Leishmaniasis and Malaria: New Tools for Epidemiologic Analysis

DYANN F. WIRTH,* WILLIAM O. ROGERS, ROBERT BARKER, JR., HEITOR DOURADO, LAKSAMI SUESEBANG, BERNARDINO ALBUQUERQUE

Parasitic diseases are still prevalent in many parts of the world, causing both human suffering and economic loss. Recent developments in biotechnology, such as the use of monoclonal antibodies and recombinant DNA, have the potential for providing both more extensive and detailed information on the parasite in the infected human and in insect vectors. New methods of detection, both in man and insect vectors, have been developed for two parasitic diseases, leishmaniasis and malaria. These new methodologies will be important in epidemiologic studies on the prevalence and transmission of these parasitic diseases.

MAJOR EFFORTS TO CONTROL AND EVEN TO ERADICATE parasitic diseases have met with some success (for example, elimination of malaria from the southern United States and Cuba), but in many developing tropical countries, parasitic diseases are still major health problems. Such diseases can pose a significant barrier to economic development, and their control is an important goal for improved world health. Intensive research is being devoted to the development of new control measures for many parasitic diseases. These control measures include development of vaccines and new chemotherapeutic agents as well as improved vector control strategies. Previous experience has demonstrated the need for extensive baseline information before a control program is introduced and the need for continued monitoring of the control program in order to assess its effectiveness. These diseases have complex life cycles involving vectors of transmission and, often, intermediate hosts, both of which have an impact on the transmission of disease and can affect the outcome of any control measure.

Recent developments in biotechnology, including the use of monoclonal antibodies and recombinant DNA, have provided new tools for the collection of information about these diseases. This article focuses on the potential impact of these new methodologies on epidemiologic studies of two parasitic diseases, leishmaniasis and malaria, for which new methods of detection, both in man and insect vectors, have been developed. These diseases are caused by parasitic protozoa of different genera, *Leishmania* and *Plasmodium*, respectively, each of which has a unique life cycle. Each poses a separate set of problems for epidemiologic studies and for eventual control.

D. F. Wirth, W. O. Rogers, and R. Barker, Jr., Harvard School of Public Health, Department of Tropical Public Health, Boston, MA 02115. H. Dourado and B. Albuquerque, Instituto de Medicina Tropical de Manaus, CEP:69,000, Manaus, Amazonas, Brazil. L. Suesebang, Malaria Division, Ministry of Public Health, Bangkok 10200, Thailand.

*To whom correspondence should be addressed.

Leishmaniasis

Human leishmaniasis is caused by at least 14 different species and subspecies of the genus *Leishmania*. The clinical manifestations of the disease depend in part on the infecting *Leishmania* organism and fall into three general categories: simple cutaneous disease, which is often self-limiting; mucocutaneous disease, which involves the destruction of nasal tissue; and visceral disease, a systemic infection that is often fatal if untreated. In fact, the diseases share relatively few properties except that they are caused by organisms of the same genus and that in humans the parasite grows in the phagolysosomal vesicles of macrophages.

Epidemiologic studies over the last 25 years have shown that, in general, leishmaniasis is a zoonotic disease; the parasite is transmitted to man from a reservoir mammalian host by a sandfly vector during a blood meal. Of the numerous *Leishmania* species infective for mammals, a subset can infect and cause disease in humans. Presumably, this selectivity is due to a combination of factors, including the intrinsic susceptibility of humans and the feeding habits of the sandfly vector. Enormous effort has been devoted to the isolation and characterization of *Leishmania* organisms that infect man and to the identification of the principal mammalian reservoirs and species of sandfly vector [for review see (*1*)]. The result of many such studies has been the correlation of particular clinical manifestations with certain species or subspecies of the parasite (see Table 1 for summary).

