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Towards a Strategic Plan for Research to Support the Global Program to Eliminate Lymphatic Filariasis

Summary of Immediate Needs and Opportunities for Research on Lymphatic Filariasis

Identified by the Filariasis Community of Scientists in Association with an "LF Research Forum"

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Executive Summary

The Global Program to Eliminate Lymphatic Filariasis (GPELF), now four years old and clearly providing enormous health benefits from its broad deworming effects in the poorest sectors of the developing world, represents a societal investment already measured in the tens of millions of dollars. Despite rapid progress in scaling up the program to more than 38 endemic countries (or perhaps even because of this rapid progress), there is one element essential for ultimate program success that is now being severely neglected—research, both operational and basic (upstream).

The dramatic research successes in developing effective tools and strategies during the 1980s and 1990s provided the foundation for the GPELF. Generous public-private partnerships enabled its implementation, in concert with the 1997 formal resolution by the World Health Assembly calling for the elimination of LF as a public health problem worldwide.

Implementation alone, however, does not guarantee success. An essential characteristic of all successful public health programs is the continuing involvement of an active research community ready to provide solutions for program problems as they arise and for anticipated problems or barriers that might appear during program activities. Indeed, such operational research must be especially vigorous and focused in programs (such as the GPELF) with a time-limited goal for disease elimination. For lessons to be learned, program strategies improved, and activities made more effective and cost-efficient, there must be a problem-solving research community actively engaged with the ongoing program initiatives and focused on their challenges. Furthermore, for diseases such as LF, the neglected 10/90 diseases of poverty, where research funds are particularly limited, it is especially critical that the most acute research needs of the program be accurately identified, effectively prioritized, and clearly laid out so that the research community and the organizations supporting it can recognize the most important opportunities available and focus their resources accordingly.

It was toward this end that efforts were made during 2003–2004 to gather diverse and valued input from a very broad representation of the filariasis community, both program and research oriented. More than 90 research, clinical, and public health experts in LF came together in meetings (Annexes 1–3) and deliberations for the purpose of creating a comprehensive, collective assessment of today's LF research horizon and research needs. While there was broad agreement that the GPELF remains very much on target, in-depth assessments were made of ways to improve program support or increase understanding for each of the most important issues related to operational and basic, upstream research. For each of these domains, needs and opportunities were first defined and then prioritized.

For program-oriented, operational research the greatest needs fall into four clusters:

1) to establish the tools and measures of program success by
   a) evaluating comparatively the diagnostics and sampling strategies available, both in humans and in vectors,
   b) testing the endpoints for declaring transmission interruption,
   c) creating/testing sets of indicators developed to monitor
      i) morbidity-control/disability-prevention efforts,
      ii) multi-disease integrated program activities,
      iii) the GPELF impact on national health systems,
2) to enhance current program effectiveness by
   a) identifying adjunctive measures that could reduce the number of mass drug administrations (MDAs) required to achieve success (e.g., vector control, modified regimens of available drugs),
   b) refining predictive models for decision-making,
   c) improving methods and tools to treat difficult populations (especially urban and *Loa*-endemic communities),
   d) integrating LF programs with others having cost-effective complementarities,
   e) optimizing social mobilization techniques, and
   f) developing creative advocacy and fundraising strategies,
3) to ensure good clinical/morbidity management by
   a) standardizing clinical terminology and technical approaches to patient assessment,
   b) establishing best practices for home-based care for lymphedema, surgical care for hydrocoele/lymphocoele, and treatment for individuals with LF infection,
   c) assessing reversibility of clinical/subclinical LF disease,
4) to protect effectiveness of drug-based PELFs by
   a) establishing a definition for decreasing drug sensitivity,
   b) developing parasite repositories and surveillance for genotypic signs of drug resistance,
   c) continuing new and alternative drug development.

Particularly important for upstream research study are those issues defining the

1) effect of LF co-infections on clinical expression of other diseases and on responsiveness to routine vaccines,
2) mechanisms that determine the pathogenesis of lymphatic disease and its clinical expression,
3) susceptibility and resistance to LF and the effect of MDA on natural immunity to LF in treated populations,
4) genomics and proteomics of filarial parasites

It is clear that public health programs require both implementers and problem solvers. When problems loom large, society invests greatly in problem solving (i.e., research). When solutions are found, investments appropriately shift towards implementation. It is essential to recognize, however, that the need for problem solving (even to develop increased program efficiencies or cost-effectiveness) remains, and if not supported, threatens the very success of the program, putting at risk not only society's initial investment but also the health and welfare of the underserved populations for whom the program was created. The LF Research Community and their programmatic colleagues have deliberated extensively to define how best to strengthen the GPELF to ensure its immediate success and to enhance its research base to ensure long-term availability of problem solving research to provide solutions for program needs that are sure to arise. The clearer understanding that has emerged now promises to create a much stronger, more effective partnership between the implementing and research communities of the GPELF and the public and private funding organizations whose support is so essential for program success.

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1. INTRODUCTION

Eric A. Ottesen and Gary J. Weil

1.1 The Need for Research—The Need for a Strategic Plan for Research

The GPELF, now four years old and clearly demonstrating its enormous potential value to the poorest sectors of the developing world, already represents a societal investment measured in the tens of millions of dollars. Despite great progress in scaling up this program (currently active in 38 of the 80 endemic countries), or perhaps even because of this progress, there is one critical element for ultimate program success that is increasingly being neglected; namely, research.

It was the dramatic research success in developing effective tools and strategies during the 1980s and 1990s that provided the foundation and rationale for the GPELF, and it is widely appreciated that a mark of all successful public health programs is the continuing involvement of an active research community capable of providing solutions both to program problems as they arise and to anticipated problems or barriers that might arise during program activities. Indeed, such research, while essential for any successful control program, must be especially vigorous and focused in programs (such as the LF program) with a time-limited goal of disease elimination. Furthermore, for diseases such as LF, the neglected 10/90 diseases of poverty, where research funds are particularly limited, it is especially critical that research needs and research initiatives be clearly identified and effectively prioritized.

Research in filariasis is currently supported to various degrees by a number of funding organizations, but there is general agreement that better definition and prioritization of the research needs and opportunities (essential for the development of a strategic plan) would be extremely helpful, not only to the GPELF for developing the solutions it needs, but also both to researchers for identifying research opportunities and to funding agencies for understanding exactly how their investments fit into the overall horizon of LF research needs. Therefore, with the encouragement and support of a number of concerned organizations and agencies a Forum for LF research was organized (Annex 1) to review ongoing filariasis research, to identify promising research opportunities (both practical and fundamental) not currently addressed, and ultimately to provide the basis of a sound and widely agreed strategic plan for LF research to be developed by the World Health Organization (WHO) during 2005.

1.2 GPELF's Strategic Plan for Program Activities and Its Achievements to Date (2004)

In 1997, the World Health Assembly adopted a formal Resolution (50.29) calling on all Member States to work "toward eliminating lymphatic filariasis as a public health problem" and requesting the Director-General "to mobilize support for global and national elimination activities."

In 1999, a Strategic Plan for implementing this Global Program was agreed by the community of partners supporting the initiative (later affirmed at the First Global Alliance meeting in 2000), with the following focus:

- Goal: Elimination of LF as a public health problem by 2020
- Aims:
  - To reduce and eliminate transmission of LF,
  - To reduce and prevent morbidity (suffering and disease) in affected individuals,
  - To provide, through use of albendazole, a deworming benefit to endemic populations,
  - To provide a strengthening benefit to the national health services,
- Targets:
  - Transmission (five-year target): annual MDA increasing progressively to cover 200 million people by 2004,
  - Morbidity (two-year target): training materials, activities and networks in place in all countries with on-going LF elimination programs,
  - Ancillary benefits (two-year target): well-monitored pilot programs linking LF elimination with intestinal parasite control, immunization and other health-delivery programs.

In 2002, the Strategic Plan for program activities was reviewed at the Second Global Alliance meeting and revised as follows:

- Targets:
  - Transmission (by 2005): annual MDA scaled-up to cover 350 million people in 46 countries,
  - Morbidity (by 2005): 50% of programs (i.e., 23 countries) have strategy in place for disability prevention,
  - Program (by 2005): technical and management capacity in place to support ELF activities; monitoring, evaluation, and impact assessment complete in 15 countries; resources sufficient for meeting targets.

In 2004, the Strategic Plan was reviewed at the Third Global Alliance meeting and again updated, as follows:

- Targets:
  - Transmission (by 2010): annual MDA initiated or completed in all at risk areas of all 80 endemic countries; (by 2015) transmission interrupted in all endemic countries,
  - Morbidity (by 2010): disability programs established in all endemic countries; (by 2020) home-based self-care for all patients with lymphedema or surgery for all with hydrocoele,
  - Program (by 2010): interruption of transmission verified in 10 countries; (by 2020) surveillance in place for children born after 2015 to verify absence of transmission in all formerly endemic countries.

Progress by 2004 towards achieving the targets of numbers of countries and people undergoing annual MDAs as part of the GPELF is shown in Figure 1.

1.3 Research Activities Necessary to Support the GPELF

There are two, and only two, essential elements in determining whether an infection (or disease) is eradicable. Nei-

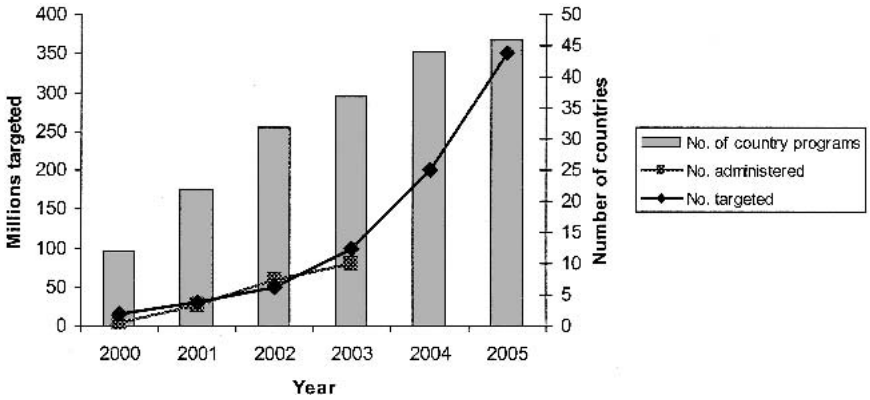

FIGURE 1. Progressive increase in the number of countries with active programs to eliminate lymphatic filariasis, the number of two-drug treatments delivered by mass drug administration each year, and the number of individuals projected for inclusion in the Global Program through 2005.

ther is a biologic determinant; instead, both are tools that can be created through research efforts.* The first is an effective intervention tool (to get rid of the infection) and the second is an effective diagnostic tool (to recognize when the infection is gone and to detect it if it begins to reappear).

Therefore, in the following consideration of the most important research needs and opportunities related to filarial disease, these two issues are presented first, followed by those research needs related to implementing the LF elimination program, evaluating it, and integrating it with other public health initiatives. Finally, since a firm science base of related, but more upstream, research must underlie all such program-oriented acquisition of knowledge (operational research), the more fundamental issues of pathogenesis, protective immunity, and genomics/proteomics are addressed as well. Each section begins with a summary of the prioritized research needs followed by an overview of the issue and the rationale underlying those identified needs.

*Ottesen, E.A., Dowdle, W.R., Fenner, F., Habermehl, K.-O., John, T.J., Koch, M.A., Medley, G.F., Muller, A.S., Ostroff, S.M., Zeichhardt, H. (1998) Group Report: How is eradication to be defined and what are the biological criteria? in Dowdle, W.R. and Hopkins, D.R., *The Eradication of Infectious Diseases,* John Wiley & Sons, Chichester, U.K. pp. 47–59.

2. RESEARCH DIRECTLY LINKED WITH GPELF ACTIVITIES (OPERATIONAL RESEARCH)

2.1 ESSENTIAL TOOLS—DIAGNOSTICS

Patrick J. Lammie**

Summary of Prioritized Research Needs

1) Develop effective, practical strategy for defining in field settings the areas and individuals with levels of *L. loa* microfilaremia so high as to be dangerous if MDA for concurrent LF were to be initiated (novel tool; novel approach with available tools),
2) Define the comparative accuracy of available diagnostics (antigen, antibody, DNA) and strategies for monitoring the progress of LF elimination programs and for deciding both when to stop MDAs and how to initiate surveillance to detect potential recrudescence;
   a) involves determining limits of sensitivity and specificity of available tests,
   b) requires longitudinal studies using all diagnostic tools concurrently and in both high- and low-prevalence areas where LF elimination programs are underway,
3) Take advantage of new technologies to improve user-friendliness and efficiency of the LF diagnostics currently available (e.g., isothermal polymerase chain reaction [PCR], multiplex antibody or PCR kits, use of oral fluids or urine for diagnostic tests),
4) Validate sampling strategies for testing both vector and human populations for LF infection or exposure to infection.

2.1.1 Overview

Principal challenges for LF diagnostics.

Diagnostic tools are essential at each step of the GPELF, initially for defining areas in need of MDA through mapping activities, then for monitoring the progress of programs following implementation of MDA, and finally for verifying the absence of infection both in areas where MDA has been conducted and in settings where LF was present historically. Existing tools are thought to be adequate for these purposes, but validation, refinement, and additional practical experience with the available assays are clearly needed.

As LF programs approach their planned end points (five or more years with greater than 80% MDA coverage), it will be necessary to determine whether transmission has been interrupted and whether MDA can be stopped. Parasitologic assessment, whether by microfilaremia or antigenemia, will require testing thousands of persons to demonstrate that infection levels are below 0.1%, the level targeted by WHO.[1] Current test strategies represent a best guess about approaches likely to be effective based on detection of microfilaremia or use of the immunochromatographic test (ICT) for antigenemia. To introduce use of entomologic tools or antibody testing for this type of decision-making will require an understanding of how these new tests perform. Intensive data collection, in the context of ongoing programs, will be needed to determine how these different tests for antigen, antibody, and vector infection correlate and compare with microfilaremia as a programmatic endpoint. Age- and sex-specific longitudinal data collections (in a variety of epidemiologic settings) are the key to developing the ability to recognize with confidence when transmission has been interrupted.

Preliminary guidelines for verifying the absence of LF transmission have been set by WHO (www.filariasis.org). Testing the effectiveness of the available diagnostic assays in the application of these guidelines also should be a priority. Since test specificity is of greatest concern for this issue, algorithms used to confirm suspected infection or exposure must be developed and validated. In addition, population movement from endemic to nonendemic regions carries with it the risk of possible introduction of LF transmission. Surveillance methods to address these situations, based on diagnostic and serologic methods, should be evaluated.

LF diagnostics today.

To date, LF infections have been diagnosed principally by direct demonstration of the parasite, initially through microscopic detection of microfilariae (mf) in the blood of infected persons and more recently by detection of parasite antigen or DNA.

While microscopic detection of parasites will continue to play an important role for demonstrating the impact of community-wide interventions, measurement of microfilaremia is not an ideal tool for program monitoring or surveillance, both because of 1) the need to examine nocturnal blood to find the parasites in most areas of the world and 2) the relative insensitivity of the commonly used methods for mf detection.

For *Wuchereria bancrofti*, assessment of antigenemia offers the convenience of any-time-of-day testing and greater sensitivity than testing for mf; however, current tests are specific for *W. bancrofti* and do not detect *Brugia* (*B. malayi* or *B. timori*) infections. Polymerase chain reaction–based methods can be used to detect infection in humans and to monitor filarial infection in mosquitoes with exquisite specificity and a sensitivity greater than that of direct microscopic detection of mf (or, in the mosquito, other larval stages), but current tests require a relatively sophisticated laboratory infrastructure. Also, since the technique identifies DNA still in the mf in blood samples from humans (i.e., not DNA freely circulating in the blood), its diagnostic usefulness is still constrained by microfilarial periodicity.

Antibody assays, though not detecting the parasite directly, in principle provide sensitive and relatively inexpensive tools to measure filarial exposure; however, the application of an-

** Other contributors in this working group are listed in Annex 2.

tibody tests to precise program monitoring still needs further development.

2.1.2 Research Needs

Improvements in existing tools.

Antigen detection.

Adult *W. bancrofti* release antigens that can be detected in human blood, plasma or serum by immunoassay. Unlike mf, circulating antigen can be detected with blood collected during the day or night. There are currently two antigen detection tests on the market. An enzyme-linked immunosorbent assay (ELISA) (which detects Og4C3 antigen and is produced by TropBio, Townsville, Queensland, Australia) is highly sensitive and specific; however, this test requires a back-up laboratory infrastructure and is mainly used in research projects.[2] The other antigen test (the ICT filariasis test, which detects AD12 antigen and is produced by Binax, Portland, ME) is a rapid-format ICT.[3] It can directly test blood, serum or plasma in the field and provides a result in 10 minutes. This test is widely used around the world to identify or map endemic areas for inclusion in MDA programs.[1] This antigen card test has provided much better information on the distribution of filariasis than traditional testing of night blood for mf. Indeed, card testing has identified many highly endemic areas that were not previously recognized as being endemic for LF.[4]

One important limitation of antigen testing is that it currently does not detect *Brugia* infections (which account for ~10% of the world's burden of LF). Another is that antigen test results often remain positive after treatment with DEC/albendazole or DEC/ivermectin.[5,6] This is probably because these drug regimens are not completely effective in killing adult worms, but it is also possible that even when all adult worms are killed, antigen clearance from the blood takes some (still undefined) period of time. Furthermore, when antigen prevalence rates are low in young children, as they are in some LF-endemic areas, measuring incidence rather than reductions of antigen positivity may be more valuable for monitoring changes in transmission of filariasis following implementation of MDA.

The ICT is now an essential tool for mapping the prevalence of filariasis. Should it fail to continue working for any reason or become unavailable for commercial reasons, the operation of the GPELF would be seriously compromised. The ELISA, which is slightly more sensitive than the ICT and provides a quantitative result, is widely used in filariasis research. Because both tests recognize the same circulating antigen, a strategic and sensible research goal would be

- the development of a complementary, alternative antigen-detection test.

Neither the ICT nor the Og4C3 ELISA can detect *Brugia* antigens. Consequently, for defining areas where *B. malayi* or *B. timori* are endemic and for defining changes in infection post treatment, antigen detection is not yet possible. Therefore, an important diagnostic initiative would be

- the development of an antigen assay that could be used in *Brugia*-endemic areas.

