Cercaria–schistosomulum surface transformation of *Trichobilharzia szidati* and its putative immunological impact

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SUMMARY

Schistosome cercariae of the genus *Trichobilharzia* are the causative agent of swimmers' itch. In order to characterize the changes in parasites during and after the penetration of the host skin, *in vitro* and *in vivo* (in ducks and mice) transformations of *T. szidati* cercariae to schistosomula were performed. Ultrastructural observation revealed that cercariae possess a simple outer tegumental membrane with a thick glycocalyx. As with human schistosomes, the latter structure disappears during transformation and a new double membrane with putative protective function is formed. Our biochemical and immunological observations showed that the carbohydrate-rich glycocalyx of cercariae is readily bound by lectins and antibodies. The *in vitro* transformation to schistosomula can be detected by enhanced reactivity of 2 lectin probes (PNA and ConA) with the surface. The *in vivo*-transformed (skin and lung) schistosomula appear to have few surface ligands for the 12 lectin probes being tested. Similarly, the cercarial surface and its remnants on the *in vitro*-produced schistosomula is recognized by sera from immunized mice and humans with cercarial dermatitis; the tissue schistosomula fail to react with these antibodies. The loss of surface targets as a part of parasite immune evasion within the host is discussed.

Key words: *Trichobilharzia*, schistosome, cercarial dermatitis, tegument, glands, penetration.

INTRODUCTION

Penetrating cercariae of the genus *Schistosoma* undergo rapid surface transformation in order to become schistosomula and, as a consequence, to resist host immune reactions (complement attack, cytotoxic reactions, etc.) (for reviews see Pearce & Sher (1987); Abath & Werkhauser (1996)). The parasite surface changes at both the ultrastructural and molecular levels (for review see Wilson (1987)). These changes enable the establishment of the parasite within the host. From this viewpoint, the cercaria/schistosomulum transformation and the early developmental adaptations within the host seem to be the crucial steps for parasite survival. Cercariae of another schistosome genus – *Trichobilharzia* – emerge from the snail intermediate host and enter the skin of the definitive host, usually a water bird, where they mature. The cercariae can also invade an accidental human host causing cercarial dermatitis. In this case, the parasite usually dies soon after the penetration.

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Although migration and developmental success of the parasite in the duck after primary infection and reinfection has been described (Bourns, Ellis & Rau, 1973; Ellis, Bourns & Rau, 1975; Rau, Bourns & Ellis, 1975) and some data on migration (Haas & Pietsch, 1991) or immune (mainly cell) response against larvae (Haemmerli, 1953) in an abnormal host exist, the precise role of parasite evasion/host immunity in *Trichobilharzia* infections needs to be clarified. The penetration of *Trichobilharzia* cercariae including the action of penetration glands seems to be essentially the same as in *S. mansoni* (Bourns *et al.* 1973). Both genera are able to secrete similar types of eicosanoids, products of fatty acid oxidation involved probably in vasodilatation and immunosuppression (Nevhutalu *et al.* 1993). Similarly, based on serological tests, both genera share certain antigenic epitopes triggering production of host antibodies (Kolářová, Sýkora & Bah, 1994). On the other hand, *T. ocellata* in an abnormal host stimulates, mainly after the second challenge (Haemmerli, 1953), a pronounced skin inflammatory reaction unlike *S. mansoni* which produces an anti-inflammatory factor (Ramaswamy *et al.* 1996). Measuring the speed of tissue migration in the vertebrate hosts, schistosomula of *T. ocellata* evade

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the host immune response probably within a shorter time and migrate faster than do those of *S. mansoni* (Haas & Pietsch, 1991).

In Central Europe, *T. szidati* is the most common species of the genus (Kolářová, Horák & Fajfrlík, 1992). As the parasite can easily be maintained in the laboratory, we used this schistosome in our experiments. In order to study the host–parasite interactions in cercarial dermatitis and keeping in mind that the parasite's surface may serve as an important target of host immune reactions, we focused our attention on surface characteristics. Three criteria for cercaria/schistosomulum developmental changes have been adopted: the ultrastructure of the tegument, the presence of carbohydrates (lectin targets) and antigenic epitopes (immunoglobulin targets) on the parasite's surface.

MATERIALS AND METHODS

Trichobilharzia szidati Neuhaus, 1952 (strain SB-II) was isolated in South Bohemia in 1996 and subsequently maintained as a laboratory model organism in the duck *Anas platyrhynchos f. domestica* and the snail *Lymnaea stagnalis* as definitive and intermediate hosts, respectively (Meuleman, Huyer & Mooij, 1984). In order to facilitate detachment of cercarial tails, the emerged cercariae were repeatedly passed through a syringe needle (0·7 mm in diameter). Then, the *in vitro* cercaria/schistosomulum transformation was performed in 300 mOsm phosphate buffer at 39 °C for 5 h (Samuelson & Stein, 1989). Stages showing a positive reaction with ConA/PNA probes (see below; Horák, 1995) were considered to be transformed. In this case, 5-h-old cercariae kept in water at room temperature served as a control. *In vivo* transformations were undertaken in ducks (Meuleman *et al*. 1984) or laboratory mice (BALB/c strain; Christensen, Gotsche & Frandsen (1984)) after skin penetration by parasites.

