can be achieved following immunization via mosquito bites with large numbers of radiation-attenuated *P. falciparum* sporozoites. Until recently, all attempts at reproducing this generation of immunity using immunization of volunteers with purified proteins or peptides had been relatively unsuccessful. Now however, Phase I/II safety and efficacy studies conducted by WRAIR and SmithKline Biologicals in American volunteers have demonstrated over 80% protection (6/7 individuals) against challenge with five *P. falciparum*-infected mosquitos.[19]

The promising vaccine candidate, RTS,S, consists of sporozoite coat proteins expressed in the hepatitis B surface coat and formulated in a novel adjuvant. Additional studies designed to determine the duration of immunity and the degree of protection against natural challenge in the field are currently under way, with TDR

providing independent clinical monitors for the field trial in The Gambia. Additional pre-erythrocytic candidate antigens will also be combined with the basic vaccine to form a multicomponent vaccine, with the idea of overcoming a potential immune-selection of antigenic variants of the malaria parasite. In this vein, TDR has supported recent studies on the isolation and characterization of several promising pre-erythrocytic candidate antigens expressed at the infected hepatocyte stage of the parasite cell cycle.

The implications of strain variation for vaccine production

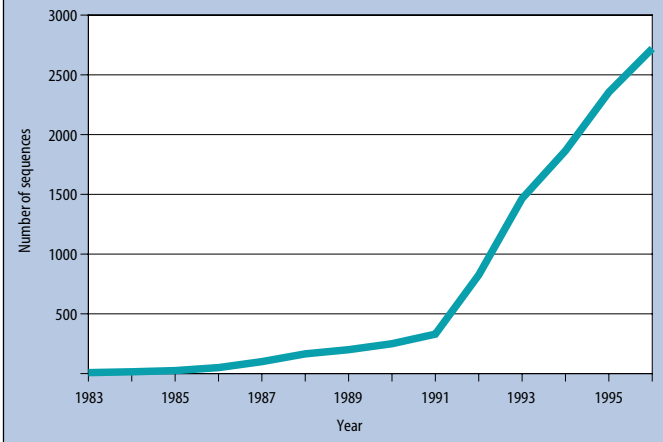
Most of the above-mentioned candidate malaria vaccines share the common feature of being directed towards a limited set of antigens and, in some instances, towards certain epitopes. However, many of these epitopes show considerable antigenic diversity between different isolates, suggesting the need for strategies aimed at circumventing this strain variation. Strategies which can be adopted include:

- The development of multistage-multicomponent vaccines, aimed at covering all the possible variants. This may be possible for certain antigens which appear to exhibit limited variation, but probably will not be feasible for antigens showing multiple variations and many gene copies (e.g. products of the recently described var/PfEMP1 genes), unless it can be shown that pathogenic strains constitute only a small proportion of all possible variants.
- Elicitation of immune responses to non-variant (sometimes non-immunogenic) portions of the candidate antigens. This can be achieved through creation of hapten-carrier conjugates carrying the specific epitopes in question or through amino acid substitution of such epitopes. Such 'second generation' vaccines hold considerable promise for inducing effective immunity against the malaria parasite.

Naked DNA vaccines

As more and more genes coding for malaria antigens and proteins (including the above-mentioned variant forms) are identified and elucidated, the acquisition rate of malaria sequences into the WHO/TDR sponsored *IMMAL Malaria Sequence Database* has followed an exponential curve (Fig. 3). Based on genetic engineering technology, encouraging preliminary results have been obtained using a promising new method for vaccination against malaria which involves the use of a DNA plasmid encoding various malaria genes, the so called 'naked DNA' vaccine strategy. This multicomponent approach would appear to have several advantages, including stimulating T cell immunity and providing a cost-effective means of presenting several malaria antigens from different stages of the life cycle (and possibly cytokines/adjuvants) by a single injection. In addition, the method is ideally suited for use in screening for promising candidate antigens, as studied in various animal models.

Fig. 3. Acquisition of sequences in the WHO/TDR IMMAL malaria DNA sequence database



The number of DNA sequences identified from the genome of *P. falciparum* is increasing rapidly as a result of the malaria genome projects supported by the Wellcome Trust and others. The development of nucleic acid based vaccine technology now makes it possible to screen such sequences rapidly in animal models in order to identify promising *P. falciparum* molecules/epitopes due to their conferring cross-protection against malaria parasites of animals. This approach should allow the number of potential vaccine candidates for falciparum malaria to considerably expand over a short period of time.

Strategic Research

Pathogenesis

Our understanding of the pathology of severe and complicated malaria, in particular of some of the events in cerebral malaria, and of other pathological results of infection, such as anaemia, has expanded during the biennium. In addition, promising new research models are being developed which should pave the way for studies under physiological conditions. Results of recent pathogenesis research are included in Box 3.

Molecular entomology

Recent biotechnological advances in the area of host-parasite relationships and molecular genetics create the possibility for new approaches to malaria control.[21] Disrupting the *Plasmodium* transmission cycle by replacing the wild *Anopheles* populations with transgenic noncompetent mosquitos might be a promising strategy for preventing malaria.[22] This is the main goal of molecular entomology research in TDR – genetically modifying mosquitos in order to disrupt the parasite's development in the vector.

Box 3. Pathogenesis research

Parasite virulence and host immune mechanisms

- The 'stickiness' of malaria-infected red cells for uninfected red cells, endothelium and other tissues is a well-known marker of virulence and cerebral involvement in falciparum malaria. Recent research has implicated an important novel class of polypeptides in contributing to this stickiness, which can be tested *in vitro* by their ability to form 'rosettes' of red cells.
- The complexity of the interaction between parasitized erythrocytes and the micro-vasculature of the brain is becoming apparent – recent evidence emphasizes the important role platelets play in modifying the surface of the endothelium expressing receptors implicated in obstructing the blood flow.
- Field studies on genetic polymorphism indicate that susceptibility to severe and complicated malaria is correlated to two alleles associated with high levels of tumour necrosis factor (TNF-α) secretion. This cytokine is implicated in serious cerebral malaria, possibly mediated by the generation of reactive nitrogen intermediates. This confirms the relationship between severe disease caused by *P. falciparum*, the intrinsic properties of infected erythrocytes and the ability of plasma factors to regulate endothelial cell receptors.
- The anaemia normally associated with malaria may be explained by residual malarial pigments in the bone marrow interfering with the differentiation of blood cells. This effect could be achieved by production of inhibitory factors or by counteracting production of growth factors, probably both. Collected evidence suggests an association between increased levels of lipophilic aldehyde, a highly toxic product of lipoperoxidation, and anaemia.

Drug and vaccine targets

- A number of dihydrofolate reductase (DHFR) mutants have been constructed using a synthetic *P. falciparum* gene and the antifolate resistance of these mutants has shown good correlation with that of wild mutants. Inhibition of the DHFR mutants by pyrimethamine and cycloguanil, and evidence that residues are selected for their synergistic as well as direct effects, suggests that drugs with a low propensity for inducing resistance can be developed.

Gene regulation

- *Plasmodium berghei* has been transfected with *P. falciparum* genes producing a stable system for the study of drug resistance and sensitivity.[20]

Research models

- Malaria parasites are strongly host specific and useful animal models are scarce. However, the development of transgenic severely-compromised immunodeficient (SCID) mouse models means that various effects of *P. falciparum* on human cells can be studied under physiological conditions. One model being developed will be suitable for adoption of human hepatocytes as host cells for human *P. falciparum* sporozoites. Another model will be capable of generating standardized *P. falciparum*-infected human erythrocytes.

Box 4. Molecular entomology research

New data have been obtained on mosquito-parasite interactions at different stages of the parasite's development in the midgut, haemocoel, and salivary glands of the mosquito:

- **Gametocyte exflagellation factor is being purified.**
- **Secretion by the parasite of a chitinase necessary for penetration of the peritrophic membrane has been shown to be activated by trypsin secreted in the mosquito midgut.**
- **Ookinetes have been shown to invade specific ion-water transport cells in the mosquito gut.**
- **Antibacterial peptides (defencins) have been isolated from the mosquito haemocoel and tested for protective action against *Plasmodium*.**
- **Sporozoite receptors in mosquito salivary glands, which can be used for blocking parasite invasion, have been identified.**
- **Genes responsible for refractoriness of mosquitos to *Plasmodium* have been identified on a genetic map by quantitative train locus (QTL) analysis.**
- **Electrophysiological studies for understanding the genetic mechanisms of mosquito olfaction for human blood have been initiated; and *A. gambiae* attractants have been discovered.**

There has been steady progress towards the objective of developing a robust technique for the transformation of anopheline mosquitos, including identification of genetic markers, vectors and delivery systems:

- **New transposable elements, mainly of the hAT-class (e.g. Huni, Ikarara, Pegasus), have been discovered in mosquitos and tested for transposition in cell lines.**
- **Pantropic retroviral elements show infectivity, integration and expression of marker genes in mosquito cell lines.**
- **Sindbis virus has been adapted as a genetic vector which can replicate in *Aedes* mosquitos and express inserted genes.**
- **Eye colour phenotype genes of *A. gambiae* (white, vermillion, cinnamon) have been characterized at the molecular level and are now available for genetic transformation experiments.**
- **Endosymbionts, *Wolbachia*, have been tested for ability to transport mosquito germlines. The resulting cytoplasmic incompatibility genes are proposed as a system for driving genes into mosquito populations.**
- **An *A. gambiae* integrated genomic map incorporating RAPD and microsatellite markers into a physical map with one centimorgan resolution has been created. It will be a valuable tool for cloning genes involved in refractoriness and the localization of probes on the cytogenetic map.[23]**
- **An *A. gambiae* genomic database has been constructed and made available through the Internet.**

Population genetics studies of several *Anopheles* species have been performed using modern molecular markers, such as RAPDs, microsatellites, and nucleotide sequences from mitochondrial and nuclear genes. These data will provide new information on vector dynamics and the potential problems and impact of releasing transgenic mosquitos into the environment.

- **Computer models and laboratory experiments have confirmed the hypothesis that novel genes loaded onto transposable elements could spread in mosquito populations.**
- **Field studies in Africa, South America and South-East Asia suggest the absence of barriers to gene flow on vast geographical territories.**
- **There are indications of a low level or even lack of genetic differentiation between distant populations of *A. gambiae* and *A. arabiensis* across the African continent. The Rift Valley appears to be a barrier to gene flow.**
- **Population genetics studies have extremely important implications for the management of insecticide resistance as well as the contemplated release of transgenic mosquitos.**

During 1995-96, research has been supported in three main areas:

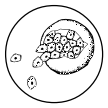
- The biochemistry of malaria parasite-vector relations and identification of parasite-inhibiting genes.
- The development of technologies for genetic transformation of mosquitos.
- The development of mechanisms for driving particular genes into natural *Anopheles* populations.

There are many problems to overcome and the time needed to achieve these goals will be at least 10-15 years. However, the results obtained to date demonstrate the feasibility of such an approach (Box 4). The intermediate goals of this research will themselves generate and provide useful tools to further our understanding of the entomology and epidemiology of malaria transmission.

Box 5. Training and capacity building in malaria

Over the 1995-96 biennium, TDR funded 122 ongoing projects in the field of malaria related to training and research capacity building in malaria endemic countries. The geographic distribution of the projects based in 42 countries was as follows: AFRO – 51 projects in 18 countries; AMRO – 13 projects in 8 countries; EMRO – 12 projects in 4 countries; SEARO – 20 projects in 4 countries; and WPRO – 26 projects in 8 countries. The activities were located in universities, research institutes, medical schools, disease control programmes and ministries of health, and included the following endeavours:

- support for formal research training to 39 PhD, 3 MD and 5 MSc projects on topics ranging from immunology of malaria to molecular entomology to health economics and financing of malaria control activities; support for local postgraduate training including collaborative small grant initiatives with China and Sudan, and with the AMRO and EMRO Regional Offices; and support to the West Africa Francophone Research and Training Network;
- support for institutional capacity building including 4 partnership grants in molecular entomology (2) and vaccine related research (2); 5 programme-based grants on operational research for malaria control; support for 4 regional linkage consortiums to promote local graduate training; and 8 re-entry grants to facilitate the return of newly-graduated scientists and initiation of their own research programmes;
- projects involving research studies on health sector reform and malaria; the integration of malaria control in primary health care activities; control of urban malaria in Africa; risk factors for severe malaria; assessment of malaria intervention strategies in South-East Asia; and the development and field evaluation of new drugs, vaccines and diagnostic assays for use in malaria control;
- projects providing training and technology transfer for the scale-up and production of Good Manufacturing Practices (GMP) grade recombinant antigen candidate malaria vaccines for Phase I/II testing (Hong Kong) and for DNA sequencing and combinatorial chemistry (Thailand);
- short-term group learning exercises with multicountry participation including a meeting on Health Sector Reforms and Malaria (Ghana); support for faculty/participants in a meeting on genetic transfection in malaria research, together with the European Union (Netherlands); a Training Course for Clinical Monitors (Kenya) and a Workshop on Design and Methodology of Malaria Vaccine Intervention Trials (Tanzania), conducted by the African Malaria Vaccine Testing Network (AMVTN) together with the European Union and AFRO; a Workshop on Research Data Analysis (Nigeria); a Training Course on Geographic Information Systems (Guatemala); a Workshop on Impact of Health Sector Reform on Control of Malaria (Zambia); and support for participants in two courses on Biology of Disease Vectors, together with the MacArthur Foundation (USA);
- complementary funding to ongoing projects in order to strengthen computer capabilities and Internet access in the Disease Endemic Countries.



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CHAPTER 5

Schistosomiasis

Applied Field Research

- ✓ standardization of ultrasound measurements of morbidity due to *Schistosoma haematobium* has been achieved
- ✓ pathology in young people infected with *S. japonicum* may be reversed after chemo-therapy
- ✓ a pocket chart which predicts true prevalence from the prevalence and mean egg count obtained in a single survey has been developed
- ✓ genital schistosomiasis has been shown to be a major social and medical problem in women, possibly enhancing the transmission of HIV
- ✓ a questionnaire to rapidly assess levels of urinary schistosomiasis has been validated
- ✓ the extent to which school-based inteventions affect in-school and out-of-school children differs in unexpected ways
- ✓ a guide to the development of visual aids on schistosomiasis for children is being developed

Product Research and Development

- ✓ independent trials of six priority candidate vaccine antigens in inbred mice failed to show protection levels exceeding 40%
- ✓ prospective studies to correlate egg excretion with immune responses against the six priority antigens in humans have been initiated and are well under way
- ✓ cytokine research is revealing mechanisms with the potential to modulate the immune response to schistosome infection and to purified anti-gens

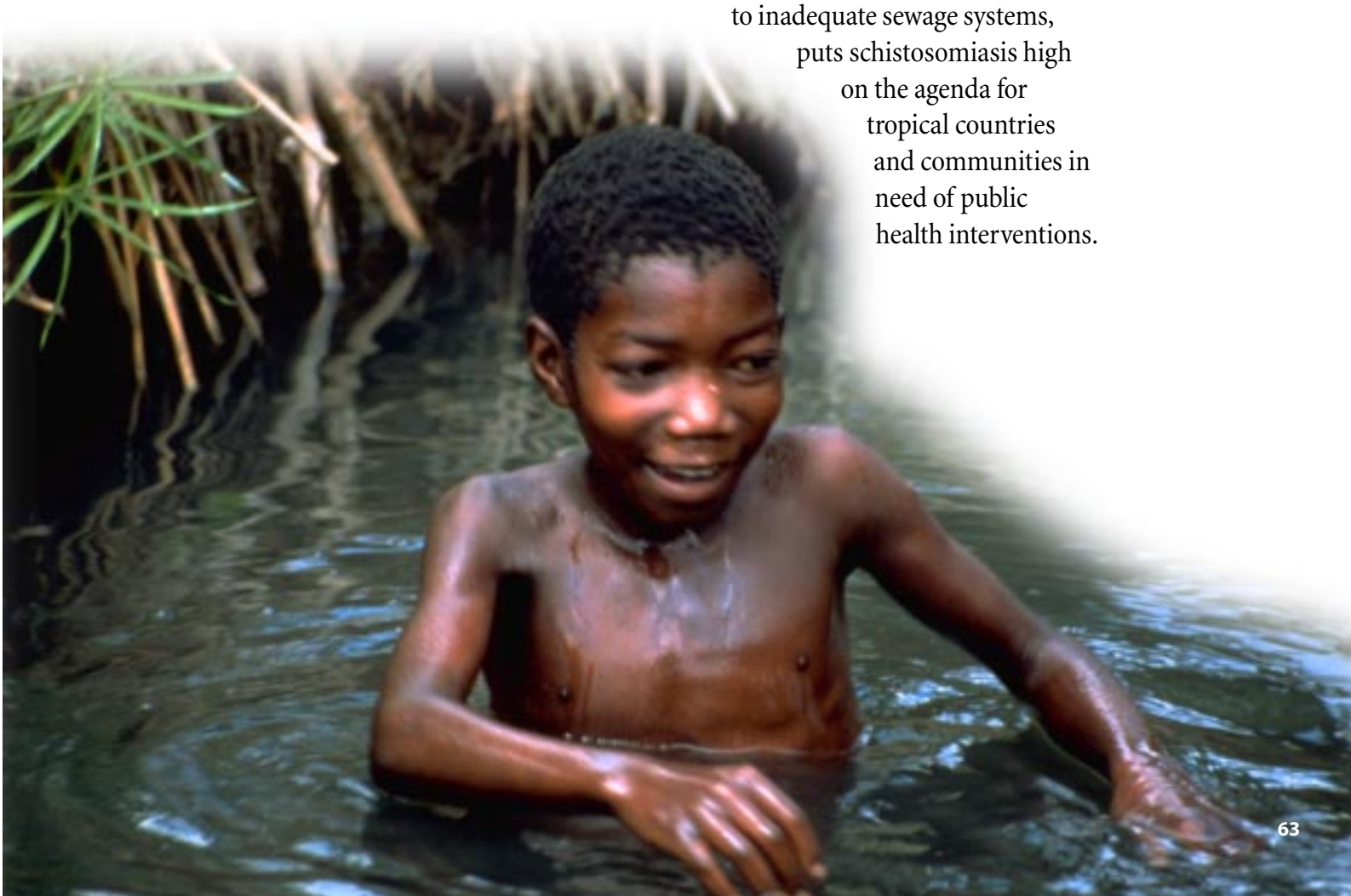
Strategic Research

- ✓ new ideas about granuloma formation and its treatment are emerging
- ✓ the possibililty of producing schistosome cell lines may be on the horizon – which could have spin-offs in many areas of research
- ✓ the *S. mansoni* genome appears to be highly conserved
- ✓ about 3000 expressed sequence tags (ESTs) have been described and up to 15% of schistosome genes (of both *S. mansoni and S. japonicum*) have been identified

Schistosomiasis is the collective name for the clinical syndromes resulting from infection with one of the five species of schistosome adapted to man. *Schistosoma mansoni* and *S. japonicum* result in intestinal schistosomiasis while *S. haematobium* produces urinary disease. *S. intercalatum* and *S. mekongi* are less common. The endemic areas, where more than 600 million people are at risk and about 200 million are actually infected, cover 74 countries in Africa, the Middle East, South America, the Caribbean, the Philippines and South-East Asia. Since the intermediate hosts are freshwater snails, the highest prevalence rates are typically found in tropical areas with lakes, streams and large rivers where up to 100% of people may be infected.

Schistosomiasis is the second most prevalent tropical disease, after malaria, and a leading cause of severe morbidity in large areas of the world (about 20 million cases). In 1990 the disease was responsible for the loss of 1.5 million Disability Adjusted Life Years (DALYs);[1] and mortality is estimated to exceed 100 000 per year.

The clinical disease (due either to intestinal or urinary lesions) varies according to the infecting species and the number of adult worm pairs harboured. Three species (*S. mansoni, S. haematobium*, and *S. japonicum*) account for more than 95% of all human cases of schistosomiasis. Various freshwater snails, depending on the species of schistosome and the geographic area, act as intermediate hosts. The high prevalence of chronic morbidity in populations associated with irrigation, fish breeding and water development projects, in addition to the rapidly increasing problems in urban areas due to inadequate sewage systems, puts schistosomiasis high on the agenda for tropical countries and communities in need of public health interventions.



The tools for controlling schistosomiasis are available, in principle, but successful and sustained eradication of the parasites has proved difficult and is limited to particular geographical areas. Although there have been important local achievements, the geographical distribution of the infection has continued to increase during the last decade due to population growth, migration and the creation of new snail habitats by large-scale irrigation projects and dam construction. In addition, schistosomiasis tends to rapidly reach epidemic proportions with very high prevalence and intensity of infection when the parasite is accidentally introduced into previously uninfected areas.

There has been a shift away from activities directed at reduction of transmission towards the control of morbidity using chemotherapy. This is partly because snail control is expensive and difficult to sustain and partly because of the availability of praziquantel. This relatively affordable drug has proved safe and highly effective against a broad range of helminths, including all schistosome species, but spiralling costs caused by high reinfection rates which commonly require repeated interventions, even after mass treatment, has limited the success of control based on this approach alone.

Research activities

TDR supports research directly but also cooperates actively with other agencies to promote research on its target diseases. With regard to schistosomiasis, TDR is regularly represented at meetings on research on tropical diseases organized by the European Union with their contract holders. TDR also collaborates with the Danish Bilharziasis Laboratory (DBL) in Denmark, in particular regarding the training of young scientists in Africa which DBL supports through an extensive programme of courses and seminars. There is close cooperation with the Swedish Agency for Research Cooperation (SAREC), a division within the Swedish International Development Agency, and TDR reviews SAREC research applications annually. Further, TDR coordinates vaccine development in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID) in the USA and the USAID-sponsored Schistosomiasis Research Programme (SRP) in Egypt and participates in grant reviews on invitation by the Ministry of Public Health in China. The latter activity concerns a 5-year (now extended for two more years) schistosomiasis control programme in China supported with a World Bank loan. A Joint Management Research Committee (JMRC), set up for the research arm of this programme, decides on the strategy and distributes grants for applied schistosomiasis research, including development of diagnostics and vaccines. TDR participates through a team of staff and experts who serve on the committee.

Applied Field Research

A variety of field research projects are being supported either directly by the Steering Committee on *Applied Field Research* or by task forces such as those on *Gender and Tropical Diseases* or *Healthy School-aged Children*. Examples include:

Morbidity assessment based on ultrasonography

The use of ultrasound to assess morbidity in schistosomiasis is an area where groundwork is still needed. The technique is field applicable, rapid and non-invasive. Its use makes possible assessment of the degree of liver fibrosis, swelling of the liver and spleen, and dilation of the portal vein in schistosomiasis. Two meetings, in Cairo, Egypt, in 1990 and in Niamey, Niger, in 1996, have achieved international standardization of *S. haematobium* examinations and it is hoped that the use of ultrasonography in patients with pathology due to *S. mansoni* and *S. japonicum* will also reach the same level of standardization and accuracy in the next few years.

An ultrasound study carried out in a *S. japonicum*-endemic area of China showed greater liver fibrosis in infected school children as opposed to non-infected school children, but no significant differences in the size of liver and/or spleen between infected and uninfected children.[2] Follow-up examinations after chemotherapy indicated that, in children, pathology induced by schistosome infection could be reversed by treatment. The results suggest that morbidity control through mass chemotherapy is more effective than selective treatment.

Differences in morbidity were also the focus of a multicountry ultrasound study in five countries of sub-Saharan Africa where *S. mansoni* is endemic.[3] The prevalence of infection in the study areas varied between 42% and 92%, and morbidity varied substantially both between and within countries. Enlargement of the spleen was the most frequent effect of schistosomiasis in all five countries, but there was no correlation between liver and spleen lesions and the intensity or prevalence of infection. Periportal thickening decreased markedly after treatment in the age group of 10-30 years, but was virtually uninfluenced in those over 40. Women were found to profit less from treatment than males. The results suggest that the intervals between praziquantel treatment interventions could be longer than at present, without losing the positive effects on morbidity. Early treatment appears to reduce early and mild hepatic morbidity but does not prevent reinfection.

Mathematical modelling

The proceedings[4] of a meeting between field researchers and mathematical modellers have stimulated wider application of mathematical modelling to various aspects of schistosomiasis, in particular vaccinology. Analyses suggest that the full impact of vaccines on reducing the intensity of infection and/or egg output will be delayed for several years and that one effect will be the relocation of peak levels of infection to older age groups.[5]

Schistosomiasis is commonly diagnosed by one examination of a single stool specimen and the level of egg excretion is generally taken to indicate the intensity of infection. However, faecal egg counts have been shown to vary enormously from individual to individual due to differences in worm load, for a given individual with a given worm load from time to time, and within one specimen. A mathematical model accounting for these variations has been developed and a pocket chart, capable of projecting how many individuals in a population living in an area endemic for *S. mansoni* are truly infected, has been produced.[6] Using the pocket chart, true prevalence can be predicted from the prevalence and mean egg count obtained in a single survey.

A similar prediction study in an endemic area of China has shown that variation of *S. japonicum* egg counts can be described by the same model. A first version of a pocket chart to predict true prevalence from the prevalence obtained by a single survey and mean egg count is being developed for this species. Data will be collected from other endemic settings to determine whether the chart is valid everywhere or if it needs to be adapted. The pocket chart will also be compared with observed prevalence using a more sensitive technique (the nylon bag filtration method as opposed to the modified Kato-Katz method).

Video production

The video is seen as a vital tool in health education, and TDR has supported the production of videos in different countries. In China, a video was made with the objective of improving compliance with examination and treatment for schistosomiasis by primary school students and to make them understand where it was unsafe to swim. The effect of the prize-winning video

'Angel's Advice' (an animated cartoon) and the booklet 'Go to Swim' on the knowledge of children in primary schools was examined with particular reference to behavioural change and the morbidity and intensity of infection. It was clear that, after viewing this material, the children knew more about infective parasite stages, symptoms and signs, snail hosts, and protective methods. They also sought out safe places for swimming more often than unsafe ones. As a consequence, stool examinations showed significantly lower egg counts.

A video entitled 'Snail Disease', produced in Brazil to give rural people the opportunity of understanding and discussing sanitation at the local level, comes with a user manual and guidelines for stimulating discussion. It was assessed for ease of comprehension of the educational messages by communities and for accessibility by health personnel through focus group discussions, interviews, and observations of comments and reactions of the target groups. Before viewing the video, most people, including health personnel, were unaware of the link between infected human faeces and the snail. With a better understanding of this they were more able to apply sanitation as a means of disease control.

Female genital schistosomiasis

Female genital schistosomiasis (FGS) has been identified as a major social and medical problem. First reported in 1899 from Egypt, FGS has more recently been shown to be associated with a broad range of pathological manifestations, particularly tumours and ulcers, in the female lower genital tract. In addition, infertility, abortion, pre-term delivery, and life-threatening conditions such as extra-uterine pregnancy, are not uncommon complications. Up to 75% of women living in *S. haemotobium*-endemic areas may suffer from genital lesions due to the infection (the total number may be as high as 2-13 million). FGS may facilitate the spread of some STDs, especially HIV, although more work is needed to understand this interaction, the relationship between urinary schistosomiasis and infertility, and the implications of the disease on the married and sexual life of women.

A pilot study,[7] conducted in an outpatient department at a district hospital in rural Malawi in order to help develop guidelines for the diagnosis of FGS, showed that 65% of women (33 out of 51) of child-bearing age had parasite ova in the lower part of the reproductive tract. In most cases, microscopic examination of fresh cervical biopsy material (obtained by an almost painless procedure) together with histopathological examination of formalin-fixed cervical biopsy material ensured diagnosis. The majority of 14 divorced women participating in the study showed this kind of pathology. In addition, the majority of infertile women with FGS in the study admitted that their partner had children with other women.

Healthy school-aged children

A number of countries in Africa, Latin America and Asia have recently decided to develop or renew interventions aimed at improving the health of school-aged children. Most interventions involve health education and many seek to provide treatment for common conditions and deliver micronutrient supplementation. The control of intestinal helminths and schistosomiasis has received considerable attention, partly because of increasing evidence that treatment can improve the physical and cognitive development of school-aged children. However, in order to plan programmes, it is necessary to know what and where the major health problems affecting school-aged children are (the prevalence of helminth infections is often very focal) and to know what structures in the community would enable cost-effective interventions to be delivered. With the support of the Edna McConnell Clark Foundation and in collaboration with the Partnership for Child Development, a series of 'situation analyses' or 'opportunity analyses', aimed at developing a method of providing this information rapidly, at the national, regional or district level, have been carried out in Africa. These studies have not focused on any single disease but attempt to build an overall picture of the health status of children and the opportunities for improvement.

Preliminary results suggest that routine data sources and unpublished reports provide a good overall guide to the development of interventions. However, important data, such as the local prevalence of diseases and the local rates of school enrollment and attendance, are missing. This information must be obtained using other techniques. Steps towards the development of low-cost ways of providing this information are now being explored with TDR, focusing on schistosomiasis.

