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# Antibody responses to experimental *Brugia malayi* infections in Patas and Rhesus monkeys

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## Summary

Humoral antibody responses in experimental infections with *Brugia malayi* (subperiodic strain) were compared in two primate species, *Erythrocebus patas* and *Macaca mulatta*. Antibody responses were related to the infection protocol and the duration and magnitude of microfilaremia. Patas monkeys were uniformly susceptible to infection and characteristically exhibited prolonged microfilaremia; infections in Rhesus monkeys produced low and usually transient microfilaremia. Antibody, measured by enzyme linked immunosorbant assay with extracts of adult *Brugia* and microfilariae as antigens, declined at patency in Patas monkeys and there was an inverse relationship between serum antibody concentration and the number of circulating microfilariae. Rhesus monkeys generally had high, sustained antibody levels relative to Patas monkeys, but antibody levels were comparable in the two species when the numbers of circulating microfilariae were similiar. By fluorescent antibody technique, antibodies reactive with somatic antigens of microfilariae were detected in all infected monkeys; antibodies reactive with the cuticle of infective larvae were also present in both primates and were consistently detected in monkeys receiving multiple infections. Antibodies (IgG, IgM) reactive with the sheath of microfilariae were detected only in certain Rhesus monkeys which were essentially amicrofilaremic and sera with antibodies specific for microfilarial sheath promoted in vitro microfilarial agglutination and leukocyte adherence.

**Key words:** *Brugia malayi;* Patas monkey; Rhesus monkey; immunoglobulins; fluorescent antibody; adherence; agglutination; peripheral blood leukocytes; enzyme-linked immunosorbant assay; radial immunodiffusion.

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## Introduction

Immune responses in Brugian and Bancroftian filariasis are considered to be major factors in the pathogenesis and the varied clinical manifestations of infection (Ottesen, 1980). These immune responses and their roles in pathogenesis or protection are not well characterized and cannot be studied under controlled, experimental conditions in human infections. *Brugia malayi* (subperiodic strain), a lymphatic dwelling filariae naturally infecting man and other primates (Denham and McGreevy, 1977) in a susceptible primate host could prove a valuable experimental model for the study of immune responses applicable to human infection with lymphatic dwelling filariae. Little information is available on the immune responses of non-human primates to *Brugia* infection with the exception of a few studies in the Rhesus monkey. The purpose of the present, initial study was to examine the antibody responses during experimental *B. malayi* infections in the Patas monkey, a host reported to be highly susceptible to the infection (Orihel, personal communication) and to compare the antibody responses to those in infected Rhesus monkeys.

### Materials and Methods

*Sera and experimental infections.* Serum samples were obtained from a study of experimental infections in 14 female Patas monkeys carried out at the Walter Reed Army Institute of Research from 1972–1974 by Dr. B. Redington. Sera were also obtained from 3 experimental and 2 control, male Patas monkeys infected at the University of Florida and still under study. Sera from 8 experimentally infected male, Rhesus monkeys were obtained from a study by Dr. W. Kozek at the Primate Center, Davis, CA during 1974–1977. Table 1 summarizes the infection protocols and microfilaremia in these monkeys.

All experimental monkeys were inoculated with the infective larvae of the subperiodic strain of *B. malayi.* Infective larvae were obtained from mosquitoes supplied by Dr. J. McCall (University of Georgia, Athens, GA) through the US-Japan Cooperative Program in Medical Sciences (National Institute of Allergy and Infectious Disease) to infect monkeys at the University of Florida. Bleeding of animals was routinely done in the morning at monthly or biweekly intervals. Microfilaremia was determined by a modified Knott's technique or Nuclepore filter procedure employing 0.5 to 2 ml of blood obtained from the femoral or saphenous vein. In the Patas monkeys, housed at the University of Florida, total and differential blood leukocyte counts were routinely made on blood samples. Ig (IgG, IgM) concentrations in serum samples from all monkeys during the first year of infection were measured by radial immunodiffusion with commercial plates (Meloy Labs., Inc., Springfield, VA) designed for measurement of human Igs. Results were expressed as a percentage of a standard prepared from pooled sera of uninfected monkeys.