DNA Detection.

Humans.

Filarial parasites contain repeated DNA sequences that can be amplified and detected in humans or vectors by PCR. Currently, assays exist for screening blood samples and vectors for *W. bancrofti*, *B. malayi*, *B. pahangi*, *B. timori*, *L. loa*, and *Mansonella streptocerca.*[7–11] The sensitivity and specificity of the PCR have also made these tests particularly useful for validating the results of conventional parasitologic tests where doubts about species identification arise. Although PCR assays are widely used, their utility could be enhanced by

- improving the availability of standard kits for blood-sample collection, DNA isolation, PCR amplification, and DNA product detection. For use in the widest range of field settings, kits that permit isothermal amplification and visual detection of PCR products without instrumentation would be ideal.
- developing a multiplex PCR that could be used to detect and differentiate *Brugia* spp. and *Wuchereria* from other filarial parasites. If such an assay included *Plasmodium* or other pathogens, this would provide added information that could increase the value of the PCR tests and improve linkages to other health programs.

Mosquitoes.

The PCR is much more sensitive than traditional dissection and microscopy for detecting filarial parasites in mosquitoes.[12] Studies in a number of settings have shown that the percentage of PCR-positive mosquito pools decreased dramatically following MDA.[13,14] In addition, standardized protocols for detecting DNA in vectors have been developed through multicenter collaborations.[12] However, a number of practical challenges must be overcome before the PCR can be used with entomologic techniques for xenomonitoring the program effects on LF transmission; particularly,

- since different vector species are responsible for transmission of LF around the world, appropriate techniques for trapping mosquitoes to sample the mosquito populations accurately must be developed,
- sampling protocols need to be validated to define how or if these protocols should change as infection prevalence decreases following MDA.

Antibody detection.

In principle, antifilarial antibody responses can serve as very sensitive markers of filarial exposure and transmission, providing evidence of infection in an individual long before the development of antigenemia or microfilaremia, since antibody responses may develop within weeks to months following exposure to infective larvae. Thus, assays for antifilarial antibodies should be able to be used for program monitoring following initiation of filariasis elimination programs. Furthermore, compared with parasitologic or entomologic methods that estimate transmission at a single point in time, antibody responses represent a cumulative measure of the experience of infection, an important advantage for a monitoring or surveillance tool.

Native antigens isolated from filarial worms frequently cross-react with antigens from other nematode parasites. However, two recombinant-antigen (Bm14 and Bm-R1)–based antibody tests have been shown to be sensitive and specific for LF infection/exposure. The Bm14 antigen is equally sensitive for both *Wuchereria* and *Brugia* infection or exposure.[15–19] This antigen has some cross-reactivity with sera from patients with other filarial infections (loiasis and onchocerciasis), but not with sera from people with non-filarial nematode infections.[15] Field studies in Egypt showed that prevalence rates of antibody to Bm14 prior to initiation of MDA were much higher than antigen or mf prevalence rates in young children.[16] In addition, follow-up studies have shown that antibody prevalence rates in children decreased rapidly in the years following implementation of MDA.[20,21] The Bm-R1 antigen performs well in antibody tests for *B. malayi* infection/exposure (sensitivity > 95% and specificity = 100%), but it has limited sensitivity for *W. bancrofti* infection.[22] The Bm-R1 antibody test detects IgG4 antibodies (generally, though not always, associated with active infection) and is available commercially in ELISA and rapid-format versions (Malaysian Diagnostic Research Sdn. Bhd., Kuala Lumpur, Malaysia). It is currently being evaluated as a potential tool for mapping and monitoring program activities in *Brugia*-endemic areas. Additional studies are needed to define the relationship between its seroprevalence and either microfilaremia or antigen prevalence.

Even as antibody assays are developed and validated, especially for use as potential surveillance tools to detect exposure to infection, there are a number of important research questions that should be answered. Most current assays have focused on detecting antifilarial IgG4 because of the greater specificity of the IgG4 (vis a vis IgG1) response or because of the lack of an IgG4 response to the fusion partner used to express the recombinant antigen. In principle, IgG1 responses should develop sooner after exposure than IgG4 responses; however, it is not clear whether this difference is of practical significance in the field. Therefore, it is important

- to determine whether IgG1-based assays can be more effective diagnostics than those currently based on IgG4.

Since experience is lacking in the practical use of antibody assays in the context of LF programs, it is also necessary

- to assess whether children provide the best sentinel population; is there a preferred sampling strategy?
- to determine how the incidence of antibody responsiveness and the prevalence of antibody responses change in the aftermath of MDAs.

New diagnostic tools.

Diagnostic tests for *L. loa* infection.

The occurrence of serious complications from ivermectin administration, including fatal encephalopathy, in areas co-endemic for onchocerciasis and *L. loa* infection presents a major obstacle to LF elimination in Africa since the distribution of LF overlaps that of loiasis. Indeed, the extension of the GPELF into areas co-endemic for loiasis has been completely halted.[23] Since current data indicates a relationship between high *L. loa* blood mf levels and the likelihood of an adverse reaction, the decision to extend treatment into a given area should depend not only on whether loiasis is present, but also on whether there is high-intensity *Loa* microfilaremia or whether individuals with high levels of *Loa* microfilaremia can be effectively excluded from MDA activities.

Although a number of epidemiologic tools, including geographic information systems and rapid assessment procedures for loiasis (RAPLOA), have proven useful in identifying regions in which *L. loa* is endemic, difficulties remain in defining those areas where individuals at greatest risk of adverse outcomes (i.e., those with very high levels of microfilaremia) are found.[24–26] Currently available tools for diagnosing individuals include microscopy, a *Loa*-specific PCR, and serologic tests.[10,27] Of these, only microscopy is able to provide a quantitative estimate of the level of microfilaremia, and even this approach is hampered by the periodicity of the *L. loa* mf, the technical expertise required to distinguish *L. loa* from *Mansonella perstans* in the blood, and the time required to prepare, stain, and read the slides. Loa-specific PCR assays and serologic tests using recombinant *Loa* antigens have been developed, but are neither quantitative nor field-adapted at this time. To address these issues, research is needed at two levels.

- To evaluate currently available technologies (including quantitative thick smears, RAPLOA, and *Loa*-specific PCR) for their usefulness in identifying regions and individuals at highest risk; this will require epidemiologic studies in areas of low and high prevalence *L. loa* infection in the presence and absence of co-endemic *M. perstans*.
- To develop novel diagnostic assays and/or adaptations of currently available assays that can rapidly identify individuals with high intensity *Loa* microfilaremia in a field setting.

New diagnostic tests for LF.

Although a recent multicenter evaluation identified several promising candidate antigens for serologic assays to monitor LF, none was specific for *W. bancrofti*.[22] Because LF elimination programs may have limited effect on persons with or exposed to *Mansonella*, *Loa*, or *Onchocerca volvulus* infections, the absence of a specific for assay *W. bancrofti* will make it difficult to use current antibody tests for program monitoring in areas where these infections are co-endemic with LF. For example, testing young children in Egypt for antifilarial antibody using the Bm14 antigen is useful as a method to detect recent exposure to *W. bancrofti*; in contrast, an incident antibody response in Nigeria may only represent exposure to other filarial parasites. Therefore, a valuable research goal is

- the development of an antibody test that is specific for *W. bancrofti* to enhance the usefulness of 'exposure antibody' assays for program monitoring in sub-Saharan Africa.

Collection of blood specimens remains, in many places, a significant programmatic challenge. Therefore, to address this practical program concern, attempts should be made to

- adapt the existing serologic assays to work with oral fluids or urine that would provide program managers with other options to achieve complete or adequate sample collection.

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2.2 ESSENTIAL TOOLS—DRUGS AND CLINICAL DRUG TRIALS

Mark H. Bradley and V. Kumaraswami**

Summary of Prioritized Research Needs

1) Create a framework for monitoring for potential development of drug resistance, including
   a) defining *criteria* for the phenotype of reduced responsiveness and resistance,
   b) establishing a repository of mf and/or adult worms to provide base-line data on the occurrence of drug-resistant genotypes,
   c) initiating a surveillance system for diminished responsiveness to anti-filarial drugs,
2) Initiate clinical trials of available anti-filarial drugs to
   a) enhance mf reduction (through alternative dosages, frequency regimens, etc.),
   b) enhance adulticidal effectiveness,
   c) define ways to minimize patient treatment reactions at a population level,
   d) define a standard treatment for individuals with LF,
3) Ensure the safety of coordinated administration of drugs in linked public health programs (e.g., albendazole, ivermectin, azithromycin, praziquantel, etc.) or in other medical settings (e.g., used with human immunodeficiency virus/acquired immunodeficiency syndrome [HIV/AIDS] or tuberculosis [TB] multidrug therapy regimens),
4) Establish a framework for seeking a macrofilaricide,
5) Pursue discovery of an anti-*Wolbachia* agent suitable for MDAs and/or individual treatment,
6) Develop broadly applicable implementation strategies for use of DEC-fortified salt (with or without concurrent albendazole administration),
7) Evaluate use of moxidectin for LF.

2.2.1 Overview

Regimens currently in use.

A series of advances during the past decade transformed LF from a neglected disease of poor countries into a disease now recognized as potentially eradicable. Principal among the reasons for these advances were the identification of ivermectin and albendazole as new, effective anti-filarial agents and the discovery of new virtues for an old anti-filarial drug, DEC (i.e., its single-dose efficacy and macrofilaricidal action). These discoveries were essential for the subsequent creation of the Global Program to Eliminate LF,[1] whose very basis is the large-scale use of DEC, ivermectin (Mectizan®; Merck and Co., Inc., Rahway, NJ) and albendazole. Indeed, in many endemic countries literally millions of tablets of these drugs are distributed over a few short days each year (often on a single day), making GPELF the largest chemotherapy program ever undertaken.[2]

Currently it is just these three drugs that are available for use in single-dose, annual MDA programs. Albendazole is co-administered with ivermectin in areas of Africa and Yemen where LF and onchocerciasis are co-endemic. For all other LF-endemic regions, albendazole is co-administered with DEC. At the recommended dose levels, none of the drugs is completely macrofilaricidal, although all appear to inflict some lasting damage to adult worms. Both DEC and ivermectin kill mf efficiently; albendazole, on the other hand, has no direct effect on mf, but rather appears to suppress embryogenesis in the adult female worm. An alternative treatment strategy involving the use of DEC as a fortificant in table/cooking salt for a period of 1–2 years is currently used in just one country, but was a mainstay of the earlier, successful LF elimination program in China.

Modes of action of available drugs.

The mechanisms by which ivermectin and albendazole achieve parasite destruction have been well studied in other, non-filarial organisms. Briefly, ivermectin binds selectively and with high affinity to glutamate-gated chloride ion channels in invertebrate nerve and muscle cells. The molecule increases permeability of the cell membrane to chloride ions, resulting in hyperpolarization of nerve or muscle cells, causing parasite paralysis and death. The drug is also observed to affect other ligand-gated chloride channels, particularly those gated by gamma-aminobutyric acid.[3] However, it is still not certain why ivermectin shows activity only against mf, and not adult worms.

The primary target for albendazole is tubulin, and the drug is observed to have a higher affinity for parasite tubulin than for that of the host.[4] By blocking tubulin polymerization and microtubule formation, the drug inhibits mitosis, and therefore embryonation and egg hatching. It is very likely that albendazole exerts an effect on adult filariae in this manner.

In contrast, the understanding of how DEC achieves a lethal effect on either mf or adult worms is very poorly developed. There is some evidence that the drug may require cooperation with a functional host immune system to be optimally effective.[5,6]

How these drugs act in combination on different lifecycle stages is not at all understood, or even well studied.

Resistance mechanisms.

All three of these anti-filarial drugs have been used extensively in humans, and to date there has been no unequivocal identification of resistance in LF to any of the drugs. While this is clearly most encouraging, it is also not entirely surprising since currently there is no phenotypic definition of such drug resistance and no comprehensive system for monitoring and evaluating the phenomenon in humans.

The mechanisms by which resistance to albendazole is conferred are well understood in nematode parasites of other animals, and PCR-based methods for detection and analysis of the expression of the beta-tubulin genes TUB1 and TUB2 are available. Resistance to albendazole appears to be associated with a loss of high affinity receptors, resulting from neucleotide changes, predominantly in the TUB1 gene.[7,8]

** Other contributors in this working group are listed in Annex 2.

Mechanisms controlling parasite sensitivity to ivermectin appear to be considerably more complex. Studies on the nematode *Caenorhabditis elegans* show that simultaneous mutation of three genes (*avr-14*, *avr-15*, and *glc-1*) that encode glutamate-gated chloride channel (GluCl) α-type subunits confers a high-level resistance to the drug.[9,10]

A decrease in sensitivity of filariae to DEC has been suggested, particularly where the drug has been used for many years. However, whether these observations reflect a genetically conferred resistance, deficiencies at a programmatic level, or some other cause is as yet not certain.[10,11] These uncertainties are currently compounded by a lack of suitable targets to facilitate the evaluation of DEC resistance in humans.

Safety of one- and two-drug regimens.

The safety of each of the three individual drugs used in the GPELF has been well established. Diethylcarbamazine, in use for nearly half a century, has proven to be extremely safe and effective both in the clinic and the field without supervision; hundreds of millions of doses of ivermectin have been safely distributed since the drug was introduced for the treatment of onchocerciasis; and albendazole has been widely used for several decades, again in hundreds of millions of people, with a remarkable safety record. When co-administration of these drugs was initiated through GPELF, active surveillance programs were undertaken in all countries where MDAs were begun. These documented thoroughly the safety of the combinations in the field.[12–14] (Only in areas of Africa endemic for loiasis, where severe adverse reactions may occur with the administration of ivermectin, are MDAs for LF elimination now contra-indicated because of safety concerns [see below].)

2.2.2 Research Needs: Drug Development

Macrofilaricide.

While DEC and albendazole clearly have macrofilaricidal effects,[15,16] there is still recognition of the potential value a more potent macrofilaricide would bring to efforts to achieve LF elimination, not only in rapidly diminishing microfilaremia but also in halting progression of the initial morbidity associated with the infection and induced largely by the adult-stage worms. Since drug development is a long and costly exercise, with the pharmaceutical industry being generally reluctant to invest in ventures that have little or no direct financial return, any meaningful advance in the development of a macrofilaricide will likely be feasible only if an effective partnership between development agencies, academia, the pharmaceutical industry, and funding organizations can be established. Therefore,

- despite the complexity of the challenge, a working group should be established to explore the creation of an anti-filarial (and particularly, macrofilaricidal) drug development partnership, and
- this partnership should focus not only on novel drugs but also take leads from currently active drug classes and from active compounds identified in 'traditional' medicine.

Anti-Wolbachia drugs.

Over recent years, alternative approaches to classical chemotherapy have emerged as the *Wolbachia* endosymbionts of filariae have been recognized as potential drug targets.[17] Indeed, treatment with the antibiotic doxycycline for six weeks depletes *Wolbachia* from *W. bancrofti*, yielding an almost complete absence of microfilaremia one year later and suggesting effective, long-term block of embryogenesis[18] and/or macrofilaricidal activity. Since treatment of filariasis with such protracted regimens of antibiotics is not compatible with the principles of mass chemotherapy, an important challenge today is

- to identify or develop alternative antibiotics or regimens effective against *Wolbachia* that could be used in MDA programs and/or offer specific treatment to infected individuals.

Moxidectin.

Studies to evaluate moxidectin as an alternative to ivermectin for the treatment of onchocerciasis have shown moxidectin to be more effective than ivermectin in most animal models.[19] The drug has potent effects on mf and results in long-term sterilization of female adult worms, but there is no evidence as yet showing that moxidectin is macrofilaricidal. Moxidectin is currently being evaluated as a treatment for onchocerciasis through a partnership involving WHO and the owner (American Home Products [Wyeth]). If the evaluation of moxidectin for human onchocerciasis produces promising results, then

- moxidectin should be evaluated for effectiveness against LF parasites as soon as possible.

2.2.3 Research Needs: Clinical Drug Trials

Standardization of clinical trial techniques.

Clinical trials with existing and newer anti-filarial drugs are needed to address many of the issues that have arisen during implementation of the GPELF and to provide insights that will strengthen the Program's evidence base. Therefore,

- standard operating procedures need to be developed for controlled clinical trials to assess the efficacy of anti-filarial drugs and to define the methodologies used
  a) to estimate mf density,
  b) to calculate clearance of microfilaremia,
  c) to define the time points at which determinations will be made,
  d) to provide evidence of the parasite adulticidal effects of drug treatment (i.e., development of nodules post-treatment, ultrasound follow-up of identified nests of worms, and antigen detection assays at appropriate intervals),
  e) to document the side effects that may be less commonly recognized (such as proteinurea or hematuria).

Optimizing clearance of mf.

While the currently recommended two-drug regimens effectively clear microfilaremia,[16,20–22] if ways to improve them

could be found, clear programmatic benefits might result. Therefore, efforts to optimize the regimens need to be undertaken in different regions and with different strains or species of parasites

- by exploring alternative frequencies of treatment (e.g., six-monthly),
- by defining the total number of treatments required in areas with different prevalences of infection,
- by evaluating the effects of higher dosages of ivermectin (i.e., > 200 μg/kg) and/or albendazole (i.e., > 400 mg) in the two-drug co-administration regimens.

Recrudescence of microfilaremia post treatment is well recognized,[23,24] but he factors that govern it are not well understood. Therefore,

- controlled trials that follow microfilaremic individuals over extended periods of time should be undertaken to identify and define the factors that determine the reappearance of microfilaremia in treated individuals.

Diethylcarbamazine-fortified salt.