Rapid assessment

In the early 1990s, the *Steering Committee on Social and Economic Research* sponsored a multicountry study to validate a method of identifying, rapidly and at low cost, communities with a high prevalence of urinary schistosomiasis. Using a questionnaire, the proportion of children who had observed blood in their urine or who thought they had schistosomiasis, was determined. The study showed that this approach was effective in identifying high-risk communities and should be given priority in any intervention. It also showed that, in most countries, the questionnaires could be delivered and returned through the education department's normal administrative structures, at a cost around eight times lower than traditional screening methods.[8] The *Task Force on Healthy School-aged Children* subsequently developed a guide for use in control programmes which is being used in at least three African countries.

The Task Force has now extended the questionnaire method by sponsoring two indirect tests for *S. mansoni* in Africa. An Ethiopian study showed that the answers to the question 'Have you suffered from schistosomiasis in the last two weeks' can be used to classify schools into those with high and those with low prevalence, and that the negative predictive value exceeds 90%. However, the questions dealing with particular symptoms related to intestinal schistosomiasis did not prove to have particularly high predictive values on a community basis. In fact, a study in Zaire showed that no question was predictive of community prevalence for intestinal schistosomiasis, but this might be due to the fact that the area chosen for the research turned out to have unusually low prevalence. One subsequent study for *S. mansoni* is under way in Africa and another for *S. japonicum* in China. The results of all work on rapid assessment for intestinal schistosomiasis, undertaken both with and without TDR support, will be reviewed late in 1997 to determine the most promising approach.

School-based delivery of chemotherapy

Given the renewed interest in improving the health of school-aged children, it is argued that it is cost-effective to deliver interventions through schools, on the grounds that they are widely distributed and that an increasing proportion of children attend them.[9] Although school-based delivery is likely to be the option of lowest cost, it does raise some problems. Firstly, while confining helminth treatment to school children can reduce prevalence in the community as a whole, it is necessary for a high proportion of children to receive treatment.[10,11] Low school attendance, or low acceptance of treatment delivered through schools, eliminates the advantages offered by school-based delivery. Secondly, children who do not attend school regularly may well be the most disadvantaged in the community – socially, in terms of gender, and perhaps in terms of infection and health status.

The Task Force sponsored a series of studies in urban and rural areas designed to identify rates of school attendance and the extent to which children who do not attend regularly, and who would be missed by school-based interventions, have a lower health status than others. In addition, a study in Sudan included a number of areas with different disease patterns and covered not only schistosomiasis but also malaria, leishmaniasis, onchocerciasis and other intestinal worms.

An Egyptian study[12] showed that children who did not attend school regularly were more likely to be infected and to have higher intensities of infection than children who attended regularly. This was true for both the urinary and intestinal forms of schistosomiasis. In Egypt, children have been successfully treated through schools for many years, and the study confirmed that, in lower Egypt (where *S. mansoni* is the predominant species), over 80% of infected boys and almost 70% of infected girls could be reached through a school-based programme. On the other hand, in Upper Egypt (with mainly *S. haematobium* infections), 40% of infected boys and over 80% of infected girls could not be reached because they did not attend school regularly. The research team is now developing and testing sustainable methods of providing the same treatment package that is available through schools to those who do not attend classes.

In contrast, a study in a *S. mansoni*-endemic area of Ethiopia suggests the opposite – that children who do not attend school are less likely to be infected and less likely to have intense infections than those who do attend school. The team is exploring possible explanations for this, but in this situation and where attendance rates are high, school-based delivery is likely to reach the majority of infected children.

Health education

The *Task Force on Healthy School-aged Children* sponsored a number of studies on visual perceptions relating to schistosomiasis. These studies showed that many of the visual aids routinely used in health education programmes aimed at children are either not understood at all or are misinterpreted by them. This reflects the wider experience where it is now established that children perceive pictures in a different way from adults, and that people developing health education materials need to work with children to ensure that their messages are understood. A guide to the development of appropriate visual aids for children is being developed on the basis of this research and should be available for field testing in mid-1997.

Ideas book

In the belief that behavioural change can reduce the risk of infection and can improve treatment-seeking patterns, the *Task Force on Healthy School-aged Children* has funded the development of an 'ideas book'. It collates the experiences of teachers in schools with poor resources who have successfully introduced discovery-based learning techniques for health education. The primary aim is to show individual teachers that the techniques can be introduced in areas which lack resources and that they work. It is felt that this will provide motivation for teachers to experiment for themselves, based on some of the examples given. The book will be tested in the field in 1997.

Product Research and Development

Vaccines for schistosomiasis

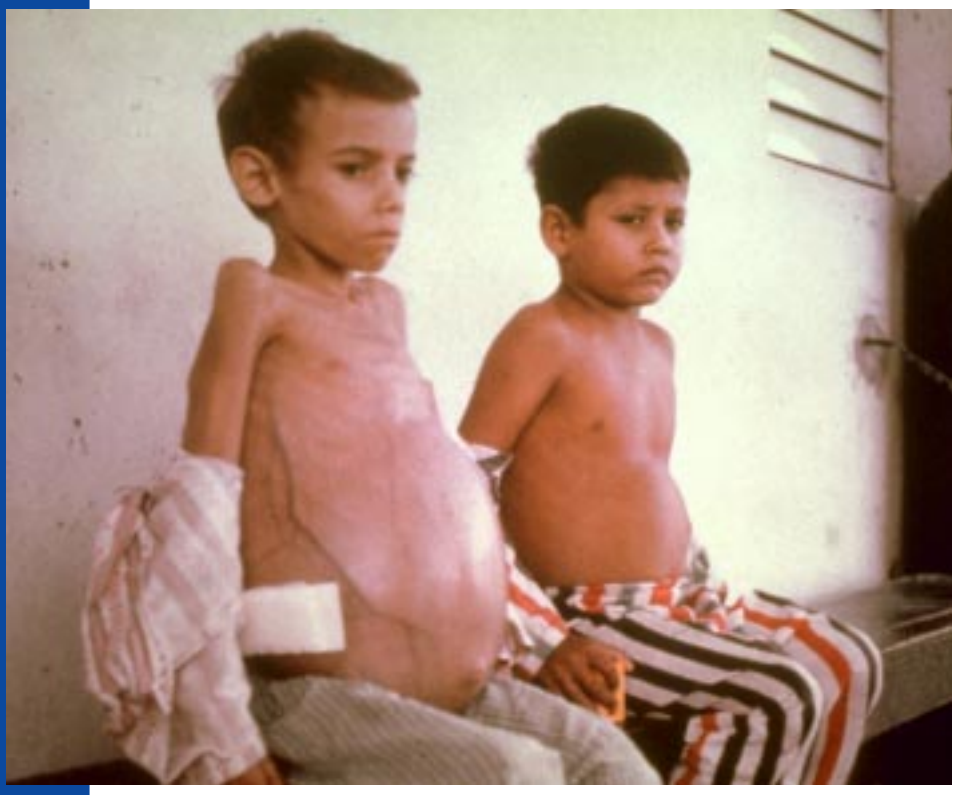
Objectives and rationale

The combined approach of chemotherapy followed by vaccination promises a formidable tool for control in endemic areas which would ensure a long-term effect. TDR's aim is primarily to produce an effective vaccine against *S. mansoni* but also to catalyse parallel research efforts directed towards the development of a *S. japonicum* vaccine. The zoonotic nature of the latter species means that in place of mice, pigs and other larger animals may be used as experimental hosts, which might reflect the host/parasite situation in humans better. In addition, a veterinary product, an obvious spin-off as research progresses, could be tested for transmission-blocking effects on human populations.

The majority of humans living in schistosomiasis endemic areas acquire relatively good degrees of protection against the infection, and the current challenge for vaccine research is to identify, mimic and improve this process. Conventional vaccines are precluded in schistosomiasis since a delicate balance between opposing immunological mechanisms must be established, i.e. the risk for reinfection must be reduced without adverse effects of egg-associated granuloma formation. Scientists are encouraged by the fact that cercariae, attenuated by sub-lethal, high-energy radiation, are capable of producing almost sterile immunity in experimental animals. Even if the potential for undesirable side effects makes attenuated vaccines unlikely, they lend themselves to the study of mechanisms of resistance and the search is on for antigens and ways of immunization that mimic this approach. Moreover, since schistosomes do not replicate in the human host, even a partially effective vaccine would prolong and reduce the development of morbidity, the signs of which do not appear until a sufficient number of egg-associated granulomas have been produced. Because natural resistance against schistosomiasis is slow to mature and does not significantly temper reinfection in younger people, a vaccine would presumably need to be relatively effective, although just how effective it needs to be can only be resolved by trials in the field. As transmission is not expected to come to a halt, natural infection may be counted on to boost vaccine-induced protection.

Candidate vaccines

Research is focused on the schistosomulum, the larval stage, and a large number of recombinant *S. mansoni* and *S. japonicum* antigens from this stage have been identified and produced. The life cycle of the former species lends itself to laboratory research, which explains why *S. mansoni* antigens have reached an advanced phase of development. It has become increasingly clear that the biochemical nature of epitopes not only has implications for immunogenicity but may also inadvertently activate granulomatous reactions, as in the host response to carbohydrate antigens which share epitopes with egg antigens.[13] The finding of enlarged granulomas in mice vaccinated with such antigens, as well as the fact that antibodies to schistosome carbohydrate antigens become down-regulated in chronic infections, supports this notion. The presence of blocking antibodies to carbohydrate epitopes, reported in infections by both *S. mansoni* and *S. haematobium*, is most probably



11-year-old Puerto Rican boys exhibit the affects of infection with *S. mansoni.*

Table 1. Schistosome priority antigens

Schistosome *(S. mansoni)* vaccine candidates						S. *japonicum* antigens	
Identity	**Stage**	**Function**	**Claimed Protection (%)**			**Identity**	**Phase**
			Mouse	Rat	Other		
Sm28-glutathione S-transferase (GST)	Somula Adult	Enzyme	30-60	40-60	40 (baboon)	Sj28-GST Sj26-GST	Animal trials
Sm97-paramyosin	Somula Adult	Muscle protein	30	–	–	Sj97-paramyosin	Animal trials
Sm62-IrV-5, myosin antigen	All Stages	Muscle protein	50-70	95	25 (baboon)	Sj62	Research
Sm28-TPI, triose phosphate isomerase	All Stages	Enzyme	30-60	–	–		
Sm23	All Stages	Membrane antigen	40-50	–	–	Sj23	Animal trials
Sm14	Somula	Membrane antigen	65 (outbred)	–	90-100 (rabbit)	Sj14	Research

a related issue. The fact that schistosome protective antigens are frequently functional and tend to be genetically conserved has led to attempts to present relevant epitopes in the form of synthetic peptides in order to avoid the potential hazard of autoimmune reactions.

The importance of certain epitopes is demonstrated by the improved protection induced by antigens such as paramyosin and IrV-5 (two muscle proteins of which the former has been implicated in the irradiated cercariae model), triose-phosphate isomerase (a glycolytic enzyme), Sm23 (a surface protein) and Sm14 (a fatty acid-binding compound). Glutathione-S-transferase (GST) is of particular interest due to its effect on schistosome fecundity through suppression of egg production. In collaboration with NIAID in the USA and the SRP in Egypt, TDR is coordinating a thorough evaluation of the most promising molecules.[14] The six antigens mentioned above have been selected, based on extensive research and reported stable induction of high levels of protection (50-70%) in the commonly-used inbred mouse model. Preliminary results of human correlate immune responses to these antigens are encouraging but independent testing in inbred mice has so far not confirmed the high levels of protection. Characteristics of the leading vaccine candidates are presented in Table 1.

A vaccine programme would be unrealistic without a commercial partner possessing the experience and economic weight necessary for large-scale vaccine production according to the standards of Good Manufacturing Practices. TDR is fortunate in having forged a liaison with Mérieux, whose firm commitment, through its American subsidiary Pasteur Mérieux Connaught, forms an integral part of the quest for a schistosomiasis vaccine, facilitating production and helping move the final vaccine through the regulatory bodies.

Cytokine regulation

The majority of research on cytokines is supported under Strategic Research but merits mentioning here as well since the immune response is largely governed by these mediators. For example, helminth infections tend to induce immunoglobulin E (IgE) and eosinophilia in response to interleukin-4 (IL-4) and IL-5, mediated by the Th-2 subset of CD4+ lymphocytes. Mice vaccinated a number of times with irradiated cercariae owe their protection to a predominantly Th-2 response, and antibodies seem also to be the main reason for the protective effects of several of the priority antigen candidates. In human schistosomiasis, reported resistance in drug-cured patients has been shown to be correlated with the presence of specific IgG, IgE and IgA antibodies, and *in vitro* experiments have emphasized the role of the eosinophil in activation of IgE or IgG_2a antibody-dependent cell-mediated cytotoxicity. On the other hand, depletion of IL-4 or IL-5, which down-regulates eosinophilia, fails to alter the development of resistance in mice. Obviously, resistance to schistosomiasis relies on both humoral and cell-mediated mechanisms, and knowledge of the exact role of key

cytokines could lead to their potential inclusion as Th-selective immunopotentiators in vaccine formulations. For example, IL-12 can enhance both humoral and cell-mediated immune responses against schistosomes but, when given together with an antigen, drives the response towards the latter (Th-1 domination).[15,16] However, at the current stage of knowledge, the ambiguity of the influence due to cytokines, in particular in relation to granuloma formation, makes it difficult to avoid exacerbation of pathology in harnessing the beneficial effects.

Approaches and prospects

Vaccine trials

Two laboratories with long experience in experimental schistosomiasis have independently tested the vaccines in two inbred mouse strains commonly used as animal models for schistosomiasis. The immunizations were carried out according to specifications provided by the investigators who produced the antigens, whilst a standardized protocol was followed for all challenges. All mice received 150 cercariae of the NMRI Puerto Rican strain of *S. mansoni* percutaneously by a 1-hour exposure of the tail or abdomen. Perfusions were uniformly carried out six weeks post-challenge. The consolidated results demonstrate that the goal of consistent induction of 40% protection or better was not reached with any of the antigen formulations tested in the trials. Possibly the instability of the formulations and inadequate control of antigens and adjuvants before and after shipping might have contributed to these results, while antigen presentation appears to play a more important role than had previously been appreciated. The immunization procedure will be further explored with reference to the stability of formulations and influence of adjuvants.

Human correlate studies

In an attempt to correlate egg excretion with immune response in humans, prospective studies are under way in endemic areas of Brazil, Egypt and Kenya. The prevalence of schistosomiasis in the study areas exceeds 50%, as determined by stool examination using two Kato-Katz smears per specimen on two separate occasions, whilst that of hepatitis B infection and HIV, as determined by serum analysis, is less than 15% and 1% respectively. In each place, well-characterized groups of about 200 individuals chronically exposed to *S. mansoni* are selected, tested and treated. To save time and money, only essential data are gathered, such as egg counts recorded along with interferon-γ (IFN-γ), IL-2, IL-4 and IL-5 responses to stimulation with the antigens under investigation and determination of the levels of the most important antibody isotypes with specificity for these antigens. Data from these studies have started to emerge but, so far, only from the baseline testing and repeat examinations three months later. However, in one of the study areas the subjects had previously been followed for several years and records of the earlier egg counts are available for consultation. In the light of this additional information, there are tentative indications of more vigorous responses, in particular from some of the antigens, which show direct correlation with resistance, as judged by the absence of egg excretion, in spite of continued contact with unsafe water.

Conclusions

In spite of the wealth of data gathered from experimentally infected animals, the mechanisms which protect humans from schistosomiasis are still largely unknown and can, in principle, only be elucidated by trials in the field, which are deemed safe thanks to access to effective drugs. Trials with single antigens could answer basic questions about human immune mechanisms, although adequate vaccination might ultimately require composite vaccines to synergistically stimulate different immune mechanisms. The need to involve multiple aspects of the immune system provides the rationale for using antigens expressing epitopes which stimulate specific T cells as well as significant antibody isotypes. Cytokines clearly have a great potential for exploitation as Th-selective adjuvants but more research is needed before practical application.

A successful vaccine cannot, unfortunately, be based solely on the most effective way of inducing protection; feasibility of production, prospects for passage through regulatory bodies, and ease of incorporation into existing immunization programmes must also be considered. In this connection it should be noted that *S. japonicum* research permits the use of farm animals, opening up the possibility of developing a veterinary transmission-blocking vaccine in parallel with the human vaccine.

Although the mouse experiments need to be repeated, the human correlate studies are sufficiently encouraging to justify continuing with the vaccine programme as planned. The full set of data permitting a firm recommendation will be available in 1997, and it is anticipated that the best antigens will again be subjected to independent testing in mice using full-length recombinant molecules, sub-units or DNA vaccination, depending on which antigen is being tested. When these results are available, the final recommendation as to whether or not to move on to limited human trials (Phase I) will be made. Realistically, taking into account the need for industrial scale-up, Phase I trials cannot be carried out before 1998.

Strategic Research

Pathogenesis

Work supported by TDR during the biennium has generated new ideas about liver granuloma formation and its treatment. In addition, the possibility of producing schistosome cell lines is now on the horizon, which could have spin-offs in all areas of research, as parasite cells would become easily available without having to resort to continuously supporting the full life cycle. Particular findings are highlighted in Box 1.

Box 1. Pathogenesis research

Parasite virulence and host immune mechanisms

- Granuloma formation has been shown to be induced by schistosome maturation and TNF-α production in the liver. This primes local immune responses, stimulating granuloma formation in response to interaction between an intercellular adhesion molecule (ICAM-1) and its cognate integrin receptor which is a leucocyte functional antigen (LFA-1). It has also been shown that a vascular cell adhesion molecule (VCAM-1) is able to act as back-up in the absence of ICAM-1.
- The restricted blood flow through the liver due to egg-associated granulomata, as in serious intestinal schistosomiasis, is being studied in a novel line of research aimed at restoring the blood flow with antifibrotic treatment.

Research models

- The discovery of a growth-enhancing factor emanating from an insect cell line but specific for *S. mansoni* cells has advanced the prospects of producing immortal schistosome cell lines which are needed in research as a substitute for costly and labour-intensive maintenance of the parasite life cycle in the laboratory. In another approach to the same goal, efforts are being made to transfer a mutant form of the ras gene into juvenile *S.mansoni* cells.

Genome

The schistosome genus consists of 19 species, five of which commonly infect man. For genome research, a consortium of seven laboratories (from Brazil, USA, UK, Egypt, Japan and Australia) was organized in 1994 and it was decided to concentrate research efforts on the two epidemiologically most important species: *S. mansoni* and *S. japonicum*. The genome is estimated to contain about 20 000 genes and have a total size of 270 megabases (Mb).

During the first two years, attention has been focused on creating resources and techniques for the development of stage-specific libraries; on identifying genes of interest; and on drawing low-resolution physical maps of the whole parasite genomes (Box 2). Priority has been given to gene discovery using the expressed sequence tag (EST) approach. About 3000 ESTs have been described, thus increasing by a factor of ten the number of new schistosome genes. Despite this significant accomplishment, less than 15% of schistosomal genes have been identified and EST sequencing will therefore continue to be the primary objective of network activities. A physical map of the schistosome genome, based on an ordered array of cloned fragments (yeast artificial chromosomes or YACs, bacterial artificial chromosomes or BACs, and cosmids) is the long-term goal, for which resources (in terms of protocols and expertise) are now being prepared.[17] A schistosome database is being developed for access via the World Wide Web.

Box 2. Genome research

Construction of libraries

- A variety of stage-specific complementary DNA (cDNA) libraries have been, or are being, developed. These include libraries of immature and mature eggs, miracidia, sporocysts, cercariae, schistosomula, juvenile worms, lung-stage worms, adult female worms, and immature and mature male worms from *S. mansoni* and *S. japonicum*.

Gene discovery

- About 3000 expressed sequence tags (ESTs) have been generated and sequenced from analysis of the cDNA libraries. Of these, 22% matched sequences from schistosome species, 19% matched sequences from other species, while 53% appeared to be unique and had no significant database match.
- Of *S. japonicum* adult worm libraries, 10% of ESTs matched existing schistosome sequencies, 30% of ESTs matched sequencies from other species, and 60% had no significant database match. About 1.5% of the estimated expressed gene content of *S. japonicum* is now tagged. Amongst the expressed genes are a drug-resistance-associated, calcium-binding protein and mobile elements which could prove to be valuable markers for physical mapping.

Chromosome (physical) mapping

- Protocols have been developed for the preparation of schistosome chromosome spreads and micro-dissection protocols are now available for the isolation of individual chromosomes (or parts thereof) from chromosome spreads. A modified fluorescent-in- situ-hybridization (FISH) protocol which works well with probes of any size has also been prepared, and the primed-in-situ-labelling technique (PRINS) has been adapted for use with schistosome chromosomes. All these techniques are free for interested investigators.
- The genome of *S. mansoni* appears to be highly conserved. Four strains of *S. mansoni* have been examined – two from the Old World and two from the New World – which have been in laboratory passage for 2-30 years. No significant genomic variation has been detected using a variety of techniques. Thus there is no evidence for gross chromosomal rearrangements in either *S. mansoni* or *S. japonicum*, although some minor ones might occur.

WWW site: http://www.nhm.ac.uk/schisto/

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CHAPTER 6

Lymphatic Filariasis

Applied Field Research

- ✓ acute filarial disease causes 3-4 full days of incapacitation per episode in subsistence farming communities in Ghana and India
- ✓ people with chronic filarial disease spend 10-60% less time at work in a subsistence farming community in Ghana
- ✓ the productivity of South Indian male weavers with chronic lymphatic filariasis is reduced by about one third
- ✓ sexual disability associated with the genital manifestations of lymphatic filariasis is a primary concern of those affected
- ✓ a number of easy, non-invasive procedures have been shown to be of potential use for rapid assessment during surveys of lymphatic filariasis
- ✓ compared to DEC alone, DEC+ivermectin has a greater effect on vector infectivity, on microfilaria prevalence rates and on blood microfilaria levels
- ✓ ivermectin+albendazole may suppress microfilaraemia for at least 15 months (and could be useful where onchocerciasis and loiasis co-exist with Bancroftian filariasis)
- ✓ in the short term, hygiene and antibiotics are of greater direct benefit in preventing acute filarial disease than antifilarial drugs
- ✓ self-help support groups among patients and their families are effective in ensuring compliance with vigorous hygiene regimens
- ✓ a rapid, sensitive, specific and convenient diagnostic test is now available for Bancroftian filariasis

Product Research and Development

- ✓ See Product Research and Development section in chapter 7

Strategic Research

- ✓ the absence of circulating antibodies in lymphatic filariasis is associated with a Th-1 type of response and specific IgG4 antibody
- ✓ vaccine development is currently focused on the chitinase enzyme of microfilariae
- ✓ the antigenic elements of a protein and an enzyme associated with eye disease in onchocerciasis have been mapped
- ✓ about 20% of the total genome of *Brugia malayi* has been sequenced
- ✓ >4000 expressed sequence tags (ESTs) are now available – representing about 20% of the total number of genes
- ✓ a number of genes encoding proteins of nematological and immunological interest have been identified

Filarial disease, until recently a neglected disease of poor people in 73 countries, causes suffering to 120 million people and threatens 1100 million more. It is considered a major obstacle to economic development in endemic countries and, in 1995, was identified as the second leading cause of permanent and long-term disability.[1] The burden for 1990 has been estimated as 4 million Disability Adjusted Life Years (DALYs) – 3 million for men and 1 million for women.[2]

Filarial disease is caused by the parasitic nematodes *Wuchereria bancrofti*, *Brugia malayi* or *Brugia timori*, transmitted by several species of mosquito. The common symptom of acute filarial disease is adenolymphangitis (also known as filarial fever), which presents as recurrent attacks of pain and inflammation of lymph nodes and ducts, often accompanied by fever, nausea and vomiting. Lymphodoema affects 16.2 million people.[3] Symptoms of chronic filarial disease include elephantiasis, which presents as gross enlargement of limbs (mostly legs) and affects 15 million patients; and hydrocoele, which presents as chronic swelling of the scrotum and affects about 27 million men.

There is evidence that chronic filarial disease causes lost employment opportunities, stigmatization and reclusion; that acute filarial disease causes 30 days of 'filarial' fever per patient per year; and that asymptomatic filarial disease patients (those with circulating microfilariae but no symptoms) do in fact have appreciable hidden lymphatic

damage and renal pathology. Recent advances have revealed the role played by localized microbial infections in the pathology of the lymphatic system.

A few years ago, although effective treatment was available, in heavily infected people the principal drug (diethylcarbamazine – which is still in use) produced severe side effects. There was little treatment for chronic infections. Many cases were said to be 'asymptomatic'. The most accurate means of diagnosis was to take blood smears in the middle of the night (not popular among patients or health workers). Pathogenesis was little understood. Methods for controlling vectors were not used effectively. And the social and economic consequences of the disease were barely mentioned.

Today this picture has changed dramatically. Recent developments in understanding the pathogenesis of the disease, in identifying its hidden pathology and especially in developing new treatment and chemotherapy strategies have placed lymphatic filariasis among the list of potentially 'eradicable' diseases. The severe social and economic consequences of infection are being exposed for the first time.

Applied Field Research

The burden of filarial disease

Identifying and quantifying the social and economic costs of filarial disease, including the costs of managing presentations such as elephantiasis, and estimating the extent and patterns of the disease, have been the objects of recent operational research. TDR-initiated studies have helped underscore the high socioeconomic impact of the disease on endemic populations and the serious psychosocial problems associated with it.

Socioeconomic burden

A study carried out in northern Ghana[4] in a rural community where filarial disease is highly endemic (41% of the population aged over ten years is microfilaraemic with *W. bancrofti* and 3% has chronic disease), showed that lymphatic filariasis can be a major social and economic burden on poor communities and that the disability and indirect economic loss (through inactivity) associated with adenolymphangitis (ADL) has previously been underestimated.

The subjects of the study were members of a subsistence farming community. Treatment seeking, the costs of treatment, the burden of the disease on the community and its public health implications were investigated. Some of the findings are outlined in Box 1.

Another study,[5] carried out in a rural community in southern India, was aimed at investigating the impact of chronic lymphatic filariasis on economic output – specifically the impact of chronic disease caused by *W. bancrofti* on the productivity of male weavers.

The weavers in the study were paid according to the length of cloth woven rather than the number of hours or days worked. All the weavers worked a similar number of hours over a period of six months. However, the productivity of those with chronic lymphatic filariasis was reduced by an average of 27.4% in comparison with the matched controls.

The results of the study clearly imply that lymphatic filariasis has an adverse impact on the productivity and wage-earning capacity of this particular occupational group.

In another study, at a clinic attached to a research centre in Brazil, the important but hidden disability associated with the genital manifestations of filarial disease has become apparent.[6] In the last ten years, 1600 men have been evaluated at the clinic and it is now understood that the sexual disability associated with the disease is the primary concern of these patients. Strong feelings of shame, fear and embarrassment are common. Sexual problems, which are generally not openly

Box 1. The social and economic effects of lymphatic filariasis on a subsistence farming community in northern Ghana

Acute disease

- ADL was found to cause at least three full days of incapacitation per episode. The incidence was highest during the rainy season (perhaps precipitated by bacterial or fungal infections), the time of peak agricultural activity. In the community studied, total incapacitation during a cru-cial period in the farming year can affect food production.

Chronic disease

- As a public health problem, chronic disease is more severe than ADL. However, the disability associated with it is not so incapacitating as that associated with ADL, partly because people with chronic disease adopt coping mechanisms – elephantiasis patients may, for instance, find relatively sedentary jobs such as basket weaving. People with chronic disease have to cope with social stigma and the psychological effects of infection. Those confined to their homes find themselves an economic burden on their families. Forty-five per cent of ADL is reported from patients with chronic disease – together, the two conditions seem to be more debilitating than either condition alone.
- People with chronic filarial disease were found to spend 10-60% less time at work. About 1.5% of the potential female labour input and over 7% of the potential male labour input was lost to chronic disease. However, ADL was far more common in women than in men (partly ascribable to elephantiasis being more prevalent in women than men in the study area). In a male-dominant society such as the one studied, where women do most of the work, the incapacitation of women has a relatively large impact.
- Direct costs of treatment were found to be very low. Very few people with ADL actively sought treatment – most relied on traditional healers and knew that the episode would last only a few days. People with chronic manifestations were even less likely to seek treatment – unless superimposed with ADL.

acknowledged, include marriages devoid of physical and sexual intimacy, profound shame and suicidal thoughts.

Significant social stigmatization associated with hydrocoele has been documented in the Philippines and in Haiti and, for many of the patients, sexual disability begins when the disease is first diagnosed. It remains to determine how best to meet the profound social and psychological needs of affected men and their families.

Epidemiological burden

As well as a general lack of information about the socioeconomic effects of lymphatic filariasis, there is also a lack of information about its prevalence and distribution in most endemic societies. A prior necessity for control is adequate information.

One of the studies[7] in this area supported by TDR during the biennium was carried out in Ghana. The aim was to gather baseline information about the extent and distribution of *W. bancrofti* microfilaraemia and clinical disease prior to designing a control programme for the entire country. The information collected would define the public health importance and provide a basis for monitoring and evaluating a future control programme.

