

Immunocytochemical Localization and Distribution of Human Albumin in *Wuchereria bancrofti* Adult Worms

Christina Alves Peixoto, MSc, PhD; Joaquim Norões, MD; Abraham Rocha, MSc; Geresa Dreyer, MD

● To determine whether albumin is present on adult worms of *Wuchereria bancrofti*, thin sections of resin-embedded parasites were incubated with a specific antiserum to human albumin. With the exception of the epicuticle, all layers of the cuticle and the hypodermis were intensely labeled. Concentration of gold particles was observed within infoldings of the hypodermal membrane. Moderate labeling of the thin basement membrane that lines the pseudocelomic cavity and the gonoduct was also observed. Within the uterus, ovular membranes labeled intensely; groups of organized particles were seen below ovular membranes and also within invaginations of microfilarial embryos. In contrast, few gold particles were seen on the surface of mature intrauterine microfilariae. No labeling was observed in control sections incubated with antiserum preadsorbed with purified human albumin. The findings suggest that human albumin may be essential for the nutrition and development of *W bancrofti* microfilariae.

(Arch Pathol Lab Med. 1999;123:173–177)

W*uchereria bancrofti* is the main parasite responsible for human lymphatic filariasis in tropical and subtropical countries, causing approximately 120 million infections worldwide.¹ The adult worms reside within human lymphatics, where they can survive for many years without causing clinical disease. Thus, the parasites are well adapted to survive within the mammalian host, but the nature of the mechanisms that permit adult worms to evade protective host responses and reproduce within the lymphatic system are largely unknown. Development of immune tolerance to filarial worms has been invoked to explain the persistence of microfilaremia. However, it is unclear whether adult worms themselves induce immune unresponsiveness, because adult worm carriers without microfilaremia tend to have vigorous antifilarial immune responses.^{2,3}

The masking of surface antigens by host-derived molecules is thought to be one mechanism of immune evasion by schistosomes.⁴ Human serum albumin may play a sim-

ilar role in bancroftian filariasis, because it is a major component of the surface of *W bancrofti* microfilariae.⁵ In the present study, we examined the distribution of human albumin in adult worms of *W bancrofti* surgically removed from the intrascrotal lymphatics of a volunteer.

MATERIAL AND METHODS

Parasites

Living adult *W bancrofti* were obtained from a volunteer participant in a study of the adulticidal effect of diethylcarbamazine. Preoperative ultrasonography⁶ had shown the presence of an intrascrotal lymphatic dilation of 20 mm containing living adult worms, which remained alive after 12 days of treatment with diethylcarbamazine (6 mg/kg per day). The patient consented to surgery for the collection of lymph fluid to measure local diethylcarbamazine levels and for the removal of adult worms. The study protocol and procedures were reviewed and approved by the Ethical Committee of the Hospital das Clínicas, Universidade Federal de Pernambuco, Recife, Brazil.

Electron Microscopy and Immunocytochemistry

The living parasites were washed twice with phosphate-buffered saline (PBS) and fixed for 60 minutes in a solution containing 0.1% glutaraldehyde and 4% formaldehyde (freshly prepared from paraformaldehyde) in 0.1 mol/L cacodylate buffer, pH 7.2. After fixation, the parasites were washed twice with PBS, dehydrated in acetone, and embedded in L. R. White resin (Sigma Chemical Company, St Louis, Mo). Because of its hydrophilic nature, this resin is highly suitable for labeling thin sections with colloidal gold probes. Polymerization was done at 37°C for 5 days.