The use of DEC-fortified salt is an alternative to repeated single-dose regimens for interrupting transmission[25] and might be especially useful in those situations where reaching the entire at risk population is particularly difficult (as in urban populations). While DEC-fortified salt has documented effects on adult worms, decreased frequency of side effects, and potential prophylactic benefits, it is unclear if the presence of DEC, even at the low concentrations in fortified salt, would make it unsafe for use in urban or other areas of Africa where onchocerciasis might coexist. Also, current recommendations are for use of DEC-salt for a period of 1–2 years to achieve LF elimination, but studies to define the optimal duration of treatment have not yet been carried out. Therefore, additional studies are needed

- to evaluate the duration of use of DEC-salt yielding an optimal anti-filarial outcome,
- to determine the lowest LF-effective dose of DEC-fortified salt that could be safe for use in onchocerciasis-endemic areas, either urban or where previous treatment with ivermectin had been adminstered,
- to explore the potential for enhanced efficacy of DEC-salt when intermittent (e.g., once a year) albendazole is co-administered.

Decreasing parasitologic response to therapy.

Success of the LF elimination program could be jeopardized if a decrease in the efficacy of available drugs developed. Early identification of decreased susceptibility to currently available anti-filarial drugs is undoubtedly a most cost-effective means for ensuring the continued, long-term success of the LF elimination program. While analysis of changes in the parasite genotype has the potential for providing both baseline and situational information on the distribution and frequency of resistant genotypes,[10] a definition for resistant phenotype has yet to be agreed, and the challenge is made more difficult (particularly with respect to DEC) by the variability of responses in different individuals. For example, many observations document the persistence of microfilaremia even after individuals or populations have received several courses of DEC in single or multiple doses;[23] and, just as concerning is that only a proportion (~70%) of adult *W. bancrofti* is usually killed by a single dose of DEC, with no additional killing of the remaining worms even with additional doses of the drug (documented by ultrasound).[26] Therefore, while to date there has been no documentation of the existence of resistance to DEC, ivermectin or albendazole in LF parasites, there is now an urgent need

- to establish a working group to create a phenotypic definition of reduced responsiveness and drug resistance and to identify the defining criteria that must be assessed,
- to estimate the frequency of occurrence of such reduced responsiveness (e.g., by examining program data from around the world) and to document the existence of the problem in identified individuals who can be further studied,
- to develop a central repository of genetic material from mf and adult worms in different endemic areas to facilitate
  1) collection of base-line data on the occurrence of potential drug resistant genotypes,
  2) implementation of an effective surveillance system for drug resistance,
- to model drug resistance in LF so that the implications of the possible emergence of resistance can be predicted and deficiencies in relevant knowledge can be identified.

New drugs and drug combinations.

Endosymbiotic bacteria (*Wolbachia spp.*) within filarial parasites influence the life cycle of the parasites to such a degree that killing these bacteria produces adulticidal effects. Recent trials in onchocerciasis and *W. bancrofti* infections suggest that long-term (six-week) administration of tetracycline may have profound anti-parasitic effects on adult filarial parasites.[27] Therefore, to find drug regimens that are more practicable for public health programs,

- the adulticidal effects of various regimens of tetracyclines, rifampicin, and combinations of antibiotics and conventional anti-filarial drugs (such as DEC) should be evaluated in controlled clinical trials.

Moxidectin is currently in early clinical stages of evaluation for onchocerciasis. If this compound progresses satisfactorily in clinical development,

- moxidectin should undergo clinical trials for evaluation of adulticidal and/or microfilaricidal activity in LF.

While the safety and pharmacokinetics (PK) of the two-drug co-administration regimens used for the LF MDA programs have been well established,[12–14] increasingly the desirability of linking LF programs with other public health initiatives also based on MDA is being appreciated. Similarly, the increasingly widespread and long-term use of drugs to treat populations with HIV/AIDS or TB means that many individuals could inadvertently receive their LF MDA drugs at the same time as they are receiving other drugs as well. There is a concern that excessive changes in the metabolism of al-

bendazole and/or ivermectin might occur when these LF drugs are administered to patients on anti-retroviral or anti-TB therapy. For all these reasons,

- PK and safety studies should be conducted with the LF MDA regimens and with those drugs used in other public health initiatives that might be linked with the GPELF (e.g., praziquantel [schistosomiasis], azithromycin [trachoma]),
- surveillance should be promoted for potential pharmacokinetic interactions between LF therapy and highly active retroviral therapy for HIV/AIDS and combination therapy for TB; if evidence for such potential interactions is found, formal PK and safety studies should be undertaken.

Treating LF infection in individual patients.

Physicians and other health providers in endemic areas must care for individual patients with individual treatment regimens, but since these health providers in most countries are only peripherally involved in the GPELF, many are unaware of the recent advances in the chemotherapy of LF (particularly the efficacy of single-dose treatment in curing infection). Current physician guidelines (including the major textbooks of medicine) do not refer to these recent advances; indeed, even the WHO guidelines (last issued in 1992) do not refer to the dramatic changes that have taken place in the chemotherapy of LF. Consequently, tens of thousands of medical students today are completely unaware of the changed concepts of dosing and optimal regimens used to treat LF.

The guidelines for program managers, focused on interrupting LF transmission, are based on studies of the effects of single-dose drugs and drug combinations on decreasing microfilaremia in individuals and populations. The principal goal for treating individual patients, however, is to kill the adult parasite. Guidelines for treating individual patients and populations in endemic areas must be harmonized, and while available information suggests that the same drug regimens used in MDAs may also be optimal for the treatment of individual patients,

- clinical trials to define the optimal regimen of drugs and drug combinations for treating individuals with LF are urgently needed,
- in addition to defining the most effective drugs, clinical trials must also resolve questions about
  1) the optimal frequency, duration, and end point of treatment,
  2) the best tools for monitoring successful therapy.

Preventing LF infection (chemoprophylaxis).

The current global strategy based on annual single doses of combinations of anti-filarial drugs aims to clear mf from infected individuals. Healthy individuals in these areas may see little value in consuming the drugs in the absence of any direct personal benefits or long-term protection against the infection. Identification of agents or regimens that would afford protective benefits to exposed individuals would likely enhance the interest of people in participating and, thus, the success and sustainability of programs. Earlier studies in animal models and epidemiologic observations in human populations suggest that DEC (as individual doses or in fortified table salt) may be prophylactic in LF. Therefore, it would be valuable

- to develop a trial design that can identify prophylactic properties of LF drugs in human populations,
- to assess the prophylactic effects of DEC, ivermectin, albendazole and other newer drugs (such as moxidectin) in human populations (as well as in animal models).

Minimizing treatment reactions.

A major programmatic challenge in LF control/elimination programs based on drug administration has been the occurrence of post-treatment reactions in endemic populations, as they sometimes threaten program implementation or expansion. The advent of single dose chemotherapy has greatly improved the compliance of populations, and while post-treatment reactions are usually mild, self-limited and in most situations require only symptomatic therapy, efforts to reduce the occurrence of even these mild reactions could very much improve compliance in control programs. Therefore, trials should be undertaken

- to test novel approaches to minimizing the occurrence and intensity of post-treatment reactions (e.g., by administering the drugs in divided dose, with or without food, at different times of the day, etc.),
- to compare the frequency and intensity of post-treatment reactions following anti-Wolbachia treatment with those following conventional anti-filarial treatment.

Treating LF in L. loa*-endemic regions*

Since the current recommendation for treating populations in most LF-endemic countries of Africa calls for use of ivermectin (Mectizan®) and albendazole, and since the risk-benefit assessment of Mectizan® administration in areas where loaisis and LF coexist without onchocerciasis currently is judged to weigh against MDA, large areas of central and west Africa are excluded from the MDAs using the drug regimens now available.[28] Therefore, there is an urgent need

- to conduct clinical trials that examine the safety and efficacy of a variety of anti-filarial regimens in areas were loiasis and LF coexist, with or without concurrent onchocerciasis, including the recently initiated trials of albendazole pre-treatment to reduce *Loa* mf densities prior to treatment with ivermectin.

Lymphatic filariasis and traditional, indigenous medicine.

In many endemic countries numerous traditional and indigenous therapies are currently available and used for the treatment of LF. In almost all instances these drugs and interventions have not been evaluated in controlled clinical trials. Therefore,

- efforts must be made to evaluate the efficacy and safety of these traditional and indigenous therapies; if any is found helpful, their incorporation into the GPELF should be encouraged.

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2.3 LF DISEASE—CLINICAL MANAGEMENT

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Summary of Prioritized Research Needs

1) Investigate effects of MDA alone on progression or reversal of LF disease (lymphedema, filaricele, acute adenolymphangitis [ADL]),
2) Evaluate comparative studies of filaricele surgical techniques for a) relapse rates, b) surgical costs and duration, c) post-operative complications,
3) Refine and standardize (through a consensus conference) clinical definitions of LF disease, and describe epidemiology of LF disease with these new definitions,
4) Improve differential diagnosis of pediatric LF disease, and initiate studies to define measures to prevent its progression,
5) Investigate the value of traditional medicine approaches to lymphedema and urogenital disease,
6) Increase understanding of the skin barrier function, entry lesions, and ways of preventing the bacterial complications of LF disease,
7) Define epidemiology, risk factors, complications, and optimal management of chyluria.

2.3.1 Overview

LF disease management today.

In May, 1997, the World Health Assembly called for a global effort "to eliminate lymphatic filariasis as a public health problem." Unlike most other infectious disease eradication or elimination programs, which focus only on interrupting transmission, two essential program components, or "pillars," were envisioned for LF elimination: mass treatment with antifilarial drugs to interrupt transmission of the parasite, and care for those who already suffer from lymphedema or hydrocoele.[1] This clinical care component is believed to be important for the success of the overall programs; the antifilarial drugs themselves are thought to provide little benefit to people with chronic filarial disease, which is usually life-long;[2] and reducing the suffering of those with filarial disease enhances program acceptance and maximizes population coverage with antifilarial drugs.

Proper surgery can cure hydrocoele. For lymphedema of the leg, simple, inexpensive self-care measures have been shown to reduce the frequency of acute bacterial adenolymphangitis (a major factor in disease progression),[3,4] stop progression of lymphedema to elephantiasis, and improve quality of life.[5,6] These measures, which can be readily incorporated into the daily routine of people with lymphedema, include hygiene, skin care, range-of-motion movement or exercise, elevation of the leg, and wearing proper shoes to protect the feet from injury.[7]

Despite the availability of these simple and effective strategies for hydrocoele surgery and lymphedema management, thus far a relatively small proportion of people with filarial disease worldwide has had access to them.[8] With initiatives in several countries, this situation is beginning to change. Renewed effort is now underway at the program level to implement morbidity control interventions that are based on current knowledge of LF disease.[9] However, research—basic, clinical, epidemiologic, social science, and operational—remains extremely important.

2.3.2 Research Needs

Epidemiologic assessments based on standardized definitions and techniques.

Considerable progress has been made during the last decade in our understanding of LF disease. However, particularly in the area of urogenital disease, these new findings have not been widely applied to epidemiologic research or to clinical or surgical management. For example, the relative frequency of hydrocoele, lymphocele, chylocele, and hematochylocele, all indistinguishable by clinical examination and all characterized by fluid inside the scrotal sac, is unknown. In addition, the clinical assessment of treatment outcomes has not been standardized. For example, adult worm death has been inferred by the disappearance of filarial antigenemia,[10] detection of scrotal nodules,[11] and cessation of the filaria dance sign,[12] among other techniques. Ultrasound is being used increasingly, but techniques and procedures need to be standardized, and its potential value in women and children with *W. bancrofti* and in all individuals with *Brugia* infections still needs to be defined. Therefore,

- a consensus conference should be held both to establish case definitions for clinical and epidemiologic research in LF, and to identify and standardize the most appropriate diagnostic approaches for clinical research in individuals of all ages,
- epidemiologic studies should be carried out using the refined clinical definitions to describe the frequency, severity, geographic and age distributions of clinical conditions caused by LF (including less well-defined conditions of filarial arthritis and glomerulonephritis),
- through these studies the impact of disease should be measured, especially as it relates to disability (including psychological), since this information will be essential for planning for morbidity control, for advocacy, and for gaining further understanding of the pathogenesis and progression of filarial disease.

Effect of MDAs on filariasis morbidity.

Data on the impact of antifilarial MDAs on filariasis morbidity are inconsistent. Several studies report surprising reductions in acute attacks, lymphedema, and/or hydrocoele following MDAs,[13] but a roughly equal number of studies reports no such association. Many of these studies are limited by inadequate or non-standardized case definitions and intermittent or incomplete follow-up. Few studies included control groups. Assessing the public health impact of mass treatment

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with antifilarial drugs is a critically important issue for program advocacy and for planning morbidity control strategies. The MDAs also induce transient adverse reactions, which, when DEC is used, may include lymphatic inflammatory reactions and hydrocoele. Therefore,

- the impact of MDAs on the prevalence and incidence of ADL, lymphedema, and hydrocoele should be studied both acutely and chronically using rigorous case definitions, close clinical assessment and control groups; they should be conducted both in areas using DEC/albendazole and in areas using ivermectin/albendazole.

Pediatric LF disease.

Pediatric LF remains under-recognized, misunderstood, and misdiagnosed. Physicians in filariasis-endemic areas need to be trained in differential diagnosis of swollen limbs. Guidelines are needed for referral from the peripheral level to reference centers, particularly for children with lymphedema or hydrocoele.

Most LF morbidity control activities currently focus on reducing severity or stopping progression of existing disease, and they do not address the prevention of clinical disease in persons who may already have subclinical disease. Lack of efforts to address prevention represents a major gap in current morbidity control efforts. Therefore, studies of pediatric, endemic-area populations are needed

- to define the extent and character of subclinical disease in children,
- to assess the effectiveness of early anti-filarial treatment on the development of subclinical and clinical lesions,
- to determine the degree to which bacterial ADL and lymphedema can be prevented through education programs focused on hygiene, maintenance of the skin barrier function through good skin care, and recognition and treatment of entry lesions,
- to assess the effectiveness of educational materials and school curricula developed to help prevent pediatric filarial disease.

Urogenital disease.

Clinical research and observation in filariasis-endemic areas, especially in Recife, Brazil, has revealed that fluid inside the scrotal sac, which was considered as hydrocoele, actually is comprised of several distinct entities: true hydrocoele, lymphocele, chylocele, and hematochylocele. Indeed, the term filaricele has been suggested recently to encompass all of these conditions. The implications for surgical management and for risk of compromised testicular function vary considerably for these different conditions. Little is known about the relative frequency of these conditions, and techniques and markers to discriminate among them are currently inadequate. Therefore,

- clinical studies are needed to define the epidemiology and assess the risk factors for various causes of filaricele,
- laboratory and diagnostic studies are urgently needed to develop simple, inexpensive, easy-to-use markers to distinguish among various causes of filaricele.

In many filariasis-endemic areas, hydrocoelectomy is commonly regarded as a simple, uninteresting procedure that is relegated to junior doctors, and current techniques are regarded as adequate. However, patient follow-up is sporadic, and rates for complications following surgery and for hydrocoele recurrence in filariasis-endemic areas are unavailable. Furthermore, anecdotal reports suggest that relapse rates may be unacceptably high using current standard techniques and that the rates of infection and other post-operative complications are also quite high in some settings. To prevent recurrence of filaricele, complete excision of the tunica vaginalis is recommended by some, but this technique has not been adopted globally and multi-center studies have not yet documented its superiority to current techniques. Therefore,

- comparative studies of filaricele surgical techniques should be performed using standardized procedures and state-of-the-art diagnostics to distinguish various causes of filaricele,
- standardized outcome assessment should include
  1) relapse rate at specific post-operative intervals,
  2) surgery costs and duration,
  3) rates of infectious and other post-operative complications.

Because some hydrocoeles appear to reverse spontaneously and surgical morbidity is high in many filariasis-endemic areas, and because non-invasive physiologic measures such as deep breathing have proved useful in the management of non-filarial lymphedema,[9]

- studies should explore the role of such non-invasive measures in the management of scrotal lymphedema and other forms of urogenital disease where surgery is not required to preserve testicular function (i.e., excluding lymphocele, chylocele, and hematochylocele).

Lymph scrotum is a devastating disease of unclear etiology; however, previous hydrocoele surgery that did not involve excision of the tunica vaginalis has been postulated to be a risk factor.[14] Furthermore, techniques for surgical management of lymph scrotum and urogenital elephantiasis are not standardized and follow-up is limited. Therefore,

- clinical research is needed to determine the most effective techniques for surgical management of scrotal and penile elephantiasis and for lymph scrotum.

Lymphedema and elephantiasis.

Maintaining skin barrier function and treatment of entry lesions.

Little is known about current practices for skin care in filariasis-endemic areas, and it is unknown whether such practices serve to maintain or degrade the skin barrier function.[9] Locally made topical preparations are widely used, but little is known about them. They may be effective and inexpensive, or they may, in some cases, be harmful. Therefore,

- the safety and efficacy of current skin care practices, including the use of local topical preparations, should be determined.

The physical location and pattern of entry lesions (breaks in the skin that can serve as conduits for infection) likely reflect the anatomy of lymphatic dysfunction, and may have implications regarding its etiology (i.e., filarial or non-filarial). Furthermore, the prevalence and severity of entry lesions in various populations and their relationship to LF infection prevalence are unknown. Because prevention and treatment of entry lesions is such an important focus of recommended lymphedema management, this information is very much needed. Therefore,

- the epidemiology of entry lesions in filariasis-endemic areas needs to be described, using standardized definitions.

Although, in general, most entry lesions respond to basic topical agents, treatment failures do occur. The microbial, antimicrobial, and host factors responsible for these treatment failures are not completely understood. Therefore,

- research is needed on the bacteria and fungi involved in entry lesions in different endemic regions and on their susceptibility to topical antimicrobial agents,
- risk factors for clinical treatment failures should be assessed, especially in areas where investigations can include research on the microbial flora.

Acute attacks.

Two distinct clinical syndromes have been described for acute attacks, one putatively associated with a filarial etiology (i.e., adult worm death) and the other with a bacterial etiology.[14,15] In addition, observations that the frequency of acute attacks has decreased following MDAs, along with some data from animal models, suggest a possible role for filarial larvae in initiating inflammatory events.[13] A role for *Wolbachia* in acute attacks has also been speculated.[16] However, such events have not been adequately described clinically, thereby limiting our understanding of the etiology and frequency of such events. Therefore,

- acute attacks or inflammatory events not explained by bacterial infection or by adult worm death should be studied and case definitions developed and tested,
- the frequency of acute filarial attacks and associated acute morbidity, such as hydrocoele, following MDAs should be investigated in areas using different mass treatment drug regimens,
- longitudinal studies of patients treated with doxycycline or other drugs that target *Wolbachia* should be designed to include observation and clinical characterization of acute attacks as well as control groups coming from the same endemic area or from areas with equivalent levels of filarial transmission,
- additional placebo-controlled, blinded trials of different acute attack management and prevention strategies (including prophylactic antibiotics or even bacterial vaccines) should be carried out in areas with bancroftian filariasis.