The study indicated that the disease may be a major public health problem in the country. High prevalence was found, but with considerable regional variation, necessitating rapid assessment to identify more precisely the communities at risk before a national control programme could start.

Rapid assessment

Rapid assessment is used to estimate the burden of lymphatic filariasis at the community level. This is required before planning and monitoring of control programmes can begin.

Diagnosis of filarial disease is generally based on demonstration of the parasite in peripheral blood (in inconvenient night-time surveys) and on clinical manifestations. However, a suitable immunodiagnostic test for *W. bancrofti* has recently become available (see below). New rapid assessment methods are needed because the current methods are inconvenient, inappropriate, and/or beyond the means of ministries of health in endemic countries. TDR has been supporting studies to find more acceptable ways of rapidly assessing the burden.

Studies evaluating a variety of rapid assessment procedures (RAPs) have been supported in a number of sites. In a study in Ghana,[8] RAPs included:

- morbidity indicators of lymphatic filariasis available in health facilities (outpatient department records, admission records, laboratory records and surgical operative procedure records from district health facilities, as well as interviews with key people in hospitals);
- community key-informant reporting (community leaders and traditional health providers as key informants);
- focus group discussions (with women's groups, men's groups and groups of chronic disease sufferers);
- use of self-administered questionnaires through existing administrative systems (district assembly representatives and school teachers);
- examination of adult males for hydrocoeles (random samples in each community of 30-40 adult males above the age of 20 years).

As reported, all the RAPs were easy, non-invasive and acceptable to the community. The results obtained were comparable to those obtained by standard epidemiological diagnostic procedures for lymphatic filariasis at community level. The key informant interviews and focus group discussions gave a broad perspective of the burden of disease in general but also a specific perspective on lymphatic filariasis, while the use of self-administered questionnaires gave data comparable with those obtained from a case search on elephantiasis in the community. Examination of 30-40 adult males for hydrocoeles provided good correlation with data on community microfilaria prevalence. All the procedures tested were of potential use in rapid assessment.

The next step will be to bring together all investigators of rapid assessment procedures to consolidate their findings. After that, future objectives and use of RAPs will evolve from the review by the new Task Force on *Community-Directed Treatment*.

Chemotherapy

Diethylcarbamazine (DEC) has been available for treatment of filarial disease for almost 50 years and is still used today. It destroys larval worms and even some of the adult worms but has drawbacks if used in heavy infections, when the destruction of large numbers of worms may cause severe side effects.

Effect of drugs on microfilaraemia

TDR-sponsored studies have shown that a single dose of DEC achieves the same result as the long-recommended two-week course[9] (Fig. 1).

Today however, other effective drugs are also available. For instance, ivermectin. Although effective against lymphatic filariasis, ivermectin is not yet registered for its treatment. Assessing the safety and efficacy of newer drugs and drug combinations in comparison with DEC has been the object of recent studies.

For example, in Papua New Guinea[10] the effect of treatment with DEC alone was compared with that of

DEC+ivermectin by assessing microfilaraemia prevalence rates following treatment. The microfilaria prevalence rate decreased by 28.9% after one year in villages where DEC alone was used and by 67.2% in villages where DEC+ivermectin was used (Fig. 2).

Fig. 1 DEC: Single dose compared with 14-day dose (meta-analysis – *W. bancrofti*) blood microfilaria levels

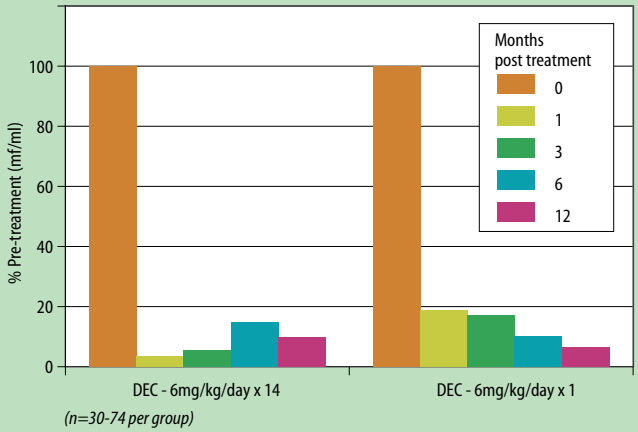
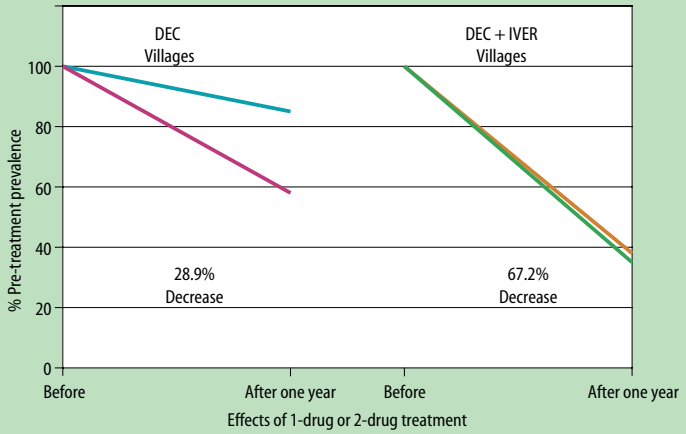


Fig. 2 Microfilaria prevalence changes Effects of 1-drug or 2-drug treatment



In a study in India,[11] three treatment schedules were compared – ivermectin alone, DEC alone, and ivermectin+DEC. All three schedules were effective to greater or lesser extents, but after 12 months, blood microfilaria levels began to rise again, although this was minimal in the combination therapy group (Fig. 3).

Other TDR-supported studies have involved other drugs, such as albendazole, a compound which is effective against adults of many species of nematode, though not *Onchocerca*, but which may have some effect against adult filarial worms. A study in Sri Lanka[12] compared the effects of four treatment regimens on blood microfilaria levels. Whereas blood microfilaria levels declined to low levels 15 months after treatment with albendazole alone or albendazole+DEC, they

Fig. 3 Ivermectin and DEC: 1- and 2-drug regimens blood microfilaria levels (*W. bancrofti*)

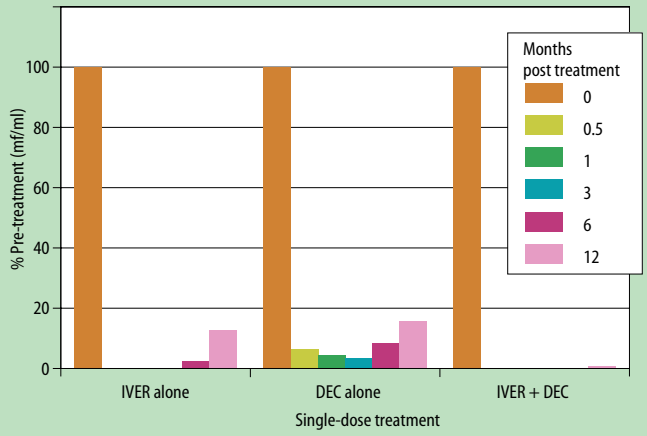
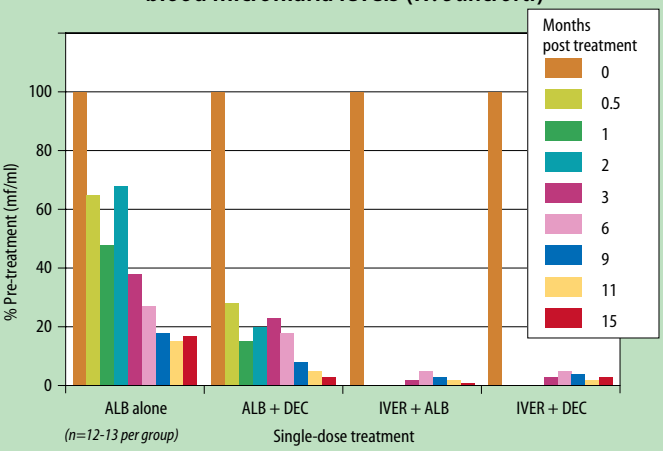


Fig. 4 Albendazole, ivermectin and DEC: 1- and 2-drug regimens blood microfilaria levels (*W. bancrofti*)



reached zero almost immediately following treatment with ivermectin+albendazole or ivermectin+DEC – and remained at this level for at least 15 months (Fig. 4). Thus it appears that single-dose ivermectin+albendazole may suppress microfilaraemia in the long term, and could be an ideal tool in regions where onchocerciasis and loiasis co-exist.

Effects of drugs on acute disease and early chronic disease

Other studies have compared the effectiveness of antibiotics with that of ivermectin and DEC, individually or in combination, in preventing lymphoedema and acute filarial attacks as well as early chronic disease in asymptomatic and symptomatic individuals. These studies have clearly shown that the major benefit in directly preventing acute disease comes not so much

from the use of antifilarial drugs but from the use of hygiene and antibiotics – indicating the importance of secondary bacterial infection in precipitating recurrent episodes of filarial disease.

Effects of drug treatment on vector infectivity and transmission indices

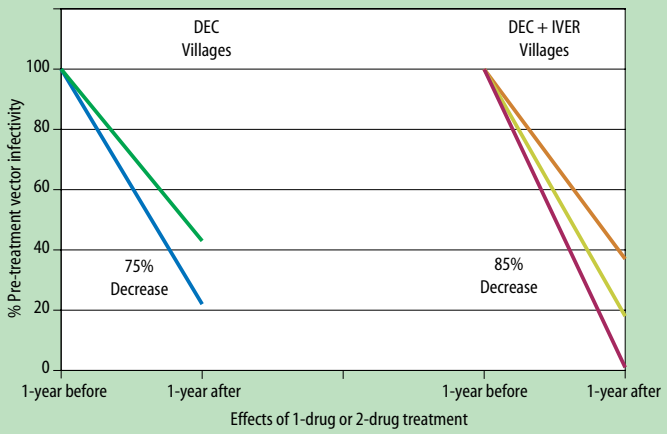
In a study in Papua New Guinea,[13] the effect on vector infectivity of DEC alone was compared with that of DEC+ivermectin. One year after treatment in villages where DEC alone had been used, vector infectivity had decreased by 75%. In villages where DEC+ivermectin had been used, vector infectivity had decreased by 85% (Fig. 5).

Supplementary interventions in control

Vector control

A variety of means for controlling the vectors of filarial disease are available today. These include *Bacillus sphaericus,* a toxin-producing bacterium; polystyrene beads, which help limit the breeding of mosquitos in certain situations; insecticide-impregnated bednets and curtains, which limit host/vector contact; indoor spraying of long-lasting pyrethroid insecticides, especially effective for adult *Mansonia* and *Culex*; and community participation in integrated vector management. All these methods help to decrease vector numbers and transmission, but exactly how and when the tools are cost-effective and useful in large-scale control programmes for lymphatic filariasis have not yet been clearly defined.

Fig. 5 Vector infectivity changes
Effects of 1-drug or 2-drug treatment



In Papua New Guinea, DEC and ivermectin proved to be a potent combination.

One study completed during the biennium indicated the value of vector control methods in filariasis endemic areas of South India. Yearly ivermectin and DEC were given to communities with or without concurrent vector control (polystyrene beads and/or *B. sphaericus*). Vector control methods were shown to be of definite value with regard to the mosquito problem, but costly. Now the emphasis is on determining whether there is also a benefit with respect to the disease itself.

Morbidity management

Today it is known that, for cases of chronic filarial elephantiasis and hydrocoele, the simple measure of keeping the affected area extremely clean can dramatically decrease the inflammation and burden on a patient's lymphatic system. Managing the morbidity of filarial disease in this way, through intense local hygiene of the affected parts, has been shown to be particularly suited to community participation – and it can be implemented simply by training the community in the importance of local hygiene of affected limbs.

Preliminary evidence indicates that self-help support groups among patients and their families, organized from within the community, are extremely effective in stimulating and maintaining compliance with the vigorous hygiene regimens necessary for morbidity control in this way. Such a strategy can be exploited worldwide. Multicentre morbidity control trials comparing the efficacy of antibiotics and hygienic practices with a placebo are ongoing.

Detection of infection in communities and vectors

There have been tremendous advances in diagnostics for filariasis in recent years, which have been supported directly or indirectly by TDR.

Detection of infection in vectors is important for assessing parasite transmission, and is essential when monitoring large-scale control programmes. Methods based on dissection of mosquitos and morphological identification, which have been available for many years and are still in use today, are time-consuming. However, DNA probes for detection of *B. malayi* and *W. bancrofti* in mosquitos are now available.

With regard to diagnosing filarial disease in humans, a number of new technologies have become available in recent years. These include lymphoscintigraphy, ultrasonography, DNA probes for blood infection, and circulating antigen assays. The use of these techniques has led to new understanding of filarial disease. For example, the sub-clinical pathology (abnormalities of lymphatic and renal function) and masses of worms found in asymptomatic individuals have been identified. Steps have been taken to transfer these new technologies to country control programmes – so far in China, Sri Lanka, India, Australia and Papua New Guinea.

There is still a major need to replace night-time blood surveys – which are not popular with either health workers or communities – and a very good test has become available in recent months for the detection of Bancroftian filariasis. This test is a card immunochromatographic test (ICT). It is easy to use, rapid (results obtained in 5-15 minutes), sensitive and specific, convenient (eliminating specimen collection at night), and cheap (much less set-up time and equipment required).

Product Research and Development

See Product Research and Development section in Chapter 7.

Strategic Research

Pathogenesis

A better understanding of the pathogenic mechanisms of onchocerciasis and lymphatic filariasis could form the basis of new therapeutic interventions to prevent the debilitating consequences of these diseases.

Highlights of recent work supported by TDR on the pathogenesis of filarial disease can be seen in Box 2.

Box 2. Pathogenesis research

Host immune mechanisms

- The absence of circulating filarial antigens in Bancroftian filariasis has been shown to be associated with a Th-1 type response (production of interleukin-2 and interferon-γ cytokines), while individuals with antigenaemia have high levels of specific IgG4 antibody.

Drug and vaccine targets

- Vaccine development in filariasis has focused on stage-specific microfilarial chitinase which has been identified as a candidate antigen for a prototype transmission-blocking vaccine against lymphatic filariasis. This enzyme is thought to be essential for the worm's development and to be involved in the exsheathment of microfilariae. The critical antigenic element at the carboxy terminus of the protein has been identified. In animal models, the vaccine is effective when given before infection but, when given to jirds with patent infections, does not seem to alter microfilaraemia levels.

Gene regulation

- Mapping of the antigenic elements of calreticulin protein and disulphide isomerase has been achieved. These filarial antigens are thought to be responsible for causing the eye disease associated with *Onchocerca volvulus* infection through an autoimmune reaction.

Genome

Filarial genome research begun in 1994 when collaboration between six laboratories in the USA, UK, India, Egypt and Uganda was initiated by TDR. *Brugia malayi* is used as the model organism and is the focus of attention for most studies. The main efforts during the first two years have been concentrated on the construction of complementary DNA (cDNA) and bacterial artificial chromosome (BAC) genetic libraries for DNA sequencing and genome mapping (Box 3).

Significant progress has been made and interesting results obtained. Before 1995, only 94 *Brugia* expressed sequence tags (ESTs) were known, whereas more than 4000 were available by the end of 1996. They correspond to about 2500 different genes, i.e. approximately 20% of the total number expected in the *Brugia* genome. These genes encode housekeeping proteins, structural proteins, and proteins of immediate immunological or drug-discovery interest, while many more sequences may prove to have a significant role in host invasion.[14]

By producing a catalogued archive of sequence-tagged cloned genes, a resource can be built which may remove much of the initial difficulty of isolating filarial drug-target or vaccine-encoding genes. Using the genome database it should be relatively simple and cost-effective to survey in depth the larvally expressed genes of *Brugia malayi* and select novel, nematode-specific and/or stage-specific clones for directed vaccine and drug-discovery efforts.

Box 3. Genome research

Construction of parasite stage-specific cDNA libraries and gene discovery

- Eight complementary DNA (cDNA) libraries of *B. malayi* have been constructed – one of microfilaria; one of L2 larvae; two of infective L3 larvae; one of L3-L4 transition larvae; one of L4 larvae; one of adult males; and one of adult females. Additionally, using funding from other sources, one cDNA library of *Onchocerca* and one of *Wuchereria* have been constructed for comparison.
- Preliminary expressed sequence tag (EST) analysis has been carried out on 200 randomly selected clones from each cDNA library. More than 4000 ESTs have been sequenced, representing some 2500 new genes – about 20% of the total genome of *Brugia*. Many interesting genes have been identified and over 100 clones are being further characterized – by laboratories both within and outside the network. There are indications that the data are reaching a wide audience for research in many areas of biology.
- The subtractive hybridization technique is used to clone genes expressed at low levels. The use of this technique on the most common clones from all of the libraries has allowed EST analysis to be performed on 800 additional clones from each library.
- An interactive DNA database and communication network has been established on the Internet – which contains DNA sequence information, map data and information on the biology of filarial parasites. All data are made available for interested scientists.

Construction of large fragment genome libraries and genome mapping

- Construction of a *B. malayi* genomic cosmid library has begun. The library will allow researchers to isolate and study their chosen gene on a relatively small DNA fragment.
- Construction of a *B. malayi* bacterial artificial chromosome (BAC) genomic library of 10 000 clones has begun. This library will be used for mapping.
- Gridding of cDNA and genomic DNA libraries has commenced. The gridded BAC library will be used for constructing a low- to medium-resolution physical map.

Individual gene analysis

- This in an ongoing study which involves screening of cDNA libraries with infected and immune sera and detailed DNA sequence analysis and mapping of genes with potential as vaccine candidates and drug targets.
- A number of genes encoding proteins of nematological and immunological interest have been identified, such as: *B. malayi* homologues of other filarial antigens, proteinases and their inhibitors; serodiagnostic antigens common to other nematodes and similar to mammalian lipid-binding proteins and insect odorant binding proteins; and thiol antioxidant proteins and mammalian macrophage inhibition factor which might play important roles in immune down-regulation.

WWW site: **http://helios.bto.ed.ac.uk/mbx/fgn/filgen.html**

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CHAPTER 7

Onchocerciasis

Applied Field Research

- ✓ TDR has now concluded the preparatory operational research required by the African Programme for Onchocerciasis Control (APOC); the results have been passed to APOC and transformed into policy
- ✓ skin disease has been shown to be a far greater burden than previously thought; results of the studies have been used for advocacy for APOC
- ✓ Rapid Epidemiological Mapping of Onchocerciasis (REMO) is used for rational planning; APOC has recommended that all countries complete REMO as the basis for national plans for onchocerciasis control
- ✓ Community-Directed Treatment with ivermectin has been shown to be entirely feasible and effective; it is now APOC strategy
- ✓ operational research has provided the first evidence that ivermectin can prevent deterioration of vision in onchocerciasis patients
- ✓ operational research has indicated that the treatment of onchocerciasis with ivermectin in loiasis endemic areas can continue but with intensified monitoring because of a low risk of severe adverse reactions
- ✓ since the preparatory operational approach to onchocerciasis control has now almost ended, at the request of APOC, TDR will next assist in the scientific aspects of evaluation and optimization of Community-Directed Treatment with ivermectin with particular emphasis on sustainability
- ✓ the exclusion criteria for pregnant and lactating women during mass treatment with ivermectin have been changed

Product Research and Development

Ivermectin

- ✓ high doses are no more effective than the standard dose
- ✓ is prophylactic in the *O. ochengi*/cattle model
- ✓ the nematode genes most commonly responsible for resistance are being identified and studied

Amocarzine

- ✓ Phase I study completed – Phase II study for lymphatic filariasis awaits government approval
- ✓ Phase II study completed – no macrofilaricidal activity on a forest strain of *O. volvulus*

UMF 078

- ✓ has good macrofilaricidal effect in the best available animal model
- ✓ preclinical studies being completed
- ✓ study of enantiomers under way

WR 251993. WR 129577. PD 105666

- ✓ selected from MACROFIL screening activities and now being evaluated in the *O. ochengi*/cattle model

ONCHOSIM (computer simulation)

- ✓ indicates a definite role for a macrofilaricide in addition to ivermectin if eradication is the object of control
- ✓ suggests that ivermectin resistance in onchocerciasis should not become a problem until 20-30 years after initiation of drug treatment
- ✓ indicates that resistant microfilariae will emerge when both the number of microfilariae in the skin and the incidence of the disease are low

Strategic Research

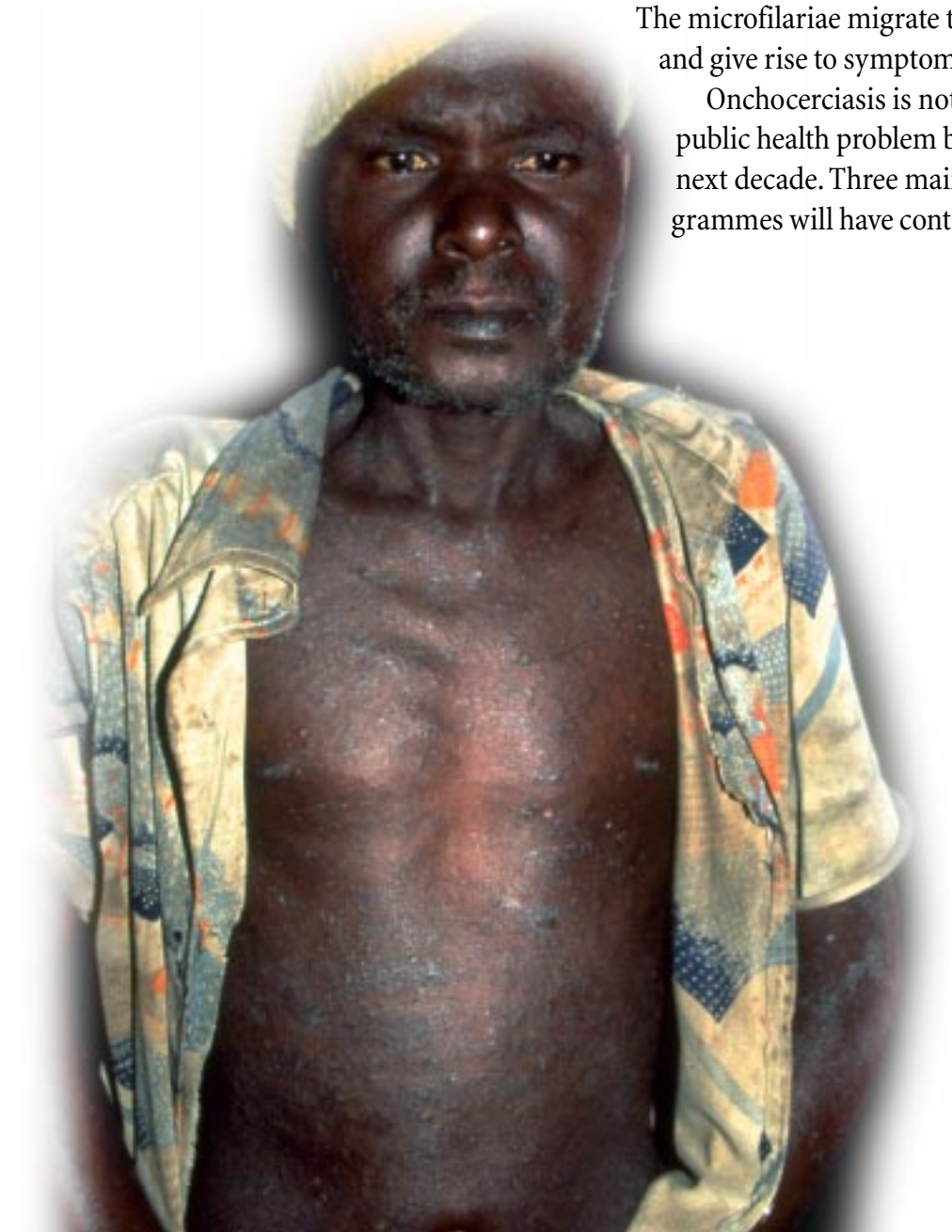
See Strategic Research section in chapter 6

Blindness; severe itching; disfiguring skin lesions; abandonment of fertile river valleys; and social ostracism are some of the consequences of infection with *Onchocerca volvulus*, the worm which causes onchocerciasis or 'river blindness'. The blackflies which transmit the worm can be an intolerable nuisance. They are found in river basins throughout tropical Africa and in isolated foci in Latin America and Yemen.

Eighteen million people are infected with onchocerciasis, 99% of whom live in Africa. Although not fatal, blindness in onchocerciasis causes lowered expectation of life (by 10-15 years). In some hyperendemic communities, every second person ultimately goes blind. In other communities, onchodermal skin lesions are an important social problem. In 1990, onchocerciasis was responsible for the loss of 884 000 Disability Adjusted Life Years (DALYs).[1]

Dozens of adult worms are present in heavily infected people. They occur in tangled masses inside fibrous nodules under the skin. The females produce microfilariae (microscopic larvae) which live mostly in the skin. The microfilariae migrate throughout the body and give rise to symptoms of the disease.

Onchocerciasis is not expected to be a public health problem beyond the end of the next decade. Three main control programmes will have contributed to this.



The African Programme for Onchocerciasis Control (APOC):

- was launched in 1996 and covers 19 countries in Africa – Angola, Burundi, Cameroon, Central African Republic, Chad, Congo, Equatorial Guinea, Ethiopia, Gabon, Kenya, Liberia, Malawi, Mozambique, Nigeria, Rwanda, Sudan, Tanzania, Uganda and Zaire;
- oversees more than 85% of the global onchocerciasis burden (an estimated 15 million infected people), including populations who suffer more from onchocercal skin disease than from onchocercal blindness (and who were previously overlooked);
- is based on treatment with ivermectin and aims to establish, within a period of 12 years, effective and self-sustainable community-based ivermectin treatment throughout the endemic areas covered by the programme and to eliminate the disease by vector control in selected foci.

During 1994, the TDR Task Force on *Onchocerciasis Operational Research* was asked to prepare the groundwork and technical basis for APOC. The activities of this Task Force therefore reflect the needs and priorities identified by those involved in the planning and control of onchocerciasis.

None of the APOC countries was included in the first large-scale onchocerciasis control initiative, the Onchocerciasis Control Programme in West Africa, which has been particularly successful.

The Onchocerciasis Control Programme in West Africa (OCP):

- is the largest and most notable human disease control programme in operation today, and covers 11 countries in West Africa;
- has, after 20 years of operation, prevented more than 100 000 people from going blind; reduced the number of infected people in the original programme area to practically nil; spared millions from the risk of blindness; opened up vast areas of fertile riverside land for resettlement; and eliminated the disease as a public health problem from all OCP countries;
- is a very cost-effective operation, giving a financial return on investment of 15-20%;
- is based on controlling the vector and interrupting the transmission cycle of the disease (for 14 years, which is longer than the lifespan of the adult worm), and is set to conclude in 2002.

Though ivermectin has become an important additional control tool, the main focus of OCP, in contrast to that of APOC, is on vector control. Vector control is only considered technically feasible and cost-effective in OCP countries (being based on aerial spraying of insecticides over blackfly breeding sites in fast-flowing rivers), except for isolated foci in other countries. Whereas vector control interrupts the transmission cycle, treatment with ivermectin does not. But treatment with ivermectin does kill the larval worms, which are responsible for skin disease and blindness in infected people.

The Onchocerciasis Elimination Programme in the Americas (OEPA):

- covers all six endemic countries in the Americas and was launched in 1993;
- is also based largely on treatment with ivermectin and is scheduled to end after 10-15 years.

Applied Field Research

Operational research

Today, onchocerciasis is still an important public health and socioeconomic problem, especially in Africa. The most serious consequence of the disease is blindness, which may afflict over one third of the adult population in severely affected communities, but the severe skin disease and associated maddening itching which causes great suffering to millions more people is also responsible for a large burden when considered in terms of DALYs.

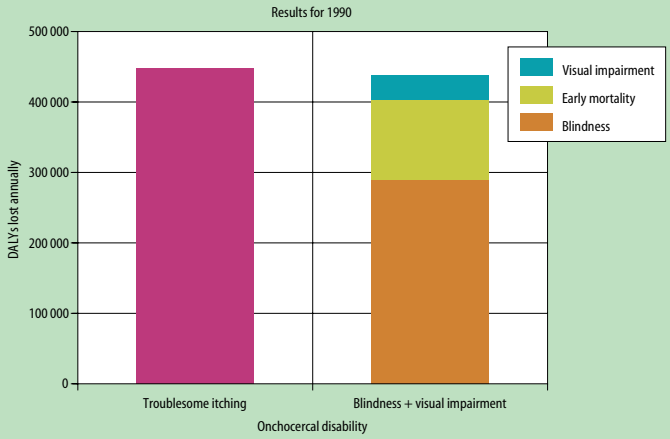
The launching of APOC in 1996 was a landmark event in the history of onchocerciasis control, covering as it does more than 85% of the global burden of the disease. TDR was requested to prepare the groundwork and oversee the operational research needed before the Programme could begin. During the biennium, therefore, the TDR Task Force on *Onchocerciasis Operational Research* has been concerned with questions of why, where and how to control the disease. The importance of onchocercal skin disease has been highlighted, a technique of rapid epidemiological mapping developed, and the effect of ivermectin on ocular disease as well as the effectiveness of community-directed treatment and focal vector eradication determined. The Task Force has been actively involved with the African Programme for Onchocerciasis Control (APOC) since the outset.