*Enzyme-linked immunosorbent assay (ELISA).* A microplate, indirect ELISA was used basically as described by Voller et al. (1976). Antigens were extracts of adult females and microfilariae of *B. malayi* recovered from the peritoneal cavity of laboratory-infected jirds. Worms were suspended in buffer (0.05 M Tris, 0.5 M NaCl, pH 7.8), homogenized in a French pressure cell (20,000 psi) and extracted at 4° C for 18 h. The soluble components were isolated by centrifugation for 30 min at 40,000 *g*, concentrated by ultrafiltration on a Diaflow YM-10 ultra filter (Amicon Corp., Lexington, MA) and protein concentration measured with a Bio-Rad Protein Assay Kit (Bio-Rad Labs., Richmond, CA). Assay conditions were established to permit comparison of antibody levels in monkeys by absorbancy (400 nm) measurements with a single dilution of sera; it was demonstrated that isolated Patas and Rhesus IgG bound equivalent amounts of enzyme labeled anti-IgG used in

Table 1. Infection protocols and microfilaremia in Patas and Rhesus monkeys infected with *Brugia malayi*

| Group | Infection protocol* | Monkey No. | Patency (week) | Microfilarial/ml (week post infection) | |
|---|---|---|---|---|---|
| | | | | Maximum | At necropsy |
| *Patas* | | | | | |
| A. | 350 $L_3 \times 1$ | 928 | 13 | 3900 (24) | 53 (66) |
| | | 927 | 11 | 350 (24) | 1 (53) |
| | | 926 | 13 | 360 (19) | 20 (53) |
| | | 925 | 13 | 1900 (24) | 75 (59) |
| B. | 150 $L_3 \times 1$ | 684 | 11 | 1260 (22) | 45 (95) |
| | and | 687 | 10 | 489 (14) | 150 (83) |
| | 100 $L_3 \times 9$ | 689 | 10 | 1896 (22) | 19 (104) |
| | (at 4 week intervals) | 691 | 10 | 1173 (22) | 254 (83) |
| C. | 75 $L_3 \times 1$ | 686 | 11 | 250 (22) | 7 (101) |
| | and | 693 | 10 | 663 (16) | 149 (110) |
| | 50 $L_3 \times 9$ | 746 | 10 | 223 (16) | 10 (96) |
| | (at 4 week intervals) | 688 | 11 | 150 (83) | 3 (111) |
| | | 690 | 18 | 46 (82) | 0 (114) |
| | | 695 | 12 | 71 (16) | 10 (101) |
| D. | 300 $L_3 \times 1$ | 780 | 12 | 1102 (22) | 26 (49) |
| | (study in progress) | 781 | 12 | 1521 (22) | 30 (138)*** |
| | | 784 | 12 | 1550 (22) | 4 (112)*** |
| *Rhesus* | | | | | |
| A. | 2000 $L_3 \times 1$ | 708 | 17 | 3.5 (24) | 0 (150) |
| | | 947 | 13 | 245 (24) | 20 (150) |
| B. | 2000 $L_3$ IP $\times 2$ | 719 | 127 | 3 (150)** | 3 (150) |
| | (weeks 0 and 34) | 970 | 44 | 4 (53) | 0 (150) |
| C. | 200 $L_3 \times 3$ | 387 | 12 | 1 (152)** | 1 (152) |
| | (weeks 0, 34 and 80) | 436 | 110 | 0.5 (110)** | 0 (152) |
| D. | 25 $L_3 \times 34$ | 980 | 20 | 5 (24)** | 0 (152) |
| | (3–4 week intervals) | 714 | 20 | 0.5 (20)** | 0 (152) |

* Number of infective larvae ($L_3$) injected and number of injections ($\times$ No.). All larvae injected SQ except Rhesus Group B injected IP

** Amicrofilaremic in >90% of periods examined using 2 ml of blood

*** Last count

the assays. Routinely, wells of a microtiter plate (polystyrene, U-form substrate plates, Dynatech Labs., Alexandria, VA) were coated by 0.2 ml of antigen (5.0 $\mu$g protein/ml) overnight at 4° C. Sera were assayed in duplicate at a 1:400 or 1:800 dilution; 0.2 ml of diluted sera was incubated for 2 h at room temperature and, after washing, 0.2 ml of alkaline phosphatase labeled anti human IgG (Miles-Yeta, Elkart, IN) at a 1:1000 or 1:800 dilution was added to the wells. After 2 h the reagent was washed out, substrate was added and optical density (OD) was measured 30 min later in a Model DB Spectrophotometer (Beckman, Fullerton, CA) using a microcuvette with a 1 mm light

path. Optical density (OD) measurements were multiplied by 10 for the units presented in the figures and for equivalence with a 1 cm light path. Preinfection sera gave OD values $<0.02$ with microfilarial and adult extracts at the serum dilutions used; these OD readings of the preinfection sera were subtracted from OD readings of the postinfection sera.