Ultrathin sections were collected on 300-mesh nickel grids and incubated for 60 minutes at room temperature in PBS, pH 8.0, containing 5% nonfat milk and 0.01% Tween 20 (PBS-MT). The sections were then incubated for 1 hour at room temperature with rabbit immunoglobulin G (IgG) anti-human albumin diluted 1:500 in PBS-MT. The sections were then rinsed in PBS and incubated for 1 hour at room temperature with gold-labeled goat anti-rabbit IgG (Sigma) diluted 1:100 with PBS-MT. The specificity of the antibody against human albumin was ascertained by incubation for 2 hours of a solution containing 2% purified human albumin in the presence of rabbit IgG anti-human albumin (Sigma) at the final dilution of 1:500 in PBS, pH 8.0. The adsorbed antibody was pelleted at 12,000g for 30 minutes, and the supernatant was used for immunocytochemical analysis. Second antibody control sections were incubated only in the presence of the gold-labeled antibody. After incubation, the sections were washed with PBS followed by distilled water, counterstained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (Zeiss Em 109, Schott-Zeiss do Brasil LTDA, Rio de Janeiro).

RESULTS AND COMMENT

In this study, human albumin was readily demonstrated in all layers of the cuticle and in the hypodermis of adult

Human Albumin in *W bancrofti*—Peixoto et al 173

Accepted for publication August 21, 1998.

From the Departments of Pathology and Cellular Biology (Dr Peixoto) and Parasitology (Drs Peixoto and Dreyer and Mr Rocha), Centro de Pesquisas Aggeu Magalhães, and the Department of Surgery, Section of Urology, Centro de Ciências da Saúde (Dr Norões), Universidade Federal de Pernambuco, Recife, Brazil.

Reprints: Christina Alves Peixoto, MSc, PhD, Department of Pathology and Cellular Biology, Centro de Pesquisas Aggeu Magalhães, Av Moraes Rego s/n, CEP 50670-420 Recife, Brazil.

