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Objekttyp: **Article**

Zeitschrift: **Acta Tropica**

Band (Jahr): **44 (1987)**

Heft 1

PDF erstellt am: **13.09.2024**

Persistenter Link: https://doi.org/10.5169/seals-313817

Acta Tropica *44*, 35–42 (1987)

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Lectin-binding characteristics of *Wuchereria bancrofti* microfilariae[1]

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Summary

The binding of 10 different lectins to the surface of microfilariae of *Wuchereria bancrofti* has been investigated. Wheat germ agglutinin (WGA) and *Helix pomatia* lectin (HPA) bound specifically to the sheathed microfilariae indicating the presence of N-acetyl-D-glucosamine and N-acetyl-D-galactosamine respectively on the surface. Exsheathed microfilariae did not react with any of the lectins. Treatment of sheathed microfilariae with proteases resulted in increased binding of WGA and HPA. Such treated microfilariae showed a weak binding of Concanavalin A (Con A), and lectins of lentil (LCH) and of *Limulus polyphemus* (LPA). Sheathed microfilariae incubated with sera of people living in endemic zones of filariasis but with no apparent evidence of infection (endemic normals), or with sera of chronic elephantiasis patients, or with their respective gamma globulin fractions, bound Con A and LCH. These lectins bound weakly to exsheathed microfilariae under the same conditions. Binding was due to the mannose components of the specific immunoglobulins of the sera which coated the microfilariae. However, microfilariae when incubated with sera or their globulin fractions from non-endemic normals (NEN), or from microfilarial carriers, did not bind Con A and LCH, suggesting that specific immunoglobulins were neither present in NEN sera nor in significant amounts in sera of microfilarial carriers.

Key words: *Wuchereria bancrofti;* microfilariae; sheath; cuticle; lectins; carbohydrates; immunoglobulins.

[1] Contribution No. 801 from Hindustan Ciba-Geigy Research Centre

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Introduction

Available evidence suggests that the surface antigens of the filarial parasites act as targets for the host's immune attack. Substantial information is available on the dynamics of the surface components (Howells, 1980) and the presence of immunogenic proteins on the surface of different filarial worms (Maizels et al., 1982). Comparative studies and ultrastructural cytochemistry of *B. pahangi* and *D. immitis* microfilariae revealed that there is a thick outer covering of carbohydrate molecules on their surfaces (Cherian et al., 1980; Hammerberg et al., 1984; Sayers et al., 1984).

Recently, lectins have been used to identify the carbohydrates on the surface of microfilariae of *B. pahangi* (Furman and Ash, 1983a, b; Devaney, 1985), *B. malayi* (Kaushal et al., 1984) and of *Onchocerca gibsoni* (Forsyth et al., 1984).

The present communication deals with the lectin-binding characteristics of the microfilariae of the lymphatic filarial parasite, *Wuchereria bancrofti.*

Materials and Methods

Parasite

Live microfilariae (mf) of *W. bancrofti* were isolated from night blood samples by iso-osmotic Percoll-sucrose gradient technique as described by Chandrashekar et al. (1984). After separation the mf were washed 3 times in RPMI 1640 medium (GIBCO Diagnostics), pH 7.2. Mf stored at –70°C were also tested for their lectin-binding activity. Mf that spontaneously lost the sheath on incubation in RPMI 1640 at 4° for 24 h or mf that were stripped of the sheath by incubation with type III protease (Sigma) (Devaney and Howells, 1979) were the source of exsheathed mf. The final concentration of mf was adjusted to 200 parasites per 50 µl of the medium.

Lectins

The lectins used and their affinity to specific carbohydrates are listed in Table 1. All the lectins and the carbohydrates were obtained from Sigma Chemical Company, USA. While WGA, Con A, SBA, DBA and UEA were obtained in the fluoresceinated form (FITC conjugates), HPA, LCH, LPA, PNA and RCA were labelled with fluorescein dye by the method of Clarke and Hoggart (1982).

