Invited Review

Functions of the tegument of schistosomes: Clues from the proteome and lipidome

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Abstract

The tegumental outer-surface of schistosomes is a unique double membrane structure that is of crucial importance for modulation of the host response and parasite survival. Although several tegumental proteins had been identified by classical biochemical approaches, knowledge on the entire molecular composition of the tegument was limited. The *Schistosoma mansoni* genome project, together with recently developed proteomic and lipidomic techniques, allowed studies on detailed characterisation of the proteins and lipids of the tegumental membranes. These studies identified tegumental proteins and lipids that confirm the function of the tegument in nutrient uptake and immune evasion. However, these studies also demonstrated that compared to the complete worm, the tegument is enriched in lipids that are absent in the host. The tegument is also enriched in proteins that share no sequence similarity to any sequence present in databases of species other than schistosomes. These results suggest that the unique tegumental structures comprise multiple unique components that are likely to fulfil yet unknown functions. The tegumental proteome and lipidome, therefore, imply that many unknown molecular mechanisms are employed by schistosomes to survive within their host.
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1. Introduction

Schistosomes are metazoan parasitic flatworms that belong to the digenean family of Schistosomatidae. This family comprises 12 genera that all infect vertebrates, mainly birds and mammals, where they inhabit the blood-vascular system. The genus *Schistosoma* comprises 18 species of which five cause the human disease schistosomiasis, also known as bilharziasis (Rollinson and Southgate, 1987). Schistosomiasis is endemic in many rural areas in tropical countries, where it has a significant effect on the economy and welfare. Infection of the mammalian host occurs via penetration of the skin by the cercarial stage, which thereafter transforms into the schistosomulum stage. These juvenile schistosomes then reside in the skin for 2–4 days before entering a blood vessel. Within the vascular system, juvenile schistosomes migrate via complex routes to their final venous destination where they mature and form male–female couples at locations from which their eggs can exit the host in faeces or urine. *Schistosoma mansoni* and *Schistosoma japonicum* adults migrate to the mesenteric veins while *Schistosoma haematobium* adults reside in the veins of the bladder (Wilson, 1987).

Schistosomes are complex multicellular eukaryotes that have co-evolved with their mammalian hosts such that adult worms are typically able to survive for 5–10 years in the vascular system of immune competent hosts (Von Lichtenberg, 1987). Clearly, these parasites had to evolve mechanisms to evade host immune effectors. These adaptations resulted in

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exposed or secreted products that influence the host and in protective armoury of the parasite itself. Schistosomes both secrete products that favourably skew or suppress host immune responses and cover themselves with an immune refractory barrier called the tegument (McLaren and Hockley, 1977; Maizels et al., 1993; Pearce and MacDonald, 2002; van der Kleij and Yazdanbakhsh, 2003). This tegumental outer-surface structure appears to be critically involved in complex host–parasite interactions. This review will focus on recent advances in the knowledge on the biogenesis and molecular composition of the schistosomal tegument in relation to its function in the interaction with the host.

2. Structure and function of the tegument

In 1977, it was demonstrated that all investigated blood-dwelling flatworms contain an outer-surface covering that is unique in nature (McLaren and Hockley, 1977). This tegument consists of a syncytium of fused cells surrounding the entire worm with a single continuous double-bilayer membrane (Fig. 1). The tegument ultrastructure has been studied in great detail in *S. japonicum*, *S. haematobium* (Leitch et al., 1984; Gobert et al., 2003) and especially *S. mansoni* (Morris and Threadgold, 1968; Silk et al., 1969; Smith et al., 1969). The host-interactive surface consists of two closely-apposed lipid bilayers that overlay the syncytium. The two outer-surface membranes of the tegument, which are detected as a heptalamellar layer by transmission electron microscopy, form many surface pits that substantially enlarge the surface area of the schistosome (Fig. 1) (Hockley, 1973; Gobert et al., 2003). The underlying syncytial-matrix contains some mitochondria and many vesicular structures, such as discoid or elongated bodies and multilamellar bodies. A basal lamina membrane separates the syncytium from a layer of muscle cells. Nuclei and ribosomes for the tegument are located in cytons, which are located underneath the muscle layer and connected by microtubule-lined cytoplasmic connections. These cytons actively synthesise membranous bodies that move along the cytoplasmic connections to the syncytium (Fig. 1) where they are suggested to have a function in