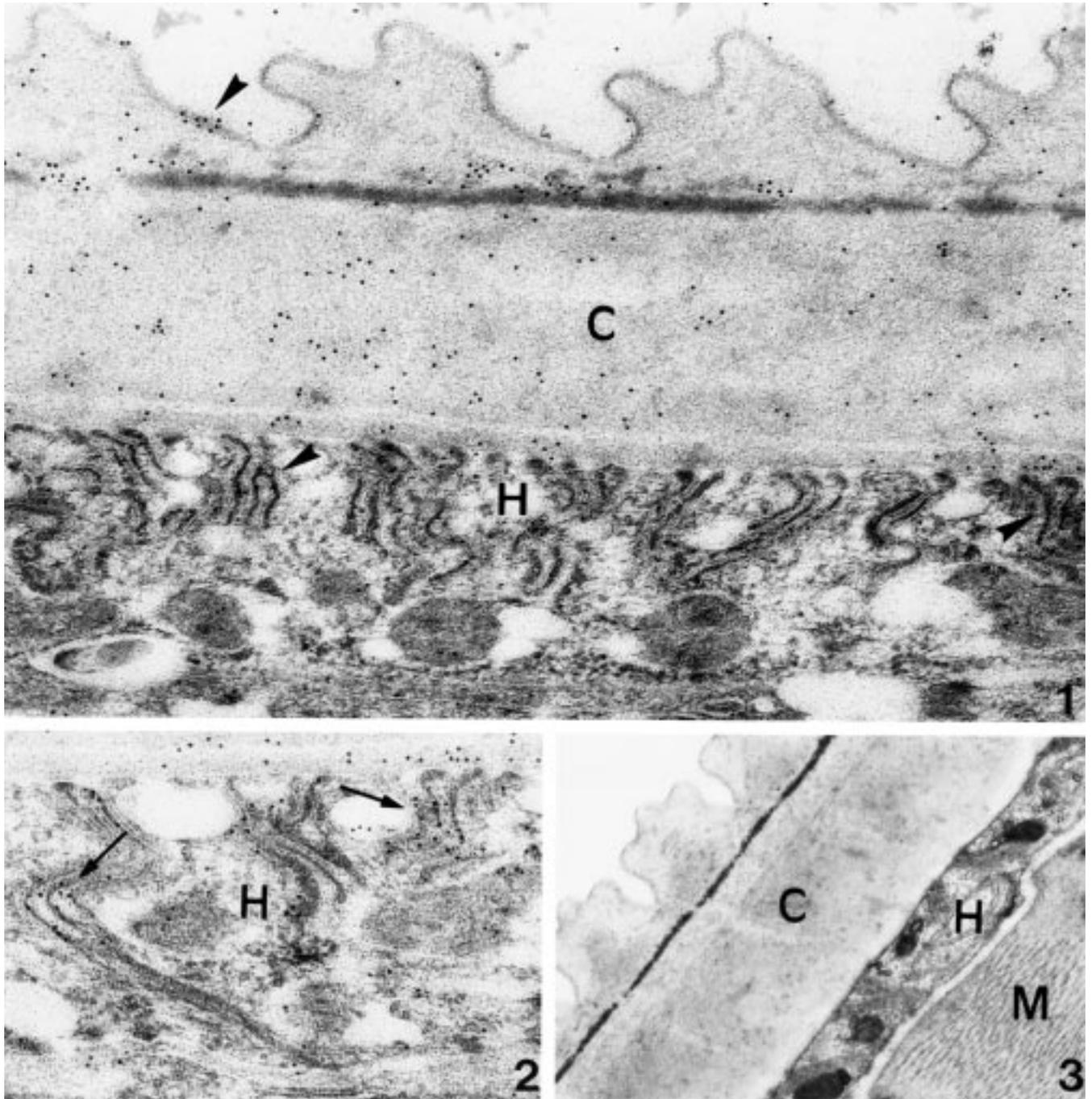


Figure 1. Localizaton of antigenic sites in thin section of L. R. White-embedded female *Wuchereria bancrofti* organism using rabbit immunoglobulin G anti-human albumin. All the cuticle layers (C) show intense labeling, except for the epicuticle (arrowhead). The hypodermis (H) also labeled strongly (arrows) (original magnification $\times 44\,000$).

