Immune Responses in Human Infections with *Brugia Malayi*

SPECIFIC CELLULAR UNRESPONSIVENESS TO FILARIAL ANTIGENS

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ABSTRACT We evaluated the cellular immune competence of 101 subjects living in an area of South Kalimantan (Borneo) where Malayan filariasis is endemic. All patients with elephantiasis but none with other clinical stages of filariasis reacted with adult worm antigens. The majority of subjects without clinical or parasitological evidence of filariasis and approximately one-half of those with amicrofilaremic filariasis reacted with microfilarial antigens. In contrast, most patients with patent microfilaremia did not respond to microfilarial antigens. The in vitro reactivity of all patient categories to nonparasite antigens was similar to that of the distant control group. These results indicate that patent microfilaremia is associated with a state of specific cellular immune unresponsiveness and are consistent with the current hypothesis that the various clinical manifestations of filariasis result from different types of immune responses to distinct antigens associated with different developmental stages of filarial worms.

INTRODUCTION

Human filariasis is a major global health problem affecting an estimated 250 million people. Disease caused by infections with the parasitic nematode *Brugia malayi* is characterized by a broad spectrum of clinical manifestations including asymptomatic microfilaremia, recurrent lymphadenitis with retrograde lymphangitis, "filarial fevers," lymphedema, and elephantiasis. This wide range of clinical disease and of the immunological changes associated with it are thought to reflect the diversity of the host's immune response to filarial infections (1), but direct proof for this hypothesis is lacking.

Serological studies reveal that infected individuals develop antifilarial antibodies of different immunoglobulin classes. The detection of these antibody responses by a variety of in vitro techniques and by immediate hypersensitivity type skin reactions forms the basis of current immunodiagnostic methods (2, 3). In contrast, few detailed studies on cell-mediated immune reactions in human filariasis have been performed to date. Ottesen et al. (4) reported a specific cellular immune unresponsiveness in human subjects infected with *Wuchereria bancrofti*. The interpretation of this finding is complicated because in vitro testing was performed with heterologous antigens prepared from the related species *B. malayi* and *Dirofilaria immitis*. The present study was undertaken to evaluate cell-mediated immune responses to microfilarial and adult worm antigens of the infecting species as well as to nonparasite antigens of individuals living in an area where *B. malayi* infections are endemic. The results indicate that patients with different stages of filariasis exhibit marked differences in their cellular response to filarial antigens. All patients with elephantiasis but none of the other subjects tested responded to adult worm antigen. The majority of patients with overt filariasis also failed to respond to microfilarial antigens, although all reacted with at least one nonparasite antigen. In contrast, subjects without clinical or parasitological evidence of filariasis were able to respond to microfilarial antigens but not to adult worm extract.

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METHODS

Study population. As part of a comprehensive demographic, epidemiological, and parasitological study in an area in South Kalimantan (Borneo), where filariasis with subperiodic *B. malayi* is endemic, 101 persons were selected for the studies reported here. Their willingness to donate blood was the main criterium for admission to the study; volunteers were chosen to represent all aspects of Malayan filariasis.

The study population lived in either one of two ecotypes: One village in an agricultural area was surrounded by wet rice fields, three other villages were located on rubber estates surrounded by scattered rice fields and dense secondary forest. The main vectors transmitting *B. malayi* in the study area are *Anopheles, Coquilletidia,* and *Mansonia* species. It is estimated that each year persons living in the area are bitten several thousand times by mosquitoes that are potential vectors for the parasite and approximately 100 times by mosquitoes harboring infective third stage *B. malayi* larvae.[1]

The control group consisted of healthy Indonesian or Caucasian laboratory personnel living in Jarkarta (Java).

Clinical and parasitological evaluations. Clinical histories and physical examinations were taken from all patients. At the time of study thin and thick blood smears were examined for presence of malaria and microfilariae. The level of microfilaremia was further determined by counting the number of microfilariae trapped on a 5-μm nucleopore filter (Nucleopore Corp., Pleasanton, Calif.) after filtration of 1 ml heparinized venous blood on at least two occasions, including once at night. None of the patients had been treated with diethylcarbamazine within 15 mo preceding the studies reported here.

The stools of a large number of patients were examined for the presence of eggs of *Ascaris, Trichuris,* and *Necator* species by a modified Kato technique (5).