Antibody in certain sera was titered by ELISA to confirm the differences in antibody levels observed by OD measurements in a single serum dilution. For titration, microtitration plates (Linbro, Flow Labs., McLean, VA), were coated by antigen suspension (3 $\mu$g protein/ml) and OD (405 nm) read with a Multiscan Titertech (Flow Labs.). The assay procedures were similiar to those used for the single serum dilution. Sera were tested in 2 fold dilutions beginning with a 1:10 to a 1:100 dilution; the end point was selected as the lowest dilution of sera giving an OD of 0.2–0.3. Preinfection sera were also titered and compared with the appropriate postinfection sera.

*Fluorescent antibody (FA).* The indirect FA test with microfilariae and infective larvae ($L_3$) of *Brugia malayi* was carried out by the tube method of Wong and Guest (1969). Microfilariae from the peritoneal cavity of jirds (Grove et al., 1977) and $L_3$ from infected mosquitoes were fixed in 2% buffered formalin overnight, washed with buffered saline and stored at 4° C. Mosquitoes infected with *B. malayi* were supplied by Dr. J. Nayar (Florida Medical Entomology Lab., Vero Beach, FL). For certain tests, microfilariae were lightly sonicated to fracture the larvae to expose somatic antigens before fixation in formalin. Routinely, larvae were incubated at room temperature in serum dilutions for 30 min, washed with buffered saline and fluorescein (Fl) labeled antimonkey IgG (Cappel Labs., Cochranville, PA), added for an additional 30 min, followed by washing and mounting on slides. Antibody was not titered but all sera were screened for antibody at a 1:20 dilution using a 1:20 dilution of Fl-labeled anti Ig. This dilution of sera and reagents did not give positive staining of microfilariae or $L_3$ with sera from uninfected monkeys. Examination was also made with Fl-labeled anti IgM and IgG for human Ig (Cappel).

*Cell adherence to microfilariae and agglutination.* Serum mediated adherence of leukocytes to microfilariae was assayed in Falcon microtiter plates (Fisher Scientific, Orlando, FL). Microfilariae were isolated from the peritoneal cavity of jirds, extensively washed and suspended in Hepes buffered (pH 7.2) RPMI-1640 (Flow Labs) containing 100 $\mu$g streptomycin and 100 units penicillin/ml. Leukocytes for all adherence experiments were obtained from heparinized blood of a single human volunteer. Mononuclear and polymorphonuclear leukocytes were separated by a Hypaque-Ficoll gradient (Ferranti and Thong, 1980) and the cell suspensions were evaluated separately for adherence to microfilariae. Monkey sera were heated at 56° C for 30 min before use in adherence assays. In each microtiter well there were approximately 50 microfilariae at a 1:4000 ratio of microfilariae to leukocytes with a final concentration of 4% sera in a total volume of 0.15 ml. Microtiter plates were incubated at 37° C in a 5% $CO_2$-air mixture and observed for adherence at 2 h. If 20% or more of the surface of a microfilariae was covered by leukocytes after 2 h of incubation it was scored as a positive adherence reaction; the results were expressed as a percentage of the microfilariae showing this adherence reaction.

Agglutination of microfilariae by monkey sera was tested by the procedures of Grove and Davis (1978). Serum dilutions (1:2–1:32) in microtiter plates with 500–1000 microfilariae, in a total volume of 0.2 ml, were incubated at 37° C and examined for agglutination at 2–3 h and again at 6 h.

## Results

*Microfilaremia.* Patas monkeys were uniformly susceptible to infection usually with a 2–3 month prepatent period followed by a prolonged microfilaremia. The microfilarial counts presented in Table 1 are from morning bleedings and higher counts were obtained in the evenings, but in only one animal did the microfilaremia exceed 5000/ml. The majority of monkeys receiving a single large inoculation of infective larvae (Groups A, D) or multiple, heavy inocula-

tions (Group B) had a similar pattern of microfilaremia characterized by maximum numbers of circulating microfilariae within 5–6 months after initial inoculation followed by a decline in numbers over a 1–2 month period to a lower but persistent microfilaremia. Monkeys of Group C which received smaller, multiple inoculations of infective larvae had a more varied pattern of microfilaremia although it was generally similar to that of other infection groups. All Rhesus monkeys became microfilaremic but only 5 of 8 monkeys became patent after the first inoculation of infective larvae. With the exception of a single monkey, No. 947, microfilaremia did not exceed 5 microfilariae/ml and there were periods of amicrofilaremia; in three monkeys, Nos. 387, 436 and 714, circulating microfilariae were rarely detected (Table 1).