formation and replacement of the outer-surface membranes (see below and MacGregor et al., 1988). The basal lamina, located between the syncytium and the muscle layer, shares many characteristics with those found in mammalian cell types and is suggested to function as a filtering barrier (Gobert et al., 2003). The syncytial matrix contains an extensive cytoskeleton structure connecting the outer-surface membranes to the basal lamina in a dynamic manner to ensure sufficient flexibility of the schistosomal surface (Jones et al., 2004). In addition, the tegumental cytoskeleton forms the basis of the spines on the surface of schistosomes (Jones et al., 2004).

Since, the host-interactive heptalaminate tegument is present in all blood-dwelling digenean parasitic worms, yet absent in those that inhabit the gut or other body cavities, it has been concluded that this structure is an important adaptation to survival in the blood stream (McLaren and Hockley, 1977; Wiest et al., 1988). Regarding immune evasion, several mechanisms mediated by the tegument have been suggested, including antigenic mimicry, proteolytic degradation of 'attacking' host proteins, rigid biophysical membrane properties and a rapid tegumental membrane turnover (Abath and Werkhauser, 1996). The recent reports on the molecular composition of the tegument provide new evidence for these proposed mechanisms of immune evasion by schistosomes (see below).

The tegument, with its numerous deep envaginations that greatly increase its surface area, has been postulated as the major site of nutrient uptake (Fripp, 1967) a concept supported by the presence of multiple transporter proteins for glucose and amino acids (Skelly and Shoemaker, 1996; Skelly et al., 1999). One of these glucose transporters (SGTP4) is expressed only in the mammalian stages of the life cycle and is specifically localised within the apical membranes of the tegument (Skelly and Shoemaker, 1996). Absorption of simple substrates that are abundant in the blood of the host, such as glucose and amino acids, occurs primarily via the tegument instead of the intestinal epithelium of the parasite (Fripp, 1967; Rogers and Bueding, 1975; Uglem and Read, 1975). In addition, cholesterol and other lipids are absorbed via the tegument as well (Moffat and Kusel, 1992; Rogers et al., 1993). The outer-
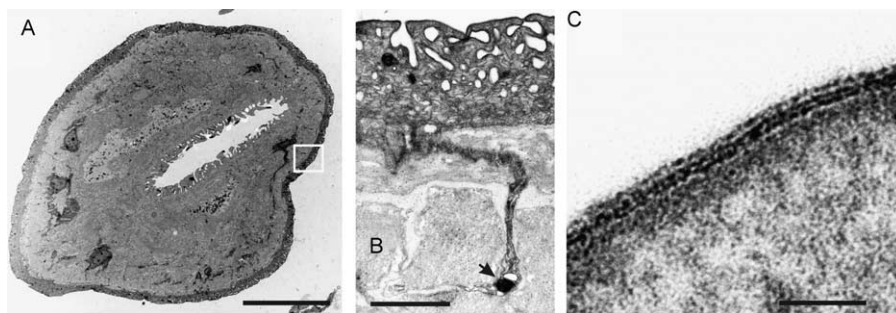

Fig. 1. Tegumental outer-surface of schistosomes. Electron micrographs were prepared from ultra-thin sections of adult *Schistosoma mansoni* parasites, which were embedded in plastic after conventional glutaraldehyde-osmium fixation. In the lowest magnification (panel A) the tegument is visible as a dark band surrounding an adult female *S. mansoni* worm. The bar represents 20 μm. Panel B shows an enlargement of the area indicated by the white rectangle in panel A. The folds in the outer-tegumental membranes (surface pits) increase the area of the epithelium. The arrow indicates a multilamellar body moving from a cyton towards the tegument in cytoplasmic connection spanning the superficial muscle layers. Bar represents 1 μm. Panel C shows a higher magnification image of the double phospholipid bilayer of the tegumental membranes known as the heptalaminar outer-surface structure (bar represents 75 nm).

membrane of schistosomes also contains receptors, which bind human low-density lipoproteins (LDL) (Rumjanek et al., 1988). However, uptake of lipids via bound LDL has never been demonstrated, and therefore, it is suggested that binding of these lipoprotein complexes to the receptors on the surface of the parasite is only used as a disguise, to shield the parasite antigens for binding by host antibodies (Xu and Caulfield, 1992).