The identification of *Leishmania* species is based on a variety of ecological, biological, biochemical, and immunological criteria [for review see (*2*)]. Each cultured isolate of the parasite has been analyzed by the use of one or more of these criteria and categorized as to species and subspecies. There remain certain controversies as to whether organisms isolated in distant geographic locations but sharing certain common properties belong to the same or distinct subspecies of *Leishmania*. For example, *L. mexicana garnhami* (*3*) isolated in Venezuela is very similar to *L. mexicana amazonensis* isolated in Brazil, as determined by isoenzyme profiles and monoclonal antibodies (*4–6*), but there are conflicting results as to their growth characteristics in sandfly vectors (*7*). Whether these organisms represent strains of the same subspecies or distinct subspecies cannot be resolved because there is no single generally accepted method for species identification in the genus *Leishmania*. This uncertainty complicates comparison of the disease epidemiology in distinct geographic locations and represents a potential limitation on the transfer of control measures from one geographic location to

Table 1. Major *Leishmania* species causing human disease.

Disease	Species	Primary location*
Cutaneous leishmaniasis	*L. mexicana mexicana*	Mexico, Central America
	L. mexicana amazonensis	Brazil Amazon region
	L. mexicana pifanoi	Venezuela
	L. major	Southern U.S.S.R., Middle East
	L. tropica	Asia, southern Europe, northern and western Africa
	L. braziliensis guyanensis	Northern, southern America
	L. braziliensis panamensis	Central America
	L. braziliensis peruviana	Peru
Diffuse cutaneous disease	*L. mexicana amazonensis*	Brazil, Amazon region
	L. aethiopica	Ethiopia and Kenya
Mucocutaneous disease	*L. braziliensis braziliensis*	Western and northern South America
Visceral disease	*L. donovani*	India
	L. donovani infantum	Mediterranean area
	L. chagasi	Northern South America

*Based on Marinkelle (*10*).

another. The World Health Organization has addressed this problem by establishing a set of reference strains for the various species and subspecies to be used for comparison and classification of new isolates.

The diverse nature of leishmaniasis clearly requires a diverse control program with specific targets for each focus of the disease. This will require more extensive collection of baseline data with regard to the infecting *Leishmania* species in humans, the relevant sandfly vector, and the principal mammalian reservoir of the particular species or subspecies. These data must be collected at each focus for proper implementation of any control program directed at either the sandfly vector or the mammalian reservoir. For control measures that involve identification and treatment of patients, accurate rapid diagnosis of leishmaniasis must be achieved before treatment with relatively toxic chemotherapeutic agents is begun (*8*).

One of the major problems in further analysis of the ecology and epidemiology of the disease is the extremely laborious task of identifying the parasite. All of the work documenting the species and subspecies of *Leishmania* is dependent on the isolation of the organism either directly in culture or after passage through a susceptible laboratory animal, most commonly the hamster. This has several limitations—namely, the number of samples that can be handled at any one time, the time it takes to grow the parasites and subsequently type them, and perhaps more important, the selection from an otherwise mixed population of parasites of those that grow either in vitro or in experimental animals. A method of direct identification of *Leishmania* parasites from lesions, sandfly vectors, and intermediate mammalian reservoirs is necessary if broader epidemiological studies on large numbers of samples are to be initiated.

Current diagnosis of leishmaniasis is achieved either by direct examination of a tissue biopsy or by means of a delayed-type hypersensitivity test referred to as the Montenegro test (*9*). Neither of these methods is able to distinguish *Leishmania* species or subspecies, and the Montenegro test cannot distinguish current from previous infections. For certain forms of leishmaniasis, the most effective control measures may be the direct treatment of infected patients (*10*) and will require specific diagnosis of *Leishmania* species or subspecies from lesion material in order to design treatment regimens that minimize morbidity and mortality [for review see (*11*)]. For example, cutaneous infection with *L. braziliensis* is often associated with subsequent mucocutaneous disease, and early diagnosis could facilitate treatment and perhaps reduce the frequency of mucocutaneous disease.

