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Analysis of *Brugia pahangi* microfilariae surface carbohydrates: comparison of the binding of a panel of fluoresceinated lectins to mature in vivo-derived and immature in utero-derived microfilariae

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Summary

A fluoresceinated lectin binding assay was employed to detect carbohydrates on the sheath and cuticle of mature in vivo-derived, and immature in utero-derived *Brugia pahangi* microfilariae. The sheath of mature microfilariae bound concanavalin A and wheat germ agglutinin, indicating the presence of N-acetylglucosamine and glucose or mannose. In addition to binding concanavalin A and wheat germ agglutinin, the sheath of in utero-derived microfilariae also bound *Limulus polyphemus* agglutinin, peanut agglutinin, *Ricinus communis* agglutinin-I, and soybean agglutinin, indicating the presence of the additional sugars galactose, sialic acid, and N-acetylgalactosamine. There was no evidence of cuticle carbohydrates, as none of the tested fluoresceinated lectins bound to either mature or immature exsheathed microfilariae. The significance of these results in terms of the survival of microfilariae in the mammalian host, and development to third-stage larvae in the mosquito vector, is discussed.

Key words: *Brugia pahangi;* carbohydrate; cuticle; lectin; microfilariae; sheath.

Introduction

During the last decade it has become increasingly evident that the surface of microfilariae is of major importance in the interaction between parasite and host, and between parasite and insect vector. A better understanding of the surface carbohydrates of the sheath and cuticle of microfilariae would facilitate

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the development of immunological and chemical measures to disrupt their normal biological function. To date, the most extensive study of the surface carbohydrates of microfilariae has been the histochemical analysis by Simpson and Laurence (1972). This study demonstrated the presence of periodate-reactive carbohydrates on both the sheath and cuticle of *Brugia pahangi* microfilariae. Due to the nature of the histochemical stains used, there was no indication of the type of carbohydrates detected, or if they were exposed to the external environment. Both of these shortcomings can be circumvented using lectin binding assays (Nicolson, 1974).

In the present report we have examined, using fluoresceinated lectins, *B. pahangi* microfilariae for the presence of surface sheath and cuticle carbohydrates.

Materials and Methods

Parasites. Adult worms of *B. pahangi* were surgically recovered from the abdominal cavities of male jirds *(Meriones unguiculatus)* 4–6 months after intraperitoneal infection with 400 third-stage larvae. Microfilariae were harvested from the peritoneal cavity of these infected jirds by lavage using a balanced salt solution. These peritoneal cavity-derived microfilariae (pd Mf) were washed and centrifuged at $600 \times g$ three times and then resuspended in balanced salt solution to a density of 2×10^4/ml.

In utero-derived microfilariae (iud Mf) were obtained from active *B. pahangi* female worms by dissection using fine needles. Active microfilariae which moved out of the uteri were then washed as described for pd Mf.

Surgical implants. Mature pd Mf were isolated from short-term adult worm-implanted jirds. Adult female *B. pahangi* removed from the peritoneal cavity of 4-months infected jirds were implanted into naive male jirds following the method of Butts and Rabalais (1974). Briefly, Ketalar (Parke-Davis, Morris Plains, NJ)-anaesthetised uninfected jirds were implanted with 10 female *B. pahangi* into the peritoneal cavity through a small incision made in the lateral abdominal wall. The incision was closed with autoclips (Clay Adams, Parsippany, NJ). Transplanted jirds were killed 3 days later and these young pd Mf were harvested as noted previously.

Short-term peritoneal implantation of immature iud Mf utilised micropore chamber implants (Weiss and Tanner, 1979). Micropore chambers (3.0 μm pore size, Type SSWP membranes, Millipore Corp., Bedford, MA) were assembled, loaded with 10^4 freshly harvested iud Mf, and implanted into the peritoneal cavity of uninfected jirds. Eight hours after implantation the chambers were removed, and the contents harvested and washed in balanced salt solution as described previously.

Lectins. Fluorescein-labeled lectins, concanavalin A (Con A), *Limulus polyphemus* agglutinin (LPA), peanut agglutinin (PNA), *Ricinus communis* agglutinin-I (RCA-I), soybean agglutinin (SBA), *Ulex europaeus* agglutinin (UEA), and wheat germ agglutinin (WGA) were obtained from E Y Laboratories, Inc., San Mateo, CA. A simplified summary of lectin specificities is listed in Table 1.

Lectin binding. Washed Mf (100 μl of a 2×10^4/ml suspension) were incubated on ice for 10 min with individual fluoresceinated lectins (50 μl of a 50 μg/ml solution). The Mf were then washed 3 times in balanced salt solution, and brought to a final volume of 100 μl. A small aliquot was mounted on a glass microscope slide, overlaid with a coverslip, and examined immediately under a fluorescent microscope. A minimum of 200 Mf were examined with each fluoresceinated lectin.