3. Biogenesis of the tegument

Any hypothesis proposing a mechanism for the biosynthesis of the schistosome outer-tegument must accommodate its peculiar double bilayer membrane, first described by Hockley and McLaren (1971), that consists of two distinct, yet closely apposed, lipid bilayers (see above). Cercariae, prior to their transformation to schistosomula, are also surrounded by a syncytial membrane, although this membrane appears to be a conventional bilayer covered by a glycan surface coat called the glycocalyx (Khoo et al., 1995). Following exposure to increased ionic strength, cercariae transform into schistosomula and the membrane is entirely replaced by a double bilayer membrane within a few hours (Hockley and McLaren, 1973). This dramatic membrane transformation offers a unique opportunity to observe the schistosome tegument biosynthesis process. Hockley and McLaren (1973) exploited this opportunity and performed a detailed electron micrographic analysis of the schistosome tegument at various times during and following the membrane transformation. These authors observed the rapid appearance of large quantities of membranous vacuoles within the first hour following the induction of cercariae to transform. The vacuoles have a double bilayer membrane containing whorls of membrane and significant quantities of granular material. Many of these vacuoles were observed joined to the outer-membrane of tegument with their membranous contents now on the surface of the worms. The cercarial bilayer membrane appears to form microvilli that are then released from the worms. Based on these observations, the authors speculated that the double bilayer outer-tegument membrane is formed by the shedding of the cercarial outer-membrane and its replacement by a burst outward of membranous vacuoles that are synthesised in the cytons, and then pass to the tegument to fuse to the tegumental surface.

The Hockley and McLaren model for the formation of the schistomula double-bilayer outer-tegument is strongly supported by subsequent studies. Skelly and Shoemaker (1996, 2001) monitored an apical tegument marker protein, SGTP4, by immunofluorescence during the transformation process. SGTP4 appears almost immediately after cercarial transformation and rapidly fills a cyton network beneath the tegument. After 30 min, SGTP4 appears to erupt onto the surface where it appears in patches which diffuse and coalesce over the next few hours to form a smooth covering.

It is now the general consensus that the apical tegumental membrane of juvenile and adult schistosomes is maintained in much the same manner as Hockley and McLaren found it was created in the transforming schistosomula. Numerous electron

microscopy studies have shown that the tegument of developing and mature worms continues to contain significant numbers of membranous vesicles or multilamellar bodies (Morris and Threadgold, 1968; Silk et al., 1969; Smith et al., 1969). Wilson and Barnes (1974b, 1977) provided evidence that these multilamellar bodies fuse to the apical membrane such that the bounding membrane contributes to the inner bilayer and its multilamellar contents are dumped onto the surface in a disorganised state where they contribute to the outer-bilayer. These authors calculate that the multilamellar body contents contribute about three times the membrane coverage as does the bounding membrane, which may suggest the outer-tegument turns over much quicker than the inner membrane.

The tegument also contains large numbers of a second membranous body, termed the elongate or discoid body, but its role in the biogenesis of the tegument membrane remains controversial. Some studies have suggested that these bodies disintegrate to become part of the tegumental ground substance (Wilson and Barnes, 1974a) while others have suggested that discoid bodies contribute to the biosynthesis of the inner bilayer of the tegument surface membrane (Zhou and Podesta, 1989). Since, discoid bodies are not observed until 3 h following schistosome transformation (Hockley and McLaren, 1973), which is well after the double-bilayer membrane has been formed, it seems unlikely that discoid bodies are essential for the synthesis of the inner bilayer. Yet, evidence that discoid bodies contain proteins also found in the tegumental membrane does support the concept that these bodies contribute to the biosynthesis or maintenance of this membrane (MacGregor et al., 1988; Jiang et al., 1996). Perhaps, discoid bodies supplement the multilamellar bodies in the production of new inner membranes to replace those lost during tegument shedding. Both discoid bodies and multilamellar bodies appear to be synthesised in the same cell bodies or cytons but by a different Golgi apparatus (Wilson and Barnes, 1974b).