*Leukocyte and Ig responses.* The peripheral leukocyte responses to infection were monitored in Group D of the Patas monkeys and were unremarkable. There was no significant leukocytosis and a marginal eosinophilia (6–12%) at 2 to 6 months after initial inoculation of infective larvae; there was a return to preinfection levels (0–4%) after this period. Serum Ig (IgG and IgM) concentrations were measured in Patas monkeys of Groups A, B and D and were little changed during the infection. The maximum observed changes in Ig were similar in control and infected monkeys: 20% from preinfection levels in IgG and up to 50% in IgM. No consistent pattern of Ig change in relation to infection data or antibody response was apparent.

*Enzyme linked immunosorbent assay (ELISA).* Serum antibody (IgG) reactive with an extract of adult *Brugia* was detected within 30 days after infection in singly inoculated and multiply inoculated Patas monkeys. A common pattern of antibody response was typically observed. Serum antibody increased during the prepatent period and then rapidly declined with the development of microfilaremia; there was an inverse relationship between maximum antibody levels and the numbers of circulating microfilariae. This contrast in antibody concentration and magnitude of microfilaremia for animals in Group A and for 2 animals in Group B with the greatest disparity in microfilaremia is shown in Fig. 1. Monkeys in Group D, each inoculated with 300 larvae, had intermediate levels of circulating microfilariae between the high and low parasitemias of Groups A and had intermediate antibody levels (data not shown). A comparison of antibody titers also demonstrated the contrast in antibody concentration and microfilaremia. At 113 days postinfection, Group A monkeys with high microfilaremia (Nos. 925, 928) had an antibody titer of 1:320 and monkeys with low microfilaremia (Nos. 926, 927) had titers of 1:2560 and 1:5120; similarly, monkeys (Group B) Nos. 687 and 689 had antibody titers of 1:5120 and 1:640, respectively. Titers of preinfection sera from these Patas monkeys were high, 1:80–1:160.

The Patas monkeys receiving multiple small inoculations of infective larvae, Group C, exhibited the same pattern of antibody response as exhibited by monkeys in the other groups. The range of antibody responses and microfilare-

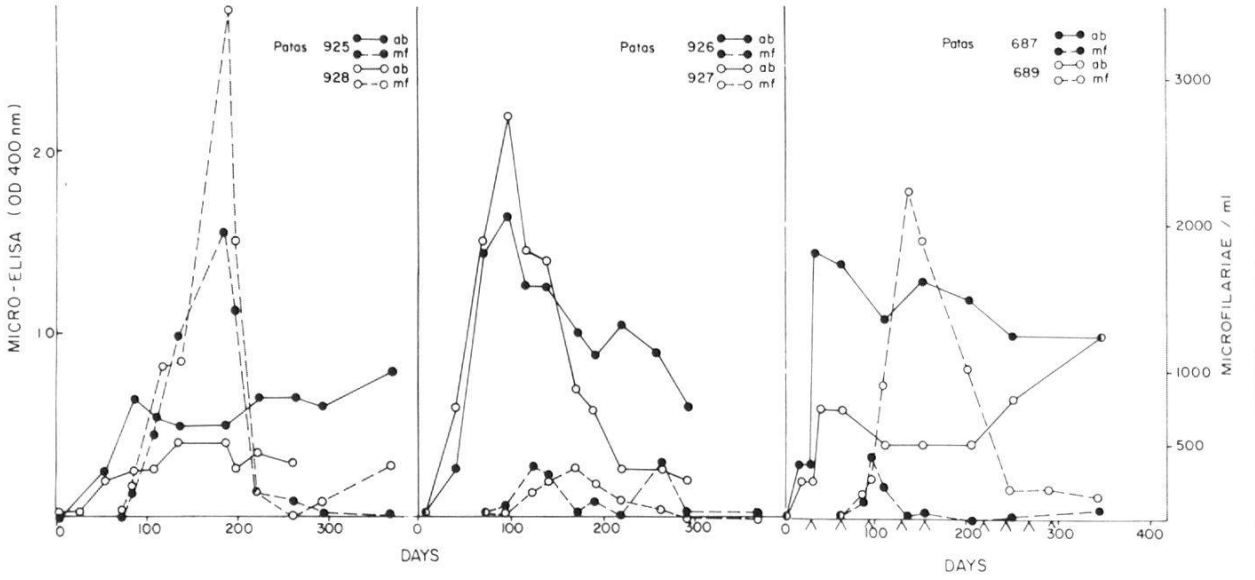


Fig. 1. Antibody and microfilaremia in Patas monkeys following infection with *Brugia malayi*. Left and middle panels, Group A monkeys inoculated with 350 infective larvae ($L_3$); right panel, Group B monkeys receiving multiple inoculations of 100 $L_3$ after an initial inoculation of 150 $L_3$. IgG antibody (ab) measured by ELISA in sera diluted 1:800. Optical density (OD), $\times 10$, measured in 1 mm light path (see methods). $\wedge$ = inoculations of 100 $L_3$.