Binding specificity experiments were performed by adding an appropriate sugar inhibitor (0.2 M) to the tube containing Mf prior to adding the fluoresceinated lectin. Hapten sugar inhibitors (Sigma Chemical Co., St. Louis, MO) used in this study were: α-methyl-D-mannoside, chitobiose,

Table 1. Lectins used for fluorescent staining

Lectin	Abbreviation	Sugar specificity
Concanavalin A	Con A	D-mannose, D-glucose
Limulus polyphemus agglutinin	LPA	Sialic acid
Peanut agglutinin	PNA	D-galactose, $\beta(1\rightarrow3)$-N-acetyl-galactosamine, >D-galactose
Ricinus communis agglutinin-I	RCA-I	D-galactose
Soybean agglutinin	SBA	N-acetyl-D-galactose
Ulex europaeus agglutinin	UEA	L-fucose
Wheat germ agglutinin	WGA	$[\beta(1\rightarrow4)$-N-acetyl-D-glucosamine$]_2$

D-galactose, N-acetyl-D-galactosamine, D-glucose, N-acetyl-glucosamine, lactose, L-fucose and sialic acid.

Exsheathment procedures. B. pahangi Mf were exsheathed by either sonication or papain digestion. Microfilariae were sonicated in a Sonifier Cell Disruptor (Heat Systems, Inc., Melville, NY) at 100 watts power for 20 sec, and then examined under light microscopy to ascertain the degree of exsheathment. Sonicated mixtures which contained at least 10% viable exsheathed nonfragmented Mf were used to assess the cuticle lectin binding.

The papain enzymatic exsheathment technique of Devaney and Howells (1979) gave a higher percentage of viable exsheathed Mf (75–92%). Papain (Type IV, Sigma Chemical Co., St. Louis, MO) at a concentration of 5 units/ml in balanced salt solution was incubated with Mf for 1 h at 37° C in humidified, 5% CO_2 air. After incubation, papain activity was quenched with 5% horse serum, and the mixture was washed in balanced salt solution.

Results

Fluoresceinated lectin binding to sheathed and exsheathed B. pahangi pd Mf. All intact sheathed pd Mf bound fluoresceinated Con A and WGA (Table 2). None of the other lectins bound to the sheath. WGA bound intensely over the entire sheath surface. Con A bound less intensely but also bound over the entire sheath surface. No movement or capping of either lectin was noted; the binding pattern on a small percentage of WGA-bound sheathed pd Mf, however, gave an uneven granular fluorescent pattern. It appeared, therefore, that only N-acetyl-glucosamine and mannose or glucose were present on the sheath surface.

The specificity of lectin binding was assessed by mixing the labeled lectins with those sugars known to bind competitively with Con A and WGA, mannose, and N-acetyl-glucosamine carbohydrates, respectively. Con A binding was easily and completely inhibited. N-acetyl-glucosamine and chitobiose, however, had a significant but incomplete inhibitory effect on WGA binding (all Mf were less fluorescent), thus raising the possibility that a significant portion of WGA binding is nonspecific.

We then used two exsheathment procedures, sonication and papain treatment, to examine the cuticular surface carbohydrates of pd Mf. None of the tested lectins bound to the cuticle (Table 2). Therefore, we cannot suggest which carbohydrates might be present on the cuticle surface.

Table 2. Fluoresceinated lectin binding of in vivo-derived *Brugia pahangi* microfilariae

Lectin	Sheathed	Exsheathed (Papain)	Exsheathed (Sonication)	Fragmented (Sonication)
Con A	+ (100%)	–	–	+
LPA	–	–	–	+
PNA	–	–	–	+
RCA-I	–	–	–	+
SBA	–	–	–	+
UEA	–	–	–	+
WGA	+ (100%)	–	–	+

Table 3. Fluoresceinated lectin binding to the sheath of in utero-derived *Brugia pahangi* microfilariae

Lectin	Sheath fluorescence	Lectin	Sheath fluorescence
Con A	+ (100%)	SBA	+ (61%)
LPA	+ (100%)	UEA	–
PNA	+ (100%)	WGA	+ (100%)
RCA-I	+ (100%)		

Internal carbohydrates were exposed on many sonication-fractured pd Mf (Table 2). All tested lectins were able to bind to the exposed fragmented ends.