The ultrastructural changes were evaluated in cercariae and *in vitro* (5 h) and *in vivo* (12 h – mouse skin, 5 days – duck lungs) transformed schistosomula. The material was fixed in 2·5 % (v/v) glutaraldehyde in Hepes buffer (150 mM NaCl, 400 mM Hepes, pH 7·8) supplemented with 1 % (w/v) tannic acid, 1 mM $MgCl_2$ and 1 mM $CaCl_2$ (overnight at 4 °C). Post-fixation was in 1 % (w/v) OsO_4 in 300 mM Hepes (pH 7·8) supplemented with 1 % (w/v) $K_4[Fe(CN)_6]$ for 1 h at 4 °C and subsequently, 1 % (w/v) uranyl acetate in 70 % (v/v) ethanol was applied for 1 h at room temperature. The material was embedded in Durcupan resin (Fluka) and thinly sectioned at 70 nm. Before evaluation, the sections were conventionally stained with uranyl acetate and lead citrate.

For histological examination, the larvae were fixed in Bouin's fixative (Danguy & Gabius, 1993) overnight at 4 °C, washed 3 times (3 × 20 min) in 70 % ethanol and after washing in TBS (Tris-buffered saline; 20 mM Tris, 150 mM NaCl, pH 7·8), the larvae were embedded in JB4-resin (Polysciences, Inc.). The following material was used for immuno- and affinity fluorescence: cercariae, *in vitro* transformed schistosomula (5 h), *in vivo* transformed schistosomula – skin of mice (1, 5 and 12 h p.i.), lungs of mice (3 days p.i.), skin of ducks (12 h p.i.), lungs of ducks (5 days p.i.). In addition, the hepatopancreas of infected *Lymnaea stagnalis* (with patent *T. szidati* infection) was used for certain immunofluorescence observations.

A panel of 12 fluorescein-conjugated lectins was used in lectin-binding studies (inhibitors in parentheses): PSA (*Pisum sativum*, methyl-α-D-mannopyranoside), BS-II (*Bandeiraea simplicifolia*, *N*-acetyl-D-glucosamine), PWM (*Phytolacca americana*, oligomeric *N*-acetyl-D-glucosamine), PNA (*Arachis hypogaea*, D-galactose), UEA-I (*Ulex europaeus*, L-fucose) and LTA (*Tetragonolobus purpureas/Lotus tetragonolobus*, L-fucose) were purchased from Sigma, while LCA (*Lens culinaris*, methyl-α-D-mannopyranoside), Con A (*Canavalia ensiformis*, D-mannose), WGA (*Triticum vulgaris*, oligomeric *N*-acetyl-D-glucosamine), RCA-I (*Ricinus communis*, lactose), HPA (*Helix pomatia*, *N*-acetyl-D-galactosamine) and SBA (*Glycine max*, *N*-acetyl-D-galactosamine) were obtained from Lectinola (Prague). The sections (3 μm thickness) were blocked with 1 % bovine serum albumin (BSA) in TBS for 1 h and subsequently lectins (100 μg/ml in TBS with/without 1 mM $CaCl_2$ and 1 mM $MnCl_2$) in the presence/absence of appropriate saccharide inhibitors were applied for 30 min (Horák, 1995).

Concerning the immunofluorescence tests, sera of humans with repeatedly diagnosed cercarial dermatitis (Czech origin) and sera from mice exposed to *T. szidati* cercariae were used. In the first case, the sera from healthy humans served as a control. In the latter case, mice (BALB/c) were repeatedly exposed to approximately 1400 cercariae of *T. szidati* on days 0, 7, 14 and 20. Sera were obtained before the first exposure (control sample) and then on days 8, 15 (2 interim samples) and 24 (final sample). Histological sections were blocked with 1 % BSA in TBS for 1 h. The sera were diluted 1:100 and applied on sections for 30 min. After washing in TBS, the fluorescein-conjugated swine anti-mouse (against total Ig obtained from Sevac, Prague; against particular Ig classes supplied by Sigma) or anti-human (Sevac, Prague) immunoglobulins were diluted 1:100 and applied on sections for 30 min. The polyclonal antibodies raised in BALB/c mice against *T. szidati* cercarial haemagglutinins (Horák *et al*. 1997) diluted 1:100 were also tested; the serum from the same mice before immunization served as a control. As the