DNA probes in the diagnosis of leishmaniasis. A new methodology based on DNA probes specific for the various *Leishmania* species was developed to provide a direct diagnosis of patients with leishmaniasis and to eliminate the need for culturing parasites before species identification (*12*). This methodology allows direct diagnosis from lesion material without requiring isolation of the parasite. Such direct diagnosis of *Leishmania* species, which had not been possible with any of the existing methodologies, provides the basis for the clinical management of this disease. In the initial studies, the DNA probes, which were based on total kinetoplast DNA (kDNA), could differentiate the major species complexes in the New World, *L. mexicana* and *L. braziliensis*. Subsequent experiments in which recombinant DNA methodologies were used resulted in the development of DNA probes that can differentiate species, subspecies, and even distinct isolates of the parasite (*12–17*).

The basis for the DNA probes is the minicircle, which is a highly repeated small circular DNA molecule found within the mitochondria of the parasite. It has no apparent function and has an apparent high rate of DNA sequence divergence as measured by restriction site polymorphism and DNA hybridization studies (*18–22*). In the New World *Leishmania sp.*, the kDNA minicircles isolated from *L. mexicana* do not share any sequence homology with those isolated from *L. braziliensis* (*12, 13*). These differences in DNA sequence have provided the basis for a DNA probe that can distinguish the two *Leishmania* species directly when material obtained from a lesion is applied to nitrocellulose.

These DNA probes have been used to diagnose leishmaniasis in patients from the Instituto de Medicina Tropical de Manaus (*23*) (Table 2). In each case, the results of the DNA probe were compared to standard diagnostic tests, including the Montenegro test (see above), histopathology, and culturing and subsequent characterization of the parasite. As can be seen in Table 2, the DNA probe detected the disease in 32 of the 43 patients who had positive results in the Montenegro test. This observation may mean that in some patients the parasite density was below the detection limit of the assay, which, based on laboratory experiments, is as few as 50 organisms in a single spot on nitrocellulose. In the sample reported here, there were three cases in which organisms were cultured from lesions that were negative in the DNA probe assay. Another possibility is that the delayed-type hypersensitivity test detected

Table 2. Results from 57 patients examined at the *Leishmania* clinic, Instituto de Medicina Tropical de Manaus during January and February 1984. Each patient was given a Montenegro test, and those with positive results in the test were given further diagnostic tests which included a tissue biopsy (4 mm) directly from the lesion. The biopsy specimen was divided in two, one half being placed directly in Schneider's solution and the other half touched to nitrocellulose, and then processed for histopathology. The hybridization was performed by standard procedures as described (*12*). The *L. mexicana* probe was a combination of DNA extracted from strain WR303 (*L. mexicana amazonensis*) and L11 (*L. mexicana mexicana*). The *L. braziliensis* probe was isolated from strain M4147. All hybridization results were read independently by two people.

Diagnostic test	No. of patients
Montenegro-positive	43
Hybridization-positive	32
L. mexicana DNA probe	2
L. braziliensis DNA probe	30
Culture-positive	27
Histopathology	17

Table 3. Hybridization specificities of cloned kinetoplast DNA fragments.

Source	Specificities	Reference
L. donovani	Species	(*28*)
L. infantum	Species	(*28*)
L. donovani	Visceral complex	(*16*)
L. chagasi	Non-Indian visceral	(*16*)
L. major	Isolate	(*17*)
L. mexicana amazonensis	Species	(*30*)
	Subspecies	(*30*)
	Isolate	(*30*)
L. major	Isolate	(*14*)
L. tropica	Isolate	(*14*)
L. aethiopica	Isolate	(*14*)

either a previous infection or a cross-reacting antigen from another type of infection (*24*). A third possibility is that the parasite in the lesion is of a type not recognized by the kDNA probe; however, every parasite that has been isolated from a lesion has reacted with either the *L. braziliensis* or *L. mexicana* kDNA probes.