Lymphedema management.

The role of co-morbid conditions and genetic factors in the progression of lymphedema, fibrosis, and acute attacks is not well defined. Additional knowledge is needed to guide clinical management. Therefore, studies should be undertaken

- to define the role of co-morbid conditions and genetic factors in the progression of lymphedema, fibrosis, and acute attacks.

Sustained patient motivation is essential for continued daily practice of lymphedema self-care. Therefore, operational research should be carried out to evaluate the effectiveness of a variety of interventions to maintain patient motivation and compliance with lymphedema management techniques.

In filariasis-endemic areas, persons with lymphedema currently seek care for lymphedema and acute attacks from a variety of sources, and traditional methods may be more or less effective. Also, engaging traditional healers in lymphedema management can lead to sustainable programs that are integrated into current health systems. Therefore,

- the effectiveness of traditional methods for lymphedema treatment should be evaluated and clinical trials performed for traditional measures that appear promising.

Chyluria.

Little is known about the epidemiology, risk factors, or complications of chyluria. The effect of diet and co-morbidities such as obesity in triggering the initial appearance of chyluria is poorly understood. In some areas, chyluria has been a relatively frequent clinical manifestation of LF, often frustrating and poorly managed.[14] In severely affected individuals, chyluria can present as a serious wasting illness. Little is known about the inflammatory properties of lymph fluid in the extra-lymphatic spaces in the urinary collection system (e.g., inside the bladder). Therefore,

- field-applicable diagnostic criteria for chyluria should be agreed upon,
- studies should be carried out to define the descriptive epidemiology, risk factors, complications, and best management approaches for chyluria,
- the usefulness (or not) of surgical approaches to management of chyluria should be assessed and compared with dietary management techniques,
- clinical studies should assess the effects of chyluria on the endothelium and function of the urinary collecting system.

Benefits and public health impact of LF morbidity control.

The importance of morbidity control activities to GPELF success is strikingly clear, particularly to program managers. To date, however, few data exist to document quantitatively the degree to which morbidity control activities enhance either the general public health and economic well-being of endemic populations or even the effectiveness of LF MDA activities (by increasing drug coverage or population compliance). This information is essential both for cost-benefit assessments and for program advocacy. Therefore, studies should be designed

- to document the impact of morbidity control on MDA drug coverage and on general public health,
- to analyze cost-effectiveness and cost-benefit of LF mor-

bidity control to guide programs in maximizing impact and minimizing cost.

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2.4 PROGRAM IMPLEMENTATION

Mwele Malecela-Lazaro and Nana Twum-Danso**

Summary of Prioritized Research Needs

1) Define the duration and coverage of MDA necessary to achieve interruption of transmission in different epidemiologic/entomologic settings,
2) Determine the effect of the rate of upscaling on
   a) duration of MDA necessary to achieve interruption of transmission,
   b) cost of the program in the short- and long-term,
3) Identify the most effective social mobilization strategies for MDA in different settings,
4) Optimize MDA strategies for urban areas with low prevalence of infection,
5) Determine the duration and coverage of DEC-salt administration necessary to interrupt transmission,
6) Identify safe strategies for LF elimination in *L. loa*-endemic areas,
7) Assess the impact of LF elimination programs on health systems,
8) Identify the complementarities of specific targeted-disease programs that can promote linkages among the programs for coordinated implementation, monitoring, and evaluation,
9) Review outcomes and best practices both from ongoing LF elimination programs and from other, similar targeted-disease programs, and
10) Develop novel means of resource mobilization for upscaling national programs.

2.4.1 Overview

Program.

The GPELF was formulated in 1998[1] with a goal to eliminate LF as a public health problem through focus on two principal targets:

1) interrupting transmission of the parasites that cause LF,
2) alleviating the suffering and preventing the morbidity caused by the infection.

For interrupting transmission, the two alternative strategies are

1) annual, community-wide two-drug distribution of albendazole with either DEC or Mectizan® (ivermectin) to at-risk populations until the criteria for stopping MDA are met (expected after 4–6 rounds of effective MDA coverage) or
2) exclusive use of table/cooking salt fortified with DEC for 1–2 years by at-risk populations.

For morbidity management, activities are designed to establish community home-based self care for people with lymphedema and to provide access to surgery for men with hydrocoeles/lymphoceles.

Progress.

By 2000, the first national PELF had been launched. By the end of December 2003, a total of 38 countries had established MDA programs based on albendazole and DEC, albendazole and ivermectin, or DEC-fortified salt; approximately 83 million people had been treated with these regimens and another 39 million with DEC alone.[2] The total number of people who have benefited from hydrocoelectomies or community-based self-care training is more difficult to ascertain at this time since many such programs are decentralized and still quite new.

The WHO estimates that by 2008 almost 800 million people in 70 countries will have been treated through MDAs.[3] If this target is achieved, it would represent approximately 85% of the countries and 64% of the total-at-risk population in the GPELF. As some countries prepare to exit the GPELF after 4–6 years of MDA and new countries join the GPELF, critical questions continue to arise regarding the most effective and efficient ways to implement and manage the PELFs; these must be articulated and addressed to ensure achievement of the goal to eliminate LF by 2020.

2.4.2 Research Needs

Defining the general measures of successful program implementation.

Gauging the success or failure of national and global LF elimination programs depends on recognizing a number of overarching issues and addressing them directly through research studies early enough in the program for any necessary corrections to be made in a timely fashion. These researchable issues (also emphasized elsewhere in this document) are essential for successful implementation of the PELFs and include

- determining the feasibility of interrupting transmission of LF *vis à vis* successful elimination of LF as a public health problem in all countries,
- defining effective strategies to prevent recrudescence,
- developing tools to measure the reduction in incidence of LF morbidity, and
- assessing the impact of MDAs on geohelminth prevalence, on anemia and general health status of the population, on national health systems, and on other impact measures,
- proving the cost-effectiveness and cost-benefits of LF elimination programs.

Optimizing effectiveness of drug distribution strategies.

Drug distribution based on annual MDAs.

The impact of MDAs on LF elimination depends directly on the proportion of the total targeted population actually ingesting the drugs, not merely receiving them, and on the

** Other contributors in this working group are listed in Annex 2.

geographic completeness of the MDA in covering contiguous areas where transmission might be ongoing. Thus, it is imperative to identify those strategies leading to maximal MDA effectiveness by addressing, and in different epidemiologic settings, certain particularly critical issues, including

- the cost-effectiveness of directly observed drug administration compared to other indirect approaches to drug administration,
- the rates of MDA coverage as a function of the types of personnel distributing drugs, and
- the most effective techniques for communicating and managing adverse events related to the MDA.

Drug distribution based on DEC-fortified salt.

Demonstration of the effectiveness of annual single-dose treatment with antifilarial drugs opened the door to new programmatic options for the elimination of LF, but barriers to the successful implementation of programs based on annual mass treatment still include 1) the difficulty of developing an infrastructure capable of distributing the drugs; 2) the need to achieve very high coverage levels; and 3) diminished compliance because of adverse reactions associated with the death of the parasite.

Use of salt fortified with DEC is an alternative program strategy avoiding many of the difficulties associated with tablet-based MDAs. Fortified salt (as recognized already in salt iodization programs) can be delivered to populations without developing new, dedicated drug distribution systems, and the DEC-salt (generally 0.2–0.3% w/w) is an effective microfilaricide that is rarely associated with adverse reactions.[4] Furthermore, since fortification techniques for DEC and iodine are similar, incorporation of DEC-fortification into existing salt iodization programs appears very feasible. Finally, DEC-fortified salt represents an especially attractive programmatic option in situations where traditional approaches to tablet distribution are likely to be problematic, e.g., in rapidly expanding urban areas where health infrastructure and social services are particularly strained.

Because of the potential value of a DEC-fortified salt strategy, operational research studies are very much needed to address a number of important issues, including the

- duration and population-coverage of DEC-fortified salt required to achieve interruption of transmission,
- cost-effectiveness of DEC-fortified salt in urban areas compared to traditional MDA strategies to achieve interruption of transmission, and
- best strategies to address the regulatory and supply issues associated with the use of DEC-salt.

Program implementation in urban areas.

Both the strategy and details of program implementation in urban environments are likely to be very different from those in rural settings. The experience to date with MDA activities in cities is limited, and the challenges for achieving success are great. Therefore innovative research efforts will be required

- to devise approaches to assess potential ongoing transmission in highly urbanized areas with low LF prevalence before an MDA is initiated and to monitor it afterwards,
- to develop techniques for appropriate sentinel site selection in highly urbanized areas,
- to compare the cost-benefit of conducting an MDA in highly urbanized areas with low LF prevalence with conducting a focal, targeted approach to pockets of endemicity.

Program implementation in L. loa*-endemic areas.*

Loiasis is endemic in forested areas of west and central Africa.[5] While classical presentation includes transient, localized angioedema and occasional migration of a worm subconjunctivally, the majority of infected persons have no recognizable signs of infection. Because central nervous system reactions (encephalitis/encephalopathy) may rarely occur in highly microfilaremic (> 30,000 mf/mL of blood) persons with loiasis after treatment with microfilaricidal drugs (including ivermectin),[6] use of such drugs for onchocerciasis or for LF in areas coendemic for loiasis provides a major (or in some instances, a currently insurmountable) programmatic challenge. Since this situation is posing an effective block to MDA implementation for LF in vast areas of Africa,[7] research is urgently needed for

- a rapid individual diagnostic tool for *L. loa* that gauges the risk for post-treatment serious adverse events,
- a safe and effective pre-treatment strategy for slow reduction of *L. loa* mf levels in endemic countries,
- assessing the potential for insecticide-treated nets to promote LF elimination in *L. loa*-endemic areas in the absence of a safe chemotherapy for MDA,
- defining the risk-benefit of MDA for LF elimination in *L. loa*-endemic areas.

Program upscaling/downscaling.

For most national LF elimination programs, upscaling from pilot activities to programmatic coverage of their entire at-risk populations has provided enormous implementation challenges. While many of these challenges are purely programmatic, there are still important researchable issues whose resolution would greatly aid these expanding programs, including

- the effect of the rate of upscaling on
  a) the number of MDA rounds required to achieve success,
  b) the cost implications in the short-term and long-term,
- the number of rounds of MDA needed to achieve interruption of transmission in settings with different initial mf prevalences,
- the most effective training materials to support upscaling,
- the most effective advocacy and fundraising strategies to link program needs with sources of support.

Although GPELF efforts to date have focused principally on the initiation or upscaling of programs, soon countries or regions of countries will be nearing the end of their projected 4–6 annual rounds of MDA. Assessment of the LF situation at that point might identify residual pockets of infection and lead to activities that might be termed mopping up, consolidation, termination of residual foci, or downscaling. Operational research should be undertaken in mature programs

- to determine the safest and most effective strategies for judging when to stop the MDAs,
- to identify residual foci of transmission and to implement appropriate, targeted intervention,
- to assess consequences of terminating the LF elimination program on the entomology, epidemiology, and clinical aspects of other endemic diseases in the community.

Disability prevention (disease management).

Many of the general issues and researchable questions dealing with morbidity management and disability prevention activities have been outlined elsewhere (Section 2.3), especially relating to the most suitable indicators for monitoring effectiveness of compliance, coverage, physical health outcomes, psychological health outcomes and socioeconomic outcomes. Additionally important, however, especially for its effect on the overall success of PELF implementation would be information derived from research to determine the

- impact of having or not having a morbidity/disability component in the national PELF on the success of LF elimination efforts, and
- impact of providing or not providing hydrocoele/lymphocoele surgery on the success of LF elimination efforts.

Social mobilization.

The majority of people infected with LF have no visible manifestations, and as a result, community perceptions of how people get affected are distorted. Therefore, the majority of the people in the communities where MDA is targeted are often indifferent and not motivated to take the drugs, believing they are neither affected nor at risk of being affected. Social mobilization is therefore of paramount importance to get communities to participate in the MDA. Model simulations indicate that high coverage, likely more than 80% of the total population, is essential during each annual MDA for interruption of transmission to be attained after five years;[8] indeed, most areas in countries where social mobilization has not been accorded much importance have shown poor treatment coverage. It is therefore important that effective social mobilization is maintained throughout the duration of MDA.

To address this challenge, WHO introduced in some countries a social-mobilization approach termed COMBI (Communication for Behavioral Impact), based on a mixture of five communications interventions: public/government mobilization, community mobilization, interpersonal communication, advertising, and point-of-service promotion.[9] It is meant to be a planning tool and serves as a framework for guiding the planning and development of appropriate, behaviorally orientated communications and social mobilization strategies that are culturally acceptable and country specific. A key aspect of the approach is the recruiting of private advertising agents to work on the communications while volunteer drug distributors are trained to be motivators for drug administration.

High drug coverage rates have been attained in countries where COMBI has been applied, but at in other places where classic social mobilization has been conducted based on knowledge, attitudes, and perceptions (KAP) and other social science studies, results have also been good. Therefore, it is important that social mobilization research be carried out

- to assess and compare cost-effectiveness, usefulness, and value-for-investment between the classical social mobilization methods and the more costly COMBI approach,
- to determine the factors that motivate populations to accept the drugs in various geographic, social, and cultural settings,
- to determine the factors that ensure or promote the maintenance of the motivation to continue taking the drugs each year of the annual MDA in various geographic, social, and cultural settings, and
- to develop effective social marketing strategies to maximize acceptance of DEC-fortified salt in countries where it is used.

Integrating LF elimination with other disease control programs.

Many parasitic and other infectious diseases have overlapping spatial distribution, with similar vectors and environmental determinants. Such scenarios are particularly common in the tropics and subtropics, home to the world's least developed countries, where these overlapping, pervasive infections play an essential role in perpetuating the vicious cycle of poverty. Historically, to address these infections, disease-specific control programs (vertical programs) have been set up, most operating within the national health system, and thus drawing from the same pool of available resources and personnel for their field-level operations. What have become obvious are not only the great opportunities but also the compelling need of the national health systems to decrease the costs and burden of the multiple vertical programs by integrating or packaging as many of these activities as possible.

Successful recent precedents for effective integration of programs focused on specific diseases include the programs for Integrated Disease Surveillance (to coordinate surveillance of communicable diseases), Integrated Vector Management (aiming at the control of vector-borne diseases by combined approaches that are sustainable, cost-effective and have an impact on transmission), a new targeting of neglected diseases by WHO through providing neglected communities with an integrated solution to their disease-control problems, and a number of national programs now in the early stages of integrating control of such diseases as LF, onchocerciasis, geohelminths, schistosomiasis, trachoma, and others.

Many uncertainties remain, about which control programs can be effectively linked or packaged, depending particularly on the geographic and age distributions of the diseases and on programmatic similarities or complementarities. Because, however, the potential savings to the health systems and the populations they serve are so great, research to address those uncertainties should be urgently addressed by

- determining the specific program components that can be feasibly linked in implementing multiple disease-targeted programs,
- identifying the most effective tools and methodologies for monitoring integrated health interventions,
- determining the cost-effectiveness of integrated disease control programs,

- assessing the feasibility and impact of integrating targeted disease programs with the primary health care system.

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2.5 MONITORING AND EVALUATION

John O. Gyapong**

Summary of Prioritized Research Needs

1) Initiate multi-center, longitudinal studies to define the relationship and comparative effectiveness of the available diagnostic monitoring tools (antigen, antibody, PCR) used in human or vector populations, to determine
   a) when to stop MDA,
   b) how to perform surveillance to ensure absence of resurgence,
   c) how to verify absence of transmission,
2) Use data from such multi-center studies to refine predictive models,
3) Study the role of mobility and migration both in populations and by modeling to define their effects on LF transmission dynamics,
4) Investigate both the effects of non-compliance on the LF Elimination Program and the sociologic reasons underlying it,
5) Refine and validate tools for monitoring morbidity management programs,
6) Develop guidelines for monitoring multi-program effectiveness and outcome in situations where LF elimination is coordinated (integrated) with other public health interventions (e.g., with onchocerciasis, intestinal parasite, trachoma, or malaria control; and/or with vector control activities).

2.5.1 Overview

Monitoring is a systematic, repeated assessment of the progress of a piece of work over time; it is also a basic management tool for identifying a program's strengths and weaknesses. While such monitoring may involve a wide variety of issues (including finance, personnel, vehicles, etc.), the LF Elimination Program focuses particularly on monitoring program outcomes and on pursuing research needed to ensure that the monitoring approach has a sound scientific basis. For the GPELF both process and impact monitoring are essential for achieving the Program's two principal goals: interruption of transmission and diminishing disease morbidity.[1]

Process monitoring provides information on the progress of the activities being carried out. For LF programs, the main process indicator relating to transmission interruption is MDA coverage, which can be operationally defined as reported coverage, surveyed coverage, or geographic coverage, each determined differently and each with a specific program implication.[2] For diminishing disease morbidity the potential process indicators have yet to be evaluated.

Impact monitoring provides information on progress towards achieving the objectives of a program and on the impact the program is having in relation to these objectives. For LF programs the principal impact indicator relating to transmission interruption is the prevalence of infection in humans as defined by microfilaremia; serum antigen (ICT) positivity and incidence of infection (antibody positivity) are also assessed.[2,3] Sentinel-site populations are followed serially from baseline observations to monitor program impact on transmission. For diminishing disease morbidity the principal impact indicators remain to be defined.

2.5.2 Research Needs

Tools for monitoring.

Impact.

With respect to GPELF's goal of interrupting transmission, it is generally agreed that the diagnostic tools now available to assess the presence of infection or the exposure of humans to infection are very good. High sensitivities and specificities have been recorded for most of the available assays, and their successful use for mapping the geographic distribution of LF and monitoring the early stages of program progress have proven them to be of practical value.[4] However, what has not yet been done is to define the comparative sensitivities and specificities of the various available diagnostics when they are challenged to identify extreme endpoints such as the absence of transmission. Since findings might be expected to differ for the different LF parasite-vector complexes, such assessments need to be performed in multiple regions of LF endemicity. Therefore,

- a multi-site assessment of the comparative effectiveness (including other parameters such as ease-of-use, cost, reliability, etc.) of all monitoring tools available (MF detection, ICT, DNA [in human blood and in mosquitoes], antibodies [especially those detecting exposure to infection]) is essential and should be undertaken immediately through
  1) laboratory-based testing of existing serum collections,
  2) field-monitoring of sentinel sites in countries with active LF elimination programs.