mias are shown in Fig. 2 and include monkeys Nos. 690 and 693 with the lowest and highest microfilaremias, respectively, in Group C. The antibody responses in the two Rhesus monkeys (Group D) also receiving multiple small inoculations of infective larvae are shown for comparison; both Rhesus monkeys developed high sustained levels of antibody. Rhesus monkey No. 714 was amicrofilaremic except for one period at 20 weeks post initial inoculation and No. 980 had only 3 brief periods of low microfilaremia at 20, 40 and 50 weeks.

The antibody response in Rhesus monkeys following a single inoculation of infective larvae is illustrated in Fig. 3. Group C monkeys (Nos. 387, 436), inoculated with 200 larvae, developed relatively high antibody levels and were amicrofilaremic most of the time. Group B monkeys, inoculated intraperitoneally, did not develop antibody following initial inoculation and were not included. Group A monkeys (Nos. 708, 947) received a large single inoculation of infective larvae and became patent; No. 947 was the only Rhesus monkey with a level and persistence of microfilaremia comparable to the Patas monkeys. Fig. 3 also includes the antibody response of Patas monkey No. 780 (Group D) for comparison; the antibody levels in this monkey were typical of the Patas monkeys with moderate microfilaremia. Comparison of antibody titers paralleled the differences shown in Fig. 3 with the assay of a single serum dilution; for example, at 16 weeks post infection antibody titers were 1:12,800 for No. 387, 1:5120 for No. 708, 1:1280 for No. 947 and 1:640 for No. 780. Antibody titers of preinfection sera from Rhesus monkeys were uniformly low, $<1:10$.