Schistosomes become refractory to immune effectors within days following transformation from cercariae and a number of laboratories have sought to identify changes to the tegument that may be responsible. Many different research groups have shown that schistosomes rapidly acquire host antigens, which may mask parasite antigens and that this acquisition correlates well with the immune resistance of the worms (Smithers et al., 1969; McLaren et al., 1975; Simpson et al., 1983). Some of these antigens may be acquired by fusion of the schistosome tegument to host immune cells (Caulfield et al., 1980). Other researchers have found evidence that rapid turnover of the tegumental membranes takes place and postulated that this shedding process may also shed host immune effectors (Kusel et al., 1975; Samuelson et al., 1982; Roberts et al., 1983; Ruppel and McLaren, 1986). Strong electron-microscopy evidence was reported that the presence of anti-tegumental antibodies (anti-rbc Ags) and complement actually stimulates schistosomes to shed membrane (Perez and Terry, 1973). Apparently, the host has a significant influence on the tegument, as it not only contributes antigens but also affects the turn-over rate.

4. The tegumental proteome

In 1994 the *S. mansoni* genome-sequencing project, initiated by the World Health Organisation, aimed to catalogue new parasite genes through a gene discovery program and to develop universally accessible databases. These databases (www.schistodb.org) provide tools for studies on the molecular basis of host–parasite interactions, for identification of novel targets for immunological and pharmacological intervention, and for the development of improved diagnostics (Wilson et al., 2004). This genome-sequencing project opened the way for proteomic analysis of the parasite (Ashton et al., 2001), and recently, several reports on the proteome of soluble or excreted fractions of several life cycle stages of *S. mansoni* have been published (Curwen et al., 2004; Knudsen et al., in press; Cheng et al., 2005).

Two recent studies analysed the protein content of the tegument and aimed at the identification of tegument-specific proteins (van Balkom et al., 2005), and of surface exposed proteins (Braschi and Wilson, in press). The study by van Balkom et al. identified 740 expressed schistosomal proteins, of which 179 were detected in both the tegument and the worm body and 43 were specifically detected in the tegument (Fig. 2) (van Balkom et al., 2005). These 740 identified proteins represent less than 10% of the total predicted *S. mansoni* proteome so it seems likely that many additional, less abundant, proteins are expressed in adult worms. The identified tegumental proteins are most likely also only a fraction of the total number of tegumental proteins.

Several broad generalisations can be discerned by the functional classification of the proteins identified in the schistosome tegument (van Balkom et al., 2005). First, there are relatively few proteins that play a role in DNA-, RNA- or protein-synthesis, which correlates with the absence of nuclei in the distal cytoplasm of the tegument. Second there are a relatively high number of membrane proteins and proteins involved in (vesicular) trafficking, which correlates with the extensive transport of protein-containing vesicles from the cytons to the outer-surface membranes (Skelly and Shoemaker, 2001). Thirdly, there are relatively many proteins that form, organise or influence the cytoskeleton, which correlates with the extensive and dynamic cytoskeletal structures in the tegument (Jones et al., 2004). Finally, there are an unusually large number of proteins (28%) that lack sequence similarity to any other protein in any species other than schistosomes, which correlates with the unique structure and function of the schistosomal tegument (van Balkom et al., 2005) (Table 1). These unique tegumental proteins, without significant similarity to proteins of the host, might be expected to be highly antigenic, because of their presence in the tegument, the contact site interacting with the host.

The other study on the tegumental proteome characterised surface exposed proteins (Braschi and Wilson, in press). In total this study identified 24 exposed schistosomal proteins, among which were found one secreted protein (Sm 29), 11 membrane proteins, three cytoskeleton proteins, three cytosolic proteins and six proteins with no significant homology to any other sequenced protein (Table 1). Apart from one not annotated protein, all these exposed proteins were also detected in the above described study by van Balkom et al. (2005), reinforcing the evidence for the tegumental localisation of these proteins. The identification of multiple organic phosphatases and membrane transporters as surface exposed, fits very well with the suggested tegumental function in nutrient uptake. Substrates diffuse into the porous membranocalyx, after which dephosphorylation can occur to facilitate uptake via selective membrane channels.

