

Immune Reactions in Human Filariasis

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Sera from cases of elephantiasis due to *Wuchereria bancrofti* infection promoted an intense adhesion of peripheral blood leukocytes to *W. bancrofti* microfilariae in vitro. A similar adhesion was also seen using sera from some normal persons living for several years in areas where filariasis is endemic. No such adhesion was evident with sera from microfilaria carriers or from normal subjects from nonendemic areas. The adhesion was complement independent and was associated with the immunoglobulin G fraction of serum. ⁵¹Cr release studies suggested the occurrence of cell-mediated cytotoxicity to *W. bancrofti* microfilariae in the presence of elephantiasis serum. Microfilariae of *Litomosoides carinii* could be isolated, free of blood cells, from the blood of infected rats. In the presence of serum, or its immunoglobulin G fraction, from patients with elephantiasis, *L. carinii* microfilariae adhered to human peripheral blood leukocytes or rat spleen cells.

Epidemiological studies in areas where filariasis is endemic revealed differential susceptibilities to infection in the population (4). King et al. (6) and, more recently, Srivastava and Prasad (12) observed that not all members in a family acquired the infection even though they were uniformly exposed to the infective mosquito bites. Furthermore, in patients with elephantiasis due to *Wuchereria bancrofti*, microfilariae are not usually seen in the peripheral blood even after concentration techniques, although adult worms may be present (5). Information is lacking on the possible mechanisms, immunological or otherwise, to account for these observations. In an attempt to throw light on these problems, the immune reactions operating in normal persons living for many years in endemic areas and in patients with elephantiasis due to *W. bancrofti* have been studied. The methods used stem from the finding that rats recovering from the microfilaremic stage of *Litomosoides carinii* infection produce antibodies that promote the adhesion and cytotoxicity of spleen cells and other cells to *L. carinii* microfilariae (14).

MATERIALS AND METHODS

***W. bancrofti* microfilariae.** Nocturnal blood samples from carriers of *W. bancrofti* microfilariae were collected by venipuncture into tubes containing heparin (10 U/ml) and diluted with an equal volume of tissue culture medium (HEPES [*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid]-buffered RPMI 1640 [Grand Island Biological Co.] containing 100 U of penicillin per ml and 100 µg of streptomycin per ml;

referred to hereafter as medium). The diluted blood was taken into a syringe and filtered through a Nucleopore filter (3.0 µm) fitted to it. The filter was washed free of blood cells by passage of medium. The filter containing the microfilariae was dipped in medium at room temperature for a few minutes to allow the microfilariae to pass into the medium. The medium was centrifuged, and the microfilariae were resuspended in medium and counted in a hemocytometer.

***W. bancrofti* infective larvae (L₃).** A colony of *Culex pipiens fatigans* was infected by feeding on a volunteer with microfilaremia. The mosquitos with stage 3 larvae (L₃) were dissected under a microscope, and the L₃ were isolated and suspended in medium.

***L. carinii* microfilariae.** Blood obtained by cardiac puncture of infected albino rats was placed in screw-capped vials and defibrinated with glass beads. The blood was diluted with an equal volume of medium, layered onto a Ficoll-Hypaque solution (specific gravity, 1.05), and centrifuged at 400 × *g* for 25 min. Under these conditions, the mixture separated into two layers, with erythrocytes and leukocytes sedimenting at the bottom of the tube. The microfilariae, free of blood cells, were found largely at the interface but also were found suspended in the lower layer. The top layer was discarded, and the lower layer with the microfilariae was collected, diluted with medium, and centrifuged at 500 × *g* for 5 min. The microfilarial pellet was washed twice and resuspended in medium. This method provided pure microfilariae with more than 95% recovery.

Sera. Serum was separated in the cold from venous blood of healthy subjects, microfilaria carriers, and elephantiasis patients. Samples were stored at -20°C.

Isolation of cells. Peripheral blood leukocytes (PBL) from normal subjects and from patients with eosinophilia were isolated from heparinized blood by

sedimentation of erythrocytes with dextran (molecular weight, 200,000 to 275,000). They were washed and resuspended in RPMI 1640. Animal spleen cells were obtained by teasing in medium and were washed with medium twice before use.