The DNA probe clearly detects infections in more patients than either histopathology or culturing. Although this probably results from the greater sensitivity of the DNA probe in comparison to these other methods, it may also represent false positives in the DNA probe method. Because there is no single "gold standard" for the detection of parasites, the exact determination of false positives is not possible. However, in ten cases in which histopathology failed to detect parasites and the result of the DNA probe was positive, the culturing of parasites clearly demonstrated their presence. The question of the false positive reaction with DNA probes has been tested experimentally. These kDNA probes did not hybridize with touch preparations of uninfected tissue from several animals and tissue from a limited number of human lesions that subsequently proved not to be due to leishmaniasis. In addition, these DNA probes did not react with *Trypanosoma cruzi*, malaria, or *Escherichia coli*. Further evidence that the number of false positives with the kDNA probes is relatively low was obtained by testing duplicate touch preparations of each lesions with kDNA from both *L. mexicana* and *L. braziliensis*. In every case in which there was a reaction with the kDNA probe, it was specific for either *L. mexicana* or *L. braziliensis*, and the duplicate lesion showed no reaction above background. When parasites were isolated from these lesions and subsequently tested by kDNA hybridization, the original identification was confirmed. An alternative method of limiting the number of false positive reactions due to nonspecific binding of labeled DNA to tissue or blood is to perform in situ hybridization and examine each preparation under the microscope, as suggested by Barker *et al.* (*15*). This is a very time-consuming and expert process not easily adapted to large numbers of samples.

One of the limitations of the kDNA minicircle as a hybridization probe is that although it can distinguish the major species complexes of New World cutaneous leishmaniasis, the kDNA minicircle from each complex is homologous to all the subspecies and it is therefore impossible to distinguish subspecies (*12, 13*). This identification of subspecies is important clinically because certain manifestations of the disease are specific to the parasite subspecies and is also important in the description of any intermediate host or insect vector carrying a particular subspecies. In addition, our work and the work of others has indicated that in the Old World *Leishmania* species causing cutaneous and visceral disease, kDNA sequence homology occurs among different species (*12, 16, 17, 24–27*). For example, kDNA isolated from *L. major* hybridizes with both of the other cutaneous species, *L. tropica* and *L. aethiopica*, and with the visceral strain *L. donovani* (*17*).

Therefore a new approach is required for the development of DNA probes that can distinguish subspecies in New World cutaneous leishmaniasis and differentiate the species complexes of the Old World isolates. Several groups have used recombinant DNA methods to develop such DNA probes with these narrower specificities (see Table 3) (*14–17, 26, 28–30*). The method has been to clone restriction fragments of a kDNA minicircle and to use these cloned subfragments as more specific probes for species, subspecies, and even isolates. The general observation from this body of work is that within the minicircle population, there are DNA sequences that have undergone rapid sequence divergence and can thus serve to differentiate even closely related organisms (*28*). In addition, we have shown that this sequence divergence can occur within a single minicircle. A nested set of deletions of a single cloned minicircle fragment from *L. mexicana amazonensis* was generated and then tested for hybridization specificity. The full-length minicircle had a similar specificity to total kDNA, and two deletions demonstrated species and isolate specific hybridization patterns (*30*).

The next step for the utilization of DNA probes will be in the detection of infected insect vectors and intermediate hosts. Preliminary laboratory experiments show that parasites can be detected in infected sandflies that have been squashed directly on nitrocellulose (*31*). This approach must now be tested in the field. Similarly, laboratory-based experiments have demonstrated that parasite infections can be detected in tissue touch preparations from animals experimentally infected (*12*); however, both the intensity of infection and the target tissue in natural hosts will be different and thus the DNA probes must be tested directly in field-extracted material.

The DNA probe methodology should be readily adaptable to field situations. Once the tissue biopsy or sandfly vector is obtained, it is applied directly to nitrocellulose or other solid supports and is stable in this form indefinitely. Thus samples could be collected from distant sites and returned for processing. The major disadvantage of this methodology is the requirement for a radioisotope. Alternative methods of labeling DNA, which are being developed and tested for such biological specimens, should be useful in field situations.