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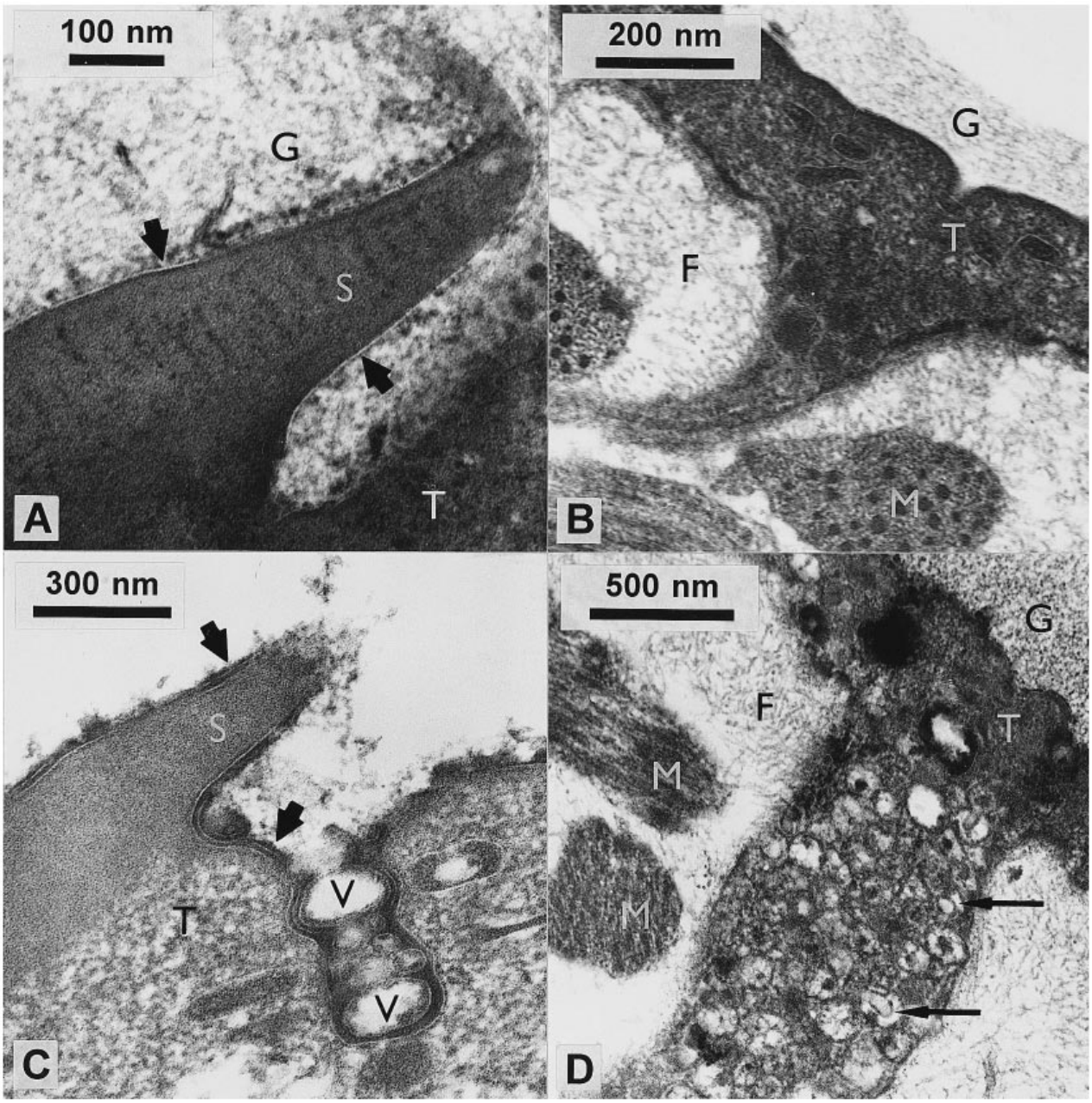

Fig. 1. Transmission electron micrographs comparing the tegument of *Trichobilharzia szidati* cercariae (A and B) and *in vitro* transformed schistosomula (C and D). The simple outer membrane (short arrows) of the cercariae (A) is gradually replaced by a double membrane (short arrows) in schistosomula (C). This is caused by fusion of membrane vesicles with the outer membrane; numerous multimembrane vesicles (long arrows) are transported through cytoplasmic connections of schistosomula (D), whereas the connections of cercariae are either free of vesicles or filled with few electron-dense bodies (B). G, glycocalyx; T, tegument; F, fibrous layer; M, muscle layer; S, spine; V, vesicle.

cercarial glands contain a lectin which is able to bind certain host immunoglobulins *via* their carbohydrate residues and interfere in this way with immunodetection (Horák *et al*. 1997), all incubations in our immunoassay were done in the presence of 2·5 mg/ml laminarin (a ligand blocking the lectin binding sites).

All fluorescence experiments were performed 3 times at room temperature. All chemicals were purchased from Sigma Chemical Company unless stated otherwise.

RESULTS

Ultrastructural observations revealed that the surface of cercariae (Fig. 1A,B) is covered by a trilaminate outer membrane which is protected from the environment by a thick glycocalyx. Tegumental spines, discoid bodies and electron-dense vesicles can be found within the tegument. Cytoplasmic processes are either free of vesicles or contain 1 or 2 morphological types of electron-dense bodies.

During transformation *in vitro* (Fig. 1C,D), the

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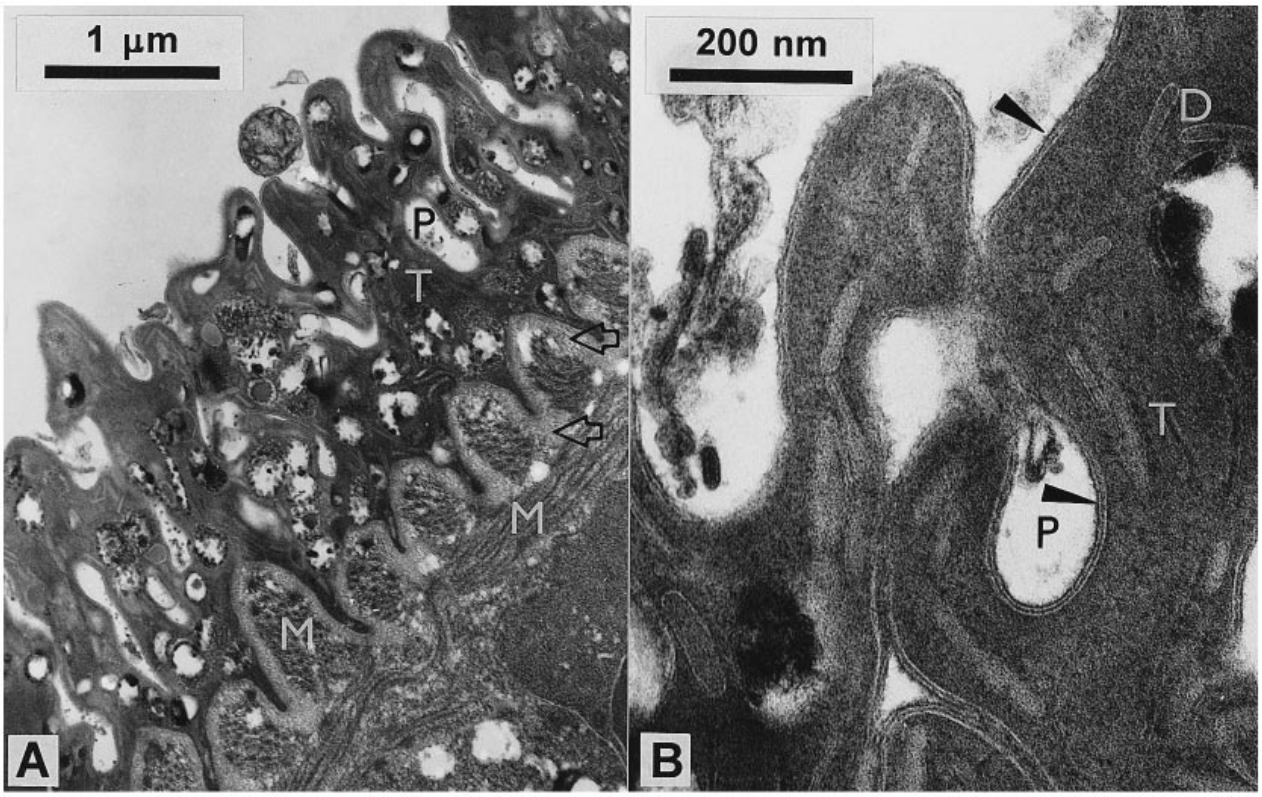