Column chromatography. Sera from elephantiasis cases were fractionated on columns of diethylaminoethyl-cellulose (Whatman DE52) (2). The column was prepared and equilibrated with 0.015 M sodium phosphate buffer, pH 8.2. Serum was dialyzed against three changes of the buffer before application to the column. Fractions, 3 ml, were collected, and the protein content of the eluates was monitored. After the first protein peak was eluted, the separation was continued with 0.3 M potassium phosphate buffer (pH 8.2) until a second protein peak was eluted. The fractions were examined for the presence of immunoglobulins, by immunodiffusion and immunoelectrophoresis, with monospecific anti-immunoglobulin antisera (Wellcome Research Laboratories).

Serum-dependent adhesion. For adhesion experiments the reaction mixtures contained parasites and cells in a ratio of 1:500 to 1:2,000 and 30% serum in a final volume of 0.4 ml, made up with medium (14). The mixtures were incubated in a humid chamber at 37°C for 16 h with occasional shaking. They were examined microscopically after 6 and 16 h.

Serum-dependent cytotoxicity. *W. bancrofti* microfilariae were labeled with ⁵¹Cr, as described by Subrahmanyam et al. (14), to give an activity of about 30 cpm/microfilaria. They were incubated with PBL and 30% serum. The release of ⁵¹Cr was measured at intervals during incubation (14).

RESULTS

Sera from elephantiasis cases promoted intense adhesion of PBL to *W. bancrofti* microfilariae (Fig. 1, Table 1). Of the 23 sera examined, 20 caused 80 to 100% of the microfilariae to adhere to the cells. Significant adhesion was observed with these sera at 1:5 dilutions (i.e., at

a final concentration of 6%). In these cases serum-dependent adhesion of cells was also seen with stage 3 larvae (L₃). Somewhat less adhesion (25%) was evident with two of the remaining sera, whereas one sample did not react. In most cases the microfilarial surface was largely covered with cells. After adhesion, the parasites became slow in movement and showed surface changes such as fragmentation. Strong adhesion was, in general, seen with sera from chronic cases with elephantiasis of more than 3 years' duration. Five of these cases had hydrocele for 1 to 7 years.

A similar adhesion was seen with many serum samples from healthy people living in endemic areas for more than 5 years. Of the 17 such cases, 8 promoted strong adhesion (60 to 100%) and 5 had moderate (25 to 40%) activity. Adhesion was also seen to L₃ with positive sera from these individuals.

Of the nine sera from microfilaria carriers, seven had no significant activity, whereas the rest had a marginal effect (20%). Twenty serum samples from normal humans living in nonen-

TABLE 1. Serum-dependent adhesion of PBL to *W. bancrofti* microfilariae

Serum source	No. of sera tested	No. of sera that caused adhesion of PBL to >50% of the microfilariae
Elephantiasis cases	23	20
Normal humans from endemic area	17	8
Microfilaria carriers	9	0
Normal humans from nonendemic areas	20	0

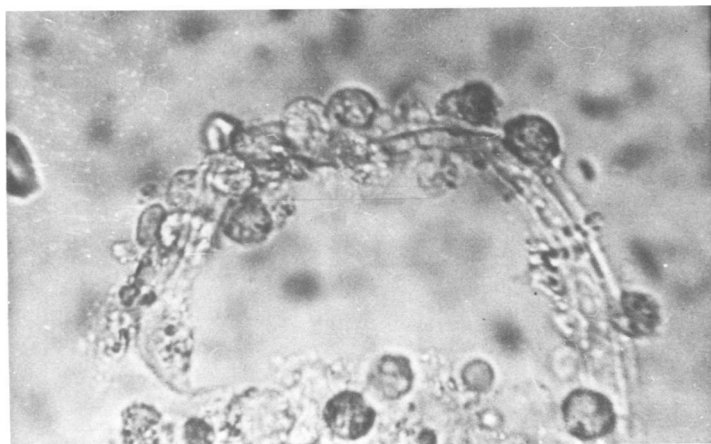


FIG. 1. Adhesion of PBL to *W. bancrofti* microfilariae after incubation with serum from a patient with elephantiasis.

demarc areas and having no history of exposure to the infection caused no adhesion.