Antigen preparations. Purified derivative (PPD),[2] was provided by the U. S.-Japan Cooperative Medical Science program, National Institutes of Health, Bethesda, Md. Streptokinase-streptodornase (SK-SD, Varidase) was obtained from Lederle Laboratories (Pearl River, N. Y.) and used after dialysis against distilled water.

Saline extracts from *B. malayi* microfilariae and adult worms (maintained in jirds) were prepared as described by Ottesen et al. (4), sterilized by filtration through a 0.45-μm filter (Millipore Corp., Bedford, Mass.) and stored at −20°C until used. The protein content of the extracts was measured by the method of Lowry et al. (6). It should be noted that, to avoid contamination with putative microfilarial antigens, only male worms were used to prepare the adult worm extract.

Lymphocyte transformation assay. 10 ml of heparinized venous blood was collected early in the morning from the patients in Kalimantan and from the laboratory controls in Jakarta. The patients' blood was then transported by road and air to Jakarta where the cultures were set up during mid-afternoon. All blood samples were kept at ambient temperature until used.

Mononuclear cells were isolated by centrifugation of the heparinized whole blood on Ficoll-Hypaque gradients (6.5% Ficoll, Pharmacia Fine Chemicals, Piscataway, N. J., and 0.7% Hypaque, Winthrop Laboratories, New York), washed three times with RPMI 1640 medium supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and Hepes buffer (25 mM, Microbiological Associates, Walkersville, Md.) and counted in a hemocytometer. Cell suspensions prepared in this manner from blood of patients with microfilaremia were found to be devoid of contaminating parasites.

The lymphocyte transformation assay was carried out in a microculture system as follows: 2×10^5 mononuclear cells were cultured in 0.2 ml of medium in round bottom microtiter plates (Cooke Laboratory Products Div., Dynatech Laboratories, Inc., Alexandria, Va.) at 37°C in tightly sealed candle jars. The culture medium consisted of RPMI 1640, supplemented with antibiotics and Hepes buffer as described above and made to contain 15% heat-inactivated human AB+ serum from a single pool. The final concentrations of antigens were 10 μg/ml of PPD, microfilarial or adult worm extract and 25 U/ml of SK-SD. Other concentrations were used as indicated in the results. All cultures were done in quadruplicate.

After 6 d of incubation, 20 μl of medium containing 1 μCi ^{125}I-deoxyuridine (>5,000 Ci/mg, The Radiochemical Centre, Amersham, England) and 10 μl of a 10-μM solution of fluorodeoxyuridine (Sigma Chemical Co., St. Louis, Mo.) were added to the cultures. 12 h later the cultures were harvested with a multiple automatic sample harvester (MASH-2, Microbiological Associates) on glass filters. The filters were then counted in a Siemens well-type manual gamma counter.

The results are expressed as mean disintegrations per minute of quadruplicate samples ± SD, or as a stimulation index (SI): SI = (mean dpm antigen stimulated culture/mean dpm nonstimulated control culture). An index ≥2 was considered a positive response because it represents the 95% confidence limit of the reactivity of lymphocytes from control donors measured in preliminary experiments. Using this cut-off point, no false-negative or false-positive reactions in vitro were observed in 25 individuals whose skin test reactions to PPD were known.

Statistical analysis was done using Student's *t* or chi-square tests as indicated in the text.

RESULTS

Clinical and parasitological observations. Because of clinical and parasitological findings, each individual could be assigned to one of five patient groups defined by the following criteria: (*a*) No microfilaremia, no symptoms of filariasis; (*b*) no microfilaremia, symptoms of filariasis other than elephantiasis; (*c*) no microfilaremia, elephantiasis; (*d*) microfilaremia, no symptoms; and (*e*) microfilaremia with symptoms. The term "symptoms" refers to the periodic occurrence of fevers associated with typical lymphadenitis and retrograde lymphangitis for which no other obvious cause was apparent. Relevant demographic, parasitological, and clinical data are summarized in Tables I and II. There were no significant differences between the various patient groups with regard to age or sex distribution or the mean number of years of residence in the study villages (Table I).

The frequency of positive blood smears for malaria prepared from blood used for immunological testing was low and did not significantly differ between patient groups. None of the subjects had clinical evidence of acute malaria at the time of testing.

The prevalence of intestinal nematode infections, determined from egg counts on stool specimens ob-

[1] Manuscript in preparation.

[2] *Abbreviations used in this paper*: PPD, purified derivative; SK-SD, streptokinase-streptodornase; SI, stimulation index.