Fig. 2. Transmission electron micrographs of the tegument of *Trichobilharzia szidati* lung schistosomula isolated from the duck host. (A) The gross morphology of the tegument shows pronounced tegumental ridges and pits which markedly enlarge the surface. The basal tegumental membrane is also invaginated and lined by the fibrous layer (open arrows). (B) The entire surface is covered by a double membrane (arrowheads). T, tegument; P, pit; M, muscle layer; D, discoid body.

Table 1. Reaction of fluorescein-labelled lectins with surfaces of different larval stages of *Trichobilharzia szidati*

(++ Strong fluorescence; + moderate fluorescence; (+) weak fluorescence; − no fluorescence; ++/− (+/−) a part of larvae, depending on the transformation rate, exhibited different reaction.)

			Schistosomulum						
	Cercaria		*In vitro*	In mouse				In duck	
Lectin	Body	Tail	5 h	1 h*	5 h*	12 h*	3 day†	12 h*	5 day†
PSA	++	++	++	+/−‡	−	−	−	+	(+)
LCA	+	+	−	−	−	−	−	−	(+)
ConA	−	−	++	(+)	−	−	−	+	−
BS-II	−	−	−	−	−	−	−	+	−
PWM	(+)‡	(+)‡	+‡	−‡	−	−	−	+‡	−
WGA	++‡	−	++‡	(+)‡	−	−	−	(+)‡	−
RCA-I	++‡	++‡	+‡	+/−‡	−	−	−	(+)	−
PNA	−	−	++	+/−	−	−	−	+	−
HPA	−	−	−	−	−	−	−	+‡	−
SBA	+	−	−	−	−	−	−	+	−
UEA-I	++	++	++	++/−‡	−	−	−	+‡	−
LTA	++	++	++	+/−	−	−	−	(+)	−

* Skin schistosomula.
† Lung schistosomula.
‡ The appropriate (specific) carbohydrate did not block the lectin binding.

glycocalyx disappears. Many vesicles with different degrees of compaction, apparently formed in the cytons, emerge in cytoplasmic connections; most of them are limited by a double membrane and some contain additional membrane structures (Fig. 1D). These vesicles migrate into the syncytial tegumental

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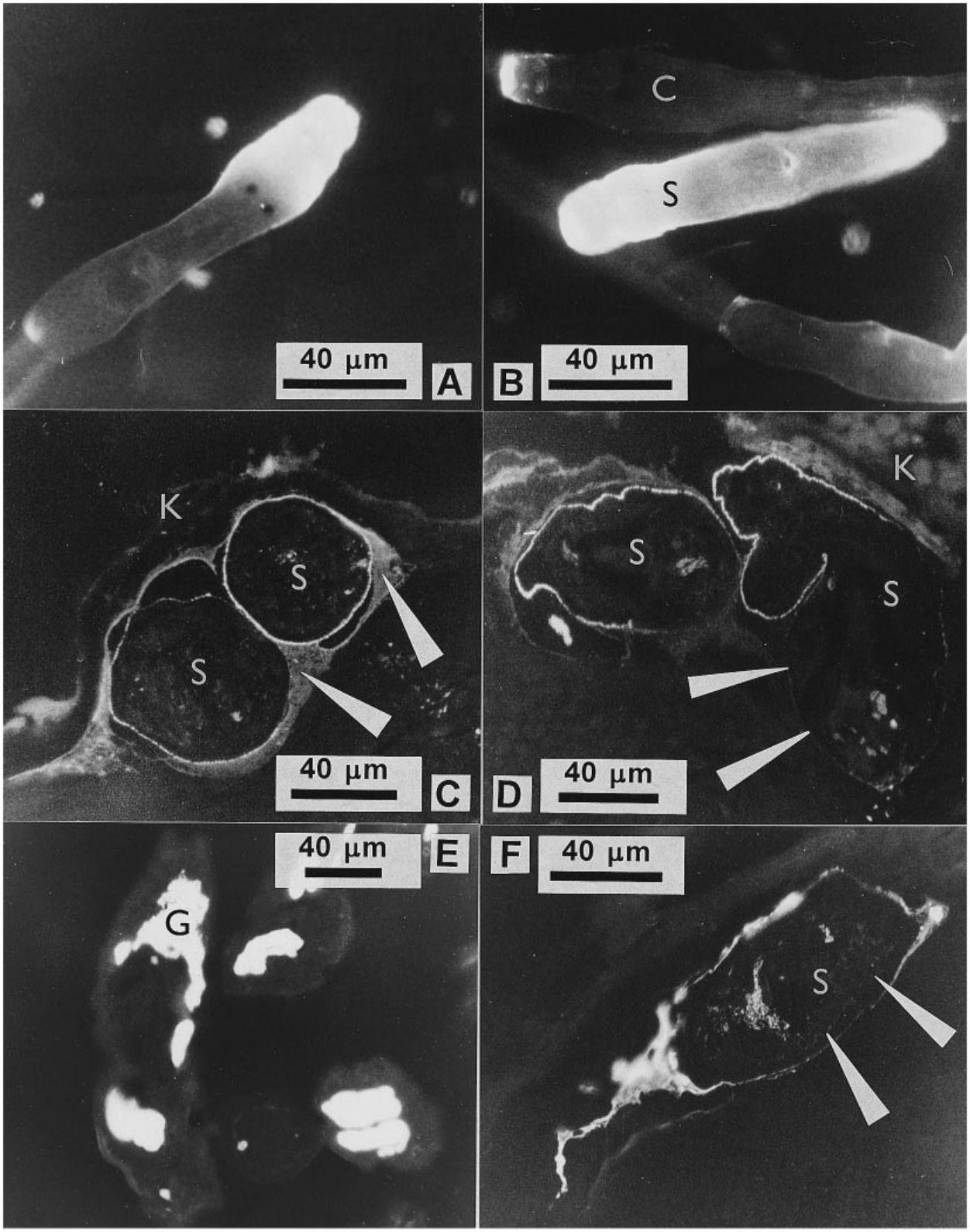