The results reported in the following experiments were obtained with pooled sera from elephantiasis cases exhibiting strong adhesion activity. The reactivity of cells from different sources is shown in Table 2. Active adherence to *W. bancrofti* microfilariae was seen with cells from normal or infected humans. Very strong adhesion was seen when eosinophil-rich cell suspensions were used. However, spleen cells from mice, rats, or guinea pigs were not effective. The role of complement in the adhesion reaction is shown in Table 3. Heating serum at 56°C for 30 min (to inactivate complement components C1 and C2) did not significantly affect the adhesion reaction, although more prolonged heat treatment resulted in a gradual loss of activity (data not shown).

The release of ^{51}Cr from labeled microfilariae in the presence of PBL and elephantiasis serum is illustrated in Fig. 2. There was clearly significant and substantial release, which did not occur in the presence of normal serum.

Our earlier data (13) suggested a sharing of antigens between the rat parasite *L. carinii* and *W. bancrofti*. Experiments were carried out to determine whether *L. carinii* could substitute for *W. bancrofti* in adhesion reactions. It was found that serum from elephantiasis patients had a strong agglutinating effect on *L. carinii* microfilariae. It also promoted the attachment of human PBL to the parasites.

In an attempt to characterize the nature of

TABLE 2. Adhesion of cells from various sources to *W. bancrofti* microfilariae in the presence of elephantiasis serum

Source of cells	Microfilariae (%) with cells adherent
PBL	
Normal human	80
Elephantiasis patient	85
Patient with hypereosinophilia	90
Microfilaria carrier	80
Rat spleen	0
Mouse spleen	0
Guinea pig spleen	0

TABLE 3. Role of complement in cellular adhesion to *W. bancrofti* microfilariae

Treatment of elephantiasis serum	Microfilariae (%) with cells adherent
None	90
Heated (56°C, 30 min)	77
Heated + normal human serum	75

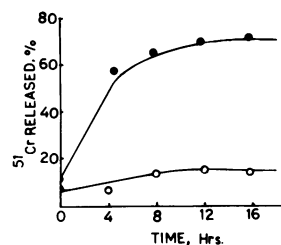


FIG. 2. Release of ^{51}Cr from labeled *W. bancrofti* microfilariae after incubation with PBL and serum from a patient with elephantiasis (●) or serum from a normal subject from a nonendemic area (○).

the antibody causing adhesion, the serum was fractionated on diethylaminoethyl-cellulose (Fig. 3). Most of the immunoglobulin G (IgG) eluted in the first protein peak, whereas the rest of the immunoglobulins were in the second peak. The adhesion-promoting activity resided in fractions 6 and 7 when tested with either *L. carinii* or *W. bancrofti* microfilariae. This fraction contained IgG and no other immunoglobulin detectable by gel diffusion or immunoelectrophoresis. The second peak had strong agglutinating activity against *L. carinii* microfilariae. This activity seems to be due to IgM, since it was mercaptoethanol sensitive and was inhibited by anti-IgM but not by anti-IgG, anti-IgA, or anti-IgE antisera.

The influence of the source of cells on the adhesion-promoting activity of the first (IgG) serum fraction with *L. carinii* microfilariae was studied. The results are presented in Table 4. PBL from a patient with hypereosinophilia were most active, whereas PBL from normal humans were less effective. Spleen cells from rats, mice, and guinea pigs were still less effective.