The identification of several cytosolic and cytoskeletal proteins at the tegument surface was not expected. Braschi and Wilson (in press) suggest that detection of these proteins may have resulted from small disruptions in the outer-surface membranes, as the detected cytosolic proteins are very abundant proteins and because the detected cytoskeletal proteins are part of the tegumental spines that are located just underneath the outer-surface membrane. The lipid-binding protein, annexin, that was identified as surface-exposed could be involved in anchoring the two bilayers of the tegumental outer-surface side by side. Twenty-five percent of the surface-exposed proteins showed no homology to any other protein sequence in the databases, which is similar to that percentage in the study of van Balkom et al. (2005).

Braschi and Wilson (in press) also found host immunoglobulins IgM, IgG1 and IgG3 and complement C3 proteins bound to the exposed surface of the schistosome tegument. Schistosomes are suggested to have Fc receptors for IgG at their tegument surface (Kemp et al., 1977), which could explain the presence of IgG1 and IgG3. Finding bound IgM was surprising and may indicate that this host protein binds via its Fab-region to parasite antigens (Braschi and Wilson, in press). Most bound C3 was detected as the alpha chain
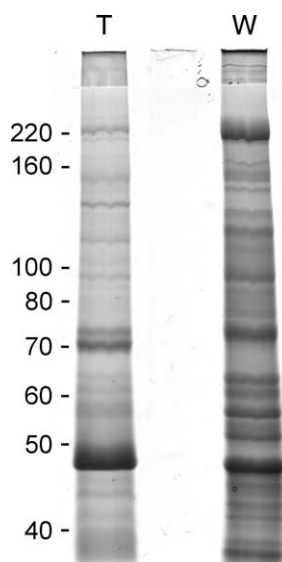

Fig. 2. The protein composition of the tegument of *S. mansoni*. This Commassie stained one-dimensional SDS-PAGE protein gel shows the protein pattern of the isolated teguments (T) and of worms from which the tegument was removed (W). Numbers indicate molecular mass of marker proteins (kDa). See van Balkom et al. (2005) for experimental details.

Table 1
Identified surface-exposed and schistosome-specific tegumental proteins

Accession no. or SMGI identifier	A Schistosome specific	B Tegument specific	C Surface exposed	D Protein or homologous to (protein function)
Sm03865	Yes	No	Yes	200 kDa surface protein (1)
Sm00962	No	No	Yes	Alkaline phosphatase (2)
Sm03987	No	Yes	Yes	Annexin (1)
Sm08542	No	No	Yes	Calpain (2)
Sm10433	No	Yes	Yes	Dysferlin (1)
Sm08331	No	No	Yes	Na/K transporter (3)
AI882660	Yes	Yes	No	No significant homology (4)
BF936329	Yes	Yes	No	No significant homology (4)
Sm00749/TC6921	Yes	No	Yes	No significant homology (4)
Sm01030/TC14173	Yes	No	Yes	No significant homology (4)
Sm07392/TC8556	Yes	Yes	Yes	No significant homology (4)
Sm11517/TC19519	Yes	Yes	Yes	No significant homology (4)
Sm11921/CD192195	Yes	Yes	Yes	No significant homology (4)
Sm13096/CD084385	Yes	No	Yes	No significant homology (4)
TC13203	Yes	Yes	No	No significant homology (4)
TC13851	Yes	Yes	No	No significant homology (4)
TC14238	Yes	Yes	No	No significant homology (4)
TC18339	Yes	Yes	No	No significant homology (4)
TC19226	Yes	Yes	No	No significant homology (4)
TC7948	Yes	Yes	No	No significant homology (4)
TC9174	Yes	Yes	No	No significant homology (4)
TC9780	Yes	Yes	No	No significant homology (4)
Sm03458	No	Yes	Yes	Phosphodiesterase (2)
Sm04463	No	No	Yes	Tetraspanin B (1)
Sm12366	No	No	Yes	Tetraspanin D (1)
Sm00707	No	No	Yes	Voltage-dependent anion channel (3)

Column A, 'Schistosome specific', refers to tegumental proteins, which do not demonstrate sequence similarity to any other protein in any species other than schistosomes; column B, 'tegument-specific', refers to proteins, which were only detected in the isolated ftegument fractions in the study by van Balkom et al. (2005); column C, 'surface exposed', refers to proteins, which were detected as surface exposed in the study by Braschi and Wilson (2005); column D, numbers refer to the following functions: (1) structural membrane protein, (2) membrane enzyme, (3) transporter, and (4) unknown function.