Fluoresceinated lectin binding to the sheath of B. pahangi iud Mf. In addition to binding Con A and WGA, every sheathed iud Mf bound LPA, PNA, and RCA-I (Table 3). Only approximately 61% of the iud Mf, however, bound SBA. As was found with Con A and WGA binding, the fluorescence pattern with these additional lectins was dispersed over the entire sheath surface. The iud Mf appeared to have a new mosaic of exposed sheath carbohydrates. Sialic acid, galactose, and N-acetyl-galactosamine now appeared to be exposed on the sheath. Inhibition experiments further substantiated these conclusions.

The presence of new sugars on the sheaths of iud Mf spurred us to examine their cuticle surface. Exsheathment experiments using the techniques employed with pd Mf were used for iud Mf. As was found with pd Mf, iud Mf cuticle did not bind any of the tested fluoresceinated lectins. It appeared, therefore, that only the sheath carbohydrate composition was different for iud Mf.

Fluoresceinated lectins binding to young pd Mf. To examine whether newly released pd Mf might bind to a fluoresceinated lectin other than Con A or GWA, we surgically implanted adult *B. pahangi* worms into the peritoneal cavities of uninfected male jirds. Microfilariae harvested from these jirds 3 days after implantation bound only Con A and WGA.

Stability of iud Mf sheath carbohydrates. To ascertain whether the difference in iud Mf and pd Mf sheath carbohydrate composition was due to a host-induced alteration, we implanted iud Mf, within millipore chambers, into the peritoneal cavity of an uninfected male jird. These millipore chamber iud Mf were harvested 8 h later, and assayed with the panel of fluoresceinated lectins. These iud Mf had the same lectin binding pattern as when they were harvested from the uteri of female *B. pahangi* adults; there was no loss or gain of sheath carbohydrates that we could measure.

Discussion

The binding of the fluoresceinated lectins, Con A and WGA, to the sheath of *B. pahangi* pd Mf strongly suggests the presence of N-acetyl-glucosamine, and glucose or mannose carbohydrates. The exposed cuticular surface of ex-sheathed pd Mf failed to bind any of the tested lectins. This lack of correlation with the histochemical procedures, which had indicated the presence of carbohydrates (Simpson and Laurence, 1972) may indicate that the cuticle contains sugar moieties which are not readily exposed to the external environment. It is also possible, however, that the histochemical techniques employed may have been harsh enough to expose internal subcuticular carbohydrates, which have been shown in this study to readily bind to all the tested fluoresceinated lectins.

The greater heterogeneity of iud Mf sheath surface carbohydrates was evident by the binding of additional lectins, LPA, PNA, RCA-I and SBA. These additional bound lectins indicated that sialic acid, galactose, and N-acetyl-galactosamine residues were correlated with immature microfilariae. The binding of SBA to only 61% of iud Mf indicated that terminal N-acetyl-galactosamine residue alteration is a separate event from galactose sugar alteration. There is no evidence, however, indicating the time scale of these events.

The sheath surface carbohydrates of iud Mf appeared to be stable after being surgically implanted into the peritoneal cavity of uninfected jirds. Any biochemical alteration must have therefore occurred before their actual release from the adult worm.

Previously, only electron microscopic morphologic changes have been reported (Rogers et al., 1976); in comparison to fully mature *B. pahangi* Mf, iud Mf had no regular particulate outer layer on the sheath. It is possible that a lack of binding of LPA, PNA, RCA-I and SBA to the sheath of fully mature pd Mf might be the result of the deposition of only Con A and WGA sheath material just before their release from the female. This deposited material might block lectin access to galactose, sialic acid, and N-acetyl-galactosamine sugars. There is no evidence available, however, to determine the possible role of either biochemical alteration or deposition on the availability of exposed sheath carbohydrates.

The mechanisms by which *B. pahangi* microfilariae evade the jird's im-

mune responses are not well understood. Most information on the response mechanisms of the host have utilised in vitro systems based on serum-mediated adherence of cells to the parasite surface (Mackenzie et al., 1981). In particular, adherence assays reveal information about the surfaces of parasites (Mackenzie et al., 1978). Recent evidence based on serum-dependent adherence between cat granulocytes and *B. pahangi* microfilariae has implicated a profound dependence on the stage of microfilarial maturation (Johnson et al., 1981). Microfilariae obtained from adult *B. pahangi* uteri did not possess the ability to adhere to complement-adherence-dependent granulocytes. Blood microfilariae, however, were fully able to participate with this class of serum-dependent granulocytes. Furthermore, in vivo-derived microfilariae bound the C3 component of complement, while in utero-derived microfilariae lacked C3 binding.