Use of monoclonal antibodies in detecting leishmaniasis. A second method for the identification of *Leishmania* species and subspecies that should also facilitate the collection of epidemiologic data is the development of monoclonal antibodies specific for *Leishmania* species and subspecies (*32*). Cultured promastigotes were used in most of the reported work with *Leishmania*-specific monoclonal antibodies, but in principle an immunofluorescent antibody (IFA) test could be developed analogous to those described for several viral and bacterial systems that could be used directly on lesion material either from human patients or other mammalian hosts. Preliminary work on the use of IFA in the identification of promastigotes directly from laboratory infected sandflies has been reported (*33*).

Malaria

Human malaria is caused by the four major *Plasmodium* species: *P. falciparum, P. vivax, P. malaria*, and *P. ovale*. In most parts of the world, the prevailing parasite species is *P. falciparum*, which causes the most severe form of the acute disease that is often fatal in children (*34*). *Plasmodium vivax*, the next most prevalent disease is characterized by relapses caused by parasites that remain in the liver in a latent form. The parasite is transmitted by various species of the anopheline mosquito to the human host. The sporozoite, the infectious form of the parasite released from mosquito salivary glands, initiates the exoerythrocytic cycle in the liver. Subsequently developed merozoites invade erythrocytes, and the asexual cycle continues through the course of the infection. A subset of the

infected erythrocytes develop into gametocytes. This form can develop in mosquitoes and results in disease transmission. There is no significant animal reservoir for this disease.

Malaria is one of the major infectious diseases in the world, with acute clinical malaria affecting some 90 million to 100 million people per year according to World Health Organization estimates (*35*). In addition, there is a large reservoir of chronic infection. The World Health Organization estimates that more than 40 percent of the world's population is at risk for malaria infection and that some 365 million people live in areas where malaria endemicity has remained unchanged despite intensive world efforts at malaria eradication (*35*).

Elimination of malaria worldwide has proved to be difficult to achieve, and thus current efforts are devoted to the control of malaria (*36*). During the eradication program, several problems arose that will have an impact on any control program. Among these problems is the widespread resistance of anopheline vectors to insecticides (*37*) and the emergence of *P. falciparum* strains resistant to chloroquine, the primary chemotherapeutic agent, and the subsequent development of multidrug-resistant parasite strains (*38*). New approaches to control measures include improved conventional methods of vector control and chemotherapy and the development of innovative measures including vaccines for the malaria parasite and biological control of anopheline vectors.

The parameters of malaria transmission and disease prevalence have been studied for the last 40 years in many parts of the world. Mathematical models (*39, 40*) of disease transmission based both on entomologic factors and human factors such as immunity have been developed [for review see (*41*)]. In addition, epidemiologic studies have demonstrated an association of malaria prevalence with certain variants in erythrocytes, sickle cell trait (*42*), glucose-6-phosphate dehydrogenase deficiency (*43*), Duffy blood-group antigens (*44*) and recently, α-thalassemia (*45*). These studies have shown that malaria is a complex and dynamic disease that is varied throughout the world. Thus any control program must take into consideration the multiple factors that can affect malaria transmission and must measure these factors in each situation. For this to be achieved, it is imperative that new and efficient means of measuring both entomologic and human factors on a large scale be implemented.

Detection of malaria infection. Both vaccine and future drug trials will require a sensitive and rapid method for detecting parasites. Currently, malaria infection is determined by the use of a thick smear stained with Giemsa. This method is both specific and sensitive for the diagnosis of malaria but has severe limitations when large numbers of samples must be handled in a timely fashion, as will be the case for the collection of baseline data for many of the vaccine trials and chemotherapy studies. A trained microscopist is required for each determination, and this is time-consuming, tiring, and potentially subject to reader bias, especially when large numbers of slides must be read in a short time period. Thus alternative methods for handling large numbers of samples are necessary.