Tools (and indicators) for assessing progress towards morbidity control goals have been proposed but not yet agreed. Therefore,

- the proposed, still untested, indicators (and tools for defining them) to assess the impact of morbidity control efforts should be evaluated at multiple sites and in multiple programs

Process.

For efforts to interrupt transmission, reported coverage from MDAs is confirmed by independent coverage surveys that depend for their accuracy on rigorous sampling methods. Currently recommended is the Expanded Program on Immunization–cluster survey technique, which is both time-consuming and expensive.[5] If acceptable alternative sampling techniques were available, programs could effect savings and increase the extensiveness of their coverage estimates. Therefore,

** Other contributors in this working group are listed in Annex 2.

- alternative sampling techniques to determine surveyed coverage values for MDA activities should be tested for cost-effectiveness in comparison to the standard cluster-survey techniques now being used.

Tools (and indicators) for monitoring morbidity control activities have been proposed but not yet tested. Therefore,

- the proposed process indicators for monitoring the morbidity component of LF elimination programs should be evaluated at multiple sites, so that selection of the most effective indicators can be finalized

Setting thresholds—testing strategies.

Impact.

Strategies have been defined for GPELF that use the existing diagnostic tools to reach decisions about when to stop the MDAs, how to define the absence of transmission, and how to conduct surveillance for possible recrudescence of transmission.[1,3] However, these strategies have not yet been tested, so it is not clear which indicator or combination of indicators will best address these issues or even whether different indicators could be more efficient in determining the status of endemicity in new areas being considered for targeting with MDA. Therefore, it is essential

- to test as soon as possible the protocols now recommended to determine whether or not it is safe or appropriate to stop the MDAs (then modify the protocols as/if necessary),
- to evaluate both the current guidelines for verifying absence of transmission and the different diagnostic tools that could be used,
- to develop background (or other) surveillance strategies and test them for use in early detection of recurrent transmission in areas where MDA activity has stopped.

Concern has been raised about the threat of introduction of LF into previously non-endemic areas or re-introduction into areas where elimination has been achieved, principally because of inter-country or intra-country migration of workers or other populations or because of a region's proximity to an LF-endemic area. Research is needed, therefore,

- to define the prevalence of infection in migrating expatriate or other populations and evaluate the threat posed by them in settings that were previously either endemic or never-endemic.

Process.

To eliminate LF by the end of the predicted 4–6 rounds of yearly MDA, it has been estimated that a minimum MDA coverage of 70–80% of the total population is required. However, it is not clear what happens if a certain proportion of the population never participates in taking the drugs during the repeated MDAs (i.e., there is systematic non-compliance), even if a targeted 80% coverage is achieved.[6] Therefore, it is very important to study populations in ongoing LF elimination programs

- to determine if such systematic non-compliance exists,
- to identify particular characteristics of these non-compliers, the reasons for their non-compliance and whether community mobilization efforts can overcome it,
- to gauge the effects of systematic non-compliance on the effectiveness of the LF elimination program.

Monitoring integrated or linked programs.

There is no question but that linking or integrating public health programs is of high priority today.[7] The GPELF has already built a strong infrastructure for health care delivery based on once-yearly contact with communities that targets MDA to everyone in the enormously large at-risk populations (often whole countries). Because of this infrastructure, along with the popularity of the program's beneficial free drugs and a good monitoring framework to assess program effectiveness and impact, the GPELF is a good match for other programs with similar needs to develop partnerships. Programs already actively engaged in trying to develop integrated or coordinated activities with LF programs include those focused on malaria, trachoma, onchocerciasis, intestinal parasites, and schistosomiasis.

Such program linkages can and will operate at many functional levels, including capacity-building, social mobilization, program implementation, monitoring, evaluation and others. While the principles underlying each of these activities are the same for each of the different programs, the individual details are unique. However, as the pay-off for successfully exploiting the complementarity of these programs and activities is so great, it is very important

- to conduct the operational research necessary to identify those elements of program monitoring and evaluation that can be effectively coordinated or linked with those of other similar or complementary public health programs,
- to develop appropriate protocols and initiate collaborative studies between LF and other large-scale national or global programs to assess the cost-effectiveness of sharing or integrating their activities.

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2.6 EPIDEMIOLOGY, PARASITE BIOLOGY, MODELING

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Summary of Prioritized Research Needs

1) Define transmission dynamics for the various vector-parasite complexes, including the estimated reproductive life span of the adult worms,
2) Determine end points at which MDA can be stopped with low probability of recrudescence, under different epidemiologic settings and using different diagnostic tests,
3) Develop mathematical models to support decision-making on the duration of local MDA programs in different epidemiologic situations (e.g., initial endemicity, coverage rates, migration patterns, drug combinations, etc.),
4) Define the comparative effectiveness of available tools and indicators for monitoring the progress of LF elimination programs and incorporate such indicators as model outputs to enable models to help decide when to stop MDAs and when to initiate surveillance to detect recrudescence.

2.6.1 Overview

The identification of LF as a potentially eradicable disease by the International Task Force for Disease Eradication[1] was based on the development of new tools and strategies deemed effective for the successful intervention against LF. These strategies and operational criteria for eliminating LF were very much influenced by China's earlier successful experience in eliminating the infection.[2,3] Whether this experience can be directly extrapolated to all remaining LF endemic regions, however, is a matter that requires further scrutiny.

Although much work has already been carried out in different geographic areas to define the local epidemiology of LF and the nature of the vector/parasite complexes involved in its transmission, many gaps in our knowledge still remain. Indeed, the exact distribution and transmission dynamics of different LF parasite/vector complexes are still not well documented in many parts of the world, especially Africa. Such knowledge is essential, however, not only to gauge the feasibility of elimination and to design appropriate interventions, but also to parameterize the mathematical models describing the population biology and control of LF. Such epidemiologic models can themselves be important intervention tools, playing a role both in offering insight for decision-making and in helping to understand the processes regulating parasite population abundance and the effects of control interventions on the dynamics of these processes. Furthermore, when the development and calibration of these models go hand-in-hand with the design and implementation of public health programs, they can also be valued tools for monitoring and evaluating these programs.

2.6.2 Research Needs

Transmission dynamics.

Knowledge of the heterogeneity in LF epidemiologic patterns across geographic regions and of the vector-parasite complexes involved in its transmission is crucial to defining the spatial ecology of transmission and understanding the processes regulating population dynamics. Particularly important are the intensity and prevalence of infection, including their age-specific profiles; the vector competence for local parasite species/strains, including issues relating to the periodicity of the parasites and vectors; the vector preferences and biting rates on humans; and the distribution of parasite numbers among different hosts and vectors.[4,15] In addition, information on the reproductive biology of the adult worms, their sexual systems, sex ratios, and distribution among different individuals are all factors important for the estimation of mating probabilities, transmission breakpoints, and the spread of potential anthelminthic resistance alleles. Estimations of the reproductive life expectancy and the distribution of survival times underpin all epidemiologic projections for control scenarios and the expected durations of intervention. Therefore, comprehensive biological studies are needed

- to document the spatial ecology and transmission dynamics of LF by different vector-parasite complexes, including estimation of the reproductive lifespan of the worm.

Transmission breakpoints—when to stop/when to start an MDA.

In a chemotherapy-based elimination program such as the GPELF, a crucial issue to resolve is the level to which the parasite population density must be reduced to safely stop the MDA, having minimized the risk of recrudescence (i.e., having reached the transmission breakpoint). In ecologic terms, the aim of the program is local (and eventually global) extinction of the parasite population. Clearly, treatment for too short a period or MDAs with inadequate duration, frequency, and coverage will lead to parasite population recovery and occurrence of recrudescence. Treatment for too long a period will be wasteful of resources and inappropriate for those being treated.

Transmission breakpoints are those parasite densities in the host and vector populations that correspond to unstable equilibria; i.e., parasite densities below which the basic reproduction number, R_0, of the parasite in that particular environment is less than 1 and, therefore, if driven below this level the parasite may undergo local extinction. Above such parasite densities R_0 will be greater than 1, and therefore the parasite population will eventually return to its original (stable) endemic state.[5–7] The nature and magnitude of these unstable equilibria will vary according to the vector-parasite complex, the type and severity of regulatory processes operating on the parasite population, the reproductive biology of the parasite, and the distribution of parasites within the host populations. Model-based predictions for reaching this transmission breakpoint will require definition and quantification of relevant demographic and environmental variables. Therefore, studies are required to define and quantify those epidemiologic variables necessary

** Other contributors in this working group are listed in Annex 2.

- to determine the end points at which MDA can be stopped with low probability of LF recrudescence, under different epidemiologic settings.

The theoretical equivalent of the LF prevalence threshold for initiating MDA in endemic areas is also the transmission breakpoint (since it is assumed that prevalence below such a cut-off would drive the parasite population to local extinction). Because of the marked overdispersion usually characterizing the distribution of such parasite populations among their hosts and the impact of density-dependent mechanisms regulating parasite abundance,[4,8–10] it is likely that these cut-off thresholds will be quite low. The WHO recommendations[2] identify a prevalence threshold for initiating an MDA that was based on the LF program experiences in China,[3] without provision for possibly different cut-off points under different epidemiologic settings. Furthermore, the relationship between the prevalence of microfilaremia and the prevalence of antigenemia (which are the measures for defining the threshold values) is not yet clear. Since there are definite needs for unambiguously identifying regions that do not need MDA (i.e., where transmission is sporadic or not autochthonous), studies are necessary

- to confirm the validity of the currently recommended LF prevalence threshold for initiating MDA activities,
- to establish the relationship between these thresholds being measured in terms of microfilaremia or antigenemia.

Epidemiologic modeling.

Studies on the transmission dynamics of the *W. bancrofti–Culex quinquefasciatus* complex in India have formed the basis for the currently available epidemiologic models using either deterministic (EPIFIL)[11,12] or stochastic micro-simulation (LYMPFASIM)[13] frameworks. These models have been quantified using longitudinal data from Pondicherry[10,14], and more recently LYMPHASIM has also been calibrated with published data from French Polynesia.

Such epidemiologic models for the transmission dynamics and control of LF should, if possible, be an integral part of the GPELF. The nature of the model used, in terms of its structural assumptions, parameter estimates, and modeling approach, must be guided by the research questions of interest. These models may need to be geographically specific to address the issues of local parasite population extinction and reintroduction. Also, incorporation of population genetics into population dynamics frameworks will be of value for investigating the potential evolution and spread of resistant parasites and for the design of strategies to minimize the probability of treatment failure. Therefore, it is necessary

- to expand the range of locally relevant models available by quantifying the parameters describing LF population dynamics in areas where different vector-parasite complexes prevail, and in particular those for Africa,
- to use the models available for supporting decision making on the duration of local MDA activities in different epidemiologic circumstances and in relation to
  a) initial endemicity (force of infection),
  b) coverage levels,
  c) compliance patterns (systematic versus random),
  d) migration issues (of infected people and vectors; of naive populations into endemic areas),
  e) different drugs and their combinations (DEC, albendazole, ivermectin) and treatment regimens (MDA versus DEC salt) and frequency (annual versus multiple),
  f) emergence and/or spread of resistance,
  g) vector control (in isolation or in combination with MDA, and its relationship with anti-vector measures against other infections, such as insecticide-treated nets [ITNs] for malaria control),
  h) treatment of different population groups (rural versus urban),
  i) MDA in relation to seasonality of transmission,
- to test the models' predictions through their validation with results from on-going national LF elimination programs.

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2.7 VECTORS

Thomas Burkot and Moses Bockarie**

Summary of Prioritized Research Needs

1) Refine and evaluate the new tools for determining LF infection rates in mosquitoes,
2) Develop and evaluate new tools for estimating the rate of contact between human and mosquito populations,
3) Evaluate vector control strategies based on knowledge of the vectors' biology for impact on LF transmission, including
   a) insecticide impregnated mosquito nets on LF transmission by *Anopheles* and *Culex* vectors,
   b) breeding site reduction strategies on LF transmission by *Aedes* vectors,
   c) polystrene beads and *Bacillus sphaericus* on LF transmission by *Culex* vectors,
4) Conduct operational research on how to integrate successful vector control strategies for LF into ongoing programs for other vector borne diseases (malaria, dengue, etc.).

2.7.1 Overview: Vector Control for Filariasis

Until the discovery of the anti-filarial properties of DEC, filariasis control programs were based on suppressing transmission though vector control. This approach took advantage of the relatively inefficient nature of filariasis transmission and the absence of significant reservoir hosts.[1] The real challenge was in identifying control measures that targeted weaknesses in the biology of the vectors. When appropriate vector control was applied, success in controlling and even eliminating filariasis was achieved. For example, environmental sanitation reduced the breeding sites of the *Culex* vectors of filariasis in Australia[2] and Japan,[3] resulting in the eventual elimination of LF from these two countries. Further proof of the success of vector control was seen when residual wall straying with DDT for malaria control inadvertantly eliminated LF from the Solomon Islands[4,5] and parts of Papua New Guinea.[6]

The GPELF is based on annual MDA with albendazole and either DEC or ivermectin.[7] Suppression of transmission though vector control should, however, be considered an important adjunct or supplement to MDA programs. Studies have already shown that MDA programs integrated with vector control require fewer annual rounds of MDA to achieve the same level of success.[8] If the effectiveness of MDAs decreases because of poor population compliance or the development of resistance by the parasite to DEC, ivermectin or albendazole, the importance of vector control as a supplemental measure to suppress transmission and thereby ensure LF elimination would be even greater.[9]

The challenge for applying vector control to filariasis elimination programs lies principally in knowing where and when it should be used for maximal cost-effectiveness. Implementation of vector control is complicated by the large number of vector species found in endemic countries.[10] Cost-effective vector control programs must be based on 1) detailed understanding of the ecology of the major vectors in each area, as well as 2) methods for monitoring the cost and impact of vector control. Furthermore, the effective control strategies identified must be integrated with existing vector-borne disease control programs.

2.7.2 Research Needs

Indicators to evaluate transmission interruption by vector monitoring (including xenomonitoring).

Evaluating the suppression of LF transmission can be undertaken in either/both the human and mosquito populations. Because of the long persistence of the parasite and antigenemia (measured by ICT) in humans, there is particular value in assessing infection rates in the mosquito population as an almost real time indicator of ongoing transmission (especially early in MDA programs) or the absence of transmission (hopefully at the conclusion of such MDA activities).

Xenomonitoring (particularly using PCR to detect parasite DNA) is especially effective in following the rate at which people infect mosquitoes with mf, while defining the level of transmission from mosquito to humans requires detecting infective stage larvae (L3) in the mosquito.[11] Both give real time estimates of transmission and both require sensitive methods for detecting mf and L3s in mosquitoes. Also, very importantly, both approaches require careful, effective sampling strategies to define the infection levels in the mosquito populations well enough to estimate both human attack rates and LF infection rates. Important ethical concerns about the use of (potentially infectious) mosquito landing catches as measures of biting rates on humans have also been raised. There are, therefore, important areas of research to be addressed

- to develop new, standardized methods for sampling mosquito populations that will yield samples representative of the entire population and be widely applicable,
- to compare PCR and dissection-based assays to determine the basis for potentially discordant results,
- to develop new techniques for determining infection rates in mosquitoes through large-scale sampling,
- to validate new techniques to determine infection rates in mosquitoes through multicountry trials (i.e., in multi-epidemiologic settings),
- to develop new methods for estimating the size of biting mosquito populations.

Tailoring vector control strategies to the specific needs of national MDA-based LF elimination programs.

Additional methods for suppressing transmission of LF are needed as adjuncts or supplements to the MDA-based pro-

** Other contributors in this working group are listed in Annex 2.

grams.[12] Low MDA coverage of endemic populations or even possible development of resistance by the parasite to DEC, albendazole, or ivermectin could threaten the success of national LF elimination programs. In addition, the danger of applying MDA programs in loiasis-endemic areas necessitates the development of alternative strategies that can reduce microfilarial densities to levels where significant adverse reactions following MDA are not seen.[13]

The historic success of vector control programs on LF transmission argues for the evaluation of new mosquito control strategies for augmenting LF programs. Since LF is transmitted by a wide range of mosquito genera in different geographic areas, multiple control strategies will need to be developed that are tailored to different epidemiologic conditions. While ITNs are a mainstay of the Roll Back Malaria program,[14,15] their potential impact on filariasis transmission by both *Anopheles* and *Culex* vectors requires further evaluation.[15,16] The use of expanded polystyrene beads in water filled pits to act as a physical barrier to both ovipositing *Culex* mosquitoes and emerging adults has been shown to drastically reduce biting populations of *Culex* LF vectors.[17] When the use of polystyrene beads for *Culex* vector control was implemented with MDA, resurgence of LF after MDA ended was prevented. The potential of biological, chemical, and source reduction strategies to interrupt LF transmission by *Aedes* vectors has been demonstrated in limited areas, but the suitability of these approaches to other areas must be explored and the operational barriers to widespread implementation identified and overcome.[18] The potential of these and other mosquito control methods to act as adjunct transmission suppression measures to MDA to ensure LF elimination needs to be substantiated. Therefore, it is necessary that

- maps of the distribution of LF vectors be developed for regions needing adjunctive vector control,
- information on the biology of the important LF vectors be determined (breeding sites, flight range, host blood feeding preferences, resting and biting activities) where it is unknown and relevant to the control strategy to be implemented,
- the efficacy of ITNs be evaluated for night- biting *Culex* vectors,
- the reasons for use and lack of use of ITNs by LF-infected people be understood (are people who do not participate in MDA also more likely to not use an ITN?),
- the impact of community-based breeding-source-reduction campaigns on LF transmission be evaluated in areas where *Aedes* are the primary vectors,
- appropriate strategies for interrupting LF transmission by *Culex* be developed and evaluated (including use of ITNs, polystyrene beads in pit latrines, etc.),
- strategies for interrupting LF transmission by *Aedes* be evaluated (including source reduction, use of insecticide impregnated materials, etc.),
- measurement of the impact of vector control on the number of MDAs required to interrupt LF transmission be determined,
- the possibility be assessed that vector control alone or along with targeted chemotherapy can eliminate LF transmission when only residual pockets of infection remain after five or more annual MDAs.