DISCUSSION

Knowledge of the host-parasite relationship in human filarial infections is still inadequate. In areas where filariasis is endemic, humans may develop well-marked resistance to superinfection with filarial parasites (7). The general absence of microfilariae in elephantiasis cases due to periodic *W. bancrofti* in the presence of living adults suggests the existence of mechanisms, possibly immunological in nature, for clearing microfilariae from the blood. Pandit et al. (9) observed adhesion of leukocytes to microfilariae on the addition of serum from elephantiasis to blood containing the parasites. In the presence of sera from infected or immunized hosts, a similar adhesion of leukocytes to a variety of parasitic helminths has since been reported (1, 8, 11, 15). Higashi and Chowdhury (3) found

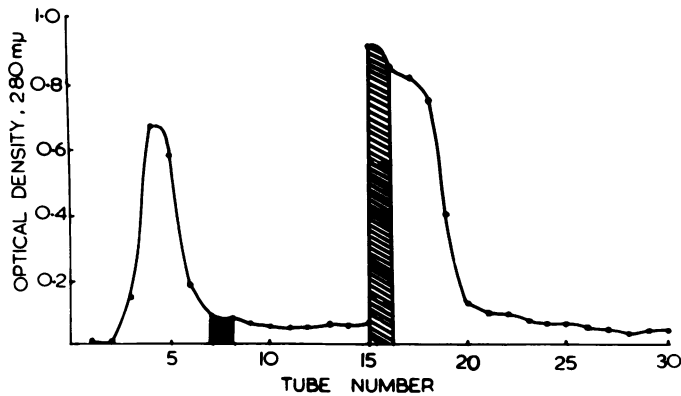


FIG. 3. Fractionation of pooled sera from elephantiasis on diethylaminoethyl-cellulose: (■) adhesion activity; (▨) agglutinating activity.

TABLE 4. Adhesion of cells from different sources to *L. carinii* microfilariae in the presence of the active fraction of serum

Source of cells	Microfilariae (%) with cells adherent
PBL	
Patient with hypereosinophilia	100
Normal human	55
Rat spleen	35
Mouse spleen	20
Guinea pig spleen	15

selective adhesion of eosinophils to the infective larvae of *W. bancrofti* in the presence of sera from filariasis patients showing microfilaremia, lymphedema, or elephantiasis. In the present study, intense adhesion of cells to *W. bancrofti* microfilariae and infective larvae was seen with sera from elephantiasis cases and from many healthy persons living in endemic areas for several years, but not with sera from microfilaria carriers. The apparent absence of adhesion-promoting activity from this last group might be due to their failure to produce the appropriate antibodies, to the presence of blocking factors, or to the absorption of the antibodies by microfilarial antigens *in vivo*.

PBL from normal and hypereosinophilic subjects were effective in this reaction. The adhesion was maximal with a homologous system, regardless of the source of human cells. The range of human cells that can function as effectors has not yet been defined, but the results with eosinophil-rich (>90%) suspensions suggest that these cells may be important effectors. The antibody responsible appears to be IgG, and the reaction is complement independent. The cells and antibodies involved are now being characterized further.

The observation that *L. carinii* microfilariae can substitute for those of *W. bancrofti* is of considerable significance in view of the fact that it is not always possible to have a source of human microfilariae. In such a situation one could screen the serum samples with *L. carinii* microfilariae, using PBL from normal or eosinophilic subjects. The reason for the reactivity of rat, mouse, and guinea pig cells with *L. carinii*, but not *W. bancrofti*, is not known.

The role of the adhesion reaction in the termination of microfilaremia, as is usually seen in elephantiasis, and in resistance to infection seen in certain populations is not clear at present. Our earlier studies with the *L. carinii*-albino rat system revealed an association between termination of microfilaremia (onset of latent infection) and the serum-dependent adhesion and cytotoxicity of cells to *L. carinii* microfilariae (14). Furthermore, induction of resistance in albino rats to *L. carinii* infection by immunization with radiation-attenuated infective larvae resulted in the appearance of cellular adhesion-promoting activity in the sera of the resistant animals (10). In the present study a similar serum-dependent adhesion and cytotoxic effect of cells on *W. bancrofti* microfilariae was seen in elephantiasis cases. In neither the rat nor the human system has cytotoxicity been seen in the absence of cells (14; unpublished data; the data presented here, however, do not formally allow the conclusion that cytotoxicity was a function of adherent cells). Serum-dependent adhesion of cells to *L₃* was also seen with sera from many clinically normal inhabitants of the endemic area. These observations suggest a possible role of these immune reactions in conferring resistance to the infection in certain populations and in the amicrofilaremic state seen in chronic elephantiasis.

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