Onchocercal skin disease – public health and socioeconomic importance

The public health and psychosocial impact of onchocercal skin disease and itching was highlighted by a TDR-supported multicountry study completed in 1994.[2] An estimated 15 million people infected with *Onchocerca volvulus* live in APOC areas, of whom some 8-9 million live where onchocercal blindness is not common and where onchocercal skin disease can be highly prevalent. Results from the study played an important role in the APOC agenda and prompted a major reassessment of the importance of onchocercal skin disease – when considered in terms of Disability Adjusted Life Years (DALYs), the burden associated with itching alone is as great as that associated with blindness alone (the number of people affected by itching being far greater than the number affected by blindness alone)(Fig.1).

Many endemic communities, particularly those with low rates of onchocercal blindness which have received little attention in the past, are now known to have a

Fig. 1 Estimated DALYs lost due to onchocerciasis



significant burden of onchocercal skin disease. Follow-up studies to determine the economic impact and social costs of onchocercal skin disease and itching were subsequently requested and are now in their final stages. The multicountry study involves sites in Ethiopia, Sudan, Uganda, and Nigeria. It is aimed at determining what effect onchocercal skin disease has on labour input, and, when present in the home, what effect it has on children's attendance at school. Results of the study will be used directly by APOC and other control programmes.

Geographical distribution of onchocerciasis in APOC countries

Rapid epidemiological mapping

Before a control programme can begin, we need to know where the high-risk communities are and who to treat. In most areas we do not yet know who is at risk. As well, the distribution of onchocerciasis is not uniform but clustered – depending on, among other things, environmental conditions and socioeconomic circumstances. In order to control the disease on a large scale, therefore, it is necessary to pinpoint the communities in greatest need of control interventions – it is necessary to diagnose communities rather than individuals. Hence the emergence of rapid epidemiological mapping techniques.

Rapid Epidemiological Mapping of Onchocerciasis (REMO), as developed by TDR, is a key tool for the control of onchocerciasis.[3] Its development was one of the first activities under TDR's Applied Field Research component. With REMO it is possible to assess quickly and cheaply which communities are at high risk of onchocerciasis and where they are located. TDR works together with the WHO Regional Office for Africa

(AFRO) and WHO's Division of Control of Tropical Diseases (CTD) on REMO activities and the tool is being used to provide basic information for rational planning of control activities in the APOC countries.

Basically, REMO works by using geographical information, particularly the presence of river basins, to identify communities likely to be at high risk. A sample of these high risk communities, representing only 2-4% of all villages in the area, is then rapidly assessed for the presence of onchocerciasis by feeling for nodules in 50 adults per village. More than 40% of 50 adults with nodules means treatment is urgent; 20-40% with nodules means that treatment is highly desirable. Results from the small village samples are then extrapolated to whole areas. The whole of Nigeria was assessed in less than eight months using REMO. Mapping has also been completed in Cameroon and is being completed in most other APOC countries.

A panel of experts from the African region was created to assist in preparing for REMO and to undertake independent validation of REMO results in a random sample of villages from all REMO countries. A workshop was held in Lagos, Nigeria, to develop the plan of activities for the panel.[4] Another workshop was held in Kinshasa, with support from AFRO, to train a group of Zairian scientists in REMO, to develop a plan for nationwide REMO and to prepare proposals for submission to the TDR Task Force.

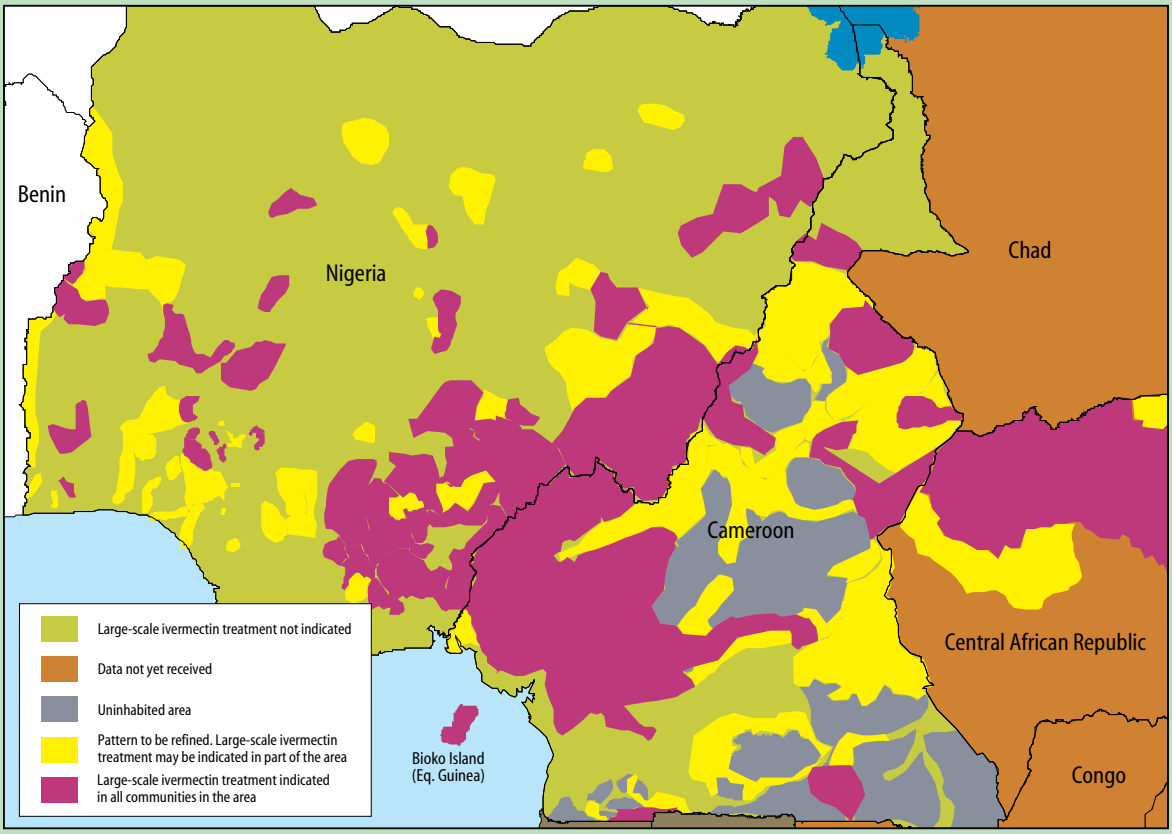
REMO does not depend on computers, making it a suitable tool for use in the field. It allows a rapid and low-cost vision of the distribution of the disease to be developed. There are limitations associated with such a system however, including for example the fact that the amount of information which can be included or integrated on a map is limited by the size and scale of the map. Results of REMO may be collated and analysed in a geographical information system (GIS).

Geographical information system

The development of a GIS for onchocerciasis in Africa is being undertaken with the Health Mapping unit of WHO/CTD. The GIS will enable identification of high-risk zones as well as estimation of the total population at risk and in need of ivermectin treatment. So far, the GISs for Nigeria and Cameroon have been completed and are being used to update national plans for onchocerciasis control (Fig.2).

Training is being organized to ensure that all REMO teams are able to use the GIS for analysis of REMO data and for planning of control. So far, two workshops have been held – one in Lagos for teams from Nigeria and

Fig. 2 Results of the Rapid Epidemiological Mapping of Onchocerciasis for Nigeria, Cameroon, Equatorial Guinea and Central African Republic – showing where high risk populations are situated



Prepared by CTD/HealthMap, in collaboration with TDR for APOC, Nov. 1996. Base maps from the African Data Sampler, World Resources Institute

Cameroon, and one in Uganda for teams from Ethiopia, Sudan, Uganda and Tanzania. Another is planned for francophone countries. Further workshops may be needed.

So far, the use of the GIS tool indicates that previous estimates of onchocerciasis prevalence in APOC countries are in fact underestimates and that the real prevalence is significantly higher than previously thought, at least in Nigeria and Cameroon. APOC has recommended that all countries complete REMO before requesting APOC funding for ivermectin treatment projects.

The effect of ivermectin treatment

Effect of ivermectin on onchocercal skin disease

Very little is known about the effect of ivermectin treatment on onchocercal skin disease, and information concerning its effect on itching is controversial. Available evidence suggests that the drug has a positive effect on onchocercal skin disease but that once yearly treatment may not be sufficient to produce this effect. Therefore a multicountry, double-blind, placebo-controlled trial is being supported to determine the effect of three-, six- and twelve-monthly ivermectin treatments on onchocercal skin disease and itching. The trial is critical for determining dosage regimens, especially in forest areas.

Four sites in Uganda, Ghana and Nigeria are involved in the trial. Both dermatological and psychosocial indicators are being used to assess the observed and perceived effects of treatment on skin lesions, itching, other symptoms, stigma and well-being. The final treatment round will take place in February 1997.

Effect of ivermectin on ocular disease

In contrast to the situation with onchocercal skin disease, a number of studies have demonstrated the effectiveness of ivermectin in preventing the development and progression of onchocercal eye lesions, and it is generally accepted (although not yet proven) that treatment once a year is sufficient to prevent onchocercal blindness.

During the biennium, follow-up studies to the TDR-sponsored community trials in Nigeria and Cameroon on the effects of seven years of annual ivermectin treatment on ocular disease and blindness were undertaken. These studies confirmed the beneficial effect of the drug in preventing anterior segment eye disease. For treatment of choroido-retinal lesions however, results from the Cameroon study were disappointing, although results from the Kaduna study in Nigeria indicated that ivermectin could significantly reduce the incidence of ocular atrophy and visual field loss. The Kaduna study provided the first evidence for a beneficial effect on vision.

Safety of ivermectin in Loa loa areas

To date, millions of people have been treated safely for onchocerciasis with ivermectin, but there remains some doubt about its safety in areas where onchocerciasis and loiasis (caused by the related filarial worm *Loa loa*) co-exist.

A study carried out in Cameroon[5] indicated that there was some risk of severe adverse reactions in loiasis endemic areas. Another study was therefore undertaken to determine the risk and appropriate management of severe neurological adverse reactions, if any, after treatment with ivermectin in an area of Cameroon where both loiasis and onchocerciasis are hyperendemic. Out of 18 000 persons treated, there were only two cases of neurological disorder in temporal association with treatment. Both patients recovered completely after hospitalization. It was concluded that ivermectin treatment of onchocerciasis could continue in endemic loiasis areas, but that intensified monitoring for adverse reactions was required for five days post-treatment.

Ivermectin delivery: Community-Directed Treatment

Before the creation of APOC, community-based ivermectin treatment was already operational in several countries. The most advanced country was Mali, where community-based delivery had been ongoing for a number of years in several hundred communities. The results of these first community-based programmes appeared very positive and suggested that communities are capable and willing to take responsibility for their own ivermectin treatment. However, no systematic evaluation of these community-based programmes had been undertaken and most of the available information was anecdotal. A multicountry study was therefore undertaken in which particular emphasis was laid on different approaches to giving communities responsibility for their own treatment.

During the study the concept of 'Community-Directed Treatment' was developed and its use came to be recommended as a principal method for onchocerciasis control. Community-Directed Treatment with ivermectin was found to be feasible and effective, and because it has been successful in a wide range of geographic and cultural settings in Africa, it is likely to be replicable in other endemic communities in Africa.

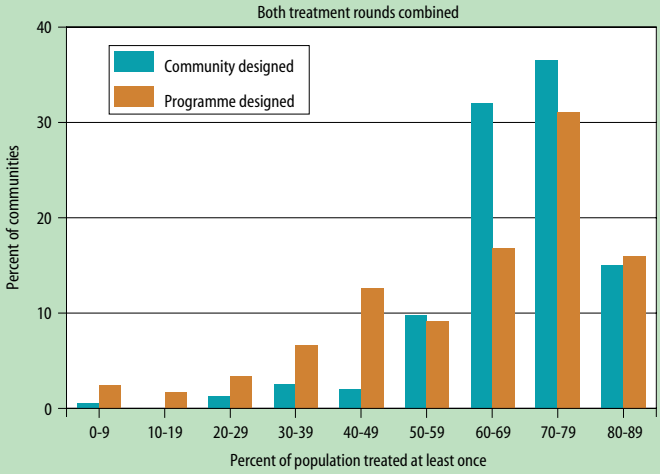
In a Community-Directed Treatment system, the community itself is in charge of delivery, including collecting the drug, treating all eligible members of the community, referring cases of severe adverse reaction, and reporting. The treatment system, characteristics of distributors, mode of drug collection, mode of distribution, cost sharing and level of supervision may all vary.

A range of different community-directed systems was employed during the study.

Two types of system design were used – systems designed by the communities themselves and systems designed for the communities by control programmes. Community-Directed Treatment systems which were designed by the communities themselves did not appear to be greatly different from those designed for them by control programmes, but the study showed consistently that community-designed systems were more effective and achieved better treatment coverage (Fig.3). The freedom of community members themselves to design their own treatment system, to select the distributors they want and to change the system when necessary were found to be important factors in the success of the community-designed systems.

A regular supply of ivermectin at the district level is critical for the success of a distribution programme. The most serious failures in the distribution system during the study were related to inadequate supply of the drug.

Fig. 3 Treatment coverage by design[6]



However, where adequate supplies were available at district level, communities were very capable and eager to collect the drug themselves for treatment of their community.

Community-directed distributors were found to adhere well to treatment procedures. The exclusion criteria were properly applied: children who were too young and too small were not treated, and pregnant women and severely ill persons appear to have been correctly excluded from treatment in all study sites. The dosage given was within half a tablet of the correct dose in over 90% of all treatments and dosage errors of more than one tablet were rare. Available information regarding referral of cases is less comprehensive but indicates that cases of severe or persistent adverse reaction were referred by the distributors to the nearest health facility. Some basic supervision by the local health service was associated with better performance in terms of treatment coverage than no supervision at all. But the inclusion of cost recovery had a negative effect on treatment coverage.

One aspect of Community-Directed Treatment with ivermectin that needs considerable improvement is that of reporting. Reporting of treatment coverage and severe adverse reactions during the study was not at all reliable. Interestingly, discrepancies in reporting were greater in those villages using systems designed by control programmes than in those using community-designed systems. This is an important observation – it indicates that we should now question the problem of reporting in general, not only that of community-designed systems. Alternative approaches to reporting, such as pictorial reporting forms, could be explored.

Since the study was difficult and some of the investigators inexperienced, the Task Force undertook several reinforcement activities. These included a workshop in Uganda to review the pilot studies and update the protocol; site visits by experts; a workshop to improve the evaluation instruments; and a final analysis and reporting workshop at the OCP and APOC headquarters in Ouagadougou, Burkina Faso.

Some of the conclusions of the study are reproduced in Box 1.

The main recommendations of the study are that:

- Community-Directed Treatment with ivermectin should become a principal means of onchocerciasis control in Africa.
- In view of their potential for increasing sustainability, preference should be given to community-designed approaches.
- Special provisions/arrangements are needed to ensure that ivermectin is available and accessible at district level.
- In view of problems with record keeping at community level, there is an urgent need to reassess both current information requirements and alternative ways of collecting needed information.
- Follow-up research is needed to validate the indicators for sustainability identified and to identify the modifications needed in Community-Directed Treatment.

Box 1. Conclusions of a TDR-sponsored study[6] on Community-Directed Treatment with ivermectin

Community-Directed Treatment with ivermectin is feasible and effective.

Community-Directed Treatment has been successful in a wide range of geographic and cultural settings in Africa and is likely to be replicable in other endemic communities in Africa.

Although sustainability can only be proven over time, the current study indicates that Community-Directed Treatment is likely to be sustainable because of the:

- **commitment of community leaders and distributors;**
- **high degree of involvement of the communities and their willingness to commit available local resources to the distribution process;**
- **ability of communities to collect ivermectin at the appropriate time from district level stores;**
- **perceived benefits of ivermectin and the high demand for it in communities where onchocerciasis is endemic;**
- **ability of communities to recognize problems with distribution methods and to modify the methods accordingly.**

Distribution systems designed by the communities themselves achieved better coverage than distribution systems designed for them by control programmes. Since community-designed systems provide more opportunities for community involvement, they appear to have greater potential for sustainability.

The performance of distributors appears to be adequate, as shown by the coverage achieved and the apparent adherence to exclusion criteria and use of appropriate dosing.

Non-availability and inadequate quantity of ivermectin impaired coverage in several study sites.

The provision of some basic supervision by the local health services was associated with better performance of the distribution systems in terms of treatment coverage. Cost recovery had a negative effect on treatment coverage.

Communities had difficulties in maintaining records as requested.

Research Capability Strengthening

A number of research capability strengthening activities were held during the above studies – for example in protocol development, skill training and data processing. The studies have been carried out almost entirely by African staff and an extensive network of researchers and institutions has been built up during the process (see Fig. 9 in chapter 1).

Other Applied Field Research studies

Gender study – exclusion of pregnant and breast-feeding women from ivermectin treatment

This topic was the subject of a study,[7] completed in 1996, in a rural area of Sierra Leone, where mass treatment for onchocerciasis takes place periodically (Box 2). Possible side effects of ivermectin on the mother and infant are the reasons for exclusion of pregnant and lactating women from treatment.

Pregnant and lactating women are advised to seek treatment one month after delivery. The study was carried out to determine how many women actually follow this advice. Few were found to do so. The most common reason given for this was that they were waiting for the next round of mass treatment. But after the next round of mass treatment, two years from when the women were first excluded from treatment, 38% of them were again denied treatment because of pregnancy/lactation. In areas of high fertility, women may thus be continually excluded from treatment and hence from the chance of a better life. Such women may also constitute a reservoir for the disease.

Also during the study, a number of women who had received treatment inadvertently, even though pregnant, were followed up. No adverse effects were apparent. Because of this and similar observations elsewhere, the manufacturer, Merck & Co., has now changed the

Box 2. Exclusion of pregnant and breast-feeding women from ivermectin treatment in Sierra Leone

A total of 534 pregnant or lactating women were excluded from treatment during the study, of whom 427 were interviewed as to why they did or did not follow the advice given to them and seek treatment one month after delivery.

The study also investigated to what extent pregnant women were treated inadvertently with ivermectin and whether they reported any side effects as a result.

Some of the findings/conclusions of the study include:

- **Only 5 of the 427 respondents sought treatment one month after delivery. Reasons given for this included lack of information, lack of economic resources and the fact that they were waiting for the next round of mass treatment.**
- **One year later, of 265 women who could be followed up, 57 had been treated. The main reason given by the 208 women who had not been treated was that they were waiting for the next mass treatment campaign.**
- **Two years after first being excluded from treatment, at the next mass treatment campaign, 64 of the 208 women who still had not received treatment were once again denied treatment because they were either pregnant or breast-feeding.**
- **Of 27 women who had been inadvertently treated during pregnancy, none reported any adverse effects to themselves or their children.**
- **The welcome decision by the manufacturer to make ivermectin available to most pregnant women and to lactating women one week after delivery during mass treatment campaigns will improve the lives of millions of women in areas where onchocerciasis is endemic.**
- **The results of the study may have implications for the routine exclusion of pregnant and breast-feeding women from treatment with other drugs.**

exclusion criteria for pregnant and lactating women. Ivermectin is now made available to pregnant women during mass treatment campaigns when the risk of complications from untreated onchocerciasis exceeds the potential risk to the foetus from treatment. Nursing mothers may also be given the drug if the benefits outweigh the potential risk to the breast-fed infant, but pregnant women are advised to delay treatment for at least one week following delivery.

Product Research and Development

The development of macrofilaricides – is eradication of filariasis possible?

A safe and effective macrofilaricide, which could kill or sterilize adult *Onchocerca, Wuchereria* and *Brugia,* would allow a greater impact on the parasite reservoir than is possible at present, and perhaps even permit eradication. It would reduce the need for 14 years of vector control or for continuous distribution of ivermectin in onchocerciasis areas.

The discovery, screening and development of potential macrofilaricides is the responsibility of TDR's Steering Committee on *Macrofilaricidal Drugs for Onchocerciasis and Lymphatic Filariasis* (MACROFIL). In drug discovery, novel drug targets are being uncovered by identifying potentially lethal molecular targets in filariae and then cloning and expressing the genes for these targets. In this way it is hoped to generate a screening assay capable of fast and reliable screening. In drug screening and development, TDR supports primary compound evaluation in two centres, contracts out secondary preclinical evaluation, and involves a network of centres in endemic countries in clinical evaluation. During the biennium, two new drug analytical laboratories were established and validated for good laboratory practice.

A computer database developed for use by the three chemotherapy projects in TDR (including MACROFIL) allows rapid retrieval of data relating to chemical structure and biological test results for all compounds tested in antifilarial, antimalarial, antitrypanosomal and antileishmanial assays. The database is of great value in drug screening and development work. During the biennium it was revised and programme modifications were initiated.

Objectives of MACROFIL include bringing to field use, as quickly as possible, drugs with macrofilaricidal activity. The possible development of resistance to ivermectin in onchocerciasis and its early detection are also being studied. A number of compounds, ranging from drugs already in clinical use to drugs still in the preclinical stages of development, are being investigated.

Drugs already in clinical use for filariasis

Ivermectin

Macrofilaricidal activity

As well as its clinical effect on microfilariae, ivermectin also has some action against adult *Onchocerca* worms.

Following reports[8,9,10] that treatment with multiple standard doses of ivermectin (150g/kg) reduces the fecundity of adult *Onchocerca* worms, or perhaps brings about their premature death, by up to 30%, clinical studies have been undertaken to investigate the possible macrofilaricidal action of ivermectin at higher than standard doses. Results from these trials became available during the biennium:

- Single doses of up to 800g/kg, and multiple doses of 950g/kg or 1600g/kg within a 2-week period, although as safe – in terms of side effects – as the single standard dose, have no greater effect on viability of adult worms than the single standard dose (150g/kg). The studies have therefore been discontinued, although the effects of multiple standard doses are still being monitored during routine community treatments.

Prophylactic activity

Another aspect of ivermectin currently of interest is its potential use as a prophylactic:

- When monthly doses of ivermectin were given by injection at two dose levels (200 and 500g/kg) in the *Onchocerca ochengi*/cattle model, all treated animals exposed to natural infections were protected over a 20-month period. Control animals developed nodules and microfilariae in the skin.

Optimal dosing regimens for prophylaxis with ivermectin, as well as with alternative avermectins/milbemycins (e.g. moxidectin), will now be determined in the cattle model. A clinical protocol to study the possible prophylactic effects of ivermectin in human onchocerciasis has also been developed.

Resistance

For drugs in clinical use, there is always the possibility that drug-resistant strains of parasite will develop. As ivermectin is the only drug now in use for control of onchocerciasis, and most control programmes are based on its use, the appearance of resistance would be a major problem.

Ivermectin resistance has already occurred in a number of gastrointestinal nematodes of veterinary importance, for which ivermectin has been in widespread use since 1981. The mechanisms by which nematodes become resistant to ivermectin, and the development of a diagnostic method to identify resistance genes in *Onchocerca* before resistance has become a problem, are therefore targets for MACROFIL research.

Initially, resistant mutants of the free-living nematode *Caenorhabditis elegans* and of the sheep parasite *Haemonchus contortus* were studied. Several resistance

genes have now been cloned and sequenced from these organisms, and the mechanisms of mutation studied. The most common mutation in ivermectin-resistant forms of *C. elegans* is a change in the properties of the amphids (chemosensory organs). The most common genes responsible for resistance in *C. elegans* and parasitic nematodes will next be identified in *O. volvulus,* with the aim of developing a diagnostic test for early detection of ivermectin resistance should it appear in *O. volvulus*. In the meantime, special attention is being paid to any apparent failures in treatment of onchocerciasis with ivermectin.

Drugs in the clinical stages of development

Amocarzine

This compound was one of several analogues patented by Ciba Geigy in 1981. Ciba Geigy has now terminated all work on the compound and, in early 1995, all documentation relating to amocarzine and all remaining drug supplies (with the shelf life extended to June 1996) were transferred to WHO. Any future development of the compound will therefore need to be carried out by the MACROFIL project.

Following a review of all chemical, preclinical and clinical data in the Ciba documentation, two further clinical trials have been undertaken, both employing maximum doses of amocarzine at 3mg/kg twice-daily after food – a dose earlier found to be optimal in studies in Latin America:

- A Phase I clinical safety and pharmacokinetic study in uninfected volunteers was carried out in Bombay using doses up to the optimum (3mg/kg, twice a day, for three days). As observed in previous onchocerciasis trials at high doses, the drug caused mild to moderate dizziness, which was dose-related, indicating a direct effect on the central nervous system. A protocol has now been prepared for a Phase II study to evaluate the effects of amocarzine on patients with Bancroftian (lymphatic) filariasis. Phase II studies will be carried out at two centres in India after government approval has been obtained.
- A Phase II study against onchocerciasis in Africa – employing the optimal dose (3mg/kg, twice a day, for three days) – was undertaken in 67 (out of a total of 100) patients at the Onchocerciasis Chemotherapy Research Centre in Ghana – to examine the safety and efficacy of the optimum dosing schedule on a forest strain of *O. volvulus*. Macrofilaricidal activity was ascertained (by histopathological examination of fixed and stained nodule material) following excision of nodules four months after treatment. In addition, in order to distinguish the adverse effects, if any, of amocarzine from the adverse effects of an amocarzine-induced Mazotti reaction, one third (34) of the patients were pretreated with ivermectin a week prior to treatment with amocarzine (to allow any immune reaction to large numbers of dead microfilariae to occur before treatment with amocarzine). The remaining one third of patients (33) were treated only with ivermectin. No unacceptable adverse effects were recorded in any patient, although the usual adverse effects related to the Mazotti reaction were seen at the time of dosing with either ivermectin or amocarzine. However, no macrofilaricidal activity was seen in amocarzine treated patients, even when combined with ivermectin. In view of this negative result, no further trials of amocarzine will be carried out in Africa.

Suramin

A further clinical trial of this compound for onchocerciasis has been completed in Nigeria. Doses optimized according to the pharmacokinetic responses of patients to the first drug dose were compared with the WHO recommended multiple-dose schedule. The sera have also been analysed for secondary parameters of macrofilaricidal action in order to develop indirect assays of macrofilaricidal action of potential drugs.

Drugs in the preclinical stages of development

UMF 078

This drug is a benzimidazole compound previously shown to have good macrofilaricidal activity by both oral and muscular routes in the *Brugia*/dog model. In 1996, its efficacy was examined in the *Onchocerca ochengi*/cattle model. Results indicated that single intramuscular doses of the drug (at 50mg/kg or 150mg/kg) are macrofilaricidal and that a single oral dose (150mg/kg) is partially macrofilaricidal. The minimum dose level and number of doses required to give complete macrofilaricidal activity will be determined in future trials. The *O. ochengi*/cattle model for onchocerciasis is the best available animal model for onchocerciasis, and the results with UMF 078 are therefore extremely encouraging.

UMF 078 exists as two enantiomers, and each may have a different efficacy or toxicity profile. All work to date has utilized a racemic mixture but the two enantiomers are now being produced by chiral column technology so that the biological properties can be studied.

Successful completion of preclinical toxicological studies will allow Phase I clinical studies to be initiated in 1998 – and, if all goes well, registration of UMF 078 could be achieved by 2002.

WR 251993, WR 129577, PD 105666

During the biennium, these three potentially macrofilaricidal compounds selected from MACROFIL screening activities all gave negative results in bacterial mutagenicity studies. Now they are being evaluated in the *O. ochengi*/cattle assay. WR 129577 is a thiadiazole compound, WR 251993 a bishydrazone, and PD 105666 a trifluoromethylguanidinopyrimidine.

Further development of any of these compounds is dependent upon good efficacy and lack of toxicity in the cattle model.

Onchocercal blindness: soon to be consigned to the history books?

Modelling

Control programmes for onchocerciasis have benefitted greatly in recent years from the development of the ONCHOSIM model – a computer programme which has been used extensively to inform decision-making (for example, about whether to stop or resume control activities). ONCHOSIM simulations have indicated:

- what the long-term impact of a macrofilaricide would be and whether the eradication of onchocerciasis would be feasible – eradication being defined as 'the attainment of an epidemiological situation in which the possibility of recrudescence of the disease after cessation of control is less than 1%';
- that, on the basis of ivermectin being predominantly a microfilaricide with some macrofilaricide activity (quantified at around 30%), it would take up to 50 years with annual ivermectin treatment alone at a coverage rate of 65% of the total population (typical for coverage with annual treatment in the OCP areas), or more than 15 years with 6-monthly treatment, to achieve eradication;
- that, when compared to ivermectin, a dramatic reduction in the required duration of control can be realized even with a drug which kills only 60% of adult worms;
- that, at a population coverage level of 65%, four years of 6-monthly treatment would be required for a drug which kills 90% of adult worms, and seven years of treatment with a drug of 60% efficacy;
- that, if certain population groups were to be excluded from the control programme – if for instance the drug were accompanied by severe side effects which would lead to systematic non-compliance at later treatment rounds – then eradication would not be attainable within a reasonable period of time.