Sera

Sera from people living in endemic areas of filariasis with no apparent evidence of infection (endemic normals, EN); from mf-carriers (mf+) and from chronic, symptomatic elephantiasis patients (EL) were collected and stored at –70°C until use. Sera from non-endemic normals (NEN) were obtained from West Germany through the courtesy of Dr. Zahner. Gamma globulins were prepared from the sera by ammonium sulphate fractionation by the method of Hudson and Hay (1980) and were made up to the original concentration with RPMI 1640. The globulin fractions were stored at –70°C until use.

Treatment of mf

Enzymes. Mf were exposed to different enzymes in order to further characterize any additional lectin binding sites. Trypsin from bovine pancreas (Serva Biochemicals), pronase (Calbiochem), protease type I or protease type VIII (*Bacillus subtilis*) (Sigma) were employed at a concentration of 50 µg/ml for 30 min at 37°C. Mf were treated with neuraminidase (*Clostridium perfringens.* Sigma) at a final concentration of 1 IU/ml in RPMI 1640, pH 7.2 for 1 h at 37°C.

Diethylcarbamazine (DEC). Mf were treated with DEC at a final concentration of 1 mg/ml for 1 h at 37°C.

Table 1. Fluoresceinated (FITC)-lectins used for binding to *W. bancrofti* microfilariae

Lectin	Abbreviation	Sugar specificity
Concanavalin A	Con A	α-methyl D-mannoside
Lentil lectin	LCH	α-methyl D-mannoside
Dolichos biflorus agglutinin	DBA	N-acetyl-D-galactosamine
Helix pomatia agglutinin	HPA	N-acetyl-D-galactosamine
Soyabean agglutinin	SBA	N-acetyl-D-galactosamine
Limulus polyphemus agglutinin	LPA	Sialic acid
Peanut agglutinin	PNA	D-galactose
Ricinus communis agglutinin	RCA	D-galactose
Ulex europaeus agglutinin	UEA	L-fucose
Wheat germ agglutinin	WGA	N-acetyl-D-glucosamine

Human sera. 1000 mf (sheathed or exsheathed) were preincubated with 500 μl of 10 different samples of undiluted sera from NEN, mf+, EN and EL cases for 1 h at 37 °C. Similarly, the mf were incubated separately with respective gamma globulins from pooled sera of NEN, mf+, EN and EL cases for 1 h at 37 °C. Serum globulins interact readily with sheathed mf within an hour as evidenced by the binding of FITC-goat anti-human immunoglobulin (Wellcome) whereas they bind very slowly to exsheathed mf. Therefore, the exsheathed parasites were incubated for up to 20 h with sera or globulin fractions. After the various treatments the mf were washed 3 times in RPMI 1640 and used in the lectin binding experiments.

Binding of lectins to mf

The mf from stock solution were centrifuged and the supernatant removed. They were incubated for 30 min at room temperature (25 °C) with 100 μl of FITC-lectins (100 μg/ml) dissolved in RPMI 1640 medium in 1 ml polycarbonate tubes in the dark. The mf suspension was then washed 3 times with RPMI 1640 and a small aliquot was examined on a slide under a Leitz fluorescence microscope. A minimum of 50 mf were observed and the experiment was done in duplicate for each lectin. The control tubes, containing mf with inhibitory sugars (Table 1) at a final concentration of 200 mM and FITC-lectins, were examined simultaneously to ensure the specificity of the lectin binding.