C3c/C3dg fragment, which means that C3 has been activated and subsequently inactivated by proteolytic cleavage, probably by a schistosomal enzyme (Braschi and Wilson, in press). Therefore, these results provide evidence for immune evasion by active degradation of 'attacking' host–defence proteins of the complement system.

5. Tegumental lipid composition

The lipid metabolism of adult schistosomes seems to be optimally adjusted to a parasitic way of life, as most lipids found in the parasite have been synthesised by the host and the few exceptions to this rule mostly result from host derived precursors to which small adaptations have been made by the parasite. Schistosomes do not synthesise cholesterol and fatty acids de novo, and therefore, they rely on uptake from the host for the supply of these lipids (Smith and Brooks, 1969; Meyer et al., 1970). Although schistosomes contain a large amount of triacylglycerol, they cannot catabolise fatty acids as the pathway of β-oxidation is not active (Smith and Brooks, 1969). Interestingly, however, schistosomes have retained the capacity to modify fatty acids, mainly by chain elongation and possibly by desaturation (Meyer et al., 1970; Brouwers et al., 1998b). These modifications result in a fatty acid profile that is substantially different from that of the host (see below).

Despite their inability to synthesise sterols de novo, adult schistosomes contain a relatively high amount of cholesterol. The molar ratio of sterols to phospholipids in adult *S. mansoni* is 0.8, whereas that in typical eukaryotic cells is only 0.3–0.5 (Furlong and Caulfield, 1988). The tegumental membranes of adult schistosomes are even more enriched and contain more cholesterol than phospholipids on a molar basis (Allan et al., 1987). Similar to other eukaryotic cells, the phospholipids phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are the major constituents of membranes of both the worm and the tegument (Rogers and McLaren, 1987), but the composition of the fatty acids present in these phospholipids is very distinct from those found in mammalian blood cells. PC in schistosomes, and in particular, PC in the tegumental membranes, contains more saturated fatty acids than most mammalian cells. For instance, nearly 50% of the acyl chains present in PC of the tegument is palmitate, a fatty-acyl chain of 16 carbon atoms without any desaturation (C16:0) (Allan et al., 1987). Furthermore, tegumental membranes are highly enriched in PC- and especially PE-species in which the fatty acid chain on the *sn*-1 position is linked by an ether bond instead of an ester bond (Fig. 3) (Brouwers et al., 1998a). The high amount of cholesterol, sphingomyelin and saturated (ether-linked) phospholipid species in the tegumental membranes results in a tight packing of the membrane, which makes the membrane more rigid in physical terms. These properties
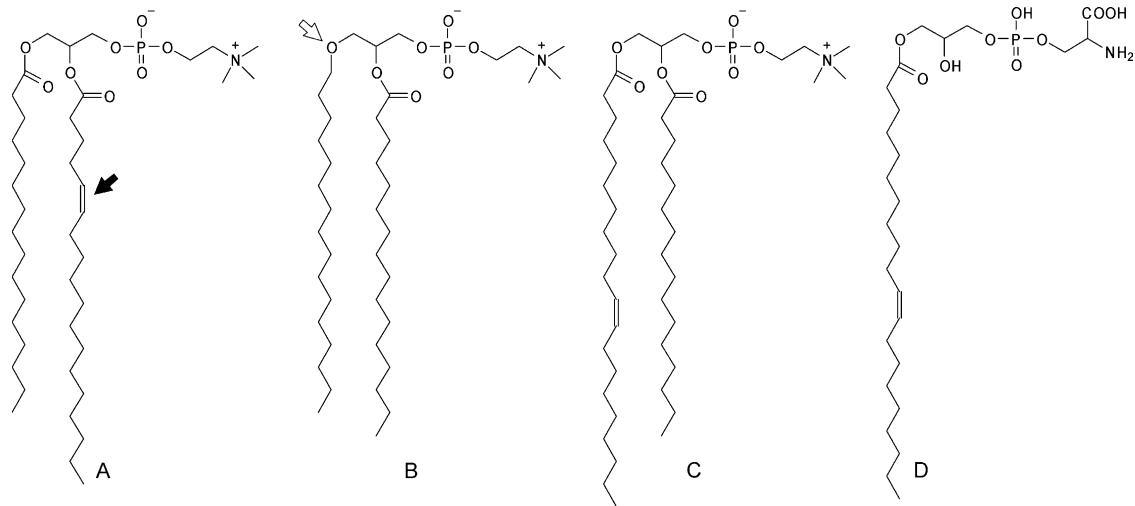
Fig. 3. Unusual phospholipid species in tegumental membranes of *S. mansoni*. Shown are the chemical structures of schistosome-specific phospholipids (see text for details and references). (A) PC with unusual position of the double bond in the unsaturated fatty acid, 1-palmitoyl-2-(5Z-octadecenoyl)-*sn*-glycero-3-phosphocholine (GPCho (16:0/18:1 (5Z)). (B) Specific ether phospholipid, 1-*O*-hexadecyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (GPCho(O-16:0/16:0)), (C) phospholipid with unusual position of the unsaturated fatty acid, 1-eicosaenoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (GPCho(20:1/16:0)) and (D) lyso-phospholipid, 1-eicosaenoyl-2-lyso-*sn*-glycero-3-phosphocholine (GPSer(20:1/0:0)). The filled arrow indicates the unusual position (5Z instead of 9Z) of the unsaturation (panel A), whereas the open arrow indicates the ether-linkage present in ether phospholipid species (panel B).