Fig. 3. Transformation of *Trichobilharzia szidati* cercariae to schistosomula detected by fluorescence reaction. (A) Binding of PNA shows that the *in vitro* transformation starts at the head end of the cercaria. (B) Subsequently, the entire surface of the parasite reacts with PNA, whereas certain cercariae are delayed in the transformation; the detached/non-detached tails show no reaction. (C) Using mouse antibodies against cercarial haemagglutinins, the skin schistosomula after 1 h show a pronounced surface reaction similar to that of cercariae; the discharged gland content also reacts with these antibodies (arrowheads). (D) The surface reaction subsequently disappears; this process starts at the head end again (arrowheads). (E) HPA reacts exclusively with cercarial post-acetabular glands and their ducts. (F) As the skin schistosomula (arrowheads show the unlabelled surface) and the host tissue are negative after HPA staining, the gland deposits lining the entry tunnel and surrounding the transforming schistosomulum can easily be detected, C, cercaria; S, schistosomulum; K, skin surface layer; G, penetration glands.

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Table 2. Reaction of sera from BALB/c mice repeatedly infected by *Trichobilharzia szidati* cercariae

(+ + Strong fluorescence; + moderate fluorescence; − no fluorescence; N.D., experiment not performed; + +/− (+/−) a part of larvae, depending on the transformation rate, exhibited different reaction; anti-CA polyclonal antibodies of BALB/c mice raised against *T. szidati* cercarial haemagglutinins (this reaction is shown for comparison).)

			Schistosomulum							
	Cercaria		*In vitro* (5 h)		In mouse				In duck	
Antibody	Surface	Glands	Surface	Glands	1 h*	5 h*	12 h*	3 day†	12 h*	5 day†
IgA‡	−	−	−	−	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
IgA§	−	−	−	−	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
IgM‡	+‖	−	+/−‖	−	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
IgM§	−	−	−	−	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
IgG‡	+ +	−	+ +/−	−	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
IgG§	+ +	−	+ +/−	−	−	−	−	−	−	−
Total Ig‡	+ +	−	+ +/−	−	+/−	−	−	−	−	−
Total Ig§	+ +	−	+ +/−	−	+/−	−	−	−	−	−
Control	−	−	−	−	−	−	−	−	−	−
Anti-CA	+ +	+	+ +	+	+ +/−	−	−	−	−	−

* Skin schistosomula.
† Lung schistosomula.
‡ Interim samples (day 8 and 15).
§ Final sample (day 24).
‖ Reaction of IgM from the second interim sample (day 15); the first interim sample (day 8) did not recognize the larvae.

layer and fuse with the surface membrane, liberating their contents (Fig. 1C). The former surface membrane is, in some areas, replaced by a double-membrane structure which appears to be either pentalaminated or heptalaminated. On the surface, among remnants of the glycocalyx, some free multimembrane blebs can be observed. At certain places, deep invaginations (pits) of the outer tegumental surface start to form.

The skin and lung schistosomula (Fig. 2A,B) possess a fully formed surface double membrane and no glycocalyx. The entire surface layer of lung schistosomula has numerous ridges and pits with a high number of inclusions, discoid bodies and multimembrane blebs.

Reaction of labelled lectins with particular developmental stages (Table 1) confirmed our former results that *in vitro* transformation can be detected by enhanced reaction of 2 probes (ConA and PNA) with the surface. As a new observation, this ConA/PNA binding appears first on the head end of the cercaria/schistosomulum body (Fig. 3A) and then it expands over the entire body (Fig. 3B). Control cercariae (i.e. incubated for 5 h in water) and detached/non-detached tails of transforming worms remain negative. The other lectin probes bind to the *in vitro*-transformed schistosomula either in the same manner or with lowered intensity as compared with the cercariae. In mice infection, a decrease in lectin ligands was detected on skin larvae after 1 h p.i. In subsequent stages (skin – 5 h and 12 h p.i.; lungs – 3 days p.i.), surface targets of lectin probes fully disappeared. In ducks, the skin schistosomula, although still retaining some lectin targets, show a tendency to reduce surface carbohydrates; the lung schistosomula are nearly unrecognizable by lectin probes.