DNA probes specific for human malaria have been developed by several groups (*46*) and recent work by Barker *et al.* (*47*) has demonstrated that the DNA probe specific for *P. falciparum* can be used to detect malaria infection directly in finger-stick blood of infected patients. The DNA probes specific for *P. falciparum* are dispersed, highly repeated DNA sequences isolated from the *P. falciparum* genome by recombinant DNA technology. Both in laboratory and field testing, the pPF14 probe (*47*) is specific for *P. falciparum* and does not react with *P. vivax*, the other major human malaria. The method compares favorably in sensitivity with routine microscopy, detecting parasite densities as low as 40 parasites per microliter of blood. The DNA probe method detected *P. falciparum* infection in 129 of 574 patients compared with the 115 detected by routine examination of Giemsa-stained thick smears (Table 4). Of the 129 samples that gave positive results with the DNA probe, 96 were also detected with routine microscopy while 33 were not. Subsequent examination of duplicate slides by expert microscopists detected *P. falciparum* parasites in 17 of these 33 samples. Of the 115 patients originally diagnosed as having *P. falciparum* infections by routine clinic microscopy, 96 were diagnosed as having *P. falciparum* infections by the DNA probe while 19 were not. In subsequent examination of duplicate slides 4 of these 19 patients were found to have *P. vivax* infections and not *P. falciparum* infections. The DNA probe method offers the advantage of a standardized procedure that can be used in a batchwise fashion on large numbers of samples. An important feature of this method is that it is reproducible in a large number of samples and should be less subject to reader bias. In addition, in our recent work, we have been able to correlate the intensity of DNA hybridization with parasite density, and thus the DNA probes may also provide information on the intensity of infection. One limitation of the correlation of hybridization intensity with parasite density is the potential for variation in the number of repeated target sequences in different *P. falciparum* strains. The DNA probe methodology now must be tested in an epidemiologic study to assess its general usefulness.

Table 4. Comparison of DNA probes specific for *P. falciparum*. The patient population consists of 574 patients examined at malaria clinics of the Malaria Division of the Thailand Ministry of Public Health in either Bangnamron or Chantiburi, Thailand, in July 1985. Blood was collected by digital puncture into a heparinized capillary and treated as previously described (*47*). Malaria thick smears were prepared in the routine manner for diagnosis at the clinic. A positive result by clinical microscopy indicates that *P. falciparum* parasites were detected, while a negative result indicates that *P. falciparum* parasites were not detected. A separate set of slides, both thin and thick smears, was prepared and subsequently analyzed by malaria experts (*47*).

Probe	Clinic microscopy		Total
	Positive	Negative	
DNA-positive	96	33	129
DNA-negative	19	426	445
Total	115	459	574

Methods for the detection of malaria-specific antigens or antibodies have also been developed and tested (*48*). A major problem with these assays has been the presence of both antigen and antibody after the malaria parasites have disappeared from the bloodstream. Malaria-specific antibody can persist for long periods and this is useful to assay initial infections in naive individuals, especially young children (this is complicated by the presence of maternal antibody) but cannot be used to determine present infection with the parasite in individuals previously infected (*49*). Extensive recent work with monoclonal antibodies has led to the identification of many different malaria antigens, and a major area for research in the future should be in the testing of specific antigens or antibodies and their correlation with active infection or protective immunity.

Detection of infective mosquito vectors. An important parameter in malaria transmission is inoculation rate, which is the number of infective mosquito bites per unit time. This rate is based both on the man-biting rate of the vector species and the fraction of infective mosquitoes. Zavala *et al.* (*50*) have developed an immunological method for the determination of infective mosquitoes that uses a monoclonal antibody specific for the major protein of the malaria sporozoite, the circumsporozoite protein. This method has been field-tested and compared with the existing method, which is the capture and dissection of mosquitoes to determine infection. The

advantage of this new method is that it can determine the species of sporozoite in the infected mosquito. Another advantage is that large numbers of mosquitoes can be tested easily. Thus vectors that have a low rate of infection and have previously been overlooked are now being discovered, and their contribution to malaria transmission is being determined (*51*).