Antibody in all groups of monkeys was also measured by ELISA with

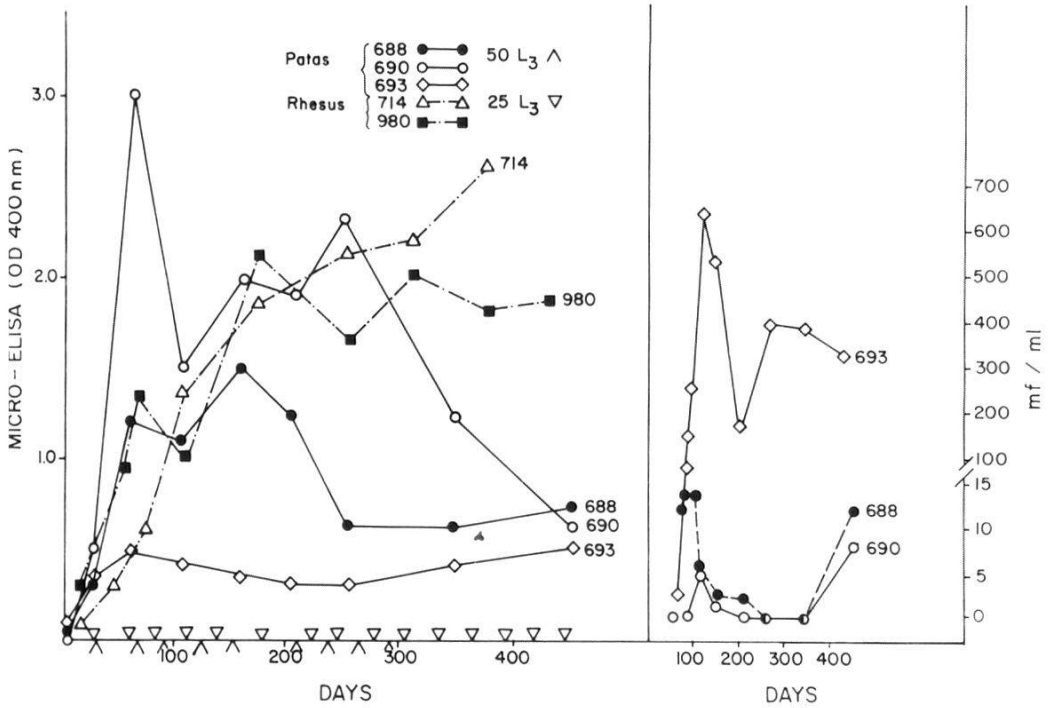


Fig. 2. Antibody and microfilaremia in Patas and Rhesus monkeys receiving multiple inoculations of infective larvae of *Brugia malayi*. Left panel, antibody (IgG) by ELISA in sera diluted 1:400; right panel, microfilaremia in Patas monkeys. ∧ = inoculations of 50 $L_3$ in Patas monkeys; ▽ = inoculations of 25 $L_3$ in Rhesus monkeys.

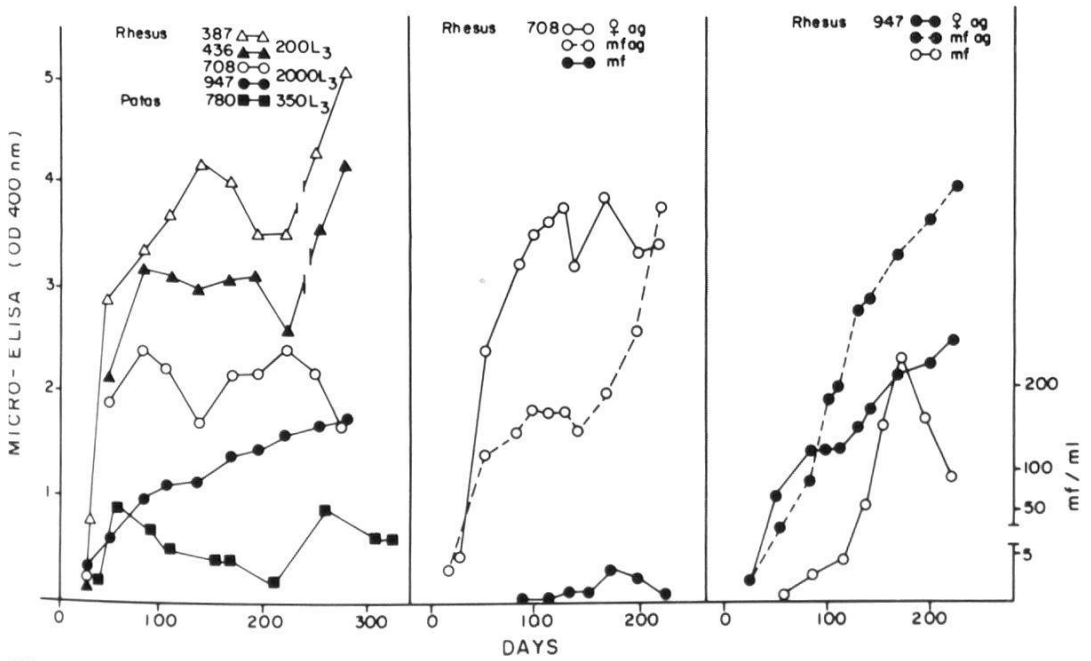


Fig. 3. Comparison of antibody responses in Rhesus and Patas monkeys following a single inoculation of infective larvae of *Brugia malayi*. Left panel, antibody (IgG) by ELISA in serum diluted 1:800 in Rhesus monkeys inoculated with 200 $L_3$ and 2000 $L_3$ and a Patas monkey inoculated with 350 $L_3$. Middle and right panels, comparison of microfilaremia and antibody by ELISA (1:400 serum dilution) in Rhesus monkeys receiving 2000 $L_3$ using female and microfilarial extracts of *Brugia* as antigens. In middle and right panel, ♀ ag = antibody response with extract of female *Brugia* as antigen; mf ag = antibody response with extract of microfilariae as antigen; mf = microfilaremia.

microfilariae extracts as antigens and in a few sera with extracts of male, adult *Brugia*. In general, the pattern of antibody response was similar with all the extracts, although minor differences in pattern with different extracts were occasionally observed among monkeys. The most striking differences observed in response to microfilariae and adult extracts are shown in Fig. 3, the middle and right panels, which compare antibody and microfilaremia during the first 8 months of infection in Rhesus monkeys of Group A. Rhesus No. 708 exhibited an early, high response with adult extract and lower response to microfilariae extract until after patency; in contrast, Rhesus No. 947 had a relatively low response to adult extract with an equal or higher response to microfilariae extract.