Concerning the mouse antibody response to *Trichobilharzia* developmental stages (Table 2), total Ig and IgG exhibit, in final as well as interim sera samples, strong reaction with the surface of cercariae and some *in vitro*-transformed schistosomula. The antigens are already present on mature cercariae within sporocysts in the infected snail hepatopancreas, as was detected by reaction with *Trichobilharzia* antiserum and FITC-labelled anti-total Ig antiserum. On the other hand, immune IgM and IgA do not bind to the above stages; only immune IgM from the second interim serum (day 15) reacts moderately/weakly with the surface of cercariae and *in vitro* schistosomula. Penetration glands or their remnants exhibit no reaction in both cercariae and *in vitro* schistosomula using immune mouse sera. Except disappearing reactivity of 1 h skin schistosomula (in mice) with immune sera (total Ig), the skin and lung schistosomula transformed either in mice or in ducks do not express any reactive epitopes for mouse immunoglobulins being tested. Antibodies raised against cercarial haemagglutinins recognize the surface and glands of cercariae, *in vitro*-transformed schistosomula, and partly the surface of skin larvae after 1 h in mice (Fig. 3C,D). The surface antigens are shed first from the anterior end of the parasite (Fig. 3D) and then, from the entire surface. After that, the anti-haemagglutinin antibodies do not react with the parasite.

In the case of humans, sera from persons with a history of repeated cercarial dermatitis and from

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Table 3. Reaction of human sera with different larval stages of *Trichobilharzia szidati*

(++ Strong fluorescence; + moderate fluorescence; (+) weak reaction; − no fluorescence.)

			Schistosomulum							
	Cercaria		*In vitro* (5 h)		In mouse				In duck	
Antibody	Surface	Glands	Surface	Glands	1 h*	5 h*	12 h*	3 day†	12 h*	5 day†
Dermatitis										
IgA	−	−	−	−	−	−	−	−	−	−
IgM	−	−	−	−	−	−	−	−	−	(+)
IgE	−	−	(+)	−	−	−	−	−	−	(+)
IgG	++	+	++	+	−	−	−	−	−	−
Ig total	++	+	++	+	−	−	−	−	−	(+)
Control										
IgA	−	−	−	−	−	−	−	−	−	−
IgM	−	−	−	−	−	−	−	−	−	−
IgE	−	−	−	−	−	−	−	−	−	−
IgG	−	+	−	+	−	−	−	−	−	−
Ig total	−	++	−	++	−	−	−	−	−	−

* Skin schistosomula.
† Lung schistosomula.

healthy humans were tested (Table 3). Little or no reaction was obtained in all developmental stages with examined sera and labelled anti-IgA, IgM and IgE antibodies. On the other hand, total Ig and IgG of humans with dermatitis specifically recognized the surface of cercariae and *in vitro*-transformed schistosomula; no reaction was obtained with the skin and lung schistosomula.

The route of penetrating cercariae in mouse skin (1 h p.i.) was detected by HPA lectin. The surface of cercariae as well as that of 1 h schistosomula does not react with this lectin; the same is true for tissue (skin) reactivity. On the other hand, the post-acetabular penetration glands show a strong reaction (Fig. 3E). The content of discharged glands lines the entry tunnel and surrounds transforming schistosomes (Fig. 3F). The released gland content can also be detected by the anti-haemagglutinin antibodies (Fig. 3C).

DISCUSSION

Ultrastructurally the *Trichobilharzia* cercaria/schistosomulum transformation resembles that of human schistosomes (see Hockley & McLaren, 1973). *T. szidati* cercariae are protected against the outer hypo-osmotic environment by a thick glycocalyx. The syncytial tegumental layer possesses a simple outer membrane. The transformation to schistosomula is a rapid process; the *in vitro* 5 h schistosomula possess a partly formed surface double membrane with traces of glycocalyx, the 12 h schistosomula in mouse skin have a fully developed double membrane and no glycocalyx. Generally, the double membrane of schistosomes should have an important immunoprotective function (Abath & Werkhauser, 1996). The morphologically complicated surface of lung schistosomula with ridges, pits and inclusions implies that this part of the parasite body possesses considerable metabolical activity.

The transformation also can occur under *in vitro* conditions, i.e. in a defined medium without influence of host factors. This experimental design allows detection of the parasite's own carbohydrates/antigens. Although transformation can be detected by lectin probes (ConA, PNA) (for *S. mansoni* see Wiest, Kossmann & Tartakoff (1989)), ultrastructural observations show that the surface layer is only beginning reconstruction and is far from being completely formed (exclusively a mosaic of simple and double membranes has been found). It can, therefore, be deduced that the chemical changes in the surface may precede the ultrastructural ones (see also below). This *in vitro* ultrastructural delay *versus* lectin binding is in accordance with findings in human schistosomes (Wiest *et al.* 1989) and underlines the limitation of simple salt-based media application. The disappearance of ConA/PNA reactivity in the later developmental stages implies that the surface reactivity is a transient phenomenon; this corresponds with the situation in ConA binding to human schistosomes (Samuelson, Caulfield & David, 1982).

Schistosome surface carbohydrates may serve as components of antigenic epitopes (Dunne, 1990). However, our tests with 12 lectin probes provided evidence that the tissue schistosomula rapidly reduce and subsequently totally eliminate carbohydrate targets for lectins on their surface. On the ultrastructural level, this process is accompanied by a loss of glycocalyx. As the glycocalyx of human schistosomes is an activator of the alternative complement

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pathway (Marikovsky, Fishelson & Arnon, 1988), its shedding (also in *Trichobilharzia*) might represent an evasion strategy. The 2 patterns of lectin binding to 12 h skin schistosomula in mice and ducks show that, depending on host species, the parasite might differ in the ability to express/shed its own surface carbohydrates or to acquire host glycoconjugates.