Determination of human genetic parameters. Epidemiologic studies have demonstrated an association between malaria prevalence and certain variants in erythrocytes. Advances in the detection of human genetic variants by the use of restriction site polymorphisms of specific DNA fragments should allow more extensive investigation of these genetically inherited diseases and, perhaps, of new diseases that have not yet been associated with malaria prevalence. An elegant study by Flint *et al.* (*45*) demonstrates the potential of this technology. A single variant of α-thalassemia has been correlated with malaria prevalence in Melanesia.

Conclusion

The application of new methods of biotechnology to the epidemiology of leishmaniasis and malaria is in its initial phases. The new tools offer distinct advantages with regard to specificity, sensitivity, and ease of use for large numbers of samples when compared to existing methodologies and have enormous potential for their contribution to new knowledge on the transmission and prevalence of these diseases. Before these methods are generally accepted for use, they must be extensively tested under field situations and modified to provide the relevant information important for epidemiologic analysis.

REFERENCES AND NOTES

1. R. Lainson, *Trans. R. Soc. Trop. Med. Hyg.* 77, 569 (1983); R. Lainson and J. J. Shaw, *Nature (London)* **273**, 595 (1978).
2. M. L. Chance and B. C. Walton, Eds., *Biochemical Characterization of Leishmania* (UNDP/World Bank/WHO, Geneva, 1982).
3. J. U. Scorza *et al., Trans. R. Soc. Trop. Med. Hyg.* **73**, 293 (1979).
4. H. Momen and G. Grimaldi, *ibid.* **78**, 701 (1984).
5. M. A. Miles *et al., ibid.* **74**, 243 (1980).
6. R. Lainson, *Proceedings of the 3rd Venezuelan Congress of Microbiology and Symposium on Leishmaniasis*, Bouquisimeto, Venezuela (1983).
7. R. Lainson and J. Shaw, in *Biology of Kinetoplastidae 2*, W. H. R. Lunden and Evans, Eds. (Academic Press, London, 1979), pp. 1–116.
8. W. Peters *et al., J. Roy. Soc. Med.* **76**, 540 (1983).
9. J. Montenegro, *An. Fac. Med. Univ. Sao Paulo* **1**, 323 (1926).
10. C. J. Marinkelle, *Bull. WHO* **58**, 807 (1980).
11. L. M. Deane and G. Grimaldi, *Leishmaniasis*, K. P. Chang and D. Bray, Eds. (Elsevier, Amsterdam, 1985).
12. D. F. Wirth and D. McMahon-Pratt, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6999 (1982).
13. D. C. Barker and J. Butcher, *Trans. R. Soc. Trop. Med. Hyg.* 77, 285 (1983).
14. W. P. K. Kennedy, *Mol. Biochem. Parasitol.* **12**, 313 (1984).
15. D. C. Barker *et al., Parasitology* **91**, S139 (1985).
16. U. G. Lopes and D. F. Wirth, *Mol. Biochem. Parasitol.*, in press.
17. D. F. Wirth and W. O. Rogers, in *Rapid Detection and Identification of Infectious Agents*, D. Kingsbury and S. Falkow, Eds. (Academic Press, New York, 1985), pp. 127–137.
18. P. T. Englund, *J. Biol. Chem.* **254**, 4895 (1979).
19. G. Z. Kidane, D. Hughes, L. Simpson, *Gene* **27**, 265 (1984).
20. P. R. Jackson *et al., Am. J. Trop. Med. Hyg.* **33**, 808 (1984).