In conclusion, if eradication is the primary object of control, there is a definite role for a macrofilaricide in addition to ivermectin. But this drug should not have major contra-indications which would lead to the systematic exclusion of certain population groups or be accompanied by severe side effects. These operational aspects are perhaps more important for the feasibility of eradication than are improvements of say 5-10% in the macrofilaricidal efficacy of the drug.

Ivermectin resistance in onchocerciasis

The ONCHOSIM model has also been used to study the result of introducing ivermectin resistance genes into the *O. volvulus* population. The outcome of such simulations is very dependent upon whether the resistance gene is an autosomal recessive or a dominant gene and, to some extent, is also dependent upon the percentage of the human carrier population which is treated annually with ivermectin.

A dominant resistance gene would spread rapidly through the worm population even when the initial frequency of the resistant allele is low, whereas a recessive allele requires a greater number of resistance genes in the population before a recrudescence due to resistance occurs.

All simulations, whether for recessive or dominant genes (usually assuming about 65% coverage), suggest that ivermectin resistance in onchocerciasis should not become a serious clinical problem until 20-30 years after drug treatment is initiated.

However, in all cases the conversion from an ivermectin-sensitive to a resistant worm population occurs when the number of detectable microfilariae in the skin is very low (<5/skin snip) and when the incidence of infection is also low. Thus detection of microfilariae of resistant phenotype will be very difficult, particularly when the gene is recessive, and 'molecular probes' for resistance genes or their products in individual microfilariae become essential for early detection of ivermectin resistance.

Strategic Research

See Strategic Research section in chapter 6.

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CHAPTER 8

Leishmaniasis

Applied Field Research

- ✓ a prediction instrument for community surveillance and screening of cutaneous leishmaniasis has been developed
- ✓ there is a tendency for infected women to wait longer than men before seeking help, and thus to be more difficult to treat because the disease is further advanced

Product Research and Development

- ✓ a single injection of killed *L. major* + BCG is safe; a trial to ascertain its efficacy against both zoonotic and anthroponotic cutaneous leishmaniasis is almost completed, and one against visceral leishmaniasis has just begun
- ✓ a preparation of *L. amazonensis* is ready for clinical trial against zoonotic cutaneous leishmaniasis in the New World
- ✓ a collection of recombinant molecules is being tested with a view to composing a 'cocktail' second generation vaccine
- ✓ topical aminosidine cured 75-83% of cases of cutaneous leishmaniasis within 2-3 months in a focus of *L. major* transmission
- ✓ injectable aminosidine, alone or in combination with antimony, has been shown to be effective against visceral leishmaniasis without major side effects
- ✓ miltefosine, an alkyl lysophospholipid with antileishmanial activity which is used orally for treatment of cancer, is to be tested for use in visceral leishmaniasis
- ✓ allopurinol was totally ineffective in a rigorously conducted and monitored double-blind, randomized, controlled trial in Colombia

Strategic Research

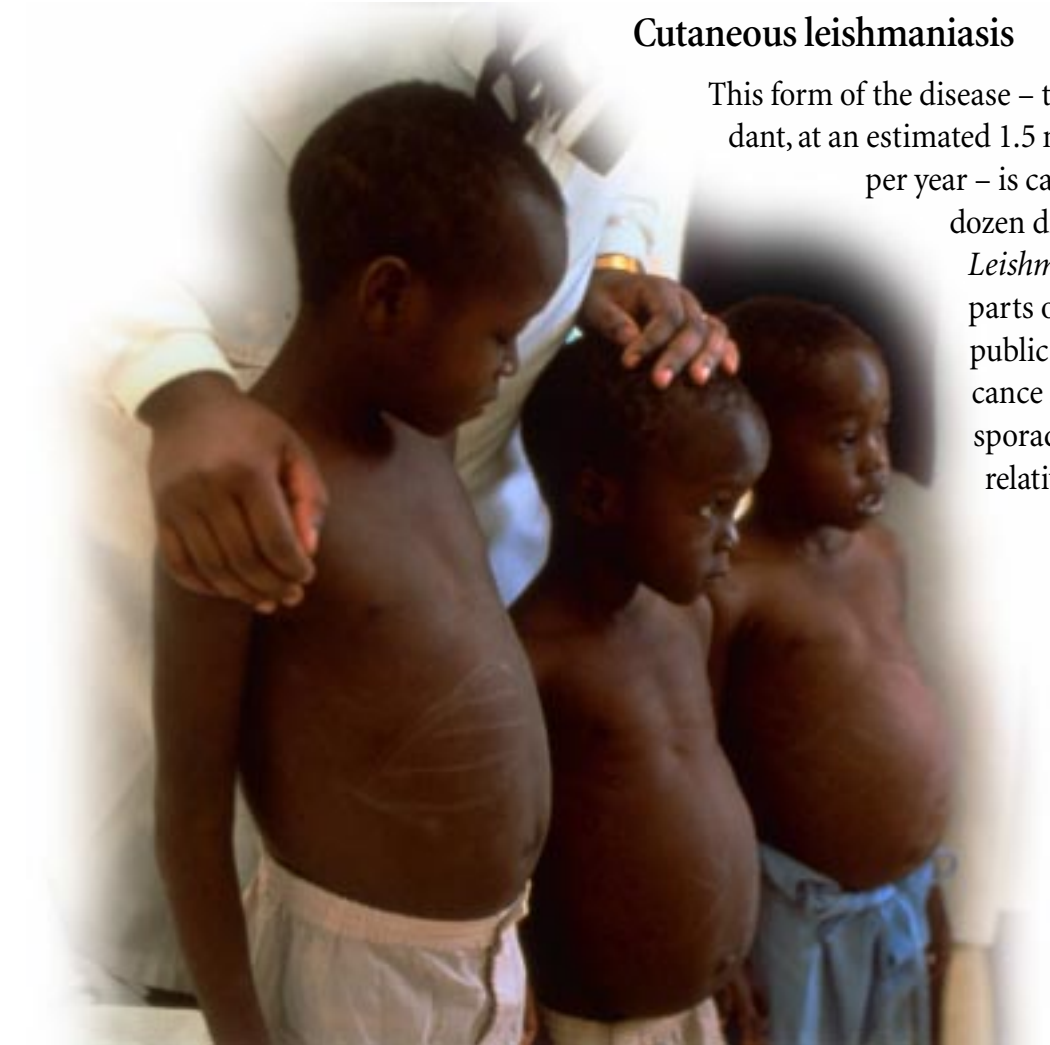
- ✓ a single strain of *L. major* is being used to unravel the genome of *Leishmania* – which will probably be the first (of the TDR parasite genomes) to be completely mapped and sequenced
- ✓ there are indications that susceptibility to leishmaniasis, in a mouse strain with high susceptibility to *L. major,* can be averted by inhibiting the pathogenic Th-2 response to a single antigen

Over 20 different species of the genus *Leishmania* are known to be pathogenic for humans. They are all transmitted by sandflies, by species of *Lutzomyia* in the Americas and species of *Phlebotomus* in the rest of the world (Europe, Asia, Africa).

The leishmaniases are divided into three general clinical patterns according to the form of the disease – cutaneous, visceral, and mucocutaneous. Transmission may be anthroponotic (from one human to another) or zoonotic (from animal to human).

Cutaneous leishmaniasis consists of skin ulcers which vary in number and are often self-healing. In diffuse cutaneous leishmaniasis, the immune system fails to act effectively and skin lesions, which are difficult to treat, develop over a large part of the body. Mucocutaneous leishmaniasis is a condition which appears subsequent to cutaneous leishmaniasis; it causes much destruction of the mucous membranes in the nasopharyngeal region. Visceral leishmaniasis is the most serious condition; it causes fever, weight loss, emaciation, and enlarged liver and spleen and is lethal if untreated. Post-kala azar dermal leishmaniasis is a condition which develops after visceral leishmaniasis and which manifests as nodules on the skin. Together these conditions accounted for the loss of 2.1 million Disability Adjusted Life Years (DALYs) in 1990.[1]

Treatment of leishmaniasis is unsatisfactory in the sense that the existing drugs require repeated parenteral administration and none of them is effective in all cases or is totally without side effects.



Cutaneous leishmaniasis

This form of the disease – the most abundant, at an estimated 1.5 million new cases per year – is caused by over a dozen different species of *Leishmania* in different parts of the world. The public health significance ranges from sporadic cases of relatively insignificant

public health importance to hyperendemic regions where almost all individuals suffer from the disease by the age of 12.

Cutaneous leishmaniasis (CL) is generally most feared in Latin America because of its association with mucocutaneous leishmaniasis, a chronic destructive condition which is difficult to treat. In hyperendemic areas there is general awareness of cutaneous leishmaniasis and many infected people do not seek treatment at all. This concurs with WHO recommendations for zoonotic cutaneous leishmaniasis, since many cutaneous lesions heal spontaneously and the patient is subsequently protected against infection.[2] However, with anthroponotic cutaneous leishmaniasis, infected humans constitute the reservoir of infection and treatment of patients is required in order to control the disease and block transmission.

On the other hand, many patients demand treatment, even in areas hyperendemic for zoonotic cutaneous leishmaniasis, obliging physicians to prescribe costly, parenteral, antimonial injections which are associated with some toxicity.

Other treatments include surgical removal of lesions, cryotherapy and measures which have not been fully established as being effective. In this regard, a form of simple, non-invasive treatment is urgently required (see below).

Visceral leishmaniasis

This potentially fatal form of the disease has more severe public health consequences than cutaneous leishmaniasis. It is known as ´killer disease` in Sudan where it is estimated to have killed more than 50 000 individuals in the southern part of the country between 1991 and 1993. Although this epidemic now seems to have subsided in its original site (the Upper Nile region), it is still raging in some other parts of Sudan. In the Indian subcontinent, where the disease is called ´kala azar` and where it has been endemic for many decades, the estimated mortality is 50 000-200 000 per year, especially during epidemics. The global estimate for new cases is 500 000 per year. Visceral leishmaniasis (VL), if untreated, is lethal in 100% of cases with developed signs and symptoms. Even with treatment, up to 15% mortality is common in some parts.

There is evidence that cases of visceral leishmaniasis with few symptoms occur in endemic areas and cure spontaneously without treatment. These individuals are presumably immune to reinfection and live a normal life unless their immune system is suppressed as a result of cancer therapy or other infections such as HIV, in which case the visceral disease will reappear. In fact, in the Mediterranean region, visceral leishmaniasis has become a problem since the advent of HIV, even in individuals with no recorded history of visceral leishmaniasis.

Following recovery from visceral leishmaniasis, or sometimes concomitant with it, the skin disease post-kala azar dermal leishmaniasis (PKDL) can develop. It is believed that PKDL is an important reservoir of anthroponotic visceral leishmaniasis in endemic foci.

Mucocutaneous leishmaniasis

With the advent of different methods of identification and characterization of *Leishmania*, it has been shown that the mucocutaneous disease may be caused by a variety of *Leishmania* species in the Latin American subcontinent (*L. braziliensis* was originally thought to be the only causative agent). However, mucocutaneous cases are seldom seen elsewhere.

This form of the disease may develop soon after cutaneous lesions heal or as long as 20 years afterwards. The condition is characterized by very few *Leishmania* organisms in the patient – it is a degenerative inflammatory disease of the nasal and oral mucous membranes, extending to the pharynx. The disease is associated with the same stigma as leprosy, due to its presentation caused by degeneration of the nasal septum and nasal-oral cavity. Treatment is very long and reconstructive surgery is required before ex-patients are accepted back into society. Patients are usually poor and refer late for treatment, by which time the destruction caused is considerable.

Fortunately the incidence of mucocutaneous leishmaniasis is low – it has been estimated as 1-2% of those who suffer from cutaneous leishmaniasis caused by *L. braziliensis*. In some societies the incidence can reach 5% or even 10%, and there may be genetic predisposition to the condition (although certainly the causative organism is also important).

Controlling leishmaniasis

Depending on the epidemiological setting, transmission may be domestic, para-domestic or sylvatic, and, as mentioned, the reservoir may be infected individuals or wild/domestic animals. Whereas it is possible to control the disease by early case detection and treatment when the reservoir is infected people, it is not possible to control the disease when the reservoir is a wild animal. And whereas vector control is feasible for domestic and para-domestic transmission, in sylvatic leishmaniasis vector control measures have not been successful.

All in all, the control of leishmaniasis of different forms requires infrastructures that are beyond the means of many countries, being costly and perhaps having adverse effects on the environment. There have been occasional success stories in the control of leishmaniasis – with the highly determined and organized programmes of some countries (such as those in Saudi Arabia and China). But the return of the disease following the relaxation of control measures indicates the need for better and sustainable measures.

Applied Field Research

Risk factors and self-protection

A study on reducing contact between humans and vectors by techniques suitable for use in the community is being carried out in Bolivia, in a very active focus of *L. amazonensis* transmission (21 of 95 inhabitants had active lesions). It is likely that the local vector (*Lutzomyia nuneztovari anglesi*), which lives in coffee plantations, flies to houses to feed as no significant genetic differences have been found in the vectors collected in these different habitats. Transmission in the area is almost exclusively domestic, and the vector also probably transmits *L. braziliensis*. Currently the use of deltamethrin-impregnated screens mounted at doors and windows to control entry of the vector is being evaluated, since the inhabitants do not use bednets.

Autodiagnosis of leishmaniasis by communities

In a study in Colombia, a historical clinical prediction rule for cutaneous leishmaniasis was developed with volunteer health workers. This has now been adapted into an instrument for community surveillance and screening of the disease. The instrument is to be used by community health workers with a low level of education, who read little and whose principle means of communication is the radio. The instrument consists of a colourful cubic box with 10cm sides, which includes variables for sandfly contact, history of trauma at lesion site, and activities which increase the risk of infection – information which is sought verbally – as well as lesion characteristics (whether they are grouped, where they are situated, and whether or not they have raised margins) – information which is obtained by observation. Methodology for applying the instrument and for functioning of the epidemiological surveillance network has also been worked out – one day per month has been allocated for leishmaniasis consultations at each health post, to which health volunteers send prospective patients.

Gender and leishmaniasis

There is growing evidence that data on the impact of tropical diseases by sex are frequently underestimations as far as the number of women infected is concerned. This is because of gender-related inequalities with respect to access to modern health services, preferences for alternate therapy or self-treatment, negative experiences at the health centre, or lack of money to purchase drugs.

A study on sex differences in cutaneous leishmaniasis in rural Colombia[3] demonstrated that the failure to focus on possible gender differences had created an inaccurate picture of the disease epidemiology in the area.

The statistics from the Ministry of Health, based on cases registered at health centres, showed that 65% of patients were men, and it was assumed that this was because they worked in the forest where the disease was thought to be transmitted. In fact, domestic transmission of leishmaniasis was not believed to exist. A community-based study in ten 'departamentos' of Colombia found, however, that these assumptions were false. Almost equal numbers of men and women were found to be infected, with slightly more female patients than males, although the differences were not statistically significant.

Interestingly, too, evidence for domestic transmission of the disease was found – there were high rates of infection among children who had never been to the forest as well as among women who stayed in the community. Moreover, different health-seeking patterns were found for men and women. Because men are more mobile, they frequently purchase drugs for treatment of household members when they go to the city – and they were found to be considerably more active in seeking modern treatment than women. Women tend to rely on self-treatment or to seek traditional cures more often than men. For women, the disease is more advanced by the time they go for treatment – they wait for almost one month more, on average, than men.

In Costa Rica, it was also found that women go to clinics for leishmaniasis treatment as a last resort,[4,5] preferring to try traditional methods of treatment such as plants, ointments, very hot baths with salt or wax, or burning with acid. The tendency for women to wait longer before going for help, and thus to be more difficult to treat because the disease is further advanced, was also noted with respect to malaria in Colombia.[6]

Product Research and Development

Activities here concern the control of leishmaniasis. Due to the complexity and variety of epidemiological settings for leishmaniasis foci, it is not possible to develop universally adaptable control measures, except perhaps for a vaccine.

Vaccine development

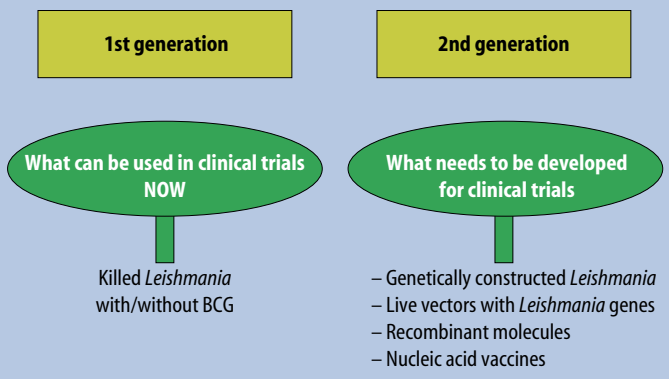
Vaccines are more cost-effective than treatment with antimonials. A rough estimate of the cost-effectiveness is shown in Table 1.

Based on this consideration and because the available drugs are inadequate (they are toxic, costly, and require repeated injection), TDR has emphasized vaccine and drug development. Currently the emphasis of the *Steering Committee on Vaccines for Leishmaniasis* is on the development of two types of vaccine – first and second generation vaccines.

First generation vaccines consist of killed *Leishmania* organisms mixed with a low concentration of bacille Calmette-Guérin (BCG, the vaccine against tuberculosis) as adjuvant. These are currently undergoing field trials for efficacy.

Second generation vaccines, which consist of genetically reconstructed *Leishmania* parasites incapable of producing disease, recombinant molecules or their corresponding DNA mixed together in a cocktail vaccine, or recombinant organisms carrying leishmanial genes, are currently undergoing predevelopment (Fig. 1).

Fig. 1 Vaccines against leishmaniasis



First generation vaccines

The injection of whole killed *Leishmania* organisms (leishmanin skin test) at very low doses (5-25µg) is used both in diagnosis and in epidemiological surveys. By this means, cell-mediated immunity in a delayed-type hypersensitivity skin reaction is detected and evidence of previous infection or exposure provided. Since the procedure has been used for decades, first generation vaccine tests commenced with larger doses of the antigen (leishmanin).

Earlier studies in mice indicated that, under certain conditions, whole killed *Leishmania* given subcutaneously can induce a harmful Th-2 type of response, which causes an exacerbation of subsequent infection.

Table 1. Cost-effectiveness of vaccines compared to antimonials[7]

Old World zoonotic cutaneous leishmaniasis	Cost (US$)	DALYs saved	Cost/DALY saved (US$)
Antimonial treatment (cost US$12-20 for mild disease; US$101-181 per course for others)			
– all cases treated	516 489–922 386	72	7173–12 811
– only very severe cases treated	70 205–125 813	44	1596– 2859
Vaccine (80% coverage with EPI, 50-90% effectiveness, cost US$0.5-2.0 per child)	4855–19 420	32–61	80–607
Old World anthroponotic cutaneous leishmaniasis			
Antimonial treatment (cost US$101-181 per course)			
– assuming passive case-finding	302 079–541 349	200	1510–2707
Vaccine (80% coverage with EPI, 50-90% effectiveness, cost US$0.5-2.0 per child)	4855–19 420	81–153	32–240

However, if the same whole antigen is given with interleukin 12(IL-12), or without IL-12 but intravenously or interperitoneally, then a protective Th-1 type immune response is induced. Therefore, to avoid inducing a Th-2 type response and since BCG is a strong inducer of cell-mediated immunity, it was decided that whole killed *Leishmania* mixed with BCG as adjuvant would be tested.

Phase I-II trials

The vaccine (killed *L. major*) was produced at the Razi Vaccine and Serum Institute, Iran, according to Good Manufacturing Practices. Phase I-II clinical trials for safety and immunogenicity began with small doses of killed parasite as vaccine mixed with different doses of BCG as adjuvant. These vaccines were tested in small groups of 10-20 adult male volunteers and the various side effects and immunological responses monitored. The immunological responses tested were the skin test to leishmanin (produced at the Pasteur Institute, Iran) and *in vitro* cytokine production (interferon-γ and IL-5) in response to leishmanial antigen.[8]

Phase III trials

Multicentre trials of a single injection (1mg of vaccine + one-tenth of the dose of BCG used for vaccination against tuberculosis) began at the end of 1994 following a workshop with participants from endemic countries of South-West Asia. Two sites in Iran were chosen to test the vaccine against: a) zoonotic leishmaniasis in people of all ages, and b) anthroponotic forms of leishmaniasis in children; and one site in Pakistan to test the vaccine against zoonotic CL in adult males. Before initiating the trials, further safety and immunogenicity studies were performed in small groups of volunteers. The trials are double-blind and randomized; BCG alone is the control.

In Isfahan, Iran, 2600 individuals between 6 and 60 years of age and leishmanin negative were selected for the trial from over 5000 people screened.[9] After one year of follow-up, when over 200 cases had been diagnosed, a data-safety and analysis committee found no indication of exacerbation of the disease in vaccinated individuals and recommended that further safety and immunogenicity trials with two and three injections should go ahead as planned. The safety trial will finish in early 1997. Should multiple injections prove to be safe, then efficacy studies with multiple injections will be initiated. Results are expected after two years of follow-up.

With respect to the trial in Bam (Iran), where the incidence of the anthroponotic form of CL is about 2%, the 2-year follow-up is expected to be completed in early 1997.

First generation vaccines in the New World

Based on the encouraging results of immunotherapy using killed *Leishmania mexicana* plus BCG, a large-scale, 2-year trial is planned in Venezuela. The trial will compare killed *L. mexicana* plus BCG with BCG as control (this follows an earlier trial involving 16 000 individuals in five different vaccine recipient groups, including *L. braziliensis,* which had to be terminated early due to reduced incidence of the disease and logistic difficulties).

Another vaccine being pursued in Latin America is produced by treating *L. amazonensis* promastigotes with merthiolate and freeze/thawing. Phase I-II trials with this vaccine (triple injection without adjuvant) proved to be safe and immunogenic in more than 80% of individuals who received three injections, as judged by skin-test conversion, but further studies were held up when the vaccine was found to be unstable, even when kept frozen. Interestingly, just like leishmanin, which also undergoes hydrolysis, this vaccine is capable of inducing a skin-test conversion after being stored for over five years at 4°C. Several preclinical studies using different preparations of a *L. amazonensis* vaccine produced by Biobras, Brazil, showed that *L. amazonensis* promastigotes killed by autoclaving constitute a stable preparation which will be further developed.

Vaccines for visceral leishmaniasis

A non-human primate model for VL has been developed in Lucknow, India, at the Central Drug Research Institute, where langur monkeys are used to evaluate various vaccine and/or drug preparations for leishmaniasis caused by *L. donovani*.

In a preliminary experiment, three doses of autoclaved *L. major* (ALM) vaccine mixed with BCG (the same vaccine as used in clinical trials for CL) were shown to be protective against a lethal challenge with *L. donovani* amastigotes given intravenously. Three of four monkeys had very low or no parasites in their livers after challenge, and one had a low parasite load compared to the placebo group in which four of four monkeys had very high parasite loads.

Further experiments are now in progress to substantiate these preliminary findings, with larger numbers of monkeys. The observation is in line with the notion that, based on the tremendous antigenic cross-reactivity amongst different species of *Leishmania*, a single vaccine could prove protective against leishmaniasis caused by different species of parasite.

ALM is being tested, on a compassionate basis, against visceral leishmaniasis caused by *L. donovani* in the Quadaref area of Sudan. The results of an ongoing prospective study have indicated that individuals exposed to *L. major* may have some immunity against VL. This is another reason for testing the *L. major* vaccine against *L. donovani* infection. Results are expected in two to three years time.

Second generation vaccines

Second generation vaccines consist of avirulent, live *Leishmania*, bacteria or viruses carrying *Leishmania* genes, or are sub-unit vaccines.

Attenuated vaccines

It was shown in earlier experiments that attenuated live *L. major* can protect mice against challenge with virulent organisms. However, the mechanism by which organisms lost their virulence was not known and therefore there was always the chance of reversion to virulence.

Cutaneous leishmaniasis was described in Iran over 1,000 years ago. Generations have been living with it ever since.

Using gene-targeting tools developed in the past few years, an attenuated strain of *L. major* has been produced[10] which lacks the gene for dihydrofolate reductase-thymidylate synthase (DHFR-TS) and requires thymidine for growth. Very large doses of the parasite do not induce lesions in BALB/c mice – the most susceptible host known. In addition, a certain degree of protection is induced following inoculation of the live organisms in BALB/c and resistant mice.[11]

Although the delivery of a live-parasite vaccine to remote areas and the standardization of such a vaccine would pose some difficulty, it is of interest to test the feasibility of this approach for vaccine development. In collaboration with Beverley, Harvard Medical School (USA), and Olobo and colleagues at the Institute of Primate Research, National Museums of Kenya, the DHFR-TS^{-} organism is being tested in primate models for safety, immunogenicity and eventually, efficacy.

Other live vaccines (organisms carrying leishmanial genes) include *Salmonella* carrying gp63, the major glycoprotein on the surface of promastigotes,[12] and *Vaccinia* carrying gp46/M2.[13] Although the recombinant *Salmonella* was shown to colonize the intestinal lymphatics of dogs, protection against leishmaniasis was not found to be significant. The *Vaccinia* construct is being further tested in primates (outside TDR).

Sub-unit vaccines

It was decided some years ago that several selected molecules would be tested in combination as a single vaccine with a view to giving a higher chance of protection and overcoming the genetic restrictions of an immune response against a single molecule.

Those recombinant molecules, which have been shown to induce protection in experimental animals and are recognized by Th-1 cells from individuals who have recovered from leishmaniasis (and are presumed immune), will be mixed to constitute a 'cocktail' vaccine. Two laboratories will independently test the cocktail vaccines in order to select suitable components for a final vaccine. Laboratories in developing countries are also being strengthened for production of candidate recombinant molecules under Good Manufacturing Practices for future clinical trials.

As most of the protective molecules known today were identified serendipitously, it may be necessary to invest in active identification of new candidate molecules for inclusion in the final cocktail vaccine.

Nucleic acid vaccines

The concept of nucleic acid vaccination has received much attention in recent years. The advantage of this approach is the continuous expression of the antigen by DNA which is incorporated into the nuclei of recipient cells. Xu and Liew,[14] with support from TDR, have reported the first DNA vaccine for leishmaniasis using the DNA encoding gp63 of *L. major*. Further studies are required to enhance the protective activity of this vaccine.

The stability of DNA vaccine is another advantage over protein vaccines. The same 'cocktail' concept as for sub-unit vaccines is envisaged for DNA vaccines.

Drug development

Aminosidine

Topical aminosidine

An ointment containing 15% aminosidine (paromomycin) and 10% urea in white paraffin oil, developed by Neal and Murphy and produced by Farmitalia Carlo Erba Srl (now Pharmacia-Upjohn), has been tested against zoonotic CL.

In the first trial, treatment for two weeks with the ointment or with placebo (white paraffin oil) was tested in Tunisia[15] and Iran.[16] The results from the Iranian study indicated that 2-week treatment significantly enhanced parasite elimination at the end of therapy although in the Tunisian study, which was terminated prior to completion, the difference between the treated and control groups was significant only at the level of $p = 0.06$.

A subsequent trial was performed by Asilian et al[17] using a randomized, double-blind protocol which compared 2-week with 4-week treatment. The results of this trial indicated that treatment for four weeks significantly enhances parasitological and clinical cure compared with treatment for two weeks. These studies indicated that 40-45% of patients treated with placebo heal spontaneously within 50 days of starting treatment, and that an additional 35-38% of patients are cured by treatment with the ointment. Thus the total cure rate of treated patients after four weeks is 75-83%.

WHO does not recommend treating zoonotic CL with the systemic drugs currently available (antimonials) unless patients have more than four lesions or have lesions close to vital organs. But since such patients usually demand treatment, and physicians feel obliged to prescribe systemic treatment or other expensive treatments not yet known to be totally effective, the use of aminosidine ointment as first line treatment is advisable – provided a patient is not amongst those who require systemic treatment as recommended by WHO.

Injectable aminosidine

The first clinical studies in VL used a combination of aminosidine (AM) and sodium stibogluconate (Sb), a standard drug. Preliminary data indicated that AM at 12mg/kg/day + Sb at 20mg/kg/day for 21 days was effective in Bihar, India, where high failure rates to Sb alone for four weeks were increasingly being reported. A study comparing AM at 12mg/kg/day + Sb at 20mg/kg/day with AM at 18mg/kg/day + Sb at 20mg/kg/day, both for three weeks, versus Sb at 20mg/kg/day alone for four weeks, is completed and results are being analysed.

Aminosidine alone has also been tested in two studies, in two different locations in Bihar. Different dosages of AM (12, 16, 20mg/kg/day) for three weeks have been compared to Sb at 20mg/kg/day for four weeks. Results are being analysed – but preliminary data indicate that AM alone is significantly more active than Sb.

