Circulating gut-associated antigens of Schistosoma mansoni : biological, immunological, and molecular aspects

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Chapter 1

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Schistosomes are blood-dwelling flukes belonging to the class Trematoda, but differ from all other trematodes in that the sexes are separate (Fig. 1). The parasites have a sexual reproduction phase in the definitive host, and an asexual reproduction phase in a snail intermediate host (Fig. 2). Definitive hosts are *e.g.* humans, cattle, rodents, primates, or dogs, depending on the *Schistosoma* species [9]. In the laboratory, hamsters and mice are often used both as definitive hosts for maintaining the parasite-cycle and as experimental animals. The *Schistosoma* species which are most important in human schistosomiasis are *S. mansoni*, *S. japonicum*, and *S. haematobium*.

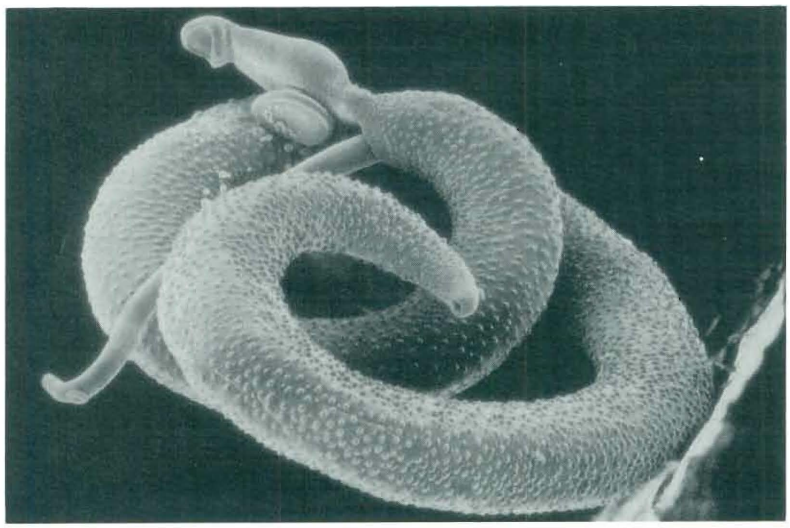

Figure 1. Paired adult *Schistosoma mansoni* worms. Clearly visible is how the male worm enfolds the female within its gynaephoric canal. Magnification approximately 40 X. *(courtesy of Dr. Ming M. Wong, U.C. Davis, U.S.A.)*

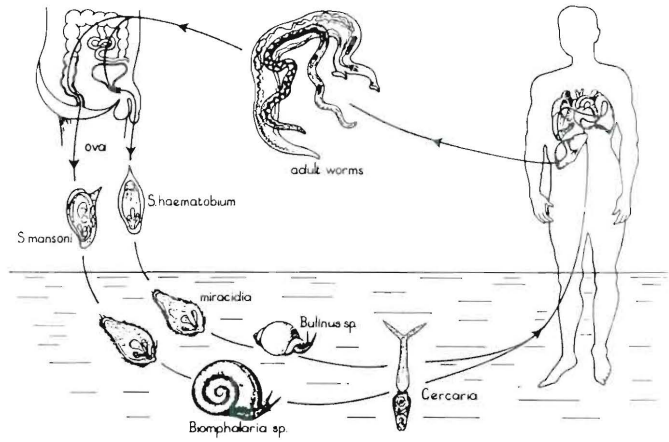

Figure 2. Life cycle of *Schistosoma mansoni* and *Schistosoma haematobium*. *(courtesy of Dr. A.M. Deelder)*