TABLE I
Clinical Characteristics of Study Population

Clinical group	Age		Number of years in village		Males	Females	Total
	Range	Mean	Range	Mean			
No microfilaremia							
No symptoms	11–78	40	1–50	20	22	14	36
Symptoms	13–70	37	1–52	19	7	4	11
Elephantiasis	11–69	39	5–44	21	10	5	15
Microfilaremia							
No symptoms	13–72	42	1–50	23	13	7	20
Symptoms	14–57	33	1–56	23	12	7	19
Distant controls	27–45	34	—	—	12	3	15

tained over a 6-mo period preceding this study, was high in all patient groups. Again, no significant differences between the various patient categories could be detected (Table II).

Several points related to filarial disease deserve further comment. First, none of the patients with elephantiasis tested had detectable microfilariae in their blood, although 13/15 had other symptoms of filariasis (recurrent fevers and/or adenitis) in addition to elephantiasis. Second, the overall incidence of typical filarial symptoms in subjects without microfilaremia was similar to that in individuals with patent microfilaremia (26/26 vs. 19/39, $\chi^2 = 0.4$, NS). On the other hand, of the 39 patients with patent microfilaremia, those with elevated microfilarial densities more often gave a history of recurrent fevers than those with low microfilarial counts. Only 4 of 20 asymptomatic patients in this group had a microfilarial density ≥ 100/ml vs. 14 of 19 symptomatic patients ($\chi^2 = 11.2$, $P < 0.001$).

Lymphocyte responses to nonfilarial antigens. Although unstimulated cultures of lymphocytes from the Kalimantan donors tended to incorporate more ^{125}I-deoxyuridine than equal numbers of cells from control donors, the difference was not statistically significant (Table III). Similarly, the in vitro response to the nonparasite antigens PPD and SK-SD lymphocytes from the Kalimantan study population was similar to that of the distant control group. There were no significant differences in the response rates or in the mean stimulation indices of the various patient groups (Table III).

Lymphocyte responses to microfilarial antigen. The scarce supply of microfilarial extract and especially the limitations on the amount of blood that could be obtained precluded extensive dose-response curve stud-

TABLE II
Parasitological Characteristics of Study Population

	Patients	Microfilariae/ml blood‡	Microfilariae/ml > 100	Malaria§	Intestinal nematodes*		
					Ascaris	Trichuris	Necator
	n	*n*					
No microfilaremia							
No symptoms	36	0	0	3/36	11/16	11/16	12/16
Symptoms	11	0	0	1/11	4/4	3/4	2/4
Elephantiasis	15	0	0	0/15	3/4	3/4	3/4
Microfilaremia							
No symptoms	20	23±13.6	4/20	4/20	8/11	7/11	9/11
Symptoms	19	211±10.7	14/19	0/19	8/9	7/9	8/9
Distant controls	15	0	0	0/16	—	—	—

* Number of positive stools per number examined within 6 mo before immunological testing.
‡ Geometric mean±SD.
§ Number of positive thick smears per number examined. Slides were made from the blood used for the immunological assay.

TABLE III
In Vitro Reactivity to Nonparasite Antigens

Patient group	Mean (±SE) dpm of unstimulated cultures	Mean SI (±SE)		Number with SI > 2/ number tested	
		PPD	SK-SD	PPD	SK-SD
No microfilaremia					
No symptoms	2,817±201	3.72±0.59	3.55±0.57	17/20	14/18
Symptoms	4,153±588	2.81±0.35	3.87±0.69	8/11	5/10
Elephantiasis	3,129±292	2.27±0.23	3.45±0.58	9/15	11/14
Microfilaremia					
No symptoms	3,524±495	3.72±0.59	3.55±0.57	17/20	14/18
Symptoms	4,051±678	3.89±0.69	6.32±1.13	13/19	16/18
Distant controls	2,589±243	3.75±0.71	4.88±1.07	11/15	11/14

ies. The results of four such experiments are shown in Table IV. Maximum transformation of patients' lymphocytes was observed when 10 μg microfilarial extract was added per milliliter culture medium. With 50 μg/ml of antigen, patients' lymphocytes responded less well, but cells from unexposed controls also reacted to this antigen concentration. For this reason 10 μg/ml microfilarial extract was used in all subsequent studies. One-half (45/90) of the Kalimantan subjects, but none of the distant controls tested, reacted in vitro to this concentration of antigen. Of 23 subjects tested with a low concentration of microfilarial antigen (1 μg/ml) only 2 had a significant response. Both were patients with elephantiasis whose lymphocytes were not stimulated by 10 μg/ml microfilarial antigen.