The results provided by these studies, as well as those from previous studies conducted by the manufacturer of aminosidine, have provided efficacy and safety data which could support the registration of the drug for visceral leishmaniasis in the near future.

As well, a clinical trial involving aminosidine in combination with pentavalent antimony is in progress in Peru, for the treatment of mucocutaneous leishmaniasis.

Allopurinol

Allopurinol alone was tested in a double-blind, randomized, placebo-controlled trial against CL in Colombia. A third group, which received treatment with systemic antimonials, was also included in the protocol. The trial was conducted with maximum scrutiny as the results of earlier trials with allopurinol alone for CL were conflicting and the outcome of treatment controversial. Results of the new trial showed unequivocally that there is no effect of allopurinol alone on the cure rate of CL in Colombia.[18] However, there are recent reports that allopurinol plus antimonials could have a profound therapeutic effect on chronic CL which is refractory to antimonial treatment.[19]

The design of the study and strict adherence to the protocol for evaluation of drugs against CL cannot be overemphasized. Much confusion in the literature stems from the inadequate design of clinical studies for a disease which is anyway self-healing in a few months. Double-blind, randomized, placebo-controlled trials are essential before any claim for efficacy of a drug against acute cutaneous leishmaniasis can be made.

Alkyl lysophospholipids

A group of four alkyl lysophospholipids has been identified with activity against *Leishmania donovani* in both tissue and mouse models. The compounds, developed by different companies, act selectively against membranes of cancer cells and *Leishmania*. They are known as ilmofosine, edelfosine, miltefosine and SR 62-834.

Miltefosine, an orally administrable agent which is in clinical trial for cancer, has been selected for a limited pilot trial at two centres in India to establish whether it has potential as a clinical agent for visceral leishmaniasis. Existing drugs are administered parenterally and the prospects of having an orally administered agent is attractive.

Strategic Research

Pathogenesis

In the human host, *Leishmania* parasites transform from promastigotes to amastigotes and multiply in cells of the mononuclear phagocyte system, cells which are part of the immune system and normally destroy foreign organisms. Although some forms of leishmaniasis are self-curing, the disease develops when the immune system fails to control the infection.

TDR has recently been supporting work in the areas of parasite virulence and drug/vaccine targets (Box 1).

Box 1. Pathogenesis research

Host immune mechanisms

- There is evidence that a single *Leishmania* antigen, known as LACK (leishmania homologue of receptors for activated C kinase), triggers a burst of IL-4 and plays a pivotal role in determining whether or not a mouse is susceptible to infection with *Leishmania major*.[20] When susceptible mice are made tolerant to LACK prior to infection (by transgenic expression of LACK in the thymus), they respond to *L. major* with Th-1 cells rather than Th-2 cells and are resistant to infection.

Parasite virulence

- Scientists in Brazil have demonstrated the presence of parasites in lymph nodes long before the development of local cutaneous signs of leishmaniasis. This indicates that *Leishmania braziliensis* spreads from the skin to the lymph nodes earlier than thought, an observation of great importance for the clinical examination of individuals with unclear symptomology.

Drug and vaccine targets

- The full-length *L. major* mrk1 gene, whose product is a kinase enzyme related to kinases in several other parasites, has been cloned and expressed. Evidence was obtained that this gene is essential for the cellular differentiation of promastigotes in the mammalian host.
- Instead of catalase and glutathione peroxidase, trypanosomatids rely on thiols for protection against reactive oxygen intermediates. The potent antioxidant ovothiol A, an important virulence factor of both amastigotes and promastigotes, has been investigated in *L. donovani* with the aim of identifying possible targets for drug development with regard to both *Leishmania* and *Trypanosoma* spp. The pathway for the biosynthesis of the compound has been partly elucidated along with possible inhibitors of the enzymes involved.
- Evidence has been obtained that a set of clustered genes in *L. donovani* which encode signal transduction receptors possessing adenylate cyclase activity (RACs), structurally similar to *T. brucei* enzymes, are likely to be involved in the switch from non-infectious to infectious stage of the parasite.

Genome

Two years ago a consortium of nine laboratories from the UK, France, USA, Canada and Brazil received TDR support to develop a low-resolution physical map of *Leishmania* parasites. Since that time, a number of stage-specific complementary DNA (cDNA) and large-fragment genome libraries have been constructed from a variety of *Leishmania* species. Although much work has been done on *L. infantum*, after thorough evaluation it was decided to focus on the *L. major* Freidlin strain, which is available to all participating laboratories as a standard. The main goal of this initiative is to sequence and map the entire *Leishmania* genome within five years. Thousands of cDNA clones have already been fingerprinted, representing 80-90% of the genome. More than 100 contigs (continuous DNA segments) are also now available for a shotgun approach to sequencing the entire genome. The 36 chromosomes have been distributed amongst networking laboratories for mapping and sequencing, and the first results are presented (Box 2). All data are available on the database page of the World Wide Web.

It is anticipated that *Leishmania* genome research will generate new data and innovative strategies for the control of leishmaniasis. The characterization of large collections of expressed sequence tags (ESTs) will be particularly valuable for understanding gene expression during the different stages of the parasite's life cycle, and will provide a large number of new and potentially unique targets for chemotherapeutic or vaccine attack.

Box 2. Genome research

- **Stage-specific cDNA libraries have been produced for *L. major* strain LV39. More than 1000 expressed sequence tags (ESTs) have been randomly sequenced – around 30% of them have known homologues in other organisms (mainly ribosomal proteins) while 70% are unique.[21]**
- **A Pulsed Field Gel Electrophoresis (PFGE) karyotype map has been created and a physical linkage map of Old World species of *Leishmania* completed. As a result, 43 genes, 66 ESTs and 177 anonymous markers are being used to delineate 36 chromosomes, giving a genome size of 35.5 Megabases (Mb). Whereas linkage groups appear to be conserved in Old World species of *Leishmania*, a number of loci are either absent or in different physical linkage groups in *L. braziliensis*.**
- **A low-resolution physical map of the entire *L. major* genome has been completed and a library of thousands of cosmid clones fingerprinted. Anchoring of fingerprinted cosmid contigs to chromosomes by end-rescue and EST content mapping is in the advanced stages.**
- **Medium-resolution restriction maps have been produced for chromosomes 1,2,3,5 and 6 of *L. infantum* strain LEM 1317, and regions of variable size close to the telomeres which are responsible for polymorphisms in chromosome size have been described.**
- **The *Leishmania* genome project has now embarked on a genome sequencing initiative. The 36 chromosomes have been distributed amongst the various laboratories involved in the network for sequencing and some of them will be completed in 1997.**

WWW site: http://www.ebi.ac.uk/parasites/leish/leishpage.html

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CHAPTER 9

Chagas Disease

Applied Field Research

- ✓ for vector control in Argentina, a combination of insecticidal paints and traditional insecticides is effective and acceptable, and decentralized strategies are more cost-effective than vertical strategies
- ✓ a Transmission Risk Index has been developed as a complementary method for assessing the impact of vector control
- ✓ communities in endemic areas of Argentina and Uruguay are very knowledgeable about Chagas disease and prefer local radio for messages about vector surveillance
- ✓ compulsory blood screening has been introduced in Mexico and Colombia where the prevalence of *T. cruzi* infected blood in blood banks is higher than that of HIV and hepatitis B
- ✓ for vector control:
  - insecticide-impregnated fabrics are proposed as a complementary method
  - paints containing fenitrothion have been adopted by the Brazilian control programme for use against two vector species that live near houses
  - standardized protocols have been developed for monitoring resistance of vectors to insecticides
- ✓ in Argentina, Brazil, Chile and Uruguay, house infestation by vectors has already been reduced below the minimum necessary for transmission of the parasite

Product Research and Development

- ✓ an inhibitor of sterol biosynthesis has been shown to clear acute and chronic infections in mice
- ✓ a randomized, placebo-controlled, multicountry trial with benznidazole produced negative seroconversion in 55.8% and 62.1% of schoolchildren aged 7-12 years in Brazil and Argentina respectively

Strategic Research

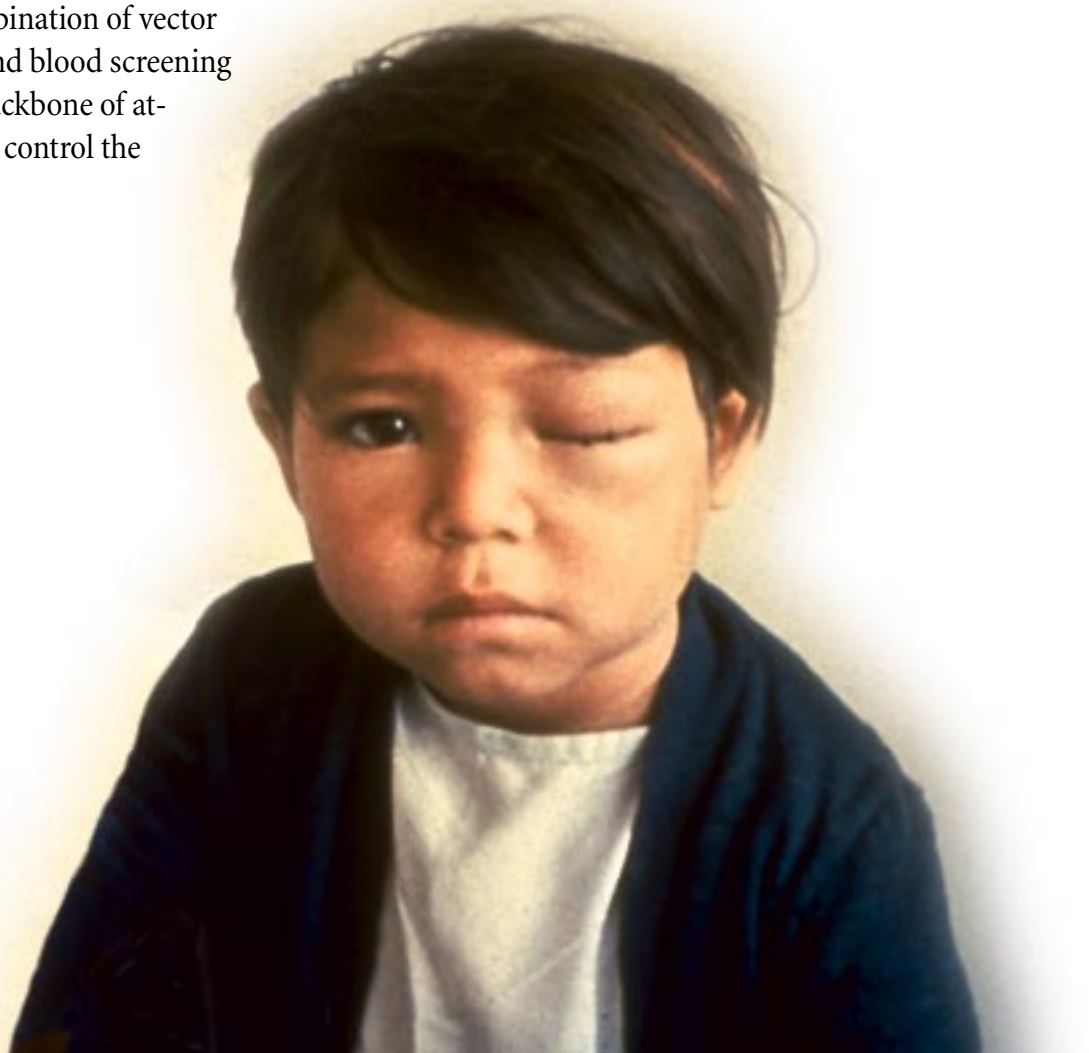
- ✓ a unique pathway has been identified in *T. cruzi* that could be exploited to reduce parasite viability and virulence
- ✓ ten laboratories in Latin America, Europe and the USA are involved in *T. cruzi* genome research
- ✓ two genes have been identified which are essential for parasite viability and virulence

As the third largest disease burden in Latin America – after malaria and schistosomiasis – Chagas disease accounts for an economic loss of US$ 6500 million, equivalent to 1.3% of the external debt of the whole of South America in 1993.[1] It caused the loss of 641 000 Disability Adjusted Life Years (DALYs) in 1990.[2]

Chagas disease is caused by *Trypanosoma cruzi,* for which many kinds of wild and domestic animals act as hosts and hence as reservoirs of the disease. Triatomine 'cone-nosed' or 'kissing' bugs are vectors of the disease. Transmission also occurs through blood transfusion (with infected blood) and congenitally. Treatment is available for acute stages of the disease only.

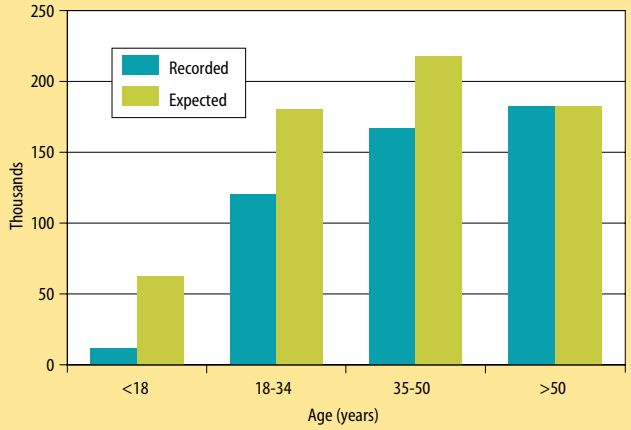
Between 16 and 18 million people in Latin America are estimated to have Chagas disease, of which 5-6 million have developed chronic complications, which are incurable. Twenty-seven per cent of chronically infected people develop cardiac lesions, 6% develop digestive disorders, and 3% develop peripheral neurological lesions. Chronic complications develop 10-20 years after the initial acute phase of the disease.

Control of Chagas disease is based on a combination of activities. It is not possible to eradicate *T. cruzi* since the parasite lives in such a large variety of mammalian hosts. However, tools such as insecticides, housing improvement and health education are available for interrupting the domestic cycle of *T. cruzi* transmission. Fumigant canisters and slow-release insecticidal paints are tools that have recently been developed for control of vectors. Strategies based on a combination of vector control and blood screening are the backbone of attempts to control the disease.



Progress towards the elimination of Chagas disease

Fig. 1 Morbidity by Chagas disease: prevented cardiomyopathy cases by age group, Argentina 1995



Major advances in the control of Chagas disease have been made during the biennium in the countries of the Southern Cone, following the initiative launched by Argentina, Brazil, Bolivia, Chile, Paraguay and Uruguay in 1991. The aim is to eliminate Chagas disease from the six countries; and epidemiological/entomological data indicate that this goal will be achieved by 2003. It is estimated that, when transmission of the disease through vectors and blood transfusion has ceased in the six countries, the incidence of the disease in the whole of Latin America will have been reduced by more than 70%.

In the countries of the Southern Cone, the main vector of Chagas disease – *Triatoma infestans* – lives inside houses (where dirt floors, thatched roofs, and cracks and crevices in walls shelter the bugs). In areas where the vector is not so 'domestic' (lives in the forest as well as in the home) the current focus of control activities is on infected blood in blood banks.

Since the beginning of the initiative in 1991, a total of US$ 206 million has been allocated from national sources of the six countries for control operations. It is estimated that this investment will reduce the economic loss due to Chagas disease by US$ 4550 million.

Impressive progress has been made since the launching of the initiative. Epidemiological and entomological data indicate progress in Argentina, Chile, Brazil and Uruguay – as reported in the TDR programme report for 1993-94. In Bolivia and Paraguay, where the control programmes began more recently, it is not yet possible to quantify the impact of operations.

Current data on house disinsectation, coverage of blood banks by screening, and serology in children, indicate that transmission of Chagas disease through vectors and

SOUTHERN CONE INITIATIVE – Elimination of transmission

Table 1. House infestation by triatomines, 1982-1994

Country	Rate (%)	Year	Rate (%)	Year	Reduction (%)
Argentina	24.5	1982	11.0	1994	55
Brazil	26.4	1982	4.8	1993	82
Chile	32.5	1982	3.2	1993	90
Uruguay	6.0	1983	0.6	1992	90

Table 2. Prevalence of human infection by *Trypanosoma cruzi*, 1981-1994

Country	Age group	Rate (%)	Year	Rate (%)	Year	Reduction (%)
Argentina	18 years	4.8	1983	1.2	1993	75
Brazil	7-14 years	4.5	1981	0.2	1994	96
Chile	< 15 years	9.1	1985	1.9	1995	80
Uruguay	< 12 years	2.4	1985	0.2	1993	92

Box 1. Argentina

The launching of an initiative by the Southern Cone countries in 1991 reinforced efforts already in progress to eliminate transmission of Chagas disease. Interruption of transmission is expected in 1998.

Since 1991, communities have been directly involved in all vector control activities. The Government has provided the necessary resources, such as 1 600 000 sensor boxes and 500 000 fumigant canisters for surveillance and control activities in 400 000 houses in 1994-95. The number of houses sprayed with insecticides has increased annually, from 80 000 to 110 000 in 1992, to 140 000 in 1994, to an estimated 170 000 in 1996. This is according to schedule. The next phase – surveillance – will begin in 1997, throughout all endemic areas.

The infestation of houses by vectors has been decreasing since 1982. In 1994, house infestation rates everywhere had been reduced by between 10.9% (in Neuquen) and 94% (in Jujuy). Across the country as a whole, house infestation had been cut by 55% (Table 1).

Between 1983 and 1993, the prevalence of *Trypanosoma cruzi* infection in 18-year-old males was reduced by 75% (Table 2).

The impact of control activities on morbidity can be clearly seen by comparing the actual number of Chagas disease morbidity cases with the number expected in the absence of control measures. In people of less than 18 years, a decrease of 81% is evident. In the age-groups of 18-34 and 35-50, decreases of 43.6% and 24.3% respectively are apparent (Fig. 1).

Monetary savings, estimated by combining the direct and indirect costs for the number of human cases prevented by the control programme, amount to US$ 2800 million – equivalent to one twentieth of the total external debt of Argentina in 1993.

Box 2. Brazil

In 1970, over 36% of Brazil was endemic for Chagas disease and 2493 municipalities (51% of all municipalities in the country) were infested with *Triatoma infestans,* the most important vector of the disease. A total of 49 million people lived in endemic areas, of whom 53% lived in rural areas.

Triatoma infestans is exclusively domestic. The infection rate of this vector with *T. cruzi* is higher than that of other triatomine species present in the same endemic areas, such as *Triatoma brasiliensis* and *Triatoma pseudomaculata,* whose vectorial capacity is much lower.

The national Chagas disease control programme was instituted in 1975 when the strategy adopted was house spraying with chemical insecticides. *Triatoma infestans* has been eliminated from houses in the state of São Paulo since 1982 and there have been no known acute cases or seropositive reactions in 1-4 year olds since 1983.

In 1983, 711 municipalities in endemic areas were infested by *T. infestans,* but in 1993 only 83 municipalities were infested – an 89% reduction in the decade.

In 1983, 84 334 *T. infestans* bugs were captured in houses by field workers of the national control programme. In 1995, only 1800 insects were found in 709 012 houses surveyed – or an average of 2.5 insects in every 1000 houses (0.0025 insects per house), *a house infestation rate far below the minimum necessary to ensure vectorial transmission of the parasite to people.*

In 10 of Brazil's 11 endemic states, reductions in house infestation rates observed between 1983 and 1993 ranged from 100% in Mato Grosso to 5% in Bahia. For the country as a whole, the average reduction in house infestation was 82%. Focal areas infested with *T. infestans* remain only in the states of Bahia, Goias and Rio Grande do Sul.

Seroepidemiological surveys carried out in 1993 in nine endemic states showed that, in eight of them, the incidence of infection in 7-14 year olds was less than 0.5%. This indicates a reduction of over 96% compared to 1980. In other words, transmission of the disease by vector has been virtually eliminated.

Similar trends have been observed in the proportion of *T. cruzi*-infected blood in blood banks between 1982 and 1992. In 1982, 6.5% of blood in the whole country was infected. In 1992, only 1% was infected.

By 1995, 98% of blood banks were covered by screening.

Vector control activities are now targeted towards eliminating *T. infestans* from the remaining focal areas in three infected states. It is estimated that this will be achieved in 1998.

blood transfusion will be interrupted in 1997 in Uruguay and Chile, and in 1998 in Argentina and Brazil. Interruption of transmission is expected in 2003 in Bolivia and Paraguay.

Tables 1 and 2 show the reductions achieved in the house infestation and human infection rates in children and young adults in Argentina, Brazil, Chile and Uruguay.[3,4,5,6]

The situation for Argentina is given in more detail in Fig.1 and Box 1 and, for Brazil, in Box 2.

An initiative by the Andean countries to control transmission through quality control of blood banks was launched in 1993. The countries involved are Colombia, Ecuador, Peru and Venezuela. The vector species in these countries are found outside houses in the natural environment, as well as inside, necessitating different vector control strategies to those used in the Southern Cone countries. These are being developed, tested and adapted to suit the local vectors.

Meanwhile, the initial focus is on controlling transmission through transfusion by instituting quality control measures in blood banks. The target in Colombia, Ecuador and Peru is to adapt existing universal blood screening infrastructure by 1997. This has already been achieved in Venezuela.

A further initiative is that of the Central American countries. This was also launched in 1993, and the countries involved are Costa Rica, El Salvador, Guatemala, Mexico, Nicaragua, Panama and Honduras. As in the Andean countries and for the same reasons, the initial emphasis is on ensuring screening in blood banks.

The target is to adapt existing universal blood screening infrastructures by 1998. This has already been accomplished in Honduras.

Box 3. Timetable towards the elimination of Chagas disease

- 1980-1985 - Cross-sectional studies on the prevalence of human infection and house infestation in nine countries.
- 1980-1985 - Standardization of serological techniques and the creation of a continental network of reference laboratories.
- 1984-1990 - Follow-up prospective studies on the course of human infection.
- 1987-1989 - Cloning of parasite genome and production of defined antigens for improved diagnostic techniques.
- 1990 - Industrial production of kits using defined antigens for control of infected blood in blood banks.
- 1988-1992 - Development of new tools for vector control (paints, fumigant canisters and sensor boxes).
- 1988-1993 - Multicountry field studies for evaluation of new vector control tools.
- 1991 - Industrial production of paints, canisters and sensor boxes.
- 1991 - Southern Cone initiative to eliminate vectorial transmission of Chagas disease.
- 1993 - Andean initiative for control of infected blood in blood banks.
- 1993 - Central American initiative for control of infected blood in blood banks.
- 1995-1998 - Evaluation of impact of control measures and projection of trends.
- 1998-2003 - Certification of interruption of vectorial and transfusional transmission in the Southern Cone countries.

Applied Field Research

Evaluating and improving vector control operations

Testing of new vector control tools

A multicountry field trial comparing fumigant canisters with insecticidal paints and traditional insecticides was completed in 1995 in Nicaragua, where the main vector is *Triatoma dimidiata*.

Insecticidal paint inside the dwelling and traditional insecticide around the outside of the dwelling was the most effective combination. This intervention remained 77.4% effective 21 months after the initial application. Insecticidal paint both inside and outside the houses was 56.6% effective after 21 months. The use of canisters inside the house and traditional insecticide outside was 5% effective after 21 months.

Two similar trials are currently being carried out in Colombia and Bolivia where the main vectors are *Rhodnius prolixus* and *Triatoma infestans* respectively.

Cost-effectiveness and acceptability of vector control tools

A study completed in Argentina in 1995 indicated that using a combination of insecticidal paint and traditional insecticides was acceptable to 94% of the population, provided that the inhabitants of the houses themselves participated in the actual application of the interventions.

A study in Argentina which compared vertical and decentralized strategies for vector control and surveillance, in the context of primary health care and community participation, showed that the annual cost of surveillance of a rural house is 3.5 times lower when the control activities are decentralized and involve appropriate technology.

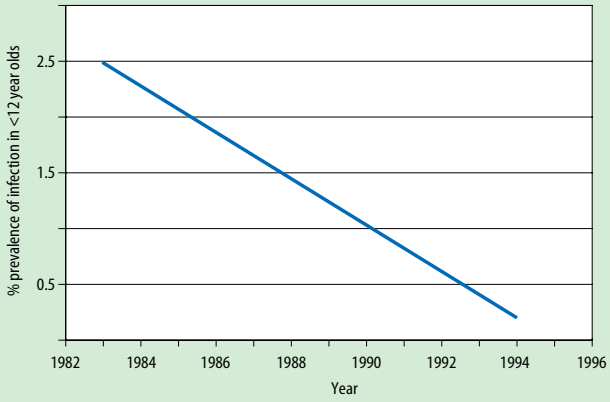
Seroepidemiological indicators for assessing vector control

A countrywide seroepidemiological survey of under 12-year-old children was completed in Uruguay in 1994. The prevalence rate was found to be 0.2%, which compares with 2.5% in 1983 (Fig. 2). Thus, vector control operations in Uruguay in the last decade have had a high impact on the transmission of Chagas disease, and it is expected that elimination of transmission will be certified in 1997.

Another similar study was completed in Chile in 1995. This study showed a prevalence rate of 1.9% in the under 15-year-old age group, which compares to 9.1% in 1983, indicating the imminent interruption of vectorial transmission in this country also[7,8] (Fig. 3).

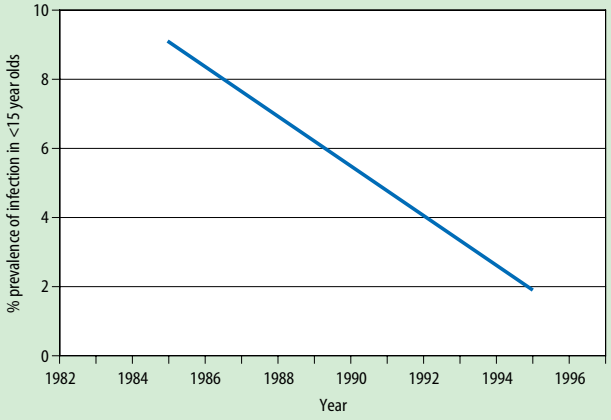
Southern Cone initiative

Fig. 2. Prevalence of *T. cruzi* infection, Uruguay 1983-1994



Source: National control programme, Montevideo 1995

Fig. 3. Prevalence of *T. cruzi* infection, Chile 1985-1995



Source: National control programme, Santiago 1996

Entomological methods for assessing vector control

Entomological indicators for evaluating the risk of human infection are the subject of study in Argentina. A transmission risk index (TRI) has been developed which estimates the probability of an infective triatomine bite per person per night. There is a linear correlation between the value of the TRI and the prevalence of infection in children. The TRI can be used as a complementary method to assess the impact of vector control activities.[9]

Geographical information system (GIS) software for evaluating triatomine control and surveillance has been developed in Argentina. It uses data that are routinely collected by the national control programme and offers several possibilities for graphical presentation and analysis. Use of the GIS has been incorporated into the regular management activities of the control programmes at provincial level, improving the planning and assessment of operations.[10]

Effect of the mass media on vector control and surveillance

Studies on the delivery of messages about vector control through the mass media are under way in a number of countries.

Initial data from a study in Argentina indicate that more than 95% of the populations in endemic areas have fairly good knowledge of the transmission of Chagas disease and the need for vector control to prevent the disease. For 93.5% of the population, the preferred means of communication is local radio. The next step is to develop education messages about detecting house infestation by bugs through the inspection of sensor boxes. The impact of the messages on behavioural change of householders will be evaluated.

A study in Uruguay has also indicated that local communities have a high level of knowledge about the transmission of Chagas disease and that the preferred means of communication is local radio broadcasting. The next step in this study will be to compare the impact of messages delivered by local radio with the impact of those delivered person-to-person through schools and community associations.

In connection with the mass media approach to vector control, a study on indigenous beliefs about household triatomine insects is being conducted in Bolivia.

Improving blood screening operations

Prevalence of *T. cruzi* infection in blood banks

Countrywide studies measuring the prevalence of *T. cruzi* infected blood in blood banks were completed in 1995 in Colombia and Mexico. The results are presented in Table 3 and show that the prevalence rates of infection of blood by *T. cruzi* in blood banks in both countries are higher than the prevalence rates of infection with HIV and hepatitis B. Screening for HIV and hepatitis B is obligatory in both countries and the findings have induced the respective ministries of health to issue regulations regarding compulsory blood screening for *T. cruzi*.[11]

Table 3. Prevalence (%) of infected blood in blood banks, 1995

Country	*T. cruzi*	HIV	Hepatitis B
Colombia	1.16	0.13	0.21
Mexico	0.8	0.08	0.48

Monitoring of quality control in blood banks

A study to monitor the quality control of rural blood banks is under way in Paraguay. Baseline data on the detection of *T. cruzi* in blood samples have been obtained – little agreement has been observed between the data produced in rural blood banks and those produced in the central reference laboratory. There will be a second round of evaluation after standardization of reagents and techniques has taken place.