The infective parasitic stage, the cercaria, enters the host through the skin, evoking an inflammatory response. In naive hamsters, neutrophils are among the first cells to attack the parasite, followed after a few days by eosinophils [37]. From this stage to about 3 weeks after infection, the parasite, present as a young schistosomulum, is most susceptible to immune damage [11,21,36,78, 79,82,97,132]. Employing a wide range of evasion mechanisms, the developing worm becomes refractory or even invisible to certain parts of the host's defense system. Consequently, the worms persist in the host for an estimated 3–5 years [55,130], although a number of reports indicated much longer times [59,127,129], and recently it has been reported that in some cases even almost 40 years after infection viable eggs were excreted [62]. Among the evasion mechanisms employed by schistosomula and adult worms are: 1. camouflage by acquisition of host antigens [35,58,114]; 2. reduction of surface antigenicity by tegument antigen shedding [49,94]; 3. modulation of the host's immune response by eliciting blocking antibodies [19,51,69]; 4. induction of cellular immunoregulatory mechanisms, e.g. downregulation of host-protective Th1-type response [95,98]; 5. induction of immunosuppressive mechanisms by excretion of specific T cell suppressor factors [2,7,22,23,77]; 6. acquisition of specific complement inhibitory membrane-bound molecules [63,96]; 7. inhibition of the immune effector cell function or lysis of cells which adhere to the parasite surface [28–31,56,102]; and 8. clearance from the surface by sloughing of antibody-antigen complexes or of molecules which are potentially harmful to the parasite [74,109,110,123].

At about 6 weeks post infection (*p.i.*) the adult worm-pairs start to produce eggs, which either penetrate the intestinal wall to be voided in the faeces (*Schistosoma mansoni* and *S. japonicum*) or the bladder wall to leave the body via the urine (*S. haematobium*). However, a considerable proportion of the eggs is not excreted but *e.g.* transported with the blood to the liver where they are retained and induce granuloma formation and subsequent liver-fibrosis [3,34,128]. *S. haematobium* induced pathology involves mainly the bladder and kidney [34,116].

A striking gross observation on the gut of schistosomes is that in healthy, freshly isolated worms the intestinal tract is filled with a dark slurry of partly digested blood. Since the gut is a cul-de-sac, the parasite has to regurgitate at regular intervals, releasing a dark cloud of undigested particulate material including parasite antigens [16,113] (Fig. 3). These relatively abundant excretory antigens may exhibit various functions and characteristics, *e.g.* in immune evasion mechanisms. A number of these antigens, which will be discussed below, can also be detected in the circulation of the host. In this review, most attention will be given to a group of highly glycosylated circulating gut-associated antigens [99].

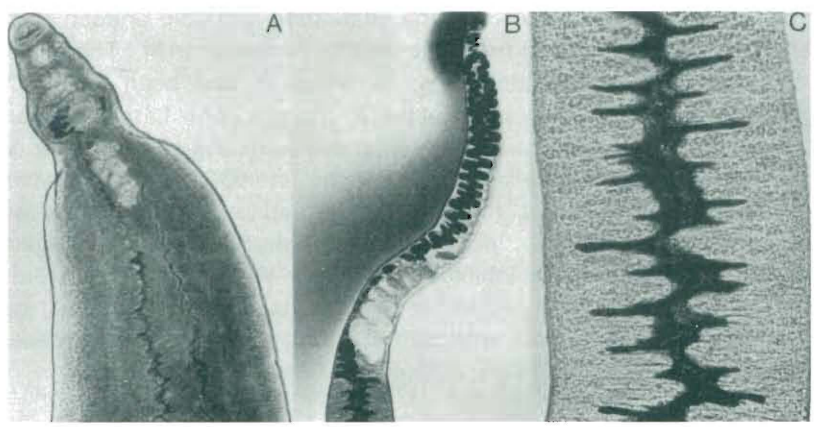

Figure 3. Anterior portion of male and female schistosome worms showing blood pigments (A) in gut and (B) in regurgitant; (C) detail of female gut at level of vitelline gland. Magnification approximately 50 X (A), 20 X (B), 100 X (C). *(photographs taken by Dr. A.W. Senft, reproduced with permission from [113])*

Other circulating antigens are found among tegument-associated antigens [38,66,115], egg antigens, *e.g.* released as hatching fluid [60,89,90,103,115] and cercarial antigens [1,61], but these fall outside the scope of this introduction.