*Fluorescent antibody (FA).* The sera examined by ELISA were also assayed by the indirect FA procedure with whole and broken microfilariae and whole infective larvae ($L_3$) as antigens. No Patas monkeys had detectable antibody in IgG or IgM to microfilarial sheath even in sera selected during periods of low or negative microfilaremia and tested at high serum concentrations. Rhesus monkeys injected with 200 $L_3$ (Group C) developed antibody to sheath in both IgG and IgM; monkey No. 436 was positive by week 10 after infection and at all subsequent periods and No. 387 was positive at weeks 23–48, became negative by week 52 and remained negative. There was no evidence of an earlier IgM than IgG antibody response. In no other monkeys was antibody to microfilarial sheath detected; however, staining of broken microfilariae (somatic antigens) was detected with sera from all infected Rhesus and Patas monkeys within 2 months after inoculation of infective larvae and this antibody response persisted. Antibody reactive with the cuticle of $L_3$ was observed in both Patas and Rhesus monkeys given multiple inoculations; antibody was detected after the second or third inoculation of larvae and persisted. Detection of antibody to the cuticle of $L_3$ varied in the single infection groups. Patas monkeys with a high antibody response by ELISA, Nos. 926, 927, were positive during early infection, 30–100 days; monkeys Nos. 780 and 784 had transient staining for antibody only in IgM during the second and third month of infection. Sera from Rhesus monkeys inoculated with 200 $L_3$ were positive for antibody to $L_3$ only after a second inoculation of $L_3$; sera from Rhesus monkeys receiving a large inoculation of $L_3$ (Group A) were positive for antibody during a limited time period around 100 days post inoculation.

*Agglutination and cell adherence.* Agglutination of microfilariae was detected only with sera positive for antibody to microfilariae sheath, i.e., sera from Rhesus monkeys Nos. 436 and 387. An apparent prozone phenomenon was observed with no agglutination at 1:2–1:4 serum dilution and positive reactions at 1:8–1:32. Patas sera were examined from Group B, receiving heavy multiple inoculations of infective larvae, and no agglutination reactions were observed. Cell adherence reactions to microfilariae were carried out with Rhesus sera used for agglutination tests. Sera positive for antibody to microfilariae

sheath gave conspicuous adherent reactions. In multiple assays, the adherence to microfilariae was approximately 50% with cell suspensions of mononuclear leukocytes and 90% with suspensions of polymorphonuclear cells; with sera taken before infection and sera from infected monkeys without detectable antibody to microfilariae sheath, the adherence varied from 1–4%.

## Discussion

The data in this study confirm the susceptibility of the Patas monkey to *Brugia malayi* infection and the development of prolonged microfilaremia. This susceptibility and extended patency have been noted previously (Orihel, personal communication) and, in fact, this primate is a suitable experimental host for a variety of helminth parasites of man including the filariae, *Loa loa* and *Mansonella ozzardi* (Orihel and Moore, 1975; Orihel et al., 1981). The reduction in number of circulating microfilariae in the initial parasitemia to a low persistent microfilaremia, exhibited by many Patas monkeys in this study, has been observed consistently by others and demonstrated to depend upon splenic trapping of microfilariae (Eberhard and Orihel, personal communication). Although this primate has been used as an experimental host for *Brugia*, there is apparently no published information characterizing the infection in the Patas monkey or describing the immune responses.

In contrast to Patas monkeys, there are several published studies on experimental infections with *Brugia malayi* in Rhesus monkeys: the distribution of the helminth in the lymphatics (El Bihari and Ewert, 1971), immune responses (Wong et al., 1977) and development of acquired resistance (Wong et al., 1969) have been investigated and clinical manifestations similar to those of acute Malayan filariasis in man have been noted (Wong et al., 1977). Comparison of experimental studies indicates variation in the host-parasite relationship established by *B. malayi* in the Rhesus monkey. Low, transient microfilaremia, as found in the present study, has been observed by investigators in this country (Orihel, personal communication); however, prolonged patency has been reported and in extensive studies carried out in Malaysia persistent microfilaremia was observed (Wong et al., 1977). The causes of the variation in experimental infections of Rhesus monkeys are unknown but inadequate nutrition of the experimental monkeys in Malaysia was suggested as a possible factor (Wong et al., 1977).