Development of better methods and procedures for vector control

New products for vector control

Initial data from an extensive field study in Argentina to test the effect of pyrethroid-impregnated fabrics in bedrooms of rural houses show that, eight months after application, there is still a triatomicidal effect of 87%. Impregnated fabrics will be proposed as a complementary measure for keeping houses free of vectors.

In Crateus, State of Ceara, Brazil, insecticidal paints containing fenitrothion were tested in 3000 houses. The two vector species in this area – *Triatoma pseudomaculata* and *Triatoma brasiliensis* – live in and around rural houses, which they are permanently threatening to invade. The paints retained 83% efficacy 24 months after application, compared to 72% efficacy retained by the traditional insecticides used in the control group of houses. This makes paints containing fenitrothion more cost-effective than traditional insecticides, and they have since been adopted for use by the national Chagas disease control programme of Brazil in areas where these two vector species infest houses.[12]

Insecticide resistance

Insecticides constitute the active principle of all currently-used tools for controlling the vectors of Chagas disease. Although there are no biological or genetic data that indicate the development of insecticide resistance, the monitoring of these parameters is of concern to national control programmes.

Standard laboratory and field techniques and procedures to monitor the development of possible triatomine resistance to insecticides were developed at a technical meeting held in Buenos Aires.[13] The protocols will be used to periodically assess the effect of insecticides in all the control programmes of endemic countries, and will allow a comparison of results from different countries. Studies have begun in Argentina and Brazil.

Alternative vector surveillance methods

Observations on vector behaviour in domestic compounds, such as the way they explore food sources, has led to improved means of surveillance and detection of house infestation. Newly-designed devices baited with chemical, faecal and sexual attractants are currently being tested in Argentina.

Gender and Chagas disease

Although it is well known that the elimination of Chagas disease depends on a safe transfusional blood supply and on vector control, the surveillance of women with respect to possible congenital transmission of Chagas disease has yet to receive much attention. Urban areas of Latin America are non-endemic for Chagas disease, and studies completed in 1995-96 indicate that not only is there a problem of congenital transmission in these areas but that there is also a problem of recognition and treatment by urban health services personnel.

The studies (Box 4) indicate that congenital transmission of Chagas disease occurs among poor families who migrate from endemic areas to the cities to look for work. Although many of the migrant mothers were found to have knowledge of the vector, they were unaware of its connection to Chagas disease. Knowledge of the disease was found to be significantly lower in infected mothers than in uninfected mothers. In urban slum areas, where the health systems are oriented more towards disease symptomatology than towards prevention and control, knowledge about the disease was also found to be low among health services staff and among the general population.

There is therefore a need to educate poor marginal populations in urban areas, especially women, about the disease, and also to raise awareness among health services staff in these areas.

Box 4. Gender and Chagas disease studies

1. **A study of selected children and their mothers[14] has been conducted in marginal areas of Asunción, the capital city of Paraguay, where many families are migrants from the interior. The study was funded by the Latin American Small Grants Programme on Social and Economic Research on Tropical Diseases.**

- **A total of 953 children less than 13 years of age were screened for the disease, and of these, 13 children, or 1.4%, were positive for *T. cruzi* antibodies.**
- **Mothers of 11 infected children who could be located were also found to be serologically positive. Nine of the mothers had migrated from endemic areas to the city for work.**
- **Seven of the infected children had been born in Asunción; none had received blood transfusions; and none had travelled to an endemic area. It is likely, therefore, that congenital transmission was the mode of infection, at least for the children born in Asunción.**

The authors of the study conclude that control programmes in Latin America have focused mainly on vector control in rural areas and that little attention has been given to congenital transmission.

2. **A similar study[15] was carried out in marginal areas of the city of Rosario in Argentina. Results indicated that:**

- **Knowledge about Chagas disease in urban slum areas is low, both in the health services and the general population.**
- **Knowledge and information is especially lacking among women infected with Chagas disease.**

Participatory research with the community and health services, completed in 1996, has made both health services staff and their patients more aware of the problem and of the special needs of women. Service provision to chagasic women in relation to ante-natal care, delivery, and post-natal check-ups has been reorganized to help shorten the time women have to wait for treatment.

Product Research and Development

Drug discovery

TDR's Product Research and Development approach to Chagas disease is focused on drug discovery and is the responsibility of TDR's steering committee on *Drugs for African Trypanosomiasis, Chagas Disease and Leishmaniasis.*

Today, Chagas disease cannot be cured in its chronic stages, but during the biennium under review a novel compound was shown to clear parasites in mice. The compound, code-named D0870 and produced by Zeneca Pharmaceuticals, is an inhibitor of sterol biosynthesis and, as such, was first identified as an antifungal agent. Inhibitors of sterol biosynthesis also affect *T. cruzi,* which has similar steroid metabolism to fungi. Earlier *in vitro* studies showed that D0870 causes the parasite's natural sterols to be replaced by 14 a-methyl sterols. Results[16] show that the compound is able to cure a large percentage of both acute and chronic *T. cruzi* infections in mice – blocking parasite growth and reproduction and penetrating cells infected by the parasites in chronic infections. D0870 has also been shown[17] to be effective against six different strains of *T. cruzi* in mice as well as against *T. brucei* (responsible for African trypanosomiasis) *in vitro*.

Further development of D0870 will depend on its safety profile. The compound is in early clinical trials by Zeneca for serious fungal disorders.

Another drug, recommended for treatment of acute and congenital Chagas disease, has now also been shown to prevent the development of chronic cardiac lesions when used in the early chronic phase of *T. cruzi* infection. A randomized, double-blind, placebo-controlled, multicountry trial carried out in a rural area of the state of Goias, Brazil, showed that a 60-day course of benznidazole treatment of early chronic *T. cruzi* infection was safe and 55.8% effective in producing negative seroconversion of specific antibodies. The results are very encouraging and would justify the recommendation of treatment for seropositive children as a public health policy.[18] In Argentina and using the same protocol, the efficacy was 62.1%.

Will this young girl's future soon be free of the threat of Chagas disease?

Strategic Research

Pathogenesis

Work supported by TDR during the biennium has focused on what makes *T. cruzi* virulent and how this virulence can be reduced. We also need to understand how the parasite causes chronic lesions of the heart and digestive system. Highlights of recent studies on the pathogenesis of *T. cruzi* are shown in Box 5.

Box 5. Pathogenesis research

Drug Targets

- **Members of the family Trypanosomatidae, in contrast to all other eukaryotic cells, have only one pathway leading to the formation of monoglucosylated oligosaccharides, some of which appear to be involved in the process of penetration of the parasite into mammalian cells. Proper folding of proteins involves glucosylation using UDP-Glc-glycoprotein glycosyltransferase followed by deglucosylation using glucosidase II. Interference with glucosylation in *T. cruzi* has been shown to slow down intracellular transport (affecting the rate of exit of cruzipain, a lysosomal enzyme, from the endoplasmic reticulum of *T. cruzi*) and this unique pathway could therefore be exploited to reduce parasite viability and virulence.**

Gene regulation

- **Two genes, present in all members of the family Trypanosomatidae and associated with the calmodulin- ubiquitin (CUB) proteins, have been shown to be required not only for viability but also for virulence of *T. cruzi*. The gene products have been shown to be transcription activators and may therefore function as regulators of gene expression, which could explain why they are essential in parasite development and viability. The genes may also be needed to produce an infection which is successful.**

Genome

T. cruzi genome research was initiated in 1995 by ten laboratories in Argentina, Brazil, USA, UK, France and Sweden.[19] A striking feature of this parasite is the heterogeneity of its biological properties and genomic variability. Therefore one clone, the CL-Brener clone, which possesses all the important and typical properties of the parasite, was selected as the reference strain for study. The genetic material of *T. cruzi* is organized in small chromosomes which are poorly condensed during cell division, making impossible its analysis by conventional cytogenetics. The molecular karyotype of the CL-Brener clone has been analysed by pulsed field gel electrophoresis (PFGE), which allows the separation of chromosomal bands. A number of complementary DNA (cDNA) libraries have been constructed from different stages of the parasite's life cycle and chromosome mapping has begun. Small sections will be mapped initially with the development of the entire map in view, using contig libraries (Box 6). A database of all available information has been organized on the World Wide Web and is available for all interested scientists.

It is expected that, through the organized network, research activity will quickly expand and will lead to greater understanding of parasite-host interactions necessary for the development of new drugs and other means of Chagas disease prevention. The identification of novel genes which could be targets for chemotherapy, and determination of the molecular bases of virulence, pathogenicity and drug resistance of the parasite are particular goals. Thus, even though Chagas disease is now under control in many countries, the *T. cruzi* genome initiative is still very useful.

Box 6. Genome research

Characterization of the reference clone

- The biological characteristics (growth, differentiation, infectivity and sensitivity to chemotherapeutic agents) and genetic markers (through zymodeme, schizodeme, ribosomal DNA sequence, and Randomly Amplified Polymorphic DNA analysis and genomic DNA fingerprinting) of the CL-Brener clone have been defined. Stability of the clone seems to be good up to 100 generations.
- Karyotype analysis by pulsed field gel electrophoresis has indicated that *T. cruzi* has a complex karyotype and great plasticity of genome. There are large differences in size among genetically equivalent chromosomes from different parasite strains. Forty-two chromosomes have been identified in the CL-Brener clone and eight linkage groups have been recognized. Indications are that chromosomal rearrangement occurs in the *T. cruzi* genome at low frequency.

Library, contig and map construction

- cDNA, yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), and cosmid libraries for epimastigote, metacyclic trypomastigote and tissue-culture-derived trypomastigote forms of the CL-Brener clone have been constructed and gridded on filters.
- Contig assembly has begun and a number of chromosomes have already been partially mapped. Sequencing of chromosome 1 has started. About 40 chromosomal bands/haploid genome-identified markers for 30 chromosomes and linkage groups for 15 chromosomes have been discovered.

Gene identification

- Expressed sequence tags (ESTs) generated from the libraries are being used to construct a physical chromosome map. Sequencing has so far indicated that very few sequences match homologues in public databases.

Genomic sequencing

- Four laboratories are sequencing ESTs, initially from the epimastigote library but also from the metacyclic trypomastigote library.
- Chromosome specific sequencing has begun, specific chromosomes having been distributed among the different laboratories.

WWW site: http://www.dbbm.fiocruz.br/genome/tcruzi/tcruzi.html

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CHAPTER 10

African Trypanosomiasis

Applied Field Research

- ✓ the Card Indirect Agglutination Test for Trypanosomiasis, a simple and rapid diagnostic test for sleeping sickness, has been shown to be specific and suitable for assessing cure following chemotherapy
- ✓ a new branched DNA technique for diagnosing sleeping sickness has many advantages; its suitability for field use remains to be determined
- ✓ community involvement in diagnosis of sleeping sickness (using the Card Agglutination Test for Trypanosomiasis) and in tsetse control has been shown to be effective
- ✓ gender differences in sleeping sickness exist, especially as regards seeking treatment
- ✓ melarsoprol treatment for sleeping sickness has been shown to significantly affect growth and academic performance

Product Research and Development

- ✓ a novel route for producing eflornithine has been developed in India to help reduce the cost of the drug
- ✓ initial follow-up of a multicentre study indicates that the duration of treatment with eflornithine can be reduced
- ✓ a preliminary study indicates that the duration of treatment with melarsoprol can be reduced (efficacy as well as adverse reactions are unchanged)
- ✓ a promising diamidine compound is undergoing preclinical studies for treatment of sleeping sickness

Strategic Research

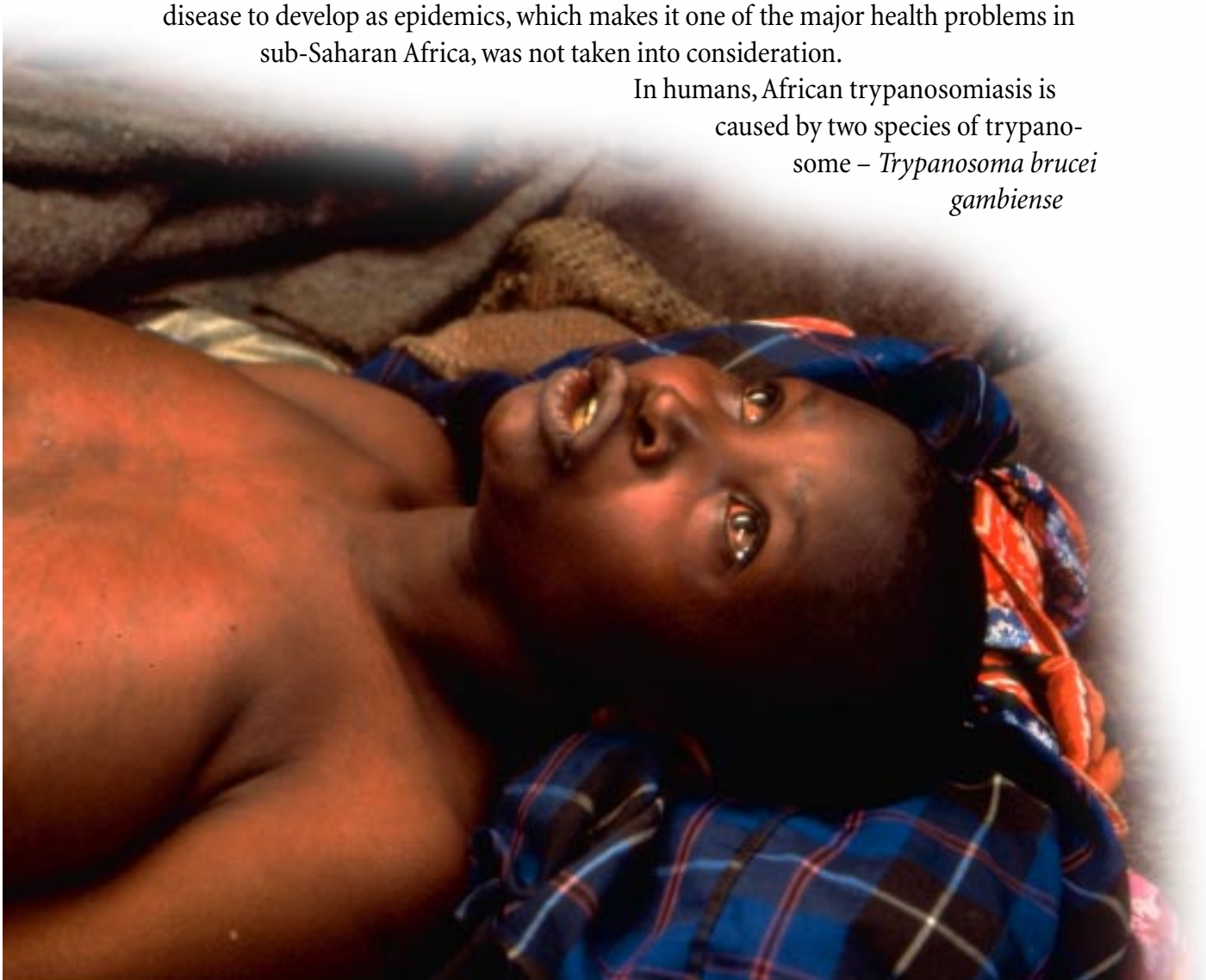
- ✓ monoclonal antibodies specific for Trypanosome Lymphocyte Triggering Factor may have potential for immunotherapy
- ✓ many new *T. brucei* genes have been discovered in the past two years – some have homologues in other organisms while others are likely to be unique

African trypanosomiasis (sleeping sickness) occurs in 36 countries in sub-Saharan Africa, where it is a public health problem with a major impact on social and economic development. In recent times there have been epidemic outbreaks in central and eastern Africa, especially in countries where there has been civil war or social upheaval. It has been estimated that about 300 000 new cases occur annually (a more realistic estimate than the previous one of 25 000).

The countries most severely affected include Zaire and Angola, where the reported prevalence in certain villages is 70-80%. Large numbers of people in these villages have either died or fled, abandoning their crops and domestic animals.[1] In Zaire, where the situation has been compared to that of 1930 (when an all-time peak of 33 562 cases was reported among a population a quarter the size it is today), 34 400 new cases were estimated to have occured in 1994. According to Ekwanzala et al,[2] the mortality rate is of the same order of magnitude as that caused by AIDS and the number of deaths in 1994 was at least 80 times higher than the total number of deaths recorded during the 1995 epidemic of Ebola in Kikwit. Other countries where there is recrudescence include Sudan, Congo, Central African Republic, Tanzania and Uganda.

The burden of African trypanosomiasis has been estimated[3] to be 1.47 million lost Disability Adjusted Life Years (DALYs). In this estimate, however, the propensity of the disease to develop as epidemics, which makes it one of the major health problems in sub-Saharan Africa, was not taken into consideration.

In humans, African trypanosomiasis is caused by two species of trypanosome – *Trypanosoma brucei gambiense*



and *T. b. rhodesiense*, which are transmitted by tsetse flies. In the early stages of infection the principal clinical manifestations of the disease are intermittent fever and signs of reticuloendothelial hyperplasia (e.g. enlarged lymph glands and spleen). In the advanced stages there are neurological symptoms and endocrine disorders. The development and progression of the disease may take several years in infections due to *T. b. gambiense*. In *T. b. rhodesiense* infections however, the disease can progress in a matter of weeks and the early symptoms are more severe and acute. These differences, however, are not absolute. Once the disease has developed, it is fatal in all cases if left untreated. Treatment is still very unsatisfactory (see Product Research and Development section below).

Wild animals act as a reservoir of infection for *T. b. rhodesiense*. With regard to *T. b. gambiense* however, the significance for humans of the infection in wild animals is not yet understood.Other species of trypanosome cause similar diseases in domestic livestock and are major obstacles to agricultural development and livestock production. The loss due to African trypanosomiasis in livestock is estimated to be US$ 5000 million a year.

African trypanosomiasis is a dynamic disease. We need simple diagnostic tests and effective and affordable drugs. Cost-effective tools for vector control which are acceptable to affected populations have been developed and optimization of the use of these tools is TDR's priority. Improved knowledge of parasite biology is being brought to bear on the development of new tools.

Applied Field Research

Surveillance and diagnosis

In collaboration with national institutions, TDR has been promoting activities aimed at integrating diagnostic tools into systems of community surveillance. Diagnostic tools fall into two categories: detection of parasite components (antigens or DNA); and detection of antibodies.

The Card Indirect Agglutination Test for Trypanosomiasis

This antigen detection test was developed initially as an enzyme-linked immunosorbent assay (ELISA) and was subsequently converted to an indirect latex agglutination assay – the Card Indirect Agglutination Test for Trypanosomiasis or CIATT – which can be performed using whole blood obtained by finger prick.

The test has been evaluated in a multicentre study in Côte d'Ivoire, Cameroon, Uganda and Malawi as a tool for the diagnosis of *gambiense* and *rhodesiense* sleeping sickness and as a potential tool for the evaluation of chemotherapeutic cure. The overall specificity of the test was shown to be 75.4% – an estimation which was based on the assumption that false positives are, in fact, false. However, the results of other tests (an antibody ELISA and the polymerase chain reaction or PCR) have indicated that 75.4% may be an underestimation, since a high percentage of false positive cases were shown to have trypanosome products and were therefore, in fact, true (not false) positives. Furthermore, 2.5% of CIATT positive but trypanosome negative subjects showed patent infection within 18 months of follow-up. Thus it is likely that an estimate of 99.4% specificity for the test, obtained from the observation that only 4 of 664 persons (0.6%) from an area not endemic for trypanosomiasis reacted with the CIATT, is perhaps more accurate.

Follow-up of 164 treated *gambiense* patients revealed that circulating antigens in 113 (69%) of the patients had disappeared by nine months, indicating that the test has potential for assessing cure following chemotherapy. A study of monkeys experimentally infected with *T. b. rhodesiense*[4] showed that the persistence of antigens in serum and/or cerebrospinal fluid would be a useful tool for evaluating the response to chemotherapy. The CIATT has the advantage of being rapid and simple to perform and is applicable in both *T. b. gambiense* and *T. b. rhodesiense* zones. It has potential for selecting high-risk groups from a population in an endemic area and for assessing cure following chemotherapy. Further evaluation of the CIATT in non-endemic areas is planned, to confirm the specificity. A pilot study is envisaged to investigate the integration of the CIATT into sleeping sickness surveillance systems.

A new branched DNA technique

Harris et al[5] have developed a non-radioactive branched DNA (bDNA)-based assay for the diagnosis of African trypanosomiasis. The technique uses simple preparations of blood and has been used successfully for detecting *T. b. gambiense* in clinical samples. Two repetitive DNA sequences specific to the *T. brucei* complex were chosen as targets of the assay, which amplifies the signal from a target molecule rather than the target itself. Analysis of human blood samples from Côte d'Ivoire indicated 100% sensitivity and specificity when compared to microscopy of buffy coat.

Branched DNA technology offers several advantages over alternative molecular techniques. These include:

- simplicity of sample preparation,
- simplicity of procedure,
- stability of reagents,
- the ability to process large numbers of samples simultaneously,
- freedom from artifacts due to cross contamination.

The suitability of this technique for use in the field needs to be studied.

The Card Agglutination Test for Trypanosomiasis

The development of cost-effective surveillance strategies and their integration into health care systems is one way to ensure early diagnosis of sleeping sickness. The Card Agglutination Test for Trypanosomiasis (CATT) is an antibody detection test.

A feasibility study was undertaken in the Sinfra sleeping sickness focus of Côte d'Ivoire, where the disease erupted in 1992. Trained community health workers collected blood on filter paper from the population, and these filter papers were subsequently analysed by two nurses in a village laboratory using the CATT. CATT positive patients were examined using parasitological tests and confirmed cases were sent to sleeping sickness treatment centres in Daloa (Projet de Recherches Cliniques sur la Trypanosomiase), and Bouafle. Altogether, 722 CATT positive cases were identified after examination of 50 375 patients from a community of 76 131 people and 238 were diagnosed parasitologically. The community health workers accomplished the task in a more cost-effective way than could be achieved by mobile teams. The community health workers also took part in tsetse control – they distributed 11 504 insecticide-impregnated screens to 5132 farmers to reduce contact between humans and

tsetse flies in resettlement areas.

The sustainability of CATT use is now under study and it is recommended that the method be applied in other foci.

The use of serodiagnostic tests in screening populations has greatly improved surveillance. However, the significance of non-demonstration of parasites in people who are serologically positive has been in question for many years. In a recent study using the PCR,[6] it was reported that 4 of 28 suspects from Mbam and 9 of 30 suspects from Fontem had trypanosome products in the blood. This agrees with the CIATT observation mentioned above.

Vector control

The social and economic aspects of community involvement in tsetse control, for example motivation and delivery systems, affect sustainability. Studies on community involvement in tsetse control and the integration of tsetse control into primary health care in Bukoli county in Uganda have shown that the Village Health Committee has the potential to mobilize community participation and can be used to integrate health services delivery.

Social impact of sleeping sickness

Gender studies

A study to examine gender differences in the detection and control of human trypanosomiasis has been conducted in South-East Uganda, where *Trypanosoma brucei rhodesiense* is endemic. Generally, symptoms such as fever, sweating, swollen cheeks, severe headaches, joint pain, dizziness and mental disturbances, as well as the mode of parasite transmission, are recognized by the community. The incidence of trypanosomiasis in the study area is equal for women and men.

Preliminary results from the study indicate that gender differences exist among this community, especially with regard to seeking treatment for sleeping sickness:

- Women do not have the same access to resources as men, and the costs of treating sleeping sickness are high – they include drugs, user fees and long hospitalization. Women rely on their husbands or families to provide funds for treatment, and for this reason often seek treatment at a late stage of the illness, if at all. In the study, men on average were found to take 1-3 months after the onset of symptoms to become aware that they were infected with sleeping sickness, while the majority of women took 3-4 months.
- Members of the community were also found to fear the consequences of treatment. They feel that a lumbar puncture (taken for diagnosis) renders one permanently weak with chronic back pain, and fear that a woman who has a lumbar puncture becomes unfit for marriage, as men cannot tolerate weak women. There is also a belief that sleeping sickness cannot be completely cured and that one remains sickly and weak. Women, therefore, who have suffered from trypanosomiasis may not readily find marriage partners or may be divorced on this ground.
- Some members of the community also believe that trypanosomiasis is closely linked to sexually transmitted diseases (STDs), especially to HIV/AIDS, and that STDs are perpetrated by women. Consequently women suffering from trypanosomiasis or related symptoms avoid seeking treatment lest STDs and/or AIDS are diagnosed. Women fear the shame, stigma and subsequent ostracization. Men, on the other hand, seek treatment promptly since they have the financial means to do so and do not have the same sociocultural constraints.
- It has also been reported that in certain parts of Cameroon, sleeping sickness patients who go to traditional healers receive scarifications on the forehead or other parts of the body. The ugliness of these scars on women often puts off potential male suitors, thus diminishing the prospects of finding a male partner.[7]

Impact of sleeping sickness on children

A case control community-based study was conducted in Cameroon recently to compare the physical growth, sexual maturity and academic performance of children and adolescents with a past history of African trypanosomiasis to a group of matched controls. The results showed that children with a past history of the disease had significantly lower weight, height and mid-upper arm circumference ($p<0.05$) than controls. Five per cent had delayed puberty against 1% of controls. It was also reported that children with a past history of the disease spent more years in school ($p<0.05$) and were academically less fit. Thirty-six per cent of previously infected children were either repeating classes or were drop-outs. Twenty-one per cent had behaviour problems. When divided into two groups according to treatment received – melarsoprol (for late-stage disease) or pentamidine (for early-stage disease) – only the growth and performance of the melarsoprol treated group (the group with late-stage disease) was significantly affected in a negative way.

These differences were not due to socioeconomic, demographic or cultural factors nor to geohelminth infections, since there were no statistically significant differences between the two groups of children with respect to each of these parameters.[8]

Product Research and Development

Treatment

The treatment of African trypanosomiasis is still unsatisfactory. The only drug developed in recent times is eflornithine, which is effective for late-stage *gambiense* disease. However, its cost makes it unaffordable. Recent shortage of melarsoprol, the first line drug for late-stage disease, apparently due to technical problems encountered by the manufacturer, meant loss of life for hundreds of patients.[9] These occurrences, as well as adverse drug effects and parasite resistance, emphasize the need for new and affordable products.

Besides eflornithine, other drugs in use for the treatment of sleeping sickness include pentamidine and suramin, which are used for the treatment of early-stage *gambiense* and *rhodesiense* disease respectively, and, in the absence of any suitable and affordable alternative, melarsoprol, which remains the first line drug for late-stage disease of both forms of sleeping sickness. All three drugs have adverse side effects. In particular, melarsoprol causes reactive encephalopathy in 5-10% of patients and is fatal for 1-5%. Nevertheless, in the absence of a safe and affordable alternative drug, melarsoprol has saved the lives of hundreds of thousands of patients over the decades it has been in use.

Eflornithine: availability and cost-effectiveness

Eflornithine (difluoromethylornithine or DMFO) was developed during the last decade and was the first new drug to be approved for use in African trypanosomiasis for over 40 years. It was first registered for use in sleeping sickness in 1990. However, the drug has a number of drawbacks: it is not effective in cases of *rhodesiense* infection; it is expensive (currently US$ 22 per vial, and 14 vials are required to treat each patient); and it can be administered only in a hospital setting due to the need for intravenous delivery. Efforts to find donors willing to guarantee supply of eflornithine have been unsuccessful.

When it became obvious that affected countries could not afford to buy the drug, TDR embarked on a two-pronged approach towards making eflornithine available at an affordable price:

- In 1994, the Indian Institute of Chemical Technology (IICT), Hyderabad, was supported to synthesize eflornithine – using a novel route developed by the Institute – which could reduce the cost of production by 50%. This technology is based on the use of benzyl methyl malonate or benzyl ethyl malonate as raw material instead of ornithine methyl ester. The IICT has successfully produced a pilot sample of eflornithine, and is now exploring methods of scaling-up production. Chemical analysis of the pilot sample by Hoechst Marion Roussel Inc. (HMRI), the developer of the drug, to compare it with the original product, indicates that it contains more impurities than the original product. The significance and implications of this are being studied.
- TDR is supporting a multicentre study to compare different durations of treatment with eflornithine – 7 days compared with 14 days. Three hundred and twenty patients were recruited into the study, which was carried out in Côte d'Ivoire, Congo, Uganda and Zaire. Follow-up of treated patients for 6 and 12 months showed no significant difference between the relapse rates of the two groups. Follow-up of treated patients will continue until the end of 1997. If these results are confirmed, it could mean a further reduction of up to 50% in the cost of treatment with eflornithine – making the drug more comparable in cost to available alternative chemotherapy.

Politi et al[10] compared the costs and effectiveness in Uganda of three alternative treatments for late-stage *gambiense* sleeping sickness infections: melarsoprol alone, combined treatment with melarsoprol and eflornithine, and eflornithine alone. The latter two alternatives, although more costly, are more effective. A decision in favour of one or the other would depend on the combined willingness of the government, donors and patients to pay. Furthermore, combined treatment with eflornithine or eflornithine treatment alone for all late-stage patients would be relatively more cost-effective than other health interventions.