Antigens present in the schistosome gut, but which have, so far, not been described as being detectable in the circulation, are proteases like hemoglobinase or cathepsin B [16,17,32,71], phospholipase A2 [105], a 68 kDa antigen which might be a vaccine candidate [13], or glutathione-S-transferases [18,81]. Although the gut proteases themselves have not been demonstrated in the circulation[1], which can be due to rapid degradation or to very low concentrations, a specific and strong antibody response to these antigens has been reported [33,107,108].

After an experimental infection with schistosomes, the first antibodies have been shown to be detectable two to three weeks *p.i.*, using an immunofluorescence assay on adult worm sections [10,46,93,100]. These antibodies were directed against the gut epithelium of the parasite. As the infection progresses, additional fluorescence has been found at the level of the tegumental membrane (4 - 5 weeks *p.i.*) and finally within the parenchyma (5 - 6 weeks *p.i.*) [93,125]. In general, the highest titres are always observed against gut antigens [45,48,53,84,87,93], probably because these antigens are excreted in relatively large quantities into the host circulation [80,131]. Earlier it had been reported that schistosome circulating antigens, detected by immunoelectrophoresis, appeared in the serum of mice 26 days after infection [12], which correlates with the time-point that the antibodies appear. By adsorption of patient serum with purified antigen preparations it has been shown that antibodies against both the circulating anodic antigen (CAA) and the circulating cathodic antigen (CCA) were responsible for the gut-associated immunofluorescence [46]. This finding was partly contrary to earlier observations, in which it had been found that only antibodies specific for CAA were detected in the gut [84]. Later studies showed that 90% of the patient IgM antibodies reactive with the schistosome gut in an immunofluorescence assay could be inhibited by anti-CCA, but not by anti-CAA or anti-cathepsin B (32 kDa) monoclonal antibodies [48], demonstrating the immunodominance of CCA in the IgM immune response against the gut-associated antigens. This inhibition was only slightly lower in more chronic infections. Antibodies to cathepsin B present in the schistosome gut were detectable from week 3 after infection [33] and seemed to increase as the infection progresses (J.P. Rotmans, unpublished observations).

Ultrastructural localization studies using monoclonal antibodies (McAbs) specific for CAA and CCA have shown that the antigens are present in the gut of adult worm, as well as in the primordial gut cells of cercariae and in 3½ week old worms [41,42]. The localization patterns indicated that the antigens may be derived from the rough endoplasmatic reticulum, and transported via the Golgi apparatus and cytoplasmic vesicles to the luminal side of the syncytium where

[1] recently, an ELISA was described in which the *S. japonicum* 31/32 kDa hemoglobinase was detected in the serum of Chinese schistosomiasis patients [76]

they finally may be released into the gut lumen [41,42]. Thus, they exhibit a biosynthesis and transport mechanism similar to that proposed for glycoconjugates associated with the gut epithelium of other trematodes or vertebrates [14,15,75]. Deelder and co-workers described the presence of CAA in epithelial cells lining the schistosome gut from the cercarial stage and onward (as also found by Andrade and Sadigursky (1978) [4]) but never on the schistosome tegument [42,46]. Likewise, CCA was primarily found in the schistosome gut, but not on the tegument. Conflicting results were obtained for CCA with respect to detectability in egg extracts [44,46], but using McAbs in immunoelectrophoresis, and in immunofluorescence and/or in dot-immunobinding assays, it was established that CCA was also present in schistosome eggs (Chapter 3, and [48]). Partly contrary to these findings, Barsoum *et al.* (1992) [8], showed the binding of a CCA-specific McAb to the schistosome tegument, although the major reactivity was to the gut epithelial cells.

The early antibody response to gut-associated antigens in comparison to tegument or other schistosome antigens might be the result of very immunodominant epitopes on the gut-associated antigens, or of the relatively large amounts of antigens which are released into the host circulation early in development of the parasite. The high excretion rate of gut-associated antigens probably means that the worms exhibit high metabolic activity starting early in life.