To evaluate which factors influence the in vitro response to microfilarial antigens of patients with filariasis, the effect of a number of clinical and parasitological parameters was examined. For this analysis the data on all noncontrol lymphocyte donors were pooled.

The following variables had no significant effect on the response to microfilarial antigens: age, sex, duration of residence in the study area, the presence of malarial parasites in thick blood smears on the day of testing, and the presence of gastrointestinal nematodes.

TABLE IV
Dose-Response Curve with Microfilarial Antigen

Antigen concentration	SI			
	Patient 1*	Patient 2	Control 1	Control 2
μg/ml				
50	2.3	3.6	4.2	3.1
10	6.1	4.2	1.8	1.2
5	3.8	2.1	1.3	0.8
1	1.8	2.1	1.5	0.9
0.5	0.9	1.6	1.2	1.1

* Both patients were amicrofilaremic: patient 1 was asymptomatic, patient 2 had elephantiasis; control 1 was an Indonesian, control 2, a Caucasian laboratory technician.

In contrast, several factors directly related to filariasis per se had a significant effect on the in vitro response to microfilarial antigen (Table V). These are: (*a*) the presence of detectable microfilaremia. Patients without patent microfilaremia had a significantly higher response rate than those with detectable microfilariae in their blood. (*b*) The presence of clinical symptoms of filariasis. Asymptomatic subjects had a significantly better response than patients with recurrent fevers, adenitis, or elephantiasis. This effect appears to be independent of the effect of patent microfilaremia per se because it persists even when microfilaremic patients are eliminated from the analysis. Thus, 26/31 asymptomatic amicrofilaremic vs. 11/26 symptomatic amicrofilaremic subjects responded to microfilarial antigen ($\chi^2 = 10.72$, $P < 0.01$). (*c*) The presence of elephantiasis. Patients with elephantiasis (all of whom were amicrofilaremic at the time of testing) had a lower response rate than amicrofilaremic subjects without elephantiasis. It should be noted, however, that if amicrofilaremic subjects who also have no clinical evidence of filariasis are eliminated from this analysis, the response rate of patients with elephantiasis is not different from that of amicrofilaremic patients with filarial symptoms other than elephantiasis (5.15 vs. 6.11, $\chi^2 = 1.16$, NS).

Lymphocyte responses to adult male worm antigen. Clear differences were found between the response rates of various patient categories to adult worm antigen. None of 42 subjects without elephantiasis and none of 4 control donors had a positive in vitro response to this antigen. In contrast, all 11 patients with elephantiasis tested had a brisk response to adult male worm antigen (Table VI). The mean (±SE) SI in this group was 4.17±0.45. Because of the limited supply of this antigen, no detailed dose-response curve studies

TABLE V
Effect of Clinical Parameters on In Vitro Response to Microfilarial Antigen

Group	Clinical status	Number with SI > 2.0/ number tested	Mean SI±SE
1	No microfilaremia	37/57	2.95±0.26
2	Microfilaremia	8/33	1.47±0.16
	No symptoms		
3	All subjects	31/50	2.05±0.29
4	Amicrofilaremic only	26/31	3.74±0.39
5	Microfilaremic only	5/19	1.61±0.24
	Symptoms		
6	All subjects	14/40	1.73±0.16
7	Amicrofilaremic only	11/26	1.97±0.20
8	Microfilaremic only	3/14	1.26±0.18
9	Elephantiasis	5/15*	1.96±0.29
10	No elephantiasis		
	No microfilaremia	32/42	3.16±0.32
11	Idem, without other symptoms	26/31	3.74±0.39
12	Idem, with other symptoms	6/11	2.00±0.31
	All noncontrol subjects	45/90	2.41±0.19
	All distant controls	0/15	1.08±0.10

* Two additional patients in this group responded to 1 μg/ml microfilarial antigen. These were excluded from the analysis. Statistical analysis of prevalence data (by χ^2 test): 1 vs. 2, 3 vs. 6, 4 vs. 7, 9 vs. 10 or 11: $P < 0.05$; 9 vs. 12 NS. Statistical analysis of SI (by t test) 1 vs. 2, 4 vs. 7, 9 vs. 10 or 11: $P < 0.05$; 3 vs. 6, 0 vs. 12: NS.

could be performed. However, no additional responders were identified when lymphocytes of 12 subjects with various stages of filariasis were cultured with 1 μg/ml adult worm antigen.