Immunofluorescence tests demonstrated that the surface of *Trichobilharzia* cercariae and its remnants on *in vitro*-transformed larvae triggers a host antibody response (total Ig, IgG); this immune response was already detected 1 day after the second exposure to cercariae. An early response to *Trichobilharzia* cercarial antigens (3 days p.i.) has formerly been detected by ELISA and IFAT technique (Kolářová *et al*. 1994). Skin and lung schistosomula showed practically no reaction with mouse/human sera. We hypothesize that either the parasite's surface molecules elicit no antibody response or the parasite masks its surface antigens by host components. In *S. mansoni*, the newly transformed schistosomula become resistant to antibody-mediated damage (Abath & Werkhauser, 1996) and, based on our data, a similar situation may be present in *Trichobilharzia* infections.

As the cercarial surface of several schistosome species contains snail and/or snail-like components (van der Knaap *et al*. 1985), we also attempted to determine the origin of the antigens. The sera of *Trichobilharzia*-infected mice recognize the surface of mature cercariae within daughter sporocysts in the infected snail hepatopancreas i.e. the stages with well-developed glycocalyx which did not come into contact with the snail plasma/tissue components. This implies that at least some antigenic epitopes on cercariae are of parasite origin.

Cercarial glands play an important role in the penetration of the host skin since their content may stimulate host immunity as referred by Linder (1990) for the *S. mansoni* cercarial 'kissing marks'. However, the immune mouse sera used in our experiment exhibit no binding to the cercarial glands; the human sera bind non-specifically with this organ. It seems, therefore, that the *Trichobilharzia* gland content triggers no specific antibody production in the infected mice within the experimental period and the reactivity of human sera with glands remains to be clarified. Localization of the released post-acetabular gland products was determined by use of FITC-labelled HPA lectin. Gland deposits line the entry tunnel made by the parasite and can also be detected around 1 h skin schistosomula; the latter might be misinterpreted as lectin surface positivity. We hypothesize, therefore, that the post-acetabular gland material has a lytic function and facilitates the migration through the skin. In *S. mansoni*, the consensus of authors now believes that both the pre-acetabular and post-acetabular gland material is able to lyse the host tissue (Fishelson *et al*. 1992; Dalton *et al*. 1997), although a secondary adhesive role of the post-acetabular glands has also been suggested (Stirewalt & Kruidenier, 1961; Linder, 1985). Moreover, the gland content (proteases) is considered to participate in shedding and transformation of the parasite surface components (Marikovsky *et al*. 1988; Fishelson *et al*. 1992) and, therefore, the same might be true for the function of *Trichobilharzia* gland deposits around the 1 h skin schistosomula. Supporting this idea, the surface transformation detected in our experiment by lectins/anti-cercarial haemagglutinin antibodies starts at the head end of the cercarial body i.e. at the place where the ducts of penetration glands open. As the full emptying of the glands is rather rare *in vitro*, the above-mentioned hypothesis might explain the incomplete ultrastructural transformation of the *in vitro*-produced schistosomula.

It can be concluded that (a) the cercaria/schistosomulum transformation of *T. szidati* is accompanied by loss of glycocalyx and formation of a surface double membrane, (b) the transformation process appears to start at the head end of the cercarial body, (c) the transformation is linked with disappearance of lectin and antibody targets on the schistosomula surface, (d) the post-acetabular penetration glands might play a role not only in tissue penetration but also in surface transformation and (e) there is no indication at present that the transformation in the inappropriate mouse host (infected for the first time) differs importantly from that in the duck. The last consideration reflects also the fact that, in primary infection, living schistosomula with haematin deposits in the gut were isolated from mouse lungs (unpublished observation). It seems that the parasite establishment in/rejection by an inadequate host depends, in the case of first infection, on yet poorly understood immunological (cellular) or physiological/nutritional factors. Like other host–parasite incompatibilities, the failure of *Trichobilharzia* maturation within a mouse/human host may be a multifactorial matter. On the other hand, during reinfection, an effective antibody response might play an important role in host immune attack against cercaria/early schistosomulum.

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REFERENCES

ABATH, F. G. C. & WERKHAUSER, R. C. (1996). The tegument of *Schistosoma mansoni*: functional and immunological features. *Parasite Immunology* **18**, 15–20.

BOURNS, T. K. R., ELLIS, J. C. & RAU, M. E. (1973). Migration and development of *Trichobilharzia ocellata*

(Trematoda: Schistosomatidae) in its duck hosts. *Canadian Journal of Zoology* **51**, 1021–1030.

CHRISTENSEN, N. O., GOTSCHE, G. & FRANDSEN, F. (1984). *Parasitological Techniques for Use in Routine Laboratory Maintenance of Schistosomes and Use in Studies on the Epidemiology of Human and Bovine Schistosomiasis*. Danish Bilharziasis Laboratory, WHO Collaborating Centre for Applied Medical Malacology and Schistosomiasis Control, pp. 40.

DALTON, J. P., CLOUGH, K. A., JONES, M. K. & BRINDLEY, P. J. (1997). The cysteine proteinases of *Schistosoma mansoni* cercariae. *Parasitology* **114**, 105–112.

DANGUY, A. & GABIUS, H.-J. (1993). Lectins and neoglycoproteins – attractive molecules to study glycoconjugates and accessible sugar-binding sites of lower vertebrates histochemically. In *Lectins and Glycobiology* (ed. Gabius, H.-J. & Gabius, S.), pp. 241–250. Springer Verlag, Berlin, Heidelberg, New York.