Integrating LF vector control activities into existing national vector control programs.

Sustained suppression of LF transmission will depend on integrating LF activities, including those for vector control, with other local public health programs. For the vector control components of LF programs, integration with malaria and dengue control programs would be logical.[14,15,18] Operational research to understand how to motivate communities to undertake vector control and strategies for health communication will be essential for successful implementation of community-based anti-mosquito interventions. To accomplish this integration, however, it is necessary

- to identify the implementable approaches to vector control that are common for LF and other vector-borne infections occurring in LF-endemic areas,
- to evaluate the impact of current vector control measures for malaria, dengue, etc. on the transmission of LF,
- to undertake KAP surveys to assess the understanding of the target population on LF transmission and the acceptability of mosquito control strategies, and
- to develop health communications strategies based on the KAP survey results with pretesting and evaluation after dissemination.

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2.8 HEALTH SYSTEMS

Deborah A. McFarland and Laura C. Barrett**

Summary of Prioritized Research Needs

1) Test the current LF/Health Systems assessment tool (matrix) in a variety of endemic countries to determine if the data necessary for each proposed indicator can be feasibly acquired,
2) Identify which health system functions represent the best opportunity for assessing LF impact,
3) Determine baselines for health systems performance in endemic countries at each level where the LF program is likely to have an impact,
4) Identify ways to disaggregate the health systems effects of the LF program from those of other concurrent disease control programs.

2.8.1 Overview

Elimination/eradication programs and the health system.

Programs against several diseases currently targeted for global elimination or eradication (EE) are operating in many of the world's least developed countries where health systems are extremely fragile. The primary goals of these EE programs are explicitly disease related and only secondarily, if at all, related to health system effects. Criticism increasingly directed at such programs is that they not only might not contribute to the strengthening of health systems or health services delivery but also might have unintended negative consequences. The experience of the Polio Eradication Initiative (PEI) is most often cited with proponents emphasizing the positive contributions of the PEI on health systems, particularly in building surveillance and laboratory capacity, while opponents emphasize the disruption in health services delivery that often accompanies national immunization days.[1] Since implementing EE strategies to meet disease control targets requires health systems to devote considerable resources to the effort, health systems advocates have begun to argue for assessing EE programs not just on their ability to reduce the burden of a disease, but also based on their ability to strengthen health systems in the implementing countries. Despite the growing recognition that the implementation of EE programs can impact health systems, there is still much that is unknown about the interaction between vertically initiated, disease-specific control programs and health systems.

The GPELF recognizes that to achieve the goals of interrupting transmission of infection and alleviating and preventing suffering and disability caused by LF, health systems in endemic countries play a critical role. It is also clear that there must be a convergence between the GPELF and global efforts to assess health systems performance; indeed, WHO has recently stressed the urgency of strengthening health systems as a *sine qua non* in accomplishing the millennium development goals as well as the ambitious 3 × 5 HIV/AIDS treatment target.[2]

Measuring the performance of health systems.

The World Health Report 2000[3] defined the boundaries, functions, and goals of health systems, but there is still controversy over the methods used to measure and assess health systems performance. Murray and Evans[4] provide a set of potential indicators to measure health system performance, but measuring health system performance is not yet standardized. Assessing the impact of a single program/intervention on a health system or on a health system's performance has not yet been achieved. The Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM) is currently engaged in an effort to monitor and evaluate the system-wide effects of its activities.[5]

Acknowledging the synergistic relationship between health systems and the implementation of disease elimination and eradication programs, the GPELF has begun to develop a tool and methodology that can be used to assess the health system-wide effects that LF program implementation has in participating endemic countries.[6] The data derived from such a tool will chart the impact that program implementation is having on the overall health system, allowing program managers to make adjustments if a negative impact occurs, or to share positive results with other countries.

2.8.2 Research Needs

Testing and improving current tools: The LF/health systems matrix.

The current LF effort to assess the GPELF's impact on health systems is structured on the conceptualization of health systems in the World Health Report 2000, which defines four functions of health systems: stewardship, financing, resource development, and service delivery. Indicators addressing each of these four functions have been drafted but not yet tested in the field. Therefore, investigative efforts are necessary

- to test the current tool to assess the LF/Health Systems interaction matrix in a variety of endemic countries to determine if the data necessary for each proposed indicator are reasonably available and accessible,
- to assess the sensitivity and specificity of each indicator with respect to the four functions of the health system,
- to identify key data gaps from the current matrix (tool) requiring further study.

Examining health system functions for their responsiveness to interventions.

Within the timeframe of LF programs, particularly the MDA, it is not clear which of the four health systems functions are likely to be most pliable/responsive to LF activities. For example, is it more realistic to expect that LF programs will strengthen the service delivery function of the health system, or the resource development function? Short run changes in health systems may be more amenable to LF in-

** Other participants in the workshop of LF and Health Systems are listed in Annex 3b.

terventions and more demonstrable by conventional monitoring techniques, but longer run effects cannot be ignored. Disaggregating the effects of LF programs from those of other disease control programs/strategies on health systems is likely to present a key methodological challenge. Therefore, research is needed

- to identify which of the health systems functions—stewardship, financing, resource development and service delivery—represent(s) the best opportunity for the LF program to show impact,
- to document the timing dimensions of health system effects due to the LF program,
- to disaggregate the health systems effects of the LF program in a dynamic environment where other disease control programs are also affecting the health system,
- to assess which changes in health systems over time will have the most effect on LF program goals.

Identifying usable indicators.

It is essential to identify indicators that can accurately and effectively demonstrate health system impacts, both positive and negative, of specific-disease, targeted programs. An essential aspect of indicator development includes determining reasonable targets for improvement (including ranges for measurement). Ordinarily, setting reasonable targets for indicators is guided by results from previous studies or programs. Since this is one of the first efforts of its kind, however, reasonable targets for health system improvement in a given district, region or nation, are unknown. Therefore, there are critical research needs

- to determine baselines for health systems performance in LF endemic countries; such baselines being necessary for each level of the health system in a given country, but with emphasis on the levels at which the LF program might be expected to have the greatest impact,
- to determine the most appropriate time interval for measuring indicators during program implementation to demonstrate effects of the LF program on health systems (e.g., annually, pre- and post-MDA?),
- to identify the ways in which the GPELF can take advantage of lessons learned from similar efforts by the GFATM and WHO to assess and monitor health systems effects of various programs.

Identifying the locus of control for health system changes.

When considering the impact of a program on health status, the program manager has ability/authority to modify a program so that the intended results can be realized. When considering health system impact, however, the program manager alone may not be in the position to alleviate any negative impact to health systems. Therefore, research is needed

- to define the most effective ways of collaborating with policy and decision makers so that any impact of the GPELF can be amplified (if positive) or mitigated (if negative), if the appropriate response to health system impact findings is beyond the control of an individual program manager.

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3. UPSTREAM RESEARCH TO SUPPORT THE GPELF (BASIC RESEARCH)

3.1 PATHOGENESIS

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Summary of Prioritized Research Needs

1) Develop revised, standardized set of definitions for lymphatic and urogenital pathology seen with LF,
2) Use animal models and clinical studies to define influence of LF parasites, molecules, endosymbionts, and host-induced factors on lymphatic endothelium,
3) Define role of co-infections (local bacterial or systemic TB, HIV/AIDS, etc.) in pathogenesis of LF disease,
4) Define effects of the LF-altered host responsiveness on reactions to vaccines or to other infectious agents (malaria, HIV/AIDS, TB, etc.),
5) Explore roles of innate and adaptive immune responses to LF in the initiation, progression or reversal of lymphatic disease and disease of other organs,
6) Define the role of the host immune response in the mechanism of action of the common antifilarial drugs (especially DEC),
7) Define differential host susceptibility to development of disease caused by genetic, endocrinologic, nutritional, and other types of host heterogeneity.

3.1.1 Overview

Why study pathogenesis?

It is, of course, knowledge that leads to solutions for problems, and nowhere is there a better example than in the management of lymphatic filarial disease. Once thought beyond repair and clinically hopeless, the pathology of LF is now approached with extremely effective tools and strategies that were all developed because of remarkable advances in understanding the pathogenesis of filarial disease (see Section 2.3). Further understanding will undoubtedly improve clinical management still more, and better definition of the underlying molecular mechanisms of disease should also lead to wholly novel strategies for both diagnosis and treatment.

Current global efforts to eliminate LF focus principally on the reduction of transmission with the progressive lowering of endemicity levels. How such changes impact on the development or progression of disease makes understanding the mechanisms of its pathogenesis extremely important. On the one hand, there have been reports of the regression of LF disease (lymphedema and hydrocoele/lymphocoele) following MDA aimed only at interrupting transmission.[1] On the other hand, potential scenarios exist for ways in which changes in LF transmission might undesirably affect the evolution of disease. For example, 1) reduction in levels of endemicity following MDA might lead to diminished immunoregulation and consequent exaggeration of disease progression after re-infection; 2) repeated treatment itself might trigger exaggerated host immune responses to filarial products that could enhance progression of disease and result in persistent lymphatic damage; and 3) even with a decrease in overall prevalence of infection persistent, low-level infections could result in altered immunoregulatory and/or inflammatory responses that could, in turn, affect an individual's susceptibility to other types of infection, reduce responsiveness to vaccines or even impair a child's growth and development. Finally, it is also important to understand the mechanisms underlying pathogenesis since the consequences of LF-initiated damage to the lymphatics may persist long after infection has been eliminated. It is unknown to what degree this lymphatic damage is reversible, and it is clear that such damaged lymphatics predispose individuals, by some undefined mechanism, to prolonged periods of increasingly damaging lymphedema and recurrent bacterial infections.

Barriers to studying lymphatic pathology.

Numerous obstacles have persistently hindered studies on LF pathogenesis. First, it has often been difficult even to establish whether a particular clinical syndrome is or is not associated with LF, principally because of the difficulty in definitively establishing current or prior infection. Confusion arising from the fact that other conditions may produce similar pathology or that multiple pathogenic pathways may lead to the same clinical endpoint often can be dispelled by longitudinal studies of patient populations,[2] but such studies are particularly difficult for LF because of the long period of time between infection and development of clinically overt disease. Second, the uncertainties regarding LF pathogenesis have been compounded by the difficulty in obtaining appropriate human tissues and by inadequate tools for detecting subclinical lymphatic abnormalities. Third, there has not been a good animal model of disease for LF, particularly for *W. bancrofti*.

Recent developments or improvements in imaging technologies have now given much better access to patient pathology, such that, at least at the organismic level models for understanding the pathogenesis of LF are being developed.[3–5] The current advances in proteomic and genomic technologies now promise to help overcome some of the remaining barriers and lead to additional refinement and progress in developing a unified model for understanding LF pathogenesis at the molecular, as well as the organismic, level.

3.1.2 Research Needs

Defining the issues.

In addition to the broad issues of pathogenesis already identified (i.e., need for improved markers of morbidity/pathology, relation between endemicity and LF disease, effect

** Other contributors in this working group are listed in Annex 2.

of MDAs or specific treatment on disease progression, role of co-infection in pathogenesis) and the micro-issues related to actual pathogenic mechanisms (described later in this report), there is one particularly important concern that transcends these issues; namely, the need for a revised and standardized set of definitions for LF disease.

For example, acute filarial lymphangitis, while thought to be the reaction to a dying or damaged adult worm, lacks a standard approach to distinguish it from an episode caused by bacterial infection in an individual chronically affected by LF.[6] Similarly better definitions for urogenital disease are needed. Hydrocoele may be mistaken for scrotal lymphedema or unreduced hernia by an inexperienced examiner, but ultrasonography can greatly improve the precision and accuracy of its diagnosis.[7] Many studies do not distinguish a simple hydrocoele from chylocele, but this difference has extremely important clinical implications. Other examples of terms that require better clinical distinctions are subclinical versus clinical disease; lymphatic disease versus lymphatic dysfunction. Better characterizations of patient populations are also needed. Therefore,

- a consensus conference should be held to establish agreed definitions for the different manifestations of LF disease and for the diagnostic criteria distinguishing them.

Identifying external factors affecting pathogenesis.

Adult worms.

The direct impact of adult worms and their endosymbiont *Wolbachia spp.* on the host is poorly understood, particularly regarding the lymphatic endothelium. A striking feature of infected individuals is the marked lymphatic dilation observed surrounding the aggregations of adult worms. This dilation can extend well beyond the location of the worms themselves, suggesting the presence of a diffusible factor produced by the worms.[6,8] An important research challenge, then, is

- to identify factors released by adult worms that cause lymphatic dilation, especially using new technologies (such as proteomics).

The recent identification of lymphatic-specific vascular endothelial growth factors (VEGF-C and VEGF-D) and molecular cell surface markers such as the VEGFR-3 receptor and podoplanin have made it possible to isolate and culture lymphatic endothelial cells (LEC) without loss of differentiated properties.[9] This could provide a powerful *in vitro* model to study the effects of excretory/secretory products of adult worms on LEC. The VEGFR-3 triggers an important signaling pathway for LEC proliferation and survival. This and related receptors would be valuable tools for studying the protein-protein interactions with excretory-secretory molecules that were identified by proteomic analysis, then screened by computational methods and tested directly.[10] *In vivo* models using severe combined immunodeficient (SCID) mice and gerbils with LF that develop lymphangeictasia could complement *in vitro* studies with the added power of being able to examine the effect of specific signaling pathways through targeted gene deletion. The degree to which lymphangiectasia is reversible following removal of the worms/growth factors could also be studied in these models. Therefore,

- *in vitro* models based on cultured lymphatic endothelial cells should be established and exploited to define the mechanisms of lymphangiectasis,
- *in vivo* models using LF-infected SCID mice and gerbils should be studied in parallel with the *in vitro* models to define mechanisms of LEC stimulation and the signaling pathways involved in causing lymphangiectasia.

Little is known about the microanatomy of lymphatic endothelium and the degree to which host inflammation contributes to lymphatic pathology, especially following death of adult worms.[8] Such studies have been severely limited by the difficulty in obtaining pathology specimens in humans. However,

- animal models and earlier-collected pathology samples from patients should now be reassessed using the newer molecular tools available.

Differences in parasite strains may contribute to differences in host pathology, but very little is known is this area. Therefore,

- identification of microsatellite or other DNA markers and statistical techniques necessary to make inferences regarding the parasite population within the host and in the human population should be undertaken, so that correlation between different parasite strains and disease pathogenesis can be investigated.

Bacterial microflora.

Bacterial infections aggravate pre-existing pathology in animal models. Lymphatic dysfunction in humans predisposes to secondary infection which can be recognized clinically to accelerate lymphatic damage,[6,11] but the degree to which bacterial microflora contributes to disease and whether this varies among populations remains poorly understood. Therefore,

- studies need to be carried out in humans and in animal models to address the important question of the role of bacterial superinfection in the pathogenesis of LF disease.

Non-infectious toxic elements.

In parts of the world with certain soil compositions, walking barefoot results in absorption of silica that can cause lymphatic damage and non-filarial elephantiasis.[12] Such soils, with silica or potentially other substances toxic to the lymphatics, might be important cofactors for the development of additional lymphatic pathology in some LF-endemic populations. Therefore,

- the mechanisms underlying this soil-induced lymphatic damage should be defined, along with its role in aggravating LF-induced lymphatic disease.

Co-infections and LF disease.

Many individuals with LF are also infected with malaria, HIV, tuberculosis, and/or other helminths. Such concurrent infections might affect the host immune response by impairing immunoregulation to LF and thereby promoting disease through chronic infection. Conversely, LF may also down-

modulate host responses to these same infections and attenuate the disease they cause. Epidemiologic studies in southeast Asia have reported that concurrent helminth infections may reduce the frequency of severe of malaria morbidity.[13,14] While such studies would suggest the possibility that control of LF and other chronic helminth infections might actually increase the frequency of severe malaria morbidity, other reports from Africa describe just the opposite; namely, that the elimination of helminth infection significantly decreases the frequency of malaria attacks.[15,16] Therefore,

- the impact of LF control on the clinical expression of other co-existing infections needs thorough investigation.

Host nutrition.

Lymphatic filariasis typically occurs among the poorest individuals in underdeveloped regions where malnutrition is rampant. Malnutrition can affect host immune responses as well as growth and repair mechanisms in tissues. Serum protein levels also decrease with malnutrition. This situation could affect intravascular and tissue oncotic pressures that would accentuate tissue fluid accumulation from lymphatic dysfunction and, additionally, provide further substrate for bacterial infection. Obesity, too, is a growing problem in some developing countries owing to a lack of available nutritious foods. The excess weight can affect the hemodynamic lymphatic flow especially in the lower extremities and contribute to a greater risk for chronic lymphedema in LF-infected individuals. Thus,

- the role of host nutrition should be investigated as a possible co-factor in the pathogenesis of lymphatic disease related to LF.

Identifying host-defense factors affecting pathogenesis.

Several stages of the parasite persist chronically within the host and continuously release antigens. The type and magnitude of the chronic immune stimulation by these antigens contribute substantially to the spectrum of disease observed in LF. The respective roles of innate and adaptive host immune responses—and their relative contributions in initiation, progression and reversal of lymphatic disease, despite being one of the most intense areas of research on pathogenesis of LF, still remains poorly understood.

Innate immune response.

The recent identification of *Wolbachia* as an essential endosymbiont of filarial parasites present in all stages has sparked greater interest in a potential role for innate immune responses in disease development.[17,18] *Wolbachia* are gram-negative bacteria that produce molecules capable of directly stimulating cells of the innate immune system and, if released in sufficient quantities, may produce symptoms that might mimic gram-negative bacterial infections. The degree to which living adult worms or mf express or release *Wolbachia* molecules remains unknown. More likely, *Wolbachia* molecules are released upon death of the parasites. Indeed, *Wolbachia* molecules may play a prominent role in acute filarial lymphangitis, presumably triggered by spontaneous death of adult worms or following treatment. Although mf appear to contain less *Wolbachia* antigen, there is likely to be some continuous destruction of mf and the consequent release of *Wolbachia* antigens. The effect of these *Wolbachia* antigens on the innate immune response may also influence the adaptive immune response to filarial antigens. Research studies are needed, therefore,

- to identify parasite or *Wolbachia* molecules responsible for inducing host innate immune responses,
- to determine the effect of these activated innate immune responses on the adaptive immune response of the host,
- to assess the role that innate or adaptive responses to *Wolbachia* have in the pathogenesis of acute filarial fevers or other inflammatory syndromes.