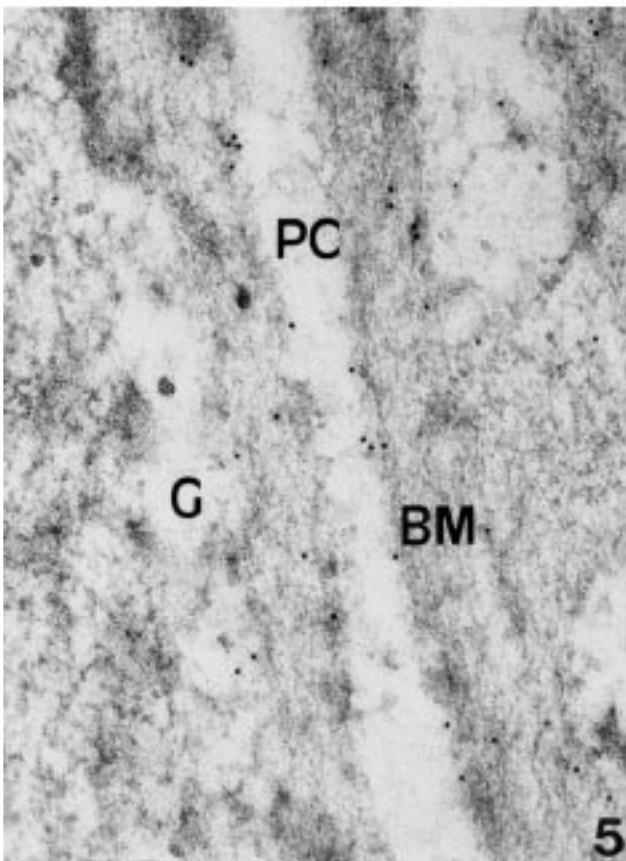
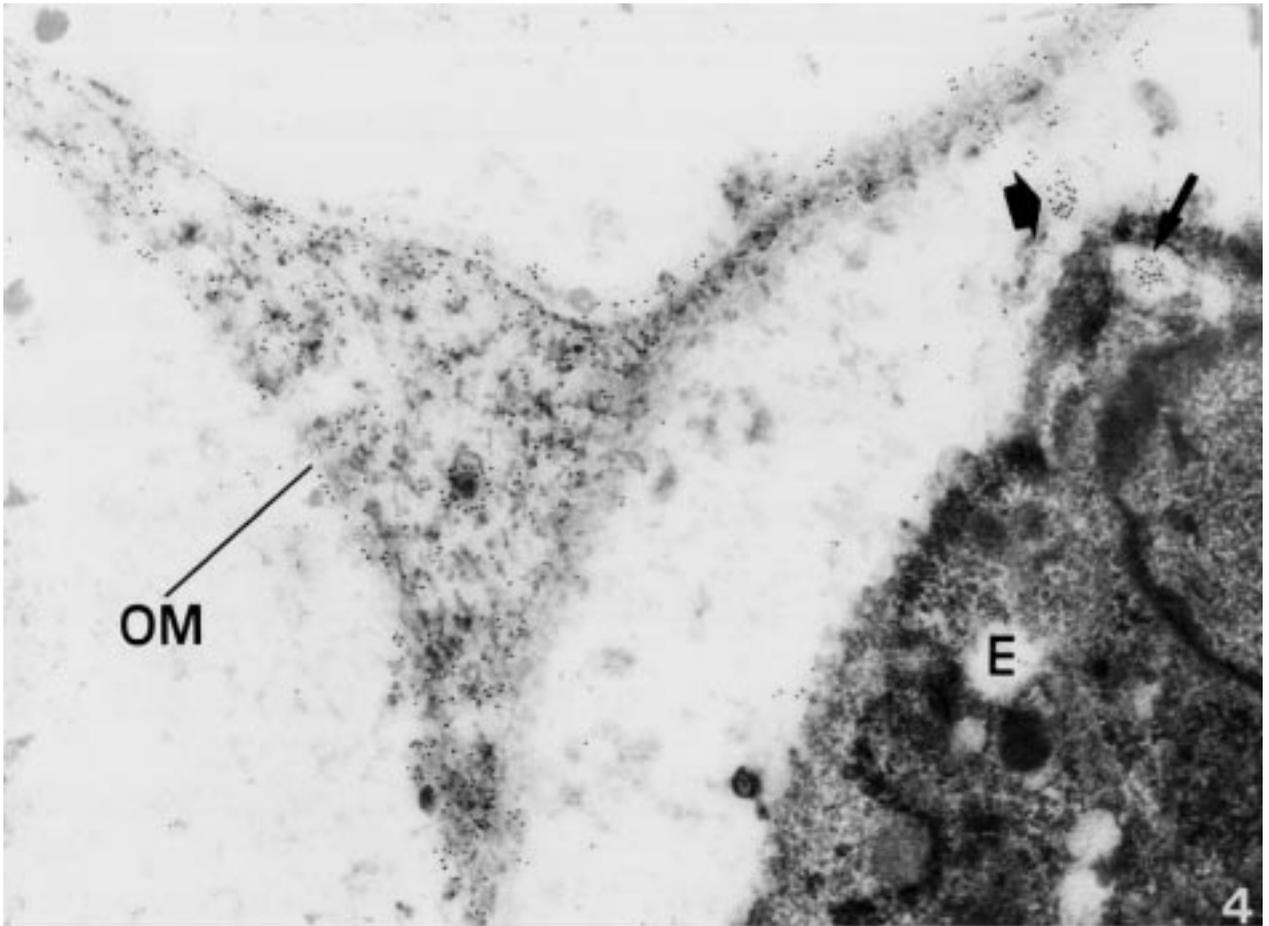
Figure 2. Thin section of the cuticle of *Wuchereria bancrofti* incubated in the rabbit anti-human albumin. Note the linear arrangement of immunogold particles centered in infoldings of the hypodermal membrane (arrows); H indicates hypodermis (original magnification $\times 65\,000$).

Figure 3. Control thin section of L. R. White-embedded *Wuchereria bancrofti* incubated in the presence of the adsorbed anti-human albumin. No labeling was observed; M indicates muscle cell (original magnification $\times 20\,000$).