Aside from indirect demonstration by the antibody response, the *circulating* antigens themselves are detectable in the *circulation* of the host. A circulating antigen in the plasma of mice or hamsters heavily infected with *Schistosoma mansoni* was first described by Berggren and Weller (1967) [12]. An anodic precipitate was observed by immunoelectrophoresis (Fig. 4), which was correlated with worm burden and duration of infection. The antigen of non-host origin was detected from day 26 *p.i.* onwards in mice infected with more than 500 cercariae [12], and from day 21 *p.i.* onwards in hamsters infected with 1100 cercariae [57]. Gold *et al.* (1969) [57] demonstrated that the antigen could also be found in urine, although in small quantities. Initial characterizations showed that this antigen was heat-stable, dialyzable with a MW < 10 kDa, and had an UV absorption maximum at 260 nm. However, enzyme incubations indicated that it was not DNA or RNA [57]. Further characterizations of this antigen, which was later named the gut-associated proteoglycan GASP [86] or the circulating anodic antigen CAA [44][1], were carried out by Nash *et al.* (1974, 1977) [86,88] and by Deelder *et al.* (1976, 1980) [44,46]. Results are summarized in Table 1.

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[1] Although a recommendation has been made to rename the antigen GASCAP [85], CAA is now widely used to denote this antigen, and throughout this thesis the acronym CAA will be used.

Table 1. Characteristics of circulating anodic antigen

Characteristics		Reference
molecular properties		
5%[a] (w/w) amino acids:		
	rich in Glycine, low proportion of Phenylalanine and Tyrosine	[86]
41%[a] (w/w) carbohydrates:		
	GalNAc : Gal : GlcNAc : Man : GlcA = 17.2 : 2 : 1.9 : 0.4 : 16.1	[86]
physico-chemical properties		
negatively charged		[86]
MW by	ultracentrifugation < 10 kDa	[57]
	gelfiltration 50 – 300 kDa	[46,88]
	polyacrylamide gel electrophoresis > 800 kDa	[44]
	ultrafiltration > 100 kDa	[88]
	ultracentrifugation of immune complexes 70 kDa	[67]
immunoelectrophoretic motility: highly anodic		[12,44,88]
resistant to	30 min boiling	[88]
	7.5% Trichloroacetic acid	[88]
	pronase, trypsin	[88]
	alkaline phosphatase, DNase, RNase, α-amylase	[88]
destroyed by periodate		[88]
no absorption peaks at 280 nm or at 260 nm		[86]
immunogenic properties		
specific antibodies demonstrated 3 – 4 weeks after infection		[46,84]
IgM levels higher than IgG levels		[46,87]
biological properties		
Schistosoma genus-specific antigen, demonstrated in *Schistosoma mansoni*, *S. haematobium*, *S. japonicum*, *S. intercalatum*, (*S. curassoni*, *S. bovis*, *S. matthee*[b])		[40,73,88]
detected in host's serum and/or urine		[44,57,88]
localization		
in the adult worm and the developing schistosomulum:		
	primarily in epithelial cells of the gut	[46,83]
	gut lumen in lysosome-like bodies, associated with host leukocytes	[42]
	intracellularly in Golgi apparatus and cytoplasmic vesicles	[42]
in cercariae:	primordial oesophagus	[4]
	in cytoplasm and surface coat of gut epithelium	[42]
also found in the host in:		
	glomerulus of kidney	[46,52]
	Kupffer cells	[46,52]
	macrophages in spleen	[52]

[a] amino acids and carbohydrates together makes 46% of the total weight of material analyzed; material resistant to hydrolysis or incompletely derivatized, salts and water may account for the remaining weight

[b] Deelder, *et al.*, unpublished observations

Table 2. Characteristics of circulating cathodic antigen

Characteristics		Reference
molecular properties		
37%[a] (w/w) amino acids:		
	rich in Glycine, Serine and Threonine, low proportion of Phenylalanine and Tyrosine	[27]
63%[a] (w/w) carbohydrates:		
	GalNAc : Gal : GlcNAc : Fuc : Man = 1 : 6.6 : 2.9 : 3.0 : 1.6	[27]
physico-chemical properties		
neutral or slightly positively charged		[27,46]
MW by	gelfiltration 10 – 300 kDa	[27,46]
	polyacrylamide gel electrophoresis > 400 kDa	[27]
	ultracentrifugation of immune complexes 