It appears, therefore, that the various filarial syndromes not only are characterized by distinct constellations of clinical and parasitological manifestations but also are associated with varying degrees of in vitro lymphocyte reactivity to microfilarial antigens. This correlation between clinical and laboratory findings is illustrated in Table VI, in which patient groups are ranked in order of decreasing response rates to microfilarial antigen. Clearly, subjects who live in an endemic area but have no clinical or parasitological evidence of filariasis exhibit the strongest, and symptomatic patients with patent microfilaremia the weakest reactivity to microfilarial antigens. In contrast, patients with elephantiasis are the only ones who react with adult worm antigens.

TABLE VI
In Vitro Reactivity to Filarial Antigens

	Number with SI ≥ 2/number tested (%)	
	Adult worm antigen	Microfilarial antigen
No microfilaremia		
No symptoms	0/21	26/31 (84)
Symptoms	0/7	6/11 (54)
Elephantiasis*	11/11	5/15‡ (33)
Microfilaremia		
No symptoms	0/8	5/19 (26)
Symptoms	0/6	3/14 (21)

* Two patients had no previous history of fevers or adenitis.
‡ Two additional subjects responded to 1 μg/ml microfilarial antigen.

DISCUSSION

Three major features of the host's immune response to Malayan filariasis emerge from the observations reported here. First, the majority of subjects living in an endemic area exhibit evidence of cellular sensitization to *B. malayi* antigens, but the degree of in vitro reactivity varies considerably with the clinical status of the individual tested. Second, immune unresponsiveness is restricted to filarial antigens; in vitro reactivity to nonparasite antigens is not altered by filarial infections. Third, there is a dissociation between the in vitro responses to microfilarial antigens and those to adult

worm antigens in individual patients. This finding suggests that different states in the parasite life cycle possess distinct stage-specific antigens.

The highest frequency and intensity of cellular reactions to microfilarial antigens was observed in subjects in whom no clinical or parasitological evidence of filariasis could be found. The serum of a large number of these individuals also contained antibodies reacting with the sheath of *B. malayi* microfilariae.[1] On the other hand, these asymptomatic individuals failed to react with adult worm antigens.

These asymptomatic persons most likely have become sensitized to filarial antigens because they bear low level subclinical infections. Observations on the infectivity rates and biting habits of the mosquito vectors clearly indicate that all individuals living in the study area are repeatedly exposed to infective third stage larvae.[1] Autopsy data also suggest that asymptomatic filarial infections can occur; in some cases the absence of filaremia can be explained by unisexual infections (7). Further, virtually all subjects living in endemic areas exhibit wheal and flare reactions to filarial extracts (3). These observations suggest that even asymptomatic individuals have ample opportunity to become sensitized to filarial antigens. Why they should react to microfilarial but not to adult worm antigens remains unclear. It is possible that those subjects have been infected with third stage larvae, but that a vigorous immune response to this parasite stage has aborted the infection by preventing further maturation of the worm. In addition, if third stage larvae and microfilariae share common antigens, such a sequence of events could account for the lack of reactivity to adult worm antigen in the face of strong reactions to microfilarial antigens. However, based on the limited number of available postmortem observations, such simple interpretation is likely to be correct only in a minority of cases (7).

In contrast to asymptomatic, amicrofilaremic subjects, the majority of patients with patent microfilaremia failed to respond to microfilarial antigens, although their response to the nonparasite antigens PPD and SK-SD was similar to that of patients with other stages of filariasis and to that of distant control subjects. Thus, we find a strong correlation between the presence of circulating microfilariae and the lack of cell-mediated responses to microfilarial antigens. A similar correlation exists between microfilaremia and absence of detectable antimicrofilarial antibodies.[1]

There are two possible explanations for this observation, based on whether one considers microfilaremia to be the cause or the result of the immune unresponsiveness.

First, it is possible to envision that a vigorous immune response during the early stages of filarial infection might prevent the normal development of the parasite and result in the absence of detectable microfilareia. Studies on the fate of experimentally transmitted infections in nonsusceptible animal hosts support this concept (8). In these animals the infection is suppressed by the host's immune response; experimental manipulations that interfere with the development of antiparasite immunity result in protracted filarial infections in an otherwise nonsusceptible host (8). By analogy, "bad" (i.e., resistant) human filarial hosts would suppress their infection and exhibit a high degree of immunity; "good" (i.e., susceptible) hosts would let the infection run its "natural" course and fail to become sensitized to filarial antigens. No evidence for such a state of tolerance exists at present.