Figure 4. Thin section of *Wuchereria bancrofti* uterus showing an ovular membrane intensely labeled (OM). Note the well-organized group of particles being directed to the embryo (E) (short arrow) and also inside endocytic invaginations of the microfilaria (arrow) (original magnification $\times 36\,000$).

Figure 5. Longitudinal thin section of the pseudocelomic cavity (PC) of *Wuchereria bancrofti* showing homogeneous labeling of the outer region of the gonoduct (G) and the basement membrane (BM) that lines the pseudocelomic cavity (original magnification $\times 55\,000$).

Figure 6. Longitudinal thin section of the *Wuchereria bancrofti* uterus showing mature microfilariae lightly labeled in the cuticle (C) and sheath (S) (original magnification $\times 50\,000$).



W bancrofti worms. Except for the epicuticle, all areas showed fairly intense labeling (Figure 1). Some gold particles were also observed in striated muscle cells. In some thin sections, the gold particles appeared to be concentrated at the infoldings of the hypodermal membrane (Figure 2). These infoldings have been described previously⁷⁻⁹ and may increase the absorptive area of the worm. Transcuticular uptake of glucose, amino acids, and adenosine is a well-known feature of filarial worms.¹⁰ However, it is unlikely that large molecules such as intact albumin are taken up via the transcuticular route. We hypothesize that human albumin is absorbed and digested by intestinal cells and that small peptides regurgitated from the worm gut are secondarily absorbed via the transcuticular route to yield the labeling pattern observed within the cuticle and the infoldings of the hypodermis. This hypothesis remains to be formally tested. It should be noted, however, that the immunostaining is specific for human albumin, because no labeling was observed in thin sections of adult worms incubated with rabbit antiserum that was preabsorbed with human albumin (Figure 3) or in control sections incubated only with gold-labeled anti-rabbit IgG (not shown).

The present study indicates that human albumin (or some fragment thereof) is present in all layers of the cuticle instead of being localized only to the surface of the nematode. The observation that fewer antigenic determinants appear to be present in the epicuticle than in deeper layers of the cuticle and in the hypodermis may signify efficient uptake and transport of albumin fragments. Alternatively, this observation may be an artifact of the immunocytochemical procedure if albumin binds weakly to the surface of the nematode and is lost during the dehydration step needed for embedding the worms in resin. Indeed, Maizels et al⁵ demonstrated that surface labeling with antiserum to secreted-excreted antigens to *Toxocara canis* was inhibited by dehydrants such as acetone and ethanol. Likewise, conventional indirect fluorescent antibody tests to detect immunoglobulin on microfilariae yield positive results with living worms but not when the microfilariae are fixed with acetone or methanol.^{11,12} Further studies in which cryosections of adult worms of *W bancrofti* are stained with immunogold are necessary to overcome the likely interference of the resin-embedding procedure with the surface properties of the cuticle.

Within the worm, the thin basement membrane that lines the pseudocoelomic cavity and the gonoduct are moderately labeled (Figure 4). This may be an intermediate step in the transport of human albumin to the reproductive system of the worm. Inside the worm uterus, the ovular membranes label intensely, and some well-organized groups of particles are seen under the ovular membrane and also inside invaginations of the microfilarial embryos (Figure 5). This is the first demonstration that a human component taken up by *W bancrofti* may be used for the nutrition and perhaps the differentiation of its embryos. In contrast, few gold particles were seen labeling the sheath or the cuticle of mature intrauterine microfilariae (Figure 6).