21. M. Steinert and S. Van Assel, *Plasmid* **3**, 7 (1984).
22. U. G. Lopes *et al., Parasitology* **70**, 89 (1984).
23. W. O. Rogers *et al.*, in preparation.
24. D. C. Aston and A. P. Thornley, *Trans. R. Soc. Trop. Med. Hyg.* **75**, 537 (1970).
25. D. F. Arnot and D. C. Barker, *Mol. Biochem. Parasitol.* **3**, 47 (1981).
26. D. C. Barker and D. F. Arnot, *Eur. J. Cell Biol.* **22**, 124 (1980).
27. T. W. Spithill *et al., J. Cell Biochem.* **24**, 103 (1984).
28. J. M. Lawrie *et al., Am. J. Trop. Med. Hyg.* **34**, 257 (1985).
29. A. C. C. Frasch *et al., Mol. Biochem. Parasitol.* **4**, 163 (1981).
30. W. O. Rogers and D. F. Wirth, in preparation.
31. P. Perkins and D. F. Wirth, unpublished observations.
32. D. McMahon-Pratt and J. David, *Nature (London)* **291**, 581 (1981); C. C. Jaffe *et al., J. Immunol.* **133**, 440 (1984); D. McMahon-Pratt *et al., ibid.* **134**, 1935 (1985); S. Frankenberg *et al., Am. J. Trop. Med. Hyg.* **34**, 266 (1985).
33. D. McMahon-Pratt *et al., Am. J. Trop. Med. Hyg.* **32**, 1268 (1983).
34. S. Cohen and P. H. Lambert, in *Immunology of Parasitic Infections,* S. Cohen and K. S. Warren, Eds. (Blackwell, Oxford, 1982), p. 422.
35. *World Health Statistical Quarterly* **37**, 130 (1984).
36. *WHO Tech. Rep. Ser. No. 640* (1979).
37. *WHO Tech. Rep. Ser. No. 655* (1980).
38. *WHO Tech. Rep. Ser. No. 711* (1984).
39. G. McDonald, *Trop. Dis. Bull.* **49**, 813 (1952); *Proc. R. Soc. Trop. Med.* **48**, 295 (1955).
40. J. A. Bruce-Chwatt, *Trop. Geogr. Med.* **28**, 1 (1976); J. A. Najera, *Bull. WHO* **50**, 449 (1974).
41. L. Molineaux and G. Gramiccia, *The Garki Project* (World Health Organization, Geneva, 1980), pp. 109–115.
42. J. B. S. Haldane, *Hereditas* **35** (suppl.), 267 (1949); J. H. Walker and L. J. Bruce-Chwatt, *Trans. R. Soc. Trop. Med. Hyg.* **50**, 511 (1956); A. F. Fleming, *Ann. Trop. Med. Parasitol.* **73**, 161 (1979).
43. L. Luzzato *et al., Bull. WHO* **50**, 195 (1974); L. Luzzato, *Blood* **54**, 961 (1979).
44. L. H. Miller *et al., N. Engl. J. Med.* **295**, 302 (1976); L. H. Miller *et al., Am. J. Trop. Med. Hyg.* **27**, 1069 (1978).
45. J. Flint *et al., Nature (London)* **321**, 744 (1986).
46. L. Franzen *et al., Lancet* **1984-I**, 525 (1984); Y. Pollack *et al., Am. J. Trop. Med. Hyg.* **34**, 663 (1985); E. McLaughlin *et al., ibid.*, p. 837.
47. R. B. Barker *et al., Science* **231**, 1434 (1986).
48. L. Mackey *et al., Bull. WHO* **60**, 69 (1980); H. Avraham *et al., J. Immunol. Methods* **53**, 61 (1982).
49. H. Avraham *et al., Am. J. Trop. Med. Hyg.* **32**, 11 (1983).
50. F. Zavala *et al., Nature (London)* **299**, 737 (1982); F. H. Collins *et al., Am. J. Trop. Med. Hyg.* **33**, 538 (1984).
51. M. Arrada *et al., Am. J. Trop. Med. Hyg.*, in press.

52. We thank R. Gonski for careful preparation of the manuscript. Supported by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, by NIH grants AI 21365 and AI 19392, by the John D. and Catherine T. McArthur Foundation, and by a Burroughs-Wellcome scholarship in molecular parasitology (to D.F.W.).