An alternative explanation is that filarial parasites cause active suppression of cellular immune responses to the worm's antigens. Because the in vitro response to the nonparasite antigens, PPD and SK-SD, was similar in all patient and control groups, the postulated immune suppression appears to be selective for parasite antigens, a conclusion also reached by Ottesen et al. (4).

Immunosuppression is a prominent feature of protozoan infections where the infecting agents are present in the circulation and an integral part of many helminth infections, including filariasis in jirds and rats (9, 10–13). What mechanism(s) might be responsible for this biological phenomenon in human filariasis cannot be determined from these studies. In Ottesen's study (4) the lymphocytes were cultured in the presence of autologous serum, leaving open the possibility that their unresponsiveness to filarial antigens was caused by the presence of suppressive factors in the patients' sera. This possibility was excluded in this study because all cultures were done in medium supplemented with AB+ serum from a single pool of unexposed donors. The spleen of infected jirds contain a phagocytic adhering cell that suppresses the in vitro reactivity of splenocytes to mitogens (13). Whether suppressor cells play a role in the selective unresponsiveness to filarial antigens in infected humans remains to be determined by additional studies.

It is of interest to note that none of the elephantiasis patients in this study had detectable microfilaremia, although the majority had an antecedent history of recurrent filarial fevers. The finding that patients with elephantiasis to a certain extent regain the ability to react with microfilarial and adult worm antigens suggests that the state of immune suppression in filariasis is reversible and that microfilariae may be the cause of the immunesuppression. Repeated exposure to infective third-stage larvae can result in the elimination of microfilariae from the blood of experimentally infected animals (14, 15). The serum of these "cured" animals contains antimicrofilarial antibodies that cannot usually be demonstrated in the serum of animals

that remain microfilaremic (16). Because all individuals living in the study area are repeatedly bitten by infected mosquitoes, it is tempting to speculate by analogy that the immune suppression in human filariasis may also be reversed by repeated reexposure to infectious larvae. Experimental evidence to support this hypothesis is lacking.

The pattern of reactivity to adult worm antigens was markedly different from that observed with microfilarial antigens. All 11 patients with elephantiasis reacted in vitro to this antigen, but none of 21 asymptomatic, and none of 21 patients with other stages of filariasis reacted to adult worm antigen. Such clear differences in the reactivity of individual patients to microfilarial and adult worm extracts were not noted by Grove et al. (3, 17), who studied the clinical usefulness of similarly prepared antigenic extracts for immunodiagnostic purposes by skin testing and measured serum antibody levels to microfilariae and adult worms. The reasons for this apparent discrepancy are not clear. However, it should be pointed out that the adult worm extract used in this study was prepared from male worms only, whereas Grove et al. (3, 17) used a mixture of male and female worms. Conceivably this extract could have been contaminated with antigens from microfilariae present in gravid females. On the other hand, the immediate hypersensitivity skin reactions observed by Grove et al. reflect the presence of humoral, antibody-mediated immunity, whereas the lymphocyte transformation assay used in our studies is an in vitro correlate of cellular immunity (17–19). It is not unreasonable to assume that the crude saline extracts used in both studies contained several antigenic materials that might preferentially elicit humoral or cell-mediated reactions. It follows that two assays measuring different parameters of the immune response might reveal two different patterns if the reaction that is measured is elicited by two distinct antigens present in a crude mixture. The presence of multiple antigenic determinants in filarial extracts has been confirmed experimentally (20). The same study also indicates that different stages in the life cycle of filarial parasites possess distinct stage-specific antigens in addition to antigens that are shared by different developmental stages.

It has long been postulated that end-stage filariasis, elephantiasis, results from cell-mediated immune reactions to dead adult worms (1, 7, 21). Our finding that patients with elephantiasis were the only ones who exhibited cellular reactivity to adult worm antigens is consistent with this hypothesis. In contrast, the symptoms of acute filariasis have been attributed to antibody-dependent "allergic" reactions elicited by living microfilariae, discarded sheaths, moulting fluids, unfertilized ova, etc. (7, 21). We find no evidence of cellular immunity to adult worm antigens and only a low incidence of cellular sensitization to microfilarial antigens in patients with these symptoms. Taken together, these observations indicate that the pathogenesis of different clinical syndromes in human filariasis may result from different types of immune reactions to antigens associated with distinct stages in the life cycle of the parasite. The exact nature of the antigens responsible for the immunity, immunopathology and immune suppression in filariasis remains to be determined by further studies.

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