Using fluorescent anti-human albumin, Kar et al¹³ detected no live microfilariae without human albumin on their sheaths, suggesting that the worms either become coated with albumin soon after birth or acquire it before birth. While it is unlikely that the whole molecule of human albumin is adsorbed onto the microfilarial surface

before birth, some digestion fragments that are antigenic appear to be present. After birth, additional intact human albumin in lymph or blood could be rapidly adsorbed onto the microfilariae surface. Host albumin is a major surface component on microfilariae of *W bancrofti* isolated from peripheral blood.⁵ It is unlikely, however, that the surface labeling methods used by these investigators would detect the small amounts of albumin we observed in the tegument of intrauterine microfilariae if the situation is analogous to what has been observed with skin and uterine microfilariae of *Onchocerca gibsoni*.¹⁴ Whether acquisition of host albumin is a means whereby microfilariae evade immune effector mechanisms and generally fail to elicit inflammatory reactions remains a matter of speculation.

The susceptibility of vertebrates to infection with filarial worms varies between and within species, but the mechanisms that make an individual a suitable host are largely unknown. Human beings are the unique host of the filarial parasite *W bancrofti* in nature, but not all humans appear to be equally susceptible to infection and subsequent development of mature adult worms. Within the past 5 years, several technical advances have opened new avenues of understanding of this host-parasite relationship. Among these, ultrasound has been used to visualize and study living adult *W bancrofti* worms in their natural habitat¹⁵⁻¹⁷ and to surgically collect these worms for studies of the morphology and ultrastructure of the adult parasite.¹⁸ The use of ultrasound has also confirmed that not all adult worms are sensitive to diethylcarbamazine, implying that 2 subpopulations of parasites exist; susceptible and nonsusceptible parasites may coexist within the same nest.¹⁹ The present study was conducted to extend these observations, to explore the biologic and immunocytochemical characteristics of *W bancrofti*, and to improve our understanding of the exquisite interplay between this organism and its human host.

Our results suggest that human albumin not only may be important for protection (of both the host and the parasite) but also could be essential for nutrition and differentiation of *W bancrofti* microfilariae. Because our study included only worms that were not susceptible to diethylcarbamazine, we cannot speculate on the status of albumin uptake and its metabolism in diethylcarbamazine-sensitive worms or on whether this substance is somehow involved in the nonsusceptibility of some adult worms to the only macrofilaricidal drug available thus far.^{19,20} Our understanding of the pathogenesis of lymphatic filariasis has changed considerably during the past few years. The mechanisms that keep the worm alive and cause lymphangiectasis, the earliest and most common lesion in lymphatic filariasis, are unknown.⁶ As long as the adult worms are alive, no inflammatory response occurs within the wall or lumen of the dilated lymphatic vessel, even at the site of the adult worm.^{15,21} Within the lymphatic vessels of human beings, the unique natural host for *W bancrofti*, the parasites find the nutritional factors and anti-inflammatory mechanisms necessary for development and survival of the adult worm. The nature of such biologic complexity between parasite and host remains a mystery. Understanding this process of parasite survival could be vital for prevention of infection and lymphatic damage.

We are grateful to Professor Willy Piessens for critical reading and helpful discussions of the manuscript. This work was sup-

ported by UNPD/World Bank/WHO Special Programme for Research and Training in Tropical Disease and Non-Governmental Organization Amaury Coutinho, Fundação de Amparo à Ciência e Tecnologia (FACEPE-APQ-0315.2.13/97), and Fundação Osvaldo Cruz (FIOCRUZ). The diamond knife was provided by the David Rockefeller Center for Latin American Studies.