Adaptive immune responses—immune regulation.

Central to the immunopathogenesis of LF is the development of immune regulation. Successful immunoregulation limits the collateral damage to the host. The mechanisms that initiate and sustain this immune regulation remain incompletely understood, but they are clearly important to consider because mass chemotherapy programs alter the dynamics of transmission and the burdens of infection in affected communities.

Hyporesponsiveness. Most studies indicate that the immune hyporesponsiveness that develops to filariae is antigen-specific, suggesting that there are either diminished frequencies of filarial antigen-specific T cells or an increase of immunoregulatory T cells (or both).[19,20] The idea of immune tolerance, either by clonal deletion or anergy of antigen-specific T cells from prenatal or early antigen exposure was first proposed more than 30 years ago,[21] but remains to be directly tested. Mass treatment that reduces infection levels in women of child-bearing age might significantly reduce this form of immune regulation. The recent identification of natural and antigen-specific T regulatory cells in humans raises the interesting possibility that these cells might be expanded in LF and contribute prominently to immunoregulation.

Some studies, particularly those in areas of high transmission, indicate a component of non-specific immunosuppression.[22] The possibility that parasite molecules actively suppress the host immune response has been shown *in vitro*,[23–25] but whether this occurs *in vivo* remains poorly understood. Some parasite-derived molecules have been identified to have homology to host immunoregulatory cytokines in different stages of the parasite. Overall, how these parasite-derived molecules affect host cell function remains unknown but is likely to be relevant to other chronic helminth infections as well. With the sequencing of the filarial genome nearing completion and annotation actively underway, the opportunity to identify additional molecules using bioinformatics or proteomics approaches should greatly enhance this line of research. The respective roles of molecules derived from different parasite stages, e.g., L3, mf, or adult worms, will be of particular interest.

The possibility that the parasite itself, particularly adult stages of the parasite, co-opts host molecules on its tegument that either disguise it from the host immune response (i.e., molecular mimicry) or impair host effector mechanisms (e.g., acquisition of decay accelerating factors that block the complement cascade) has received little study in filariasis. These and similar mechanisms help protect adult schisto-

somes within the human host and permit it to survive for decades.[26] It is conceivable that adult filarial worms may have evolved similar survival strategies.

Necessary research to address these immunoregulatory issues will require investigation

- to define the specific mechanisms underlying the immune down-regulation induced by LF infection and to gauge their effects on other concurrent infections,
- to identify the changes in immune regulation induced by MDA activities and assess their impact on the pathogenesis of filarial or other diseases,
- to define the pre-natal influence on filarial-specific immune regulatory mechanisms and the effect that MDA activities have on them,
- to characterize the parasite-derived molecules responsible for down-regulation or evasion of the host's immune response.

Hyperresponsiveness. More recognizably related to disease is the host immune response to mf that account for the vast burden of parasite material in the host. Exaggerated host response to mf is thought to be the basis of the tropical pulmonary eosinophilia (TPE) syndrome, an allergy-like clinical manifestation of LF where mf are rapidly cleared in the lung.[27,28] It is possible that even partial immunity to mf may accelerate clearance of this stage of the parasite, but that this accelerated clearance, although beneficial in reducing transmission, is achieved at the cost of an increased host pathogenic response to dying mf. Valuable research studies would include

- identification of the mechanisms involved in triggering allergic responsiveness to the parasite,
- efforts to understand the mechanisms underlying the control of allergic responsiveness to LF parasites in essentially all situations except for TPE.

Host genetics and susceptibility to disease.

Differences in response patterns among individuals could also be driven by genetic diversity in the host immune response,[29] including not only that related to the human leukocyte antigen gene complex, but also to common variants in other genes that regulate the host immune response.

Genetic diversity in the regulation of lymphatic endothelial function may also predispose an individual to lymphatic disease. Several hereditary conditions have already been linked with specific genetic defects. Milroy's disease, which is one form of hereditary lymphedema, has been associated with a missense mutation in the gene encoding VEGFR-3, demonstrating that an intact VEGFR-3 signal is required for normal lymphatic endovascular function.[9] Another form of hereditary lymphedema referred to as lymphedema-distichiasis syndrome is caused by mutations in the transcription factor FOXC2.[30] Studies should be carried out

- to examine the hypothesis that polymorphisms in these genes or their promoters would be associated with greater risk of lymphedema with LF.

Previous studies have indicated a genetic contribution to LF disease based on human epidemiologic investigations, animal models, and association studies. The recent sequencing of the human genome has provided further opportunity to explore in greater detail the genetic susceptibility to human disease. Particularly useful would be genome-wide linkage studies (family studies, disease markers) or genetic association studies using a case-control design that examines candidate single nucleotide polymorphisms or haplotypes. Such genetic information might help to identify populations at increased risk for disease that might benefit from more aggressive intervention strategies, as well as providing leads for the further development of anti-parasitic drugs and vaccines. Therefore,

- such epidemiologic studies should be undertaken to define a potential genetic determinant of LF susceptibility or disease.

Endocrinology.

Gender and age differences in rates of infection and disease suggest possible hormonal effects on LF. Most striking are the localization and concentration of adult worms in the lymphatic vessels in the spermatic cord. This concentration of parasites is likely an important factor contributing to the risk for developing hydrocoele or lymphocoele in men. Though poorly understood, adult worms also concentrate in the inguinal lymphatics of both sexes. It is possible that worms might be attracted by specific tissue derived hormones.

A number of studies in many different endemic areas indicate that men are more susceptible to infection and disease than women.[31] An increased male susceptibility to infection has also been observed in the Mongolian jird with *B. malayi*.[32] This differential susceptibility may be regulated by both immunologic and non-immunologic mechanisms; however, the reasons for these differences remain obscure. One possibility is that sex-specific hormones may influence susceptibility to infection, parasite survival, and/or disease. Therefore,

- a more detailed understanding of the role of hormones in susceptibility for infection and disease should be explored for developing novel disease interventions.

Molecular tools to assess pathogenesis.

A research opportunity with particular potential derives from the recent advances in expression and functional proteomics to identify biomarkers for disease. Specific molecules have been shown to be elevated in certain pathologic conditions, and these can be identified in serum using such technologies as protein microarrays or mass spectroscopy. Indeed these assays have been useful in studying several types of cancer and could potentially be developed for LF. A clinically relevant application of protein arrays that might apply particularly to LF is the identification of proteins that induce antibody responses in several disease states. Microarrays, produced by attaching hundreds of proteins and peptides to the surface of derivatized glass slides, could be incubated with patient serum, and fluorescent labels used to detect antibody binding to specific proteins (e.g., stage-specific proteins) that then might be associated with certain forms of disease.[10] The technologies for proteomic analysis are rapidly expanding with increasing sensitivity, reduced sample requirement, lower cost, higher through-put and more effectiveness in

identifying protein alterations (e.g., post-translation modifications). Therefore,

- studies with new molecular technologies would be extremely promising for assessing and monitoring the predisposition, occurrence or progression of LF disease syndromes.

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3.2 PROTECTIVE IMMUNITY—VACCINES

Achim Hoerauf and Cathy Steel**

Summary of Prioritized Research Needs

1) Define the changes to protective immunity among populations undergoing MDA programs (requires longitudinal cohort studies in newborn infants and in adults, identification of markers of protective immunity [sterile and concomitant], and parallel observations in experimental animal models),
2) Assess the impact of MDA-induced alteration of antifilarial immunity on efficacy of routine vaccines and on co-infections with other helminths, malaria, TB, and HIV/AIDS,
3) Work towards an LF vaccine (as a tool for use post-2010) through
   a) identification and production of protective antigens,
   b) identification of the mechanisms underlying protective immunity,
   c) "piggy-backing" observations on the anti-hookworm vaccine currently under development.

3.2.1 Overview

Why study protective immunity?

The GPELF has thus far been a success story, and because of that success the necessity of still continuing to pursue research on protective immunity or vaccines has been questioned. Valid arguments exist, however, for why such research should continue, or even be accelerated.

Most importantly, it must be remembered that none of the three anti-filarial drugs currently being used is totally curative, and numerous rounds of mass treatment will be necessary to reduce the levels of infection below those necessary to sustain transmission. Indeed, it can be anticipated that such alterations in the levels of infection in communities might have a dramatic impact on the degree of their immunity to LF, resulting in either a higher degree of protection against re-infection with filarial worms (thereby promoting success of the GPELF), or conversely, resulting in less protection (and becoming a potential impediment to elimination). Better understanding of the protective immune mechanisms active in LF-endemic populations is thus important not only to make more precise predictions about the eventual success of elimination efforts, but also to alert the GPELF to potential problems that might arise from altered immunity in treated communites. In addition, the absence of totally curative drugs also argues for sustaining the efforts to generate a vaccine that might still be a cost-effective way both to boost the effectiveness of drugs in eliminating LF and then to help to prevent its recrudescence, particularly as the force of infection (i.e., prevalence/density of infection) progressively decreases towards the end of the Global Program.

The usefulness of a vaccine, especially for the final steps of LF elimination.

A vaccine for LF has long been on the wish list of both the basic research and public health communities. Ironically, and partially because that desire has been around for so long, it has been opined that the arrival of a vaccine would come too late and at too high a cost to be of programmatic value. Clearly, it is not an easy task to generate a vaccine against a multicellular parasite that has used millions of years of co-adaptation to divert the host's immune system to ensure its long-term survival. However, it is feasible, and several examples exist of successful (i.e., marketed) helminth vaccines for live-stock,[1] as well as one example of a vaccine against human hookworm disease that is currently progressing toward human trials.[2] Furthermore, in the animal filarial infection most closely resembling human onchocerciasis (i.e., cattle onchocerciasis caused by *O. ochengi*), vaccination with irradiated L3 has resulted in highly significant protection during subsequent field exposure,[3] demonstrating clearly that an epidemiologically relevant protection in the field can be achieved through a vaccine.

From the epidemiologic standpoint of transmission interruption, a vaccine that blocks patency (microfilaremia) would be equally valuable as one that prevented initial infection. A vaccine that blocks patency in 100% of experimental animals has been available for many years in the form of injected mf in the *Litomosoides sigmodontis*–infected rat model.[4] This principle could be further developed for LF, and since a vaccine directed against MF would likely increase the proportion of non-patent infections in a population, it would work directly towards GPELF's overall goal of bringing transmission to a halt.

Finally, the two major microfilaricides DEC and ivermectin both need the host's immune mechanisms for their maximal effectiveness.[5,6] A vaccine might therefore provide highly important complementarity, enhancing the effectiveness of the drugs. Such an effect should be most important towards the end of the program when there will be low rates of infection that must be eliminated. A vaccine might also, of course, become the only useful tool, should resistance develop against one of the major drugs now used in the effort to eliminate LF.

Recent advances in understanding the mechanisms of protective immunity.

Although many hurdles, including the lack of an *in vitro* culture system for filariae and the absence of tools for easy genetic manipulation, initially led to slower progress toward a vaccine for LF than with vaccines for other infectious organisms, over the past decade there has been dramatic improvement in our understanding of the mechanisms that lead to protective immunity against filarial L3 and MF. Several factors account for this, but most important are advances in the development of animal models. While naturally occurring im-

** Other contributors in this working group are listed in Annex 2.

munity in endemic populations had been described for both humans[7–9] and cattle[3] (and likely dependent on the age of the host[10]), experimental models have also now demonstrated that protection can be induced in animals by irradiated L3s.[11–14] Indeed, many of these models have suggested that the Th2 response, most importantly working through interleukin-5 (IL-5), is essential for protection both in vaccine-induced[15–17] and in primary resistance[15,18] to infection. Other Th2-related responses (IL-4, eosinophils, and/or IgE) have also been shown to be necessary for protection to either the L3 or mf stage of the filarial parasites.[15,18–21] In fact, which immune pathway is used for protection may depend upon the stage of the parasite targeted by the host.[22]

Particularly promising for the further study of protective immune mechanisms in filariasis is the recently developed mouse/*L. sigmodontis* model[15,18–19,23] that allows development of the full filarial life cycle in inbred BALB/c mice. Work with this model confirms that a Th2 response, including IL-5-dependent mechanisms involving eosinophil degranulation in the skin,[15] is needed for L3-induced immunity, while mf patency appears also to be controlled by Th2 dependent mechanisms,[23,24] sometimes working synergistically with Th1 responses to limit filarial infection.[25]

In addition to these newly extended models, dramatic advances in molecular biology, including a now fully sequenced filarial genome (for *B. malayi*) currently being annotated, along with the advent of new technologies such as RNA interference (RNAi), protein three-dimensional imaging, novel methods of gene delivery, etc., have all led to what promises to be "a golden age for filariasis research" that provides unprecedented opportunities to build on earlier work to identify protective immune mechanisms and to develop new vaccine candidates.[26]

3.2.2 Research Needs

Effect of MDA on anti-filarial immunity in populations.

The reduction of LF transmission (with its lowered parasite exposure in individuals) will likely reduce populations' naturally acquired immunity to filariasis. This change could have unintended consequences for global elimination efforts, such as increased susceptibility to infection or to patency (microfilaremia), increased disease burden in children, or increased morbidity in both filariasis and other concurrent infections. Research that is urgently needed should focus on

- studies of human populations (stratified according to gender and age) with the generation of baseline data and a standardized profile of immune functions to be monitored serially as the MDAs progress,
- addressing the following questions in different epidemiologic settings and in programs using different MDA regimens:
  a) What are the correlates of protective (sterile vs. concomitant) immunity?
  b) How long do these markers persist after treatment?
  c) Why do some individuals become/remain patent?
     - Is there immunity against mf in humans?
     - What are the immunologic differences between microfilaremic individuals and amicrofilaremic individuals who are antigen-positive?
- studies of birth cohorts in areas with ongoing MDAs, since tolerance to filarial parasites may be acquired through prenatal antigen exposure (that would diminish as infection loads in mothers decrease, with a consequent change in immunoreactivity to filariae that might result in desirable protection or in unwanted immunopathology),
- research using the available animal models to address these same issues in a manner complementary to the studies in humans.

Impact of MDA-induced alteration of anti-filarial immunity on co-infections.

In areas endemic for LF other infections (including malaria, HIV, TB, and other helminths) are usually abundant and likely to stimulate host responses that may alter the clinical expression and state of immune protection to these infections. For example, epidemiologic studies in Thailand suggest that elimination of helminth co-infections might increase the frequency of cerebral malaria,[27] while in Africa elimination of helminth coinfections actually reduced the frequency of acute malaria attacks.[28,29] The limited success in treating and preventing TB may in part be influenced by immunodownregulation by chronic helminth infection, and similar factors may also account for the finding of reduced success of vaccination programs in the face of chronic helminth infection. There is, therefore, a clear need for studies

- to define the influence of chronic helminth infections (treated as part of the GPELF MDA) on the clinical expression of acute or severe malaria syndromes,
- to evaluate the impact of the GPELF (and consequent reduction of helminth infections) on the efficacy of all routine vaccinations,
- to develop animal models for co-infections that allow immunologic parameters and disease manifestations to be studied, and
- to permit assessment of potential causal relationships between an immune mechanism induced by one infection and its impact on a concurrent infection.

Development of animal models.

While the need for animal models to move from correlations to mechanisms is well recognized, a major problem is that no fully suitable model for LF (particularly for Bancroftian filariasis) exists in animal species that can be easily maintained in the laboratory. Therefore, data must be obtained from multiple surrogate models with experiments being designed such that complementary results will be generated, ideally by a consortium of those working with the different models. Therefore, studies should be expanded on

- models for *B. malayi* and *B. pahangi* infections that have been incompletely explored with the most recently developed immunology tools (e.g., in cats, rats, and ferrets) to address issues of immunity and re-infection,
- a model to study *W. bancrofti* in mice by adapting the chamber model already used successfully for onchocerciasis,
- the murine model of infection with *L. sigmodontis* to define more fully the components of the immune system relevant to immunity against L3, to vaccination using modern gene delivery systems, and to MF clearance and patency.

"Piggy-back" studies on hookworm vaccine development.

A hookworm vaccine, currently entering safety trials in humans, is based on recombinant antigens.[2] Given the homology of recombinant proteins used in this vaccine to key filarial molecules that have already been tested successfully for immunogenicity in animal models (e.g., *Ancylostoma*-secreted protein 2, ASP-2), a cross-over effect between hookworm and filarial infection might be expected. While synergistic effects would be welcomed, an antagonistic effect (enhancement of filarial infection due to the development of tolerance induced by the vaccine) would also be a potential concern to be evaluated. Therefore, a potentially unique opportunity (and an important research need) should be pursued

- to develop trials for LF that "piggy-back" on the current studies to develop a vaccine for human hookworm infection.

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3.3 FILARIAL GENOMICS

Steven A. Williams

Summary of Prioritized Research Needs

1) Collecting materials
   a) Before the opportunity is lost to preserve their genomes, collect geographically representative isolates of the various species and strains of the human filarial parasites,
2) Constructing libraries
   a) Construct updated and additional genomic and cDNA libraries to represent completely the different stages and species of filarial parasites,
3) Sequencing
   a) Expand expressed sequence tags (EST) sequencing from cDNA of a greater diversity of life cycle stages and parasites than currently available,
   b) Complete the sequencing and annotation of *B. malayi* genome,
   c) Expand the sequencing of *W. bancrofti* and *O. volvulus* genomes,
   d) Complete the sequencing of the Wolbachia genome from *B. malayi* and mitochondrial genomes from *B. malayi* and *O. volvulus*,
4) Technical development
   a) Develop transgenic methods useful for filarial parasites,
   b) Use RNAi techniques for functional genomics studies,
   c) Expand microarray assessments of gene expression with *B. malayi* and other filariae,
5) Repositories
   a) Develop and maintain all collections of genomes, libraries, sequences, microarrays, etc. in central repositories accessible to all interested investigators within and outside the filariasis research community.