Results

Lectin-binding to mf of W. bancrofti

Preliminary experiments revealed that the binding of lectins was apparent within 3 min of incubation with mf, reaching a maximum in 30 min. The lectins readily reacted with the parasite surface at 4 °C, 25 °C or at 37 °C. Therefore all incubations were carried out for 30 min at room temperature. Also, the lectin reactions with live or dead mf (mf stored at −70 °C) were similar. Table 2 shows the results of the binding capacity of the tested lectins to the mf. Only WGA and HPA bound to the sheathed mf suggesting the presence of N-acetyl D-glucosamine and N-acetyl D-galactosamine moieties respectively on the surface of the sheathed mf. Preincubation of WGA with N-acetylglucosamine or HPA with N-acetyl galactosamine inhibited the binding of the lectins to the mf. However, preincubation of WGA with N-acetyl galactosamine or HPA with N-acetyl

Table 2. Binding of FITC-lectins with sheathed, exsheathed and dead microfilariae (mf) of *W. bancrofti*

Lectins[1]	Degree of fluorescence[2]		
	sheathed mf (live)	exsheathed mf (live)	sheathed mf (dead)[3]
WGA	+++	–	+++
HPA	+++	–	+++
SBA	–	–	–
DBA	–	–	–
Con A	–	–	–
LCH	–	–	–
LPA	–	–	–
PNA	–	–	–
RCA	–	–	–
UEA	–	–	–

[1] Lectins were incubated with mf at 100 μg/ml for 30 min at room temperature. Experiments were done in duplicate.
[2] The degree of fluorescence has been graded by visual fluorescence rating: – = nil; + = weak; ++ = moderate; +++ = bright; ++++ = intense.
[3] Mf stored at –70°C for 1 month or longer were used in the assay.

Table 3. Binding of FTIC-lectins with *W. bancrofti* microfilariae treated with proteolytic enzymes

Enzymes*	WGA	HPA	SBA	DBA	Con A	LCH	LPA	PNA	RCA	UEA
Trypsin	++++	++++	–	–	–	–	–	–	–	–
Pronase	++++	++++	–	–	+	+	+	–	–	–
Protease type I (pancreatic crude)	++++	++++	–	–	+	+	+	–	–	–
Protease type VIII (*Bacillus subtilis*)	++++	++++	–	–	+	+	+	–	–	–
RPMI	+++	+++	–	–	–	–	–	–	–	–

* Enzymes were used at 50 μg/ml in PRMI 1640 for 30′ at 37°C.
Lectins were employed at 100 μg/ml. The experiments were done in duplicate.

glucosamine did not interfere with the binding of the lectins to the mf, confirming the lectin specificities.

Effect of enzyme treatment on lectin-binding

Microfilariae treated with proteolytic enzymes exhibited more intense fluorescence with WGA and HPA (Table 3). Furthermore, the parasites treated with pronase or proteases types I and VIII showed scattered and randomly

Table 4. Effect of various sera and their gamma globulins on lectin-binding to *W. bancrofti* microfilariae

Treatment of mf with sera	FITC-anti-human immuno-globulin	WGA	Con A	LCH	HPA	SBA	DBA	PNA	RCA	LPA	UEA
None	–	+++	–	–	+++	–	–	–	–	–	–
Nonendemic normals (NEN) sera	–	+++	–	–	+++	–	–	–	–	–	–
Gamma globulin	–	+++	–	–	+++	–	–	–	–	–	–
Endemic normals (NEN) sera	+++	++++	++++	++++	++++	–	–	–	–	–	–
Gamma globulin	+++	++++	++++	++++	++++	–	–	–	–	–	–
Mf carrier sera	–	+++	–	–	+++	–	–	–	–	–	–
Gamma globulin	–	+++	–	–	+++	–	–	–	–	–	–
Elephantiasis cases (EL) sera	+++	++++	++++	++++	++++	–	–	–	–	–	–
Gamma globulin	+++	++++	++++	++++	++++	–	–	–	–	–	–

Experiments were done in duplicate.
FITC-Anti-human immunoglobulins were used in a 1:10 dilution. Lectins were used at 100 μg/ml concentration.

distributed weak fluorescence on incubation with fluoresceinated Con A, LCH and LPA, indicating the presence of exposed mannose and sialic acid molecules. Occasionally, either excretory or anal pores showed weak fluorescence with these lectins. Neuraminidase and DEC treatments had no effect on the lectin-binding characteristics of the mf.