References

1. World Health Organization. *Lymphatic Filariasis: Fourth Report of the WHO Expert Committee on Filariasis*. Geneva, Switzerland: World Health Organization; 1994. WHO Technical Report Series 702.
2. Mahanty S, Nutman TB. Immunoregulation in human lymphatic filariasis: the role of interleukin 10. *Parasite Immunol*. 1995;17:385–392.
3. Dimock KA, Eberhard ML, Lammie PJ. Th1-like antifilarial immune responses predominate in antigen-negative persons. *Infect Immun*. 1996;64:2962–2967.
4. Sher A, Hall BF, Vadas MA. Acquisition of murine major histocompatibility complex gene products by schistosomula of *Schistosoma mansoni*. *J Exp Med*. 1978;148:46–57.
5. Maizels RM, Philip M, Dasgupta A, Partono F. Human albumin is a major component on the surface of microfilariae of *Wuchereria bancrofti*. *Parasite Immunol*. 1984;6:185–190.
6. Norões J, Addiss D, Santos A, Medeiros Z, Coutinho A, Dreyer G. Ultrasonographic evidence of abnormal lymphatic vessels in young men with adult *Wuchereria bancrofti* infection in the scrotal area. *J Urol*. 1996;156:409–412.
7. Rogers R, Denham DA, Nelson GS. Studies with *Brugia pahangi*, 5: structure of the cuticle. *J Helminthol*. 1974;48:113–117.
8. Vincent AL, Ash LR, Frommes SP. The ultrastructure of adult *Brugia malayi* (Brug 1929), (Nematoda: Filarioidea). *J Parasitol*. 1975;61:499–512.
9. Howels RE, Blainey LJ. The moulting process and the phenomenon of intermoult growth in the filarial nematode *Brugia pahangi*. *Parasitology*. 1983;87:493–505.
10. Howels RE, Chen SN. *Brugia pahangi*: feeding and nutrient uptake in vitro and in vivo. *Exp Parasitol*. 1981;51:42–58.
11. Ponnudurai T, Denham DA, Nelson GA, Rogers R. Studies with *Brugia pahangi*, 4: antibodies against adult and microfilarial stages. *J Helminthol*. 1974;48:107–111.
12. Ravindran B, Satapathy AK, Das MK, Pattnaik NM, Subramanyam VR. Antibodies to microfilarial sheath in bancroftian filariasis: prevalence and characterization. *Ann Trop Med Parasitol*. 1990;84:607–613.
13. Kar SK, Mania J, Baldwin CI, Denham DA. The sheath of the microfilaria of *Wuchereria bancrofti* has albumin and immunoglobulin on its surface. *Parasite Immunol*. 1993;15:297–300.
14. Forsyth KP, Copeman DB, Mitchell GF. Differences in the surface radioiodinated proteins of skin and uterine microfilariae of *Onchocerca gibsoni*. *Mol Biochem Parasitol*. 1984;10:217–229.
15. Amaral F, Dreyer G, Figueredo-Silva J, et al. Live adult worms detected by ultrasonography in human bancroftian filariasis. *Am J Trop Med Hyg*. 1994;50:753–757.
16. Dreyer G, Amaral F, Norões J, Medeiros Z. Ultrasonographic evidence for stability of adult worm location in bancroftian filariasis. *Trans R Soc Trop Med Hyg*. 1994;88:558.
17. Norões J, Addiss D, Amaral F, Coutinho A, Medeiros Z, Dreyer G. Occurrence of living adult *Wuchereria bancrofti* in the scrotal area of men with microfilariaemia. *Trans R Soc Trop Med Hyg*. 1996;90:55–56.
18. Araújo ACG, Figueredo-Silva J, Souto-Padron T, Dreyer G, Norões J, De Souza W. Scanning electron microscopy of adult *Wuchereria bancrofti* (nematoda: filarioidea). *J Parasitol*. 1995;81:468–474.
19. Norões J, Dreyer G, Santos A, Mendes VG, Medeiros Z, Addiss D. Assessment of the efficacy of diethylcarbamazine on adult *Wuchereria bancrofti* in vivo. *Trans R Soc Trop Med Hyg*. 1997;91:78–81.
20. Dreyer G, Addiss D, Santos A, Figueredo-Silva J, Norões J. Direct assessment in vivo of the efficacy of combined single-dose ivermectin and diethylcarbamazine against adult *Wuchereria bancrofti*. *Trans R Soc Trop Med Hyg*. 1998;92:219–222.
21. Figueredo-Silva J, Dreyer G, Guimarães K, Brandt C, Medeiros Z. Bancroftian lymphadenopathy: absence of eosinophils in tissues despite peripheral blood hypereosinophilia. *J Trop Med Hyg*. 1994;97:55–59.