Incremental cost-effectiveness analysis indicated that treatment with melarsoprol alone was associated with a cost per life and DALY saved of US$ 209 and US$ 8 respectively; whereas each additional life saved by switching from melarsoprol alone to combined melarsoprol and eflornithine would cost an extra US$ 1033 per life saved and an extra US$ 40.9 per DALY saved; while switching from the combined treatment regimen to treatment with eflornithine alone would cost an extra US$ 4444 per life saved and an extra US$ 166.8 per DALY saved.

This study however did not take into consideration the fact that eflornithine can only be administered in a hospital setting and is therefore unsuitable for mass administration.

Pharmacokinetics of melarsoprol

The treatment regimens with melarsoprol for African trypanosmiasis are based on empirical observations. Serious adverse effects and relapses often occur and, besides, a duration of treatment which can last up to 40 days is a serious drawback for patients and their families – some patients even abscond before treatment is completed. Based on recent pharmacokinetic data in humans, an alternative treatment schedule has been proposed which consists of ten consecutive injections of 2.2mg/kg melarsoprol given at 24 hour intervals. In a preliminary study carried out in Zaire,[11] 11 patients who were treated according to this protocol showed similar adverse reactions to those patients treated with the conventional regimen but the efficacy was the same and the advantage was a much shorter treatment period. Thus, the adverse effects of melarsoprol are not dose related.

Eflornithine needs to be administered in hospital. Will its promise be negated by its cost?

Development of new drugs

African trypanosomes have many biochemical features in common with *Trypanosoma cruzi* (which causes Chagas disease) and species of *Leishmania* (responsible for leishmaniasis). Within the framework of an integrated approach to the development of drugs for these three diseases, TDR supports drug screening facilities at the London School of Hygiene and Tropical Medicine, London; the Swiss Tropical Institute, Basel; and the Janssen Research Foundation, Beerse, Belgium, where hundreds of compounds provided by pharmaceutical companies and academia are screened against *T. brucei*, *T. cruzi* and *Leishmania*.

Attention is focused on three metabolic areas: polyamine/S-adenosine methionine/trypanothione (SAM) biosynthesis and function, sterol biosynthesis and protein degradation by cysteine proteases. African trypanosomes, however, do not synthesize sterol (instead utilizing cholesterol in the host's blood), so that inhibition of sterol biosynthesis, although effective in *T. cruzi* and *Leishmania*, has no effect on African trypanosomes.

These efforts have led to the identification of a number of exciting lead compounds. For African trypanosomiasis the compound CG40215, a diamidine which is an inhibitor of SAM metabolism, has been identified as a potential candidate for development. It is effective alone against *T. b. gambiense* infections in mice and, in combination with eflornithine, against *T. b. rhodesiense* infections in mice. Pharmacokinetic studies will next be carried out by the Swiss Tropical Institute in collaboration with the Institute of Primate Research, Nairobi, Kenya. CG40215 has also been shown by Ciba Geigy, the manufacturer, to be effective against opportunistic infections. Further development of the compound will depend on the outcome of ongoing preclinical studies.

Strategic Research

Pathogenesis

New strategies for managing patients with central nervous system involvement in African trypanosomiasis are urgently needed. In recent years, significant progress has been made in understanding the molecular mechanisms underlying the pathogenesis, including the brain dysfunction and neuropsychiatric symptoms associated with the disease. During the biennium, headway has been made on several fronts including immune mechanisms, virulence and new drug/vaccine targets (Box 1).

Box 1. Pathogenesis research

Host immune mechanisms

- The gene for Trypanosome Lymphocyte Triggering Factor (TLTF) has been cloned, sequenced and the product shown to be a 53kDa parasite growth-stimulating factor. It is a non-variable polypeptide of no homology with previously known proteins. It binds to CD8+ T cells, activating the secretion of interferon-γ (IFN-γ), which in turn binds to the parasite and triggers its proliferation.
- Blocking of IFN-γ has been shown to increase the survival of infected animals. Monoclonal antibodies specific for TLTF may therefore have potential for immunotherapy. TLTF-mediated triggering of interleukin-4 (IL-4) expression has only been observed in relatively disease-resistant mouse strains, indicating the emergence of an alternative protective cytokine pathway in these animals.
- Selective induction of major histocompatability class I antigens by TLTF has revealed the involvement of the hypothalamic paraventricular and supraoptic nuclei – which could account for the disruption of endogenous sleeping rhythms typical for sleeping sickness.

Parasite virulence

- The galactosyl residues of the glycosyl phosphatidyl inositol (GPI) anchor of the trypanosome variant surface glycoprotein have been shown to be of crucial importance for the modulation of parasite load through activation of Tumour Necrosis Factor (TNF).

Drug and vaccine targets

- Work on *T. brucei* has revealed a biochemical pathway thought to be essential for survival of trypanosomatid protozoa. Two methyl transferase enzymes involved in the methylation of the cap structure of the spliced leader RNA, which is essential in trans-splicing, have been characterized.
- In comparison to *T. b. rhodesiense*, a much higher accumulation of trypanosome lytic factor (TLF), which is produced by the host and protects against trypanosomes, was demonstrated in *T. b. brucei*. The latter species is non-infectious for humans and the mechanism was found not to be at the receptor level but to depend on failure to discharge TLF from the flagellar pocket. The genetic basis for this observed susceptibility of *T. b. brucei* is being investigated.

Genome

The African trypanosome genome project was initiated in 1994 with the involvement of six laboratories in USA, UK and Kenya and a view to achieving better understanding of trypanosome genetics. The range of genome analysis activities includes random sequencing of cDNAs; production of large-insert libraries of genomic DNA in vectors; long-range restriction mapping; physical mapping by construction of overlapping contigs (continuous DNA segments) of genomic clones; assignment of genes and other sequences to libraries and contigs; and production of genetic markers and a genetic map (i.e. recombination map). An interactive DNA database and communication network has been established for both EST and mapping data, and is available through the World Wide Web.

It was decided not to focus research on any particular clone of *T. brucei* because a substantial number of markers have already been developed using different isolates and can be used for physical mapping. Many large contigs of bacterial artificial chromosome (BAC) genomic DNA clones have been constructed. The large repetitive sequence and transposon content make full mapping of the chromosomes difficult, but the central gene-rich regions should be amenable to analysis in the first instance. Activities also include mapping of heritable traits, aimed at identifying the actual genes responsible for characteristics such as drug resistance and human infectivity. Highlights of genome research can be found in Box 2.

Box 2. Genome research

Gene discovery

- More than 2000 African trypanosome expressed sequence tags (ESTs) have been sequenced. About 8% of these ESTs are found to have a homologue in other organisms, and at least some of the more than 50% which lack a known homologue are likely to be genes unique to trypanosomes.[12]
- EST analysis shows that many proteins from other organisms which have been studied extensively, such as neuropeptidases and cyclophins, are also present in trypanosomes. Unexpected similarities have been discovered between trypanosome proteins and proteins from other trypanosomatids, such as *Leishmania*, which were thought to be parasite-specific.
- About 75 mRNA sequences that are expressed differently in various stages of the trypanosome life cycle have been identified, and most are being sequenced.

Genomic DNA contig mapping

- Karyotyping of *T. brucei* reference stock TREU 927 by pulsed field gradient (PFG) electrophoresis is continuing. Variations in the size of chromosomal homologues between different *T. brucei* strains and within a specific genome can be as large as one megabase (Mb), showing that the karyotype is very polymorphic.
- The previously constructed P1 library of genomic DNA (of *T. brucei* reference stock TREU 927) has been probed with several hundred ESTs and known genes. Some telomere sequences seem to be under-represented or missing from the P1 library. Most mapping activities have so far been focused on genomic DNA inserts derived from the 1.1Mb chromosome I, the smallest of the seven resolved chromosome pairs in the reference stock. More than 380 EST markers to reference stock TREU 927 chromosomal bands separated by PFG electrophoresis have so far been mapped and a number of unique markers have been identified for each chromosome.

Genetic mapping

- Genetic exchange between African trypanosomes occurs in the tsetse fly – PFG gel demonstrations indicate that trypanosome progeny can inherit a different sized chromosome homologue from each of two parental stocks. Current work is focused on identifying and mapping a few important heritable traits such as those for drug resistance, human infectivity, growth factors and transmission. It is anticipated that this will lead to identification of the actual genes responsible for these traits.

WWW site: http://parsunl.path.cam.ac.uk/newtryp/toppage.htm

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CHAPTER 11

Leprosy

Applied Field Research

- ✓ treatment of patients (who will be followed-up until 2004) in a multicentre trial of new ofloxacin- containing MDT regimens was completed in June 1996, using regimens which further reduce the duration of treatment
- ✓ evaluation of treatment of minimum duration – one day (with the three most potent drugs) – for patients with single lesions will be completed in 1997
- ✓ the role of family support has been shown to be important in determining the course of the disease, in coping and in treatment
- ✓ the introduction of user charges for MDT has been shown to reduce demand

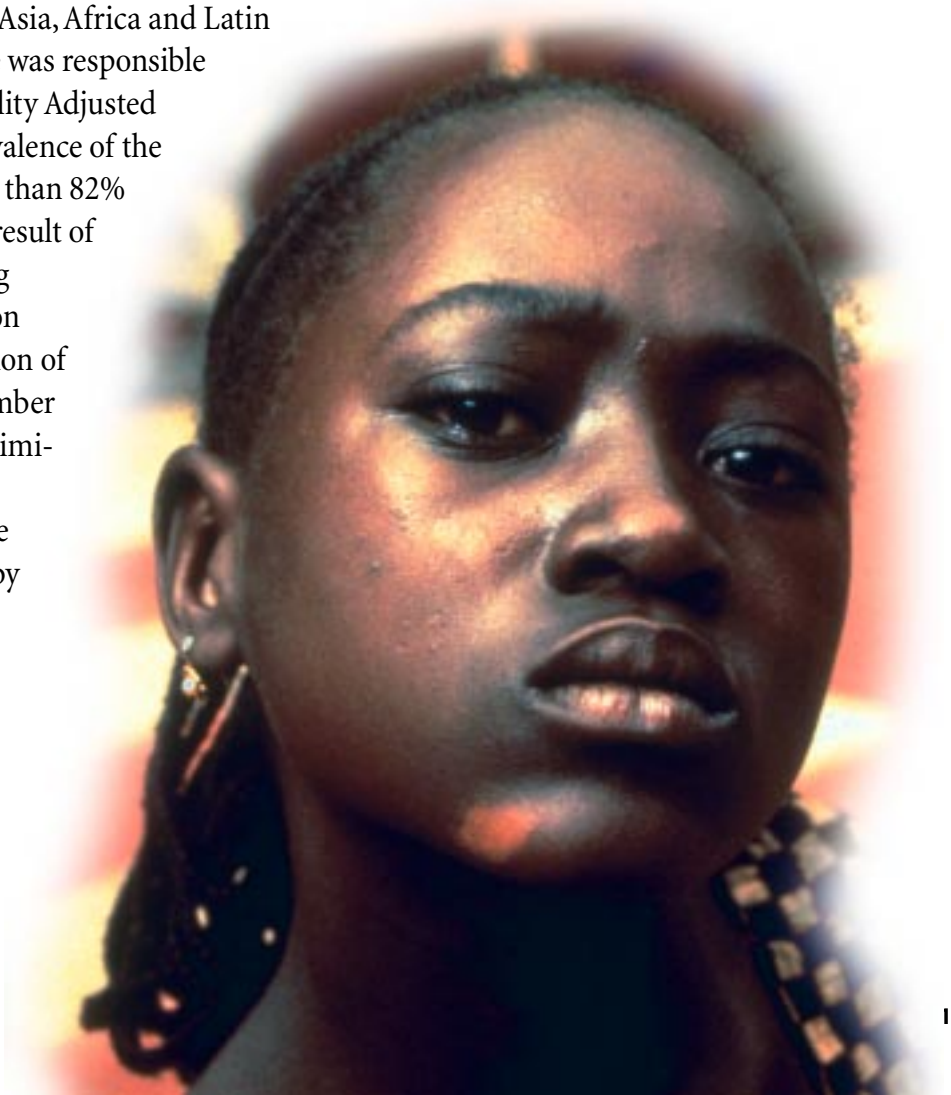
Strategic Research

- ✓ the use of peptide antigens specific to *M. leprae* is being evaluated to improve the skin tests currently available
- ✓ polymerase chain reaction technology is being simplified to make it suitable for use in the field
- ✓ the cytokine TNF-a has been shown to be a key mediator of systemic symptoms and tissue damage
- ✓ revaccination with BCG has been shown to appreciably boost protection against leprosy in Latin America and Africa

Leprosy is a chronic communicable disease caused by the bacillus *Mycobacterium leprae*. The disease can present a great variety of forms depending on the immune response to infection. At one end of the disease spectrum is lepromatous leprosy, in which cell-mediated immunity specific to leprosy is absent and the bacilli multiply uncontrolled. If untreated, this leads to damage of the mucous membranes, eyes and peripheral nerves, and ultimately causes severe deformities. Untreated lepromatous cases are thought to be the main source for transmission of the disease. At the other end of the spectrum is tuberculoid leprosy, in which the immune system is able to control the infection. In such cases, the symptoms are mild, often taking the form of anaesthetic, pale or reddish coloured skin patches. If left untreated, peripheral nerves may be damaged, leading to deformities. Leprosy is an important cause of permanent and progressive disability. The intense social stigma of leprosy appears to be decreasing but social attitudes still have considerable impact on the social and economic well-being of sufferers. In all, between 1 and 2 million individuals in the world are visibly disabled because of present or past leprosy.[1]

For practical reasons in control programmes, leprosy patients are divided into two broad groups: paucibacillary (PB) cases, which coincide with cases nearer the tuberculoid end of the spectrum, and multibacillary (MB) cases, nearer the lepromatous end.[2]

Leprosy occurs in significant numbers of people in about 60 countries or territories of Asia, Africa and Latin America. In 1990, the disease was responsible for the loss of 382 000 Disability Adjusted Life Years (DALYs).[3] The prevalence of the disease was reduced by more than 82% between 1985 and 1996 as a result of the introduction of multidrug therapy (MDT). WHO's Action Programme for the Elimination of Leprosy, established in December 1994, has made its goal the elimination of leprosy as a public health problem (less than one case per 10 000 population) by the year 2000.[4,5]



Applied Field Research

Chemotherapy of leprosy

Until the early 1980s, chemotherapy of leprosy consisted of dapsone monotherapy. This was usually administered as 100mg daily for a minimum period of five years for paucibacillary leprosy, and for life in multibacillary leprosy cases. Because dapsone is a slow acting and weak bactericidal drug, patient compliance was poor and dapsone resistant strains of *Mycobacterium leprae* emerged.[6]

Since 1982, WHO-recommended multidrug therapy (MDT) (Box 1) has been applied in the national leprosy control programmes in all leprosy endemic countries. These regimens include a combination of rifampicin, clofazimine and dapsone for multibacillary leprosy and combined rifampicin plus dapsone for paucibacillary leprosy. This therapy has proved to be reliable and practical.[2]

The introduction of MDT in 1982 resulted in a dramatic reduction in the prevalence of leprosy – from 5.4 million registered cases in 1985 to 0.9 million in 1996. By the middle of 1996 more than 8 million patients had been cured through MDT. Follow-up, based on large numbers of patients cured with MDT, has revealed very low relapse rates after completion of treatment (the cumulative risk of relapse is less than 1% over a 9-year period, for both multibacillary and paucibacillary leprosy).[7]

Box 1. WHO-MDT

For multibacillary leprosy:		For paucibacillary leprosy:	
Rifampicin	600 mg monthly	Rifampicin	600 mg monthly
Clofazimine	300 mg monthly	Dapsone	100 mg daily
Clofazimine	50 mg daily		
Dapsone	100 mg daily		
Duration of treatment: 24 months		Duration of treatment: 6 months	

Current TDR activities

These include:

- Evaluation, in field programmes, of existing MDT regimens for multibacillary and paucibacillary leprosy; an activity which was concluded in 1995.
- Screening of new drugs and drug combinations in experimental animals and short-term clinical trials.
- Evaluation of the efficacy, acceptability and feasibility of ofloxacin-containing combined regimens (see below).
- A multicentre trial for the treatment of single-lesion paucibacillary leprosy cases using a very short (single dose) treatment regimen consisting of a combination of rifampicin, ofloxacin and minocycline.

Future targets include using the single-dose combination of rifampicin, ofloxacin and minocycline (ROM) for single-lesion paucibacillary cases under routine field conditions; and using the 4-week ofloxacin-containing regimen and possibly the 12-month MDT regimen for multibacillary leprosy cases under routine field conditions.

Summary of important large-scale field trials

MDT trials

Four long-term trials established that the WHO-recommended regimens are safe (high acceptability/good compliance) and effective (very low treatment failures/relapse rates) for the treatment of leprosy:

Two trials for multibacillary leprosy:

Polambakkam, India	1983-1995
Karigiri, India	1982-1995

Two trials for paucibacillary leprosy:

South Sulawesi, Indonesia	1984-1992
Karonga, Malawi	1983-1991

Ofloxacin multicentre trial

Three additional antileprosy drugs are now available: ofloxacin (a fluoroquinolone), clarithromycin (a macrolide), and minocycline (a tetracycline). All these drugs act by different mechanisms and have potential for increasing the effectiveness and shortening the duration of antileprosy chemotherapy. In addition, the new drugs may prove useful against *Mycobacterium leprae* strains which are resistant to the drugs currently in use, especially those that are resistant to rifampicin.[8]

Therefore, WHO launched a large-scale, randomized, double-blind, multicentre field trial to evaluate the efficacy, safety and acceptability of ofloxacin-containing regimens in both MB and PB leprosy patients under routine conditions. Fifteen centres in eight countries are participating in the trial, into which about 4000 newly diagnosed leprosy patients were recruited by the end of June 1994 and in whom treatment was completed by June 1996. Patients will be followed up for

5-7 years after completion of treatment to detect relapses, if any.

Sites:

- Cebu-1, Philippines
- Bauru, Brazil
- Yangoon, Myanmar
- Hanoi, Viet Nam
- Bamako, Mali
- Karigiri, India
- Madras, India
- Manila, Philippines
- Cebu-2, Philippines
- Manaus, Brazil
- Alupe, Kenya
- Karachi, Pakistan
- Bombay, India
- Chingleput, India
- Raipur, India

Regimens:

- WHO/MDT for 2 years (MB)
- WHO/MDT for 1 year (MB)
- WHO/MDT for 1 year + ofloxacin daily for the first 4 weeks (MB)
- ofloxacin + rifampicin for 4 weeks (MB)
- WHO/MDT for 6 months (PB)
- ofloxacin + rifampicin for 4 weeks (PB)

Short-term results, which may be available in 1997, will indicate the safety of the new MDT regimens – in terms of side effects and the incidence of lepra-reactions and nerve damage during treatment.

Medium-term results, which are expected 3-6 years after initiation of the trial, will indicate the effectiveness of the combinations in arresting the progress of the disease in individual patients – in terms of clinical and bacteriological response and post-treatment complications such as late lepra-reactions and nerve damage.

Long-term results, which are expected 7-10 years after initiation of the trial (by 2004), will indicate the effectiveness of the new regimens in preventing relapses and the emergence of drug-resistant strains (i.e. their effectiveness in completely curing individual patients).

Single-lesion PB, single-dose, multicentre trial

As leprosy control services in all endemic countries are expanding their activities, and in particular are implementing WHO-recommended MDT, the number of new cases being detected is steadily increasing. In 1995, about 560 000 new cases were detected globally, of which about 85% were in India and neighbouring countries (in the WHO South-East Asia Region).[4] However, the majority of these cases are at the stage when the only visible sign of the disease is a single skin lesion.

It is well known that the specificity of diagnosis of leprosy at the stage of a single lesion is rather low. Most of these lesions may heal spontaneously without any specific treatment, and the risk of developing nerve damage is minimal. It is also known that the acceptance of diagnosis and compliance to specific antileprosy treatment among this group is far from satisfactory.[9]

Therefore, this group is being considered as a special group requiring minimal duration of treatment and a trial is being conducted at nine sites in India. About 1500 patients were recruited by July 1995 and treatment was completed by December 1995. Final results are expected in early 1997.

Box 2. Is there a need to improve the treatment of leprosy?

The approach at the moment is to encourage national programmes to implement the standard WHO recommended MDT everywhere as quickly as possible.

However, a need to develop new drug regimens for future application was identified at the beginning of the 1990s, based on promising experimental results with new drugs, for the following reasons:

- **The current duration of MDT for MB leprosy could be substantially shortened.**
- **It would be helpful if future schemes of therapy were as simple as possible for application within the general health services.**
- **A single common regimen suitable for both MB and PB leprosy, but with different duration, would considerably simplify administration of therapy by general health services.**
- **Only three antileprosy drugs – rifampicin, dapsone and clofazimine – can be safely applied in the field. If an MB patient does not accept clofazimine because of skin colouration, he/she has no access to easily applicable alternative MDT regimens.**
- **Special regimens are required for individual patients who are unable to receive rifampicin, whether because of intolerance, rifampicin-resistance or intercurrent disease (such as chronic active hepatitis).**

In other words, other drug regimens would allow leprosy control programmes and patients the opportunity to choose alternative regimens, according to the needs of the programme or the patient.

The study is a randomized, double-blind, controlled clinical trial, consisting of two groups:

- About 750 previously untreated single-lesion PB patients, who received only one dose of rifampicin 600mg + ofloxacin 400 mg + minocycline 100 mg at the time of recruitment into the study.
- About 750 previously untreated single-lesion PB patients, who received the standard WHO/MDT regimen for PB leprosy for six months.

The next steps for the Steering Committee on *Chemotherapy of Leprosy* will be to:

- review the potential of regimens which incorporate new drugs for their application in the field;
- recommend policies/strategies;
- review the potential advantages and disadvantages of introducing new regimens in national programmes close to the target of elimination of leprosy as a public health problem by the year 2000.

Millions of Indian women suffer disproportionately. MDT should improve their lives and reduce social ostracism.

Gender differences in the role of family support to leprosy patients in India

A study of the impact of leprosy, conducted between 1993 and 1996 in Pune, India, was carried out in urban and rural areas of Maharashtra State.[10] A total of 1154 patients, both current patients and those released from treatment, were interviewed to investigate various aspects of the experience of leprosy, including its detection, approaches to and compliance with treatment, the impact of the disease on social, family and personal life, and the role of the family in mediating this impact.

Four major family support indicators were considered: position as household head, sharing the diagnosis with the spouse, immediate reaction of the family after diagnosis, and suggestions for treatment.

The study clearly demonstrated the importance of family support in determining the course of disease, in coping and in treatment:

- Position as household head generally helped patients to go early for diagnosis, follow appropriate treatment and cope better with the disease. Greater decision-making power associated with headship seems to promote positive health behaviour. As women are rarely heads of household in rural India, the observation implies that empowering women with decision-making through other avenues, such as income generation or literacy, could improve their health status.
- Sharing the diagnosis with the spouse appeared to be beneficial, especially for males, as it reduced the delay in getting medical help and increased regularity in treatment. People who shared the diagnosis with their spouse tended to hide the disease from others as a coping mechanism, while at the same time seeking treatment earlier than they otherwise would have done. This suggests that counselling spouses regarding positive actions and the availability of MDT would be a powerful intervention.
- Immediate positive reaction of the family after diagnosis and positive suggestions for treatment also led to more appropriate treatment seeking and coping. In general, however, males benefitted from family support much more than females.

It is important to emphasize that family support, despite its usually positive role in health behaviour, can also be problematic in certain situations. For example, both men and women who reported that they received positive reactions from their families were also more likely to consult religious as well as medical sources. While this is not necessarily negative, such alternative advice may confound the recovery process. For example,

in an earlier study patients who visited 'jaundice specialists' were advised to stop MDT treatment, impeding their cure. Similarly, expenditures on costly offerings or pilgrimages may unnecessarily add to the costs of treatment. These considerations must be kept in mind when advocating the importance of family support to patients. Advice to religious leaders and traditional practitioners about the need for appropriate treatment and referral of patients should also be considered.

While it seems obvious that more attention to family roles and gender relations within the family could improve the effectiveness of health programmes in South Asia, more research is needed on how illness and disease are perceived and cared for by families. Priority should be given to research on the role of family members in activities that promote or hinder health, as well as in care-giving. Families are already an important health resource for their members and it is likely that they can become more effective if provided with relevant and timely information. Women are universally respected as the key health providers in the family, yet their role as potential partners is largely ignored by the formal health care sector. Women and men differ greatly in terms of the family support they receive, in their interpretation of this support, and in the use they make of it. Gender sensitivity is therefore key to understanding the influence of family dynamics in health and to designing interventions which improve the positive influence of family support on health outcomes.

Health care financing

A study in China showed that the introduction of fees for reconstructive surgery, after economic liberalization, had been associated with a sharp fall in the extent of surgery. For example, 34.1% of patients diagnosed between 1975 and 1980 had had reconstructive surgery. After 1980 however, when market reforms started and charges were introduced, no patients in the prefecture of the study had had surgery despite an apparent medically-defined need.

Another part of the same study examined the records of 67 patients diagnosed after 1980 while they were receiving treatment. Most of them (49) had received totally free treatment but the remainder (18) had been required to pay for some aspects of their care. There had been much longer delays between the onset of symptoms and the beginning of treatment in the group that had been required to make financial contributions (Table 1). Although the numbers were small, the results suggest that charges could be associated with delays in the onset of treatment which would in turn lead to an increase in the incidence of deformities. This supports the current recommendations of the Action Programme for the Elimination of Leprosy that MDT should be provided free of charge.

Table 1. – Financing of leprosy treatment

	Self-payment (18 patients)	Free services (49 patients)
Delay between onset of symptoms and consulting a doctor	233 days	138 days
Delay between first visit to doctor and start of treatment	369 days	60.3 days
Total delay	602 days	198.3 days

Strategic Research

Epidemiologic and diagnostic tools

Improved understanding of leprosy transmission will play an important role in monitoring the effect of MDT in disease control programmes and in evaluating the feasibility of a leprosy eradication campaign. An improved skin test reagent for specific detection of infection with *M. leprae* would be of considerable practical benefit in this context.

In addition to ongoing efforts to improve conventional skin tests, TDR has now taken the initiative to evaluate the potential use of peptides based on *M. leprae*-specific sequences as novel skin test reagents. Peptide antigens have two obvious advantages over conventional complex skin test antigen mixtures: (a) their supply does not depend on a biological source as they can be produced entirely by chemical synthesis; and (b) peptides can be tailored to be specific for leprosy, avoiding cross-reaction with related pathogens such as *M. tuberculosis*. These reagents are currently undergoing *in vitro* testing for reactivity and specificity, comparing T-cell responses of leprosy patients with those of tuberculosis patients.

TDR-sponsored research has shown that the polymerase chain reaction (PCR) constitutes an exquisitely specific and sensitive method for the detection of *M. leprae* in tissue samples. Technological simplification, however, is considered indispensable for the adaptation of PCR as a diagnostic field tool. Elimination of some of the most bothersome steps of PCR technology, such as the replacement of thermal cycling by an isothermal incubation procedure, has been achieved using a technology termed nucleic acid sequence-based amplification (NASBA). Promising results were obtained when clinical samples from leprosy patients were assayed. Furthermore, a NASBA-based test for mycobacterial viability has been developed with a view to its later use as a tool for following up the success of antibiotic treatment. Leprosy-NASBA is currently undergoing further development with the aim of increasing the sensitivity of detection.

Immunopathogenesis

Inflammatory reactional states and polyneuropathies are due to immune hyperstimulation triggered by antigen release from dying bacteria. These manifestations of leprosy are not necessarily accessible to or prevented by MDT.

In March 1996, an international workshop on leprosy research, held in Bangkok, Thailand, identified the prediction and prevention of reactions and nerve damage as the area in which research is most urgently needed. This issue has also been the top TDR research priority for leprosy over the last couple of years.

In TDR-sponsored studies it has been shown that the cytokine tumour necrosis factor alpha (TNF-α) is overproduced during reactional states (both type 1 and type 2) and is a key mediator of systemic symptoms and tissue damage. Levels of TNF-α in circulation were found to be elevated in all reactional patients with neuritis. Moreover, correlation between reaction and development of disability was detected.

The major challenge over the next few years will be to use these findings for the development of predictive, preventive and therapeutic intervention strategies.

Leprosy vaccine

The year 1996 witnessed the eagerly awaited publication[11] of the results of a combined leprosy/tuberculosis vaccine trial, carried out in the Karonga district of Malawi between 1986 and 1995. In this study, the efficacy of BCG alone was compared to BCG plus a preparation of killed *M. leprae* bacilli.

With regard to leprosy, two major observations were made:

i. the addition of killed *M. leprae* did not improve the 50% protection afforded by BCG; and

ii. re-vaccination of individuals who had received one dose of BCG as part of routine childhood immunization services can boost appreciably the protection against leprosy.

These results represent the second site of leprosy vaccine trials. The first study, in Venezuela, was completed earlier and gave similar results. The third and biggest trial will be completed in the year 2000 in India.

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