The limited number of animals examined in this study and the differences in infection protocols permit only a general comparison of humoral immune responses in the 2 primate species. By ELISA, an obvious difference in the response to infection was the reduction in titer of circulating antibody associated with development of microfilaremia in the Patas monkeys, in contrast to most Rhesus monkeys which showed almost no microfilaremia and maintained relatively high antibody titers. However, in the few Rhesus and Patas monkeys

in which microfilaremia was comparable, antibody titers were similar. A reduction in circulating antibody with patency has been observed repeatedly in experimental filariasis (Pacheco, 1966; Benjamin and Soulsby, 1976) and the absorbance of antibody by circulating microfilariae and their products is considered the probable cause of this reduction (Piessens et al., 1980). The present results with ELISA confirm the inverse relationship between microfilaremia and circulating antibody levels but do not indicate any basic differences in the ability of the 2 primate species to respond to antigens of the parasite. The persistence of antibody titers in Rhesus monkeys which remain amicrofilaremic or become amicrofilaremic suggests continued antigenic stimulation which is consistent with the establishment of cryptic or occult infections rather than a loss of the infection.

Comparison of antibody specificity by the fluorescent antibody procedure with stages of the parasite as antigens and by ELISA with extracts of these stages revealed few differences in the 2 primate species. The only major difference detected by fluorescent antibody was the lack of circulating antibody reactive with the microfilarial sheath in any Patas monkey. The immunofluorescent study of sera from Rhesus monkeys confirmed reports of others that antibody reactive with the surface of infective larvae was often present, regardless of clinical type of infection, but the presence of antibody to microfilarial sheath was highly correlated with amicrofilaremic infections (Wong et al., 1977). These results also apply to Brugian filariasis in man (Wong and Guest, 1969). Antibodies to somatic antigens of microfilariae were detected in all infected monkeys and these antigens, exposed by sonication of microfilariae, are considered highly effective for serologic diagnosis of human infection with lymphatic dwelling filariae (Hedge and Ridley, 1977). In the limited study by ELISA to compare the extracts of microfilariae, female and male *Brugia* as antigens there were no consistent differences in the pattern of response which suggest that antibodies in both species were reacting primarily with common antigens in the different extracts. Parasite stage specific antigens in similar extracts have been detected by ELISA (Gusmao et al., 1981) and in the current study quantitative or qualitative antigenic differences in extracts of microfilariae and adult extracts were demonstrated by the contrasting antibody responses of 2 Rhesus monkeys receiving a single large inoculation of infective larvae (Fig. 3). These results indicate that antigens from the different parasitic stages, isolated from the common cross reacting antigens, could be of value in detecting specific immune responses during development of filarial infection and possibility in different clinical types of infection.

No comparative information is available in this study on the manifestations of infection in the 2 primate species, although the infected Patas monkeys observed here, exhibited no gross signs of disease. The parasitologic data from both species indicated a control of microfilaremia but the mechanisms are probably quite different. The antibody responses of the Rhesus monkey asso-

ciated with amicrofilaremia appear identical tho those of man, i.e. antibody to the sheath of microfilariae with opsonization for leukocyte adherence; however, all infected, amicrofilaremic Rhesus monkeys did not have detectable antibody to microfilarial sheath which is also true for infections in man (Piessens et al., 1980). In man, amicrofilaremia has been associated with either antibody to microfilarial sheath or cellular immune responses to microfilarial antigens (Piessens et al., 1980). In Rhesus monkeys, cellular immune responses to microfilarial antigens have not been investigated. In Patas monkeys microfilariae are presumably selectively sequestered and destroyed in the spleen since splenectomy ablates the suppression of microfilaremia (Eberhard and Orihel, personal communication). Splenic trapping of microfilariae with suppression of microfilaremia but not complete clearance also has been described in *Loa loa* infection of baboons (Orihel and Moore, 1976) and drills (Duke, 1960; 1960a), in which microfilariae are destroyed by leukocytes within large splenic granulomata. If *Brugia* infection in Patas monkeys is similar to *Loa loa* infection in drills, microfilariae are concentrated in the lungs and only the small percentage of microfilariae circulating are subject to splenic trapping; this situation with a large pool of microfilariae retained in the lungs could explain the absorption of antibody even though the numbers of cirulating microfilariae are extremely low. This dependency on the spleen for partial clearance of circulating microfilariae is unusual in filariasis (Hawking, 1962) and there is no direct evidence that opsonizing antibody is involved (Duke 1960a). The role of the spleen in parasite infections is complex (WHO, 1978) and splenic control of parasitemia can be dependent upon immunologic and/or mechanical filtration factors (Wyler et al., 1976). An immunologic basis, if present, for splenic control of microfilaremia in these filarial infections of non human primates remains to be demonstrated.

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