Effect of sera treatment on lectin-binding

The results on lectin-binding of mf treated with sera from NEN, EN, mf+ and EL cases are shown in Table 4. Treatment of the mf with sera from EN and EL lead to increased binding of WGA and HPA. In addition, there was binding of Con A and LCH to the parasite surface, indicating the presence of exposed mannose moieties. This was further confirmed by inhibition of the fluorescence by D-mannose. The mannose residues seem to be part of the specific immunoglobulins of the sera that coated the parasite surface. Indeed, incubation of mf treated with these sera showed positive fluorescence with FITC-goat anti-human immunoglobulins. Mf+ and NEN sera did not alter the lectin-binding characteristics. Similar observations were recorded when isolated gamma globulins from these sera were incubated with the mf (Table 4). An average of 8/10 sera from EL cases and 7/10 from EN cases showed positive reactivity with Con A and LCH. When exsheathed mf were incubated with sera from EN and EL cases, even for 20 h, they only exhibited weak binding (+) with Con A and LCH lectins (data not shown).

Discussion

The mf of *Wuchereria bancrofti* possess N-acetyl glucosamine and N-acetyl galactosamine on their sheath surface, as revealed by the lectin-binding studies in the present experiments. SBA and DBA, although specific for N-acetyl galactosamine, did not react with the mf, nor did they inhibit the binding of HPA to the mf after preincubation with the parasites. Although lectins from different sources have specificity to the same carbohydrate, variations have been observed in their binding when the saccharide is presented as part of a macromolecular complex (Goldstein and Hayes, 1978; Linder and Huldt, 1982). More recently, Linder (1985) observed that SBA but not DBA bound to the secretions and the body of *Schistosoma mansoni* cercariae, although both lectins have affinity to N-acetyl galactosamine. It is interesting to note that mf treated with proteolytic enzymes showed more intense fluorescence with WGA and HPA, possibly due to the exposure of more of the carbohydrate residues. The brighter fluorescence seen when the mf were treated with sera from EN and EL cases and subsequently with WGA, Con A, LCH and HPA, might be due to the presence of glucosamine, galactosamine residues of the antibodies that coated the mf. The presence of such antibodies on the mf treated with the sera was confirmed by treatment with FITC-anti-human immunoglobulins. The absence of such an

effect, when the mf were treated with sera from NEN and mf+, suggests the lack of specific antibodies. Recently, Hammerberg et al. (1984) demonstrated the presence of neutral, negatively-charged sugars on the sheath surface of *B. pahangi* and binding of immunoglobulins to the sheath.

WGA lectin also interacts with sialic acid residues (Bhavanandan and Katlic, 1979). However, the present study revealed that WGA interaction was due to N-acetyl glucosamine since neuraminidase treatment did not affect its binding to the mf. Similar observations were made with *B. malayi* mf by Kaushal et al. (1984). The weak fluorescence seen with Con A, LCH and LPA when the mf were pretreated with pronase and protease types I and VIII, was indicative of the presence of D-mannose and sialic acid residues on the sheath. The cuticle of enzymatically or normally exsheathed mf exhibited a weak fluorescence with Con A and LCH after prolonged incubation with sera from EN and EL cases. This could be due to the low antibody levels existing in these sera against the cuticle of the mf components.

Carbohydrate residues may be part of the glycoproteins of the surface of sheathed mf. Recently, Furman and Ash (1983a, b) suggested that carbohydrates of the sheath of mf may serve as a component of the molecular trigger initiating exsheathment and development in the mosquito-vector. In such an event, proteases of the mosquito may aid in exposing the carbohydrate moieties, promoting the interaction with the lectin-like receptors in the midgut, and facilitate exsheathment (Devaney, 1985). These glycoproteins may also elicit immune responses in the host, but a more detailed knowledge of host-parasite interactions is required to determine more precisely the functions of the glycoproteins of the mf.

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