3.3.1 Overview

Genome projects were initiated for many species of parasites in the 1990s as part of a new approach to the study of these organisms that are among the most important human pathogens in tropical regions of the world.[1] The WHO and the United Nations Development Program//World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR) launched an initiative in 1994 to study the genomes of five of these parasites, including those that cause LF. The long-term goal of these genome initiatives has been to collect data that will both improve understanding of important biological problems such as parasite drug resistance, pathogenesis, and virulence and assist in the identification of new targets for chemotherapy and vaccines.[1–3]

Why genomics for LF?

For more than 10 years (approximately 1982–1994), standard molecular cloning techniques were applied to the study of filarial parasites (particularly *B. malayi* and *O. volvulus*), but few genes were cloned and identified. By the end of 1994, only 60 *Brugia* genes had been submitted to the Genbank database. It was clear that a new approach for studying the filarial genome was needed to make rapid progress in understanding the biology and biochemistry of these parasites. The genome project approach represented a complete departure from the way parasite genes had been studied in the past. Genome projects are typically not directed at the identification of individual genes, but instead at the identification, cloning, and sequencing of all the organism's genes.

At the first meeting of the Filarial Genome Project (1994), *B. malayi* was selected as the organism to be studied. This parasite was chosen over the other two medically important species of filarial parasites, *W. bancrofti* and *O. volvulus*, primarily because of the ready availability of all stages of the life cycle.[4] In addition, molecular phylogenetic studies of filarial parasites had shown that all three of these species are closely related. Thus, much of the molecular data obtained on one species would be applicable to the other two.[5]

The genome of *B. malayi*[6] is about the same size (100 million base pairs) as that of the free-living nematode *Caenorhabditis elegans*.[7] The number of protein-coding genes in *C. elegans* is estimated to be approximately 19,000 and the number of genes in *Brugia* is expected to be about the same.

Filarial genomics: The 10 most important accomplishments to date.

Filarial genomics now provide a basis for studying filarial parasites in ways that could not even be imagined only a few years ago. The 10 most important accomplishments of the first 10 years of filarial genomics include

1) the collection of sufficient numbers of parasites for the isolation of DNA and RNA from *Brugia*, Wuchereria, and *Onchocerca* from many locations around the world,
2) the construction of cDNA and genomic libraries for *Brugia*, *Wuchereria*, and *Onchocerca*,
3) sequencing many thousands of cDNA clones (EST sequencing): 24,000 from *Brugia*, 14,000 from *Onchocerca*, and 6,000 from *Wuchereria*,
4) clustering the EST data and developing user-friendly bioinformatics tools,
5) complete genomic sequencing of the *B. malayi* genome,
6) complete sequencing of the endosymbiont *Wolbachia* genome from *Brugia* and the mitochondrial genomes from *Brugia* and *Onchocerca*,
7) development of the first transgenic technique for getting DNA into a filarial parasite (*B. malayi*),
8) development of RNA interference (RNAi) technology for *B. malayi* and *O. volvulus*,
9) construction of the first microarray (gene chip) for a filarial parasite (*Brugia malayi*),
10) initiation of comparative genomics for a large number of nematode species (including *C. elegans*).

3.3.2 Research Needs

Building on the 10 most important filarial genomics accomplishments.

1) Collecting filarial parasites for the isolation of DNA and RNA.

To construct the necessary cDNA and genomic libraries for the study of filarial genomes, parasites of various life cycle stages were collected from around the world. For *B. malayi* parasites were collected as mf, second-stage larva (L2), L3, molting L3, L4, young adult, adult male and adult female stages. For *O. volvulus*, mf, L3, molting L3, adult male and adult female parasites were collected. For *W. bancrofti*, mf, L3, adult male and adult female parasites were collected. Laboratories contributing to this effort were from the United States, Egypt, Indonesia, Cameroon, India, French Polynesia, and others. This effort was time-consuming and costly, but absolutely essential; without the parasites, no work on the genomics could be undertaken.

For the future, a concerted effort must be made

- to collect large numbers of parasites of all stages of *W. bancrofti* and *O. volvulus* for future research needs; unlike *B. malayi*, there are no convenient animal models for these two species, so samples must be collected in the field; as elimination programs progress, it will become increasingly difficult to collect these parasites in sufficient quantity for research purposes,
- to collect geographic isolates of human filarial parasites from around the world and store them in a central repository; these parasites will be valuable for studying geographic variation and will prove especially important should drug resistance arise.

2) Constructing cDNA and genomic libraries.

Since fewer than 60 *Brugia* genes had been cloned by 1994, it was decided that the primary goal of the Filarial Genome Project would be the identification of at least 5,000 new *Brugia* genes.[8] These new genes would help elucidate the biology of the organism and would aid in the identification of new vaccine candidates and drug targets. The plan was to identify these new genes by randomly selecting clones for DNA sequence analysis from new cDNA libraries that were to be constructed from all life cycle stages of *B. malayi*. The construction of many cDNA libraries from all of the life cycle stages would insure that a high proportion of the expressed genes of *B. malayi* would be represented. Such EST analysis is a rapid way to identify gene sequences, since no effort is made to completely sequence each cDNA clone.

Twelve *Brugia* cDNA libraries have been constructed in the bacteriophage lambda cloning vector ZAPII (Stratagene, La Jolla, CA) representing the following developmental stages: MF, L2, L3, molting L3 (L3M), L4, young adult (YA), adult male (AM), and adult female (AF). Unidirectional cloning was chosen to facilitate the use of these libraries in EST analyses. Seven of the Brugia cDNA libraries were constructed using conventional techniques,[2] two were constructed using subtraction techniques,[9] and three were constructed using PCR and the SL1 spliced leader sequence.[10] The *Brugia* cDNA libraries (and cDNA libraries from *O. volvulus* and *W. bancrofti*) and all individual cDNA clones are available from the National Institute of Allergy and Infectious Diseases/National Institutes of Health (NIAID/NIH) Filariasis Research Reagent Repository Center (FRRRC) at Smith College (contact genome@smith.edu or www.filariasiscenter.org). For the future,

- additional libraries need to be constructed to replace old libraries with better, more complete ones using methods and reagents that were unavailable when the original libraries were constructed,
- cDNA libraries also need to be constructed for life cycle stages that are not yet represented in the current collection.

3) Sequencing cDNA clones (EST sequencing).

By March 2004, there were already 26,215 ESTs containing more than 10 million basepairs of *B. malayi* sequence data submitted to the National Center for Biotechnology Information's dbEST database (www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). In addition, for *O. volvulus*, 14,974 ESTs have been submitted, while for *W. bancrofti* more than 6,000 ESTs are now available in the database. In total, this represents almost 20 million base pairs of expressed filarial parasite DNA sequences. All of these cDNA clones are available through the NIAID/NIH FRRRC at Smith College (contact genome@smith.edu or www.filariasiscenter.org). For the future,

- additional ESTs from *W. bancrofti* should be sequenced from a greater diversity of life cycle stages, because ESTs represent the most cost-effective approach to the identification of new *W. bancrofti* genes.

4) Clustering the EST data and developing user-friendly bioinformatics tools.

Since cDNA clones are selected randomly for sequencing and since duplication (especially of highly expressed genes) is unavoidable, the 26,000 *Brugia* EST represent approximately 8,945 *Brugia* clusters as determined by The Institute for Genome Research (TIGR) gene index (www.tigr.org/tdb/tgi/) and 8,392 as determined by the Nembase clustering[11] (http://nema.cap.ed.ac.uk/fgp.html). The number of genes represented by the EST data set is determined using a clustering algorithm that groups ESTs derived from the same gene into clusters. If the total number of genes in *B. malayi* is similar to that predicted for *C. elegans*, this data set includes almost 50% of all *Brugia* genes. A unified nomenclature for naming filarial genes and gene clusters is in place[12] (http://nema.cap.ed.ac.uk/fgn/funk/funkgenenames.html). Because these data are critical to gene identification,

- such bioinformatics-based analyses need to be expanded as additional ESTs are sequenced and as new clustering algorithms are developed.

5) Completing the genomic sequencing of the Brugia genome.

The complete genome sequence of *B. malayi* has recently been obtained through an NIH-funded project carried out at TIGR.[13] The entire genome was sequenced using a whole genome shotgun strategy developed at TIGR. Approximately 1.3 million sequence reads have provided about 10× coverage (i.e., enough sequencing has been done to theoretically sequence the genome 10 times). This ensures that virtually all of the genome will be represented in the final sequence. Cur-

rently, all of the genomic data is being assembled and gaps in the sequence are being closed. The genome is also being annotated by the filarial research community; a process initiated at the "*Brugia malayi* Annotation Jamboree" held for two weeks at TIGR during January 2004. For the future,

- additional effort is required to close the remaining gaps in the *B. malayi* genome sequence and to annotate the genome,
- sequencing of the *W. bancrofti* and *O. volvulus* complete genomes should be undertaken because of its benefit for the functional analysis of these genomes (including *B. malayi*) and its providing genetic clues of potential value for developing filariasis elimination strategies.

6) Completing the sequencing of the Wolbachia genome from *B. malayi* and of the mitochondrial genomes from *B. malayi* and *O. volvulus*.

Most filarial parasites contain an obligate intracellular bacterium most closely related to the *Wolbachia* intracellular bacteria found in mosquitoes and other arthropods.[14] The genome was sequenced by a consortium of scientists led by New England Biolabs.[15] Research over the past several years has shown that killing the *Wolbachia* bacteria results in reduced fecundity and often death of the filarial parasites.[16–18] Since this intracellular bacterium contributes to the normal functioning of the filarial parasite, the bacterium itself is a target for novel chemotherapeutic and vaccination interventions. In addition, the complete sequence of the first mitochondrial genome from a filarial parasite (*O. volvulus*) was carried out at the University of Alabama Birmingham.[19] The following year, the complete sequence of the *B. malayi* mitochondrial genome was completed at the University of Edinburgh. The mitochondrial genome provides additional targets for biological study and potential chemotherapy. What still remains are for

- the *Wolbachia* genomes from *W. bancrofti* and *O. volvulus* to be completely sequenced;
- the functional/comparative analysis of the Wolbachia genomes from all three species to provide potentially valuable information for LF and onchocerciasis elimination strategies.

7) Developing a transgenic technique for Brugia.

One of the principal barriers to rapid accumulation of knowledge on the biology and biochemistry of filarial parasites has been the inability of researchers to introduce DNA and RNA into the cells of these organisms. For example, the development of a transgenic technique (the ability to insert exogenous genes into the genome of the target organism) is important for the study of many organisms including bacteria, plants, and animals. Recently, a transgenic technique using a biolistic "gene gun" has been successfully applied to filarial parasites at the University of Alabama Birmingham.[20] The development of this technique opens up the possibility of doing many gene function studies that were heretofore impossible in filarial parasites. Therefore,

- continued development of the "gene gun" and other transgenic methods for use with filarial parasites will be vital for using the genomic data now available to study gene function in these filarial parasites.

8) Developing RNAi techniques for Brugia.

Another important barrier to the rapid accumulation of knowledge on the biology and biochemistry of filarial parasites has been the inability of researchers to introduce RNA into the cells of these organisms. RNA interference has been used for the past several years in a wide variety of organisms as a means for silencing expression of specific genes. Such gene silencing is another valuable tool for studying gene function and for the identification of likely vaccine candidates and drug targets. This technique has been employed with great efficiency in *C. elegans*, a free-living nematode often used as a model system for filarial parasites. However, until recently, the technique had never been successfully applied to parasitic nematodes. However, in 2003 researchers at the University of Edinburgh successfully applied the RNAi technique in *B. malayi* adult female parasites,[21] and in 2004 scientists at the New York Blood Center successfully applied RNAi to *O. volvulus* L3 larvae. Together, the development of a transgenic technique for introducing DNA and an RNAi technique for introducing RNA will dramatically revolutionize the way gene study can be undertaken in filarial parasites. Therefore,

- continued development of RNAi technology as a functional genomics tool, along with DNA transgenic techniques, will be vital for taking advantage of the new fully sequenced genome of *B. malayi* for functional genomics studies,
- RNAi should be used (as in *C. elegans*) as a screening tool for the identification of genes critical to the development of filarial parasites. Such genes encode products that may serve as excellent drug targets or vaccine candidates.

9) Producing microarrays for filarial parasites.

Another cutting-edge molecular biology technique now widely used in the study of genes in many organisms involves gene microarrays (gene chips). These arrays include thousands of genes on a single microscope slide or on a smaller silicon chip. These arrays enable the analysis of thousands of genes in a single experiment, instead of the more traditional analysis of one or a few genes at a time. Such technology has revolutionized the study of genes and gene expression. *Brugia malayi* microarrays have now been produced by a consortium of filarial researchers (led by scientists at Washington University and Smith College) at the Washington University Genome Center. These first microarrays for a filarial parasite are now available through the NIAID/NIH FRRRC at Smith College (genome@smith.edu or www.filariasiscenter.org). The arrays have been prepared using 65-mer oligonucleotides representing more than 3,000 genes of the parasite. It is still necessary for

- an updated version of the microarray to be prepared containing even more genes (~5,000),
- sufficient numbers of parasites to be collected for performing the critical functional experiments necessary to understand filarial development, molting, response to drugs, etc.,
- the limited gene sequence data available for *W. bancrofti* and *O. volvulus* to be used in order to include these genes in subsequent versions of the filarial microarrays.

10) Initiating comparative genomics studies among nematodes (including *C. elegans*).

To take full advantage of the newly available, complete sequence of the genome of *B. malayi* this sequence must be

compared to those of other nematodes. Fortunately, the complete genomic sequence of the free-living nematode, *C. elegans*, is also available. In addition, more than 400,000 EST sequences are now available from more than 30 species of nematodes, largely through the efforts of scientists at the Washington University Genome Center and the University of Edinburgh.[22] Such genomic and EST sequence data is essential for gene identification, for studies of the conservation of gene order (synteny), and for developing generalizations concerning gene function, gene regulation, and parasite-specific pathways. Because such comparative data will be critical for identifying new genes useful in developing vaccines and new drugs for filarial parasites,

- complete sequences need to be defined and comparative studies undertaken for the *W. bancrofti* and *O. volvulus* genomes (including their endosymbiont *Wolbachia* genomes).

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ANNEX 1

The Lymphatic Filariasis (LF) Research Forum: Towards Developing a Strategic Plan for Research to Support the Global Program to Eliminate LF (GPELF)

1. Approach and Goals

The vision for an ideal Forum was one that would include all research-oriented members of the LF community from both the scientific and programmatic disciplines; subgroups would be formed to define the issues relevant to their disciplines and from these components an overall strategic plan could subsequently be developed. To come as close to this ideal as possible the LF Research Forum was held in association with two other activities where large numbers of filariasis-oriented researchers were congregating: the centennial meeting of the American Society of Tropical Medicine and Hygiene and a filariasis Research Summit where international scientists met for in-depth presentation and discussion of key, cutting-edge filariasis research issues.

During the Forum subgroups focused on clinical, epidemiologic, and other scientific research issues important to filariasis, public health and the GPELF. These issues included pathogenesis, disease management, infection in children, drug use, drug development, diagnostic tools, strategies for employing these tools, modeling to define critical epidemiologic endpoints, vector biology, and others.

The results of these Forum meetings paint a detailed picture of the filariasis research horizon today. Useful in its own right for researchers and funding agencies, it should also serve as a firm scientific base for the subsequent development by a WHO/TDR Scientific Working Group, of an agreed Strategic Plan for Research to Support the GPELF.

2. Process

Funding to Enable the LF Research Forum

The inclusiveness of researchers to participate in this Forum was limited only by the funds available to support people's necessary travel expenses. Therefore, individuals (and their home institutions) were asked to support themselves if at all possible, with the remaining costs to be covered by a pool of funds from external donors.

Rather than seeking a single donor to underwrite the costs of the Forum, it was decided to request smaller sums ($5,000 to $20,000) from several different organizations both to decrease the burden on these organizations and to broaden the Forum's support base. Indeed, nine organizations (three commercial, four non-governmental, and two governmental agencies) contributed to support this meeting; these were the Ellison Medical Foundation, The Geneva Foundation for Tropical Diseases, GlaxoSmithKline, Health and Development International, Merck & Company, Inc., New England Biolabs, The U.K. Department for International Development, The U.S. National Institute of Allergy and Infectious Diseases, and the Wellcome Trust. In addition, of the 66 participants, 44 investigators from 26 different organizations supported their own costs for meeting participation.

Participants

Sixty-six senior scientists and experts in filariasis from 21 different countries participated in the LF Forum. Another 24 invited participants from 11 countries (all but two represented by other attendees) were unable to attend but agreed to have an input into this process through review of the draft documents. An additional list of nine potential invitees from five different countries (only one not otherwise represented) was developed, but not acted upon because of budgetary constraints.

Organization

Initially an organizing committee representing six institutions (including WHO/CEE and WHO/TDR) identified 10 subtopics covering the range of scientific issues addressable at this Forum. These were the following: 1) Chemotherapy, 2) LF Infection and Drug trials, 3) LF Disease and Treatment Trials, 4) Pathogenesis, 5) Diagnostics, 6) Epidemiology and Parasite Biology, 7) Program Implementation, 8) Program Monitoring & Evaluation, 9) Protective Immunity, and 10) Vector Biology.

Prior to the Forum, each participant was asked to select two of these subtopics on which to focus in Working Groups at the Forum. Once the Groups were constituted, a chairperson was designated who then contacted the Working Group members prior to the Forum itself.

During the two-day Forum, the 10 working groups (6–15 individuals per group) met twice in five parallel sessions to determine the most important researchable questions in their topic area and what the priority of these questions should be with respect not only to the GPELF, but to other aspects of science and public health as well. Conclusions from all 10 working groups were reviewed in plenary and subsequently distributed to all forum participants.

Final Report

Summaries of the subtopics and the conclusions reached by the Working Groups were prepared by the Group chairpersons and assembled into the first draft of a comprehensive document that was sent for review by all meeting participants and invitees. The document was then redrafted into this present final report.

ANNEX 2

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ANNEX 3A

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