DUNNE, D. W. (1990). Schistosome carbohydrates. *Parasitology Today* **6**, 45–48.

ELLIS, J. C., BOURNS, T. K. R. & RAU, M. (1975). Migration, development, and condition of *Trichobilharzia ocellata* (Trematoda: Schistosomatidae) in homologous challenge infections. *Canadian Journal of Zoology* **53**, 1803–1811.

FISHELSON, Z., AMIRI, P., FRIEND, D. S., MARIKOVSKY, M., PETITT, M., NEWPORT, G. & MCKERROW, J. H. (1992). *Schistosoma mansoni*: cell-specific expression and secretion of a serine protease during development of cercariae. *Experimental Parasitology* **75**, 87–98.

HAAS, W. & PIETSCH, U. (1991). Migration of *Trichobilharzia ocellata* schistosomula in the duck and in the abnormal murine host. *Parasitology Research* **77**, 642–644.

HAEMMERLI, U. (1953). Schistosomen-Dermatitis am Zürichsee. *Dermatologica* **107**, 301–341.

HOCKLEY, D. J. & MCLAREN, D. J. (1973). *Schistosoma mansoni*: changes in the outer membrane of the tegument during development from cercaria to adult worm. *International Journal for Parasitology* **3**, 13–25.

HORÁK, P. (1995). Developmentally regulated expression of surface carbohydrate residues on larval stages of the avian schistosome *Trichobilharzia szidati*. *Folia Parasitologica* **42**, 255–265.

HORÁK, P., GRUBHOFFER, L., MIKEŠ, L. & TICHÁ, M. (1997). Lectins of *Trichobilharzia szidati* cercariae. *Parasite* **4**, 27–35.

KOLÁŘOVÁ, L., HORÁK, P. & FAJFRLÍK, K. (1992). Cercariae of *Trichobilharzia szidati* Neuhaus, 1952 (Trematoda: Schistosomatidae): the causative agent of cercarial dermatitis in Bohemia and Moravia. *Folia Parasitologica* **39**, 399–400.

KOLÁŘOVÁ, L., SÝKORA, J. & BAH, B. A. (1994). Serodiagnosis of cercarial dermatitis with antigens of *Trichobilharzia szidati* and *Schistosoma mansoni*. *Central European Journal of Public Health* **2**, 19–22.

LINDER, E. (1985). *Schistosoma mansoni*: visualization with fluorescent lectins of secretions and surface carbohydrates of living cercariae. *Experimental Parasitology* **59**, 307–312.

LINDER, E. (1990). Cercarial kissing marks – no superficial make-up. *Parasitology Today* **6**, 393–395.

MARIKOVSKY, M., FISHELSON, Z. & ARNON, R. (1988). Purification and characterization of proteases secreted by transforming schistosomula of *Schistosoma mansoni*. *Molecular and Biochemical Parasitology* **30**, 45–54.

MEULEMAN, E. A., HUYER, A. R. & MOOIJ, J. H. (1984). Maintenance of the life cycle of *Trichobilharzia ocellata* via the duck *Anas platyrhynchos* and the pond snail *Lymnaea stagnalis*. *Netherlands Journal of Zoology* **34**, 414–417.

NEVHUTALU, P. A., SALAFSKI, B., HAAS, W. & CONWAY, T. (1993). *Schistosoma mansoni* and *Trichobilharzia ocellata*: comparison of secreted cercarial eicosanoids. *Journal of Parasitology* **79**, 130–133.

PEARCE, E. J. & SHER, A. (1987). Mechanisms of immune evasion in schistosomiasis. *Contributions to Microbiology and Immunology* **8**, 219–232.

RAMASWAMY, K., SALAFSKY, B., POTLURI, S., HE, Y.-X., LI, J.-W. & SHIBUYA, T. (1996). Secretion of an anti-inflammatory, immunomodulatory factor by schistosomulae of *Schistosoma mansoni*. *Journal of Inflammation* **46**, 13–22.

RAU, M. E., BOURNS, T. K. R. & ELLIS, J. C. (1975). Egg production by *Trichobilharzia ocellata* (Trematoda: Schistosomatidae) after initial and challenge infection in ducks. *Canadian Journal of Zoology* **53**, 642–650.

SAMUELSON, J. C., CAULFIELD, J. P. & DAVID, J. R. (1982). Schistosomula of *Schistosoma mansoni* clear Concanavalin A from their surface by sloughing. *Journal of Cell Biology* **94**, 355–362.

SAMUELSON, J. C. & STEIN, L. D. (1989). *Schistosoma mansoni*: increasing saline concentration signals cercariae to transform to schistosomula. *Experimental Parasitology* **69**, 23–29.

STIREWALT, M. A. & KRUIDENIER, F. J. (1961). Activity of the acetabular secretory apparatus of cercariae of *Schistosoma mansoni* under experimental conditions. *Experimental Parasitology* **11**, 191–211.

VAN DER KNAAP, W. P. W., BOOTS, A. M. H., MEULEMAN, E. A. & SMINIA, T. (1985). Search for shared antigens in the schistosome–snail combination *Trichobilharzia ocellata–Lymnaea stagnalis*. *Zeitschrift für Parasitenkunde* **71**, 219–226.

WIEST, P. M., KOSSMANN, R. J. & TARTAKOFF, A. M. (1989). Determinants of surface membrane maturation during the cercarial-schistosomula transformation of *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **41**, 70–77.

WILSON, R. A. (1987). Cercariae to liver worms: development and migration in the mammalian host. In *The Biology of Schistosomes* (ed. Rollinson, D. & Simpson, A. J. G.), pp. 115–146. Academic Press, London.