Although this paper does not present evidence linking the presence of specific sheath carbohydrates to the in vitro adherence phenomena, it is intriguing to hypothesize that such a correlation exists. The immunodominance of exposed surface carbohydrates on other biological membranes throws added weight to the possibility that sheath carbohydrate antigenic determinants are primary targets of the host's immune cells. Surface-associated sialic acid plays a critical role in the ability to activate the alternative complement pathway. Sheep red blood cells that contain an abundance of membrane terminal sialic acids are not complement activating, while rabbit red blood cells that are deficient in sialic acid are readily complement activating (Fearon, 1978; Pangburn and Müller-Eberhard, 1978; Kazatchkine et al., 1979). Similarly, complement-dependent macrophage phagocytosis of bacteria is dependent upon lipopolysaccharide and capsular carbohydrate composition (Edwards et al., 1982; Liang-Takasaki et al., 1982).

The interaction between microfilariae and the mosquito vector is even less understood than mammalian host and microfilariae interaction. It is possible that sheath carbohydrates are a component of the molecular trigger initiating exsheathment and development once the mosquito ingests its microfilariae-laden blood meal. The role of sheath carbohydrates in the recognition and nonrecognition of microfilariae by the mosquito vector is unknown. By analogy to evidence from molluscan hemolymph lectins and larval schistosome development (Anderson and Good, 1976), however, the possible interaction between mosquito lectins and sheath carbohydrates may determine the successful maturation of the microfilariae to infective third-stage larvae. A comparative study between mature and immature microfilariae, and the cellular or humoral mosquito immune response may yield information enabling development of a biological protocol that might alter the insect-dependent stage of development.

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Anderson R. S., Good R. A.: Opsonic involvement in phagocytosis by mollusk hemocytes. J. Invertebr. Path. *27*, 57–64 (1976).

Butts J. A., Rabalais F. C.: Successful jird-to-jird transfer of juvenile *Brugia pahangi*. J. Parasit. *60*, 436 (1974).

Devaney E., Howells R. E.: The exsheathment of *Brugia pahangi* microfilariae under controlled conditions in vitro. Ann. trop. Med. Parasit. *73*, 227–233 (1979).

Edwards M. S., Kasper D. L., Jennings H. J., Baker C. J., Nicholson-Weller A.: Capsular sialic acid prevents activation of the alternative complement pathway by type III, group B streptococci. J. Immunol. *118*, 1278–1283 (1982).

Fearon D. T.: Regulation by membrane sialic acid of β1H-dependent decay-dissociation of amplification C3 convertase of the alternative complement pathway. Proc. nat. Acad. Sci. *75*, 1971–1975 (1978).

Johnson P., Mackenzie C. D., Suswillo R. R., Denham D. A.: Serum-mediated adherence of feline granulocytes to microfilariae of *Brugia pahangi* in vitro: variations with parasite maturation. Parasite Immunol. *3*, 69–80 (1981).

Kazatchkine M. D., Fearon D. T., Austen K. F.: Human alternative complement pathway: membrane-associated sialic acid regulates the competition between B and β1H for cell-bound C3b. J. Immunol. *122*, 75–81 (1979).

Liang-Takasaki C.-J., Mäkelä P. H., Leive L.: Phagocytosis of bacteria by macrophages: changing the carbohydrate of lipopolysaccharide alters interaction with complement and macrophages. J. Immunol. *128*, 1229–1235 (1982).

Mackenzie C. D., Jungery M., Taylor P. M., Ogilvie B. M.: The in-vitro interaction of eosinophils, neutrophils, macrophages and mast cells with nematode surfaces in the presence of complement or antibodies. J. Path. *133*, 161–175 (1981).

Mackenzie C. D., Preston P. M., Ogilvie B. M.: Immunological properties of the surface of parasitic nematodes. Nature (Lond.) *276*, 826–828 (1978).

Nicolson G. L.: The interactions of lectins with animal cell surfaces. Int. Rev. Cytol. *39*, 89–190 (1974).

Pangburn M. K., Müller-Eberhard H. J.: Complement C3 convertase: cell surface restriction of β1H control and generation of restriction of neuraminidase-treated cells. Proc. nat. Acad. Sci. *75*, 2416–2420 (1978).

Rogers R., Ellis D. S., Denham D. A.: Studies with *Brugia pahangi*. 14. Intrauterine development of the microfilaria and a comparison with other filarial species. J. Helminth. *50*, 251–257 (1976).

Simpson M. G., Laurence B. R.: Histochemical studies on microfilariae. Parasitology *64*, 61–88 (1972).

Weiss N., Tanner M.: Studies on *Dipetalonema viteae* (Filarioidea). 3. Antibody-dependent cell-mediated destruction of microfilariae in vivo. Tropenmed. Parasit. *30*, 73–80 (1979).