render the tegumental membrane stable and relatively inert, as it is less accessible to lipases and thereby less sensitive to degradation (Quinn, 1990; Kinnunen, 1991).

The tegument is highly enriched in 1-*O*-hexadecyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (GPCho (O-16:0/16:0)), an alkylacyl species absent in the blood of the host (Fig. 3). This PC-species may serve as a precursor for the formation of platelet activating factor (GPCho (O-16:0/2:0)), a potent signalling molecule in mammals (Brouwers et al., 1998a). One of the effects of platelet activating factor is vasodilatation, and the schistosome could profit from the increased supply of nutrients. On the other hand, activation of platelets does not seem beneficial for schistosomes, and therefore, it could be that GPCho (O-16:0/16:0), or one of its metabolites, interferes with the normal function of platelet activating factor by competitive inhibition.

Next, to the rigid biophysical properties and the high fraction of ether lipids, the lipids in the tegument are also of interest because the phospholipids contain several peculiar fatty acid species. One of the most abundant PC species in the tegumental membranes has as one of its two fatty-acyl chains the highly unusual fatty acid octadec-5-enoic acid (18:1(5Z)), a fatty acid that is absent in the host (Brouwers et al., 1998b) (Fig. 3A). Furthermore, schistosomes are relatively rich in phospholipids containing eicosaenoic acid (20:1) (Allan et al., 1987). In PC derived from adult schistosomes, eicosaenoic acid is predominantly esterified to the *sn*-1 position in combination with a saturated fatty acid (16:0) at the *sn*-2 position. This combination is very unusual as most phospholipids contain unsaturated fatty acids at the *sn*-2 position. In tegumental membranes eicosaenoic acid is predominantly present in the plasmalogen PE species (16:0/20:1) (Fig. 3), an ether–lipid species that constitutes 27% of the PE species in the outer-

surface membranes and that is absent in blood of the host (Brouwers et al., 1998a). A yet unanswered question related to the ether lipid metabolism is whether or not the specific Δ1 desaturation of plasmalogens is performed by schistosomes, or that the plasmalogen backbones are host derived. If the modification is performed by schistosomes, the Δ1 desaturase would be the second lipid desaturase (next to the Δ5 desaturase, see above) found in schistosomes.

The function of the unusual fatty-acid species 18:1(5Z) and 20:1 in schistosomes is not known, but their specific enrichment in the tegument suggests a role in the interaction of the parasite with its host. It could be speculated that these schistosome-specific lipids or their metabolites have a signalling function. Such a function could be modulation of the host response, or it could function internally, for instance if the parasite would need a lipid-mediated signal from the outer-tegumental membranes to the underlying cell layers.

The tegumental membranes are continuously renewed from membranous bodies (see above), however, the exact turnover-rate of these membranes is debated, as several studies reported half-life values for tegumental proteins or glycoconjugates ranging from a few hours to several days (Kusel and Mackenzie, 1975; Wilson and Barnes, 1977; Dean and Podesta, 1984; Saunders et al., 1987). Studies on the turnover of the phospholipids demonstrated that tegumental lipids have a shorter half-life than those in the worm body, and that lyso-phospholipids are excreted as degradation products into the environment (Furlong and Caulfield, 1989; Brouwers et al., 1999). The major lyso-phospholipid excreted by *S. mansoni* schistosomula, monopalmitoyl-PC, has been suggested to have a function in lysis of red blood cells that subsequently attach to the parasite as ghost cells (Caulfield and Cianci, 1985; Golan et al., 1986). Therefore, lyso-phospholipids could promote

the acquisition of host membrane components, resulting in surface exposure of host components that could mask antigenic epitopes of the parasite.

Another schistosome-derived lyso-phospholipid class, lyso-phosphatidylserine (lyso-PS) (Fig. 3), was recently shown to interact with the immune system of the host (van der Kleij et al., 2002). Schistosomal lyso-PS was found to activate Toll-Like Receptor 2 (TLR2) and possibly another co-receptor on dendritic cells and to suppress IL-12 production. The co-culture of lyso-PS exposed dendritic-cells with naïve T-cells resulted in the induction of Th2-response and IL-10 producing T-cells. The IL-10 producing T-cells were shown to have a suppressor function (van der Kleij et al., 2002). Regulatory T-cells are induced during chronic helminth infections (Taylor et al., 2005) and have been cloned from patients with onchocerciasis (Doetze et al., 2000). The immunosuppressive mechanisms set into action by parasitic helminths are thought to facilitate long-term survival of the parasite (van der Kleij and Yazdanbakhsh, 2003; Taylor et al., 2005). Since many lipids of pathogens, or their degradation products, are known to have a potent effect on the immune system of the host, degradation products of other (tegument-specific) schistosomal lipids might have an as yet unknown but important effect on the host, similar to the specific effect of lyso-PS and lyso-PC.

6. Conclusions and future perspectives

The tegumental outer-surface of schistosomes is a unique double membrane structure that is of crucial importance for modulation of the host response and parasite survival. Recent proteome and lipidome studies identified many tegumental proteins and lipids whose predicted functions appeared well suited for roles in known tegumental functions such as nutrient uptake and immune evasion. In addition to identifying proteins with a known function, many proteins and lipids were identified that are not present in mammals and so far have unknown functions. Most of these tegumental proteins did not demonstrate significant sequence similarity to known sequences present in other organisms. In addition, the partial characterisation of the tegumental lipids revealed several unique lipid species in the phospholipid classes PC and PE. The less abundant phospholipid classes, such as PS and phosphatidylinositol, have not been characterised in the tegument. Since these lipids are known to function as precursors for many potent signalling molecules, the number of schistosome-specific lipids found to influence the host is likely to increase.

The future challenge will be a functional characterisation of all these schistosome-specific proteins that are localised in the tegument. Until now these studies were significantly hampered by the absence of methods to manipulate gene expression in schistosomes. However, over the last few years, methods for RNA-interference (RNAi) in several schistosomal stages have been developed (Boyle et al., 2003; Skelly et al., 2003; Correnti et al., 2005; Wippersteg et al., 2005). The function of tegumental proteins can now be investigated by the identification of phenotypes resulting from RNAi 'knock-down' of their expression. The unravelling of the function of the many not yet annotated proteins in the tegument will certainly result in novel insights in the complex interaction between the blood-dwelling schistosomes and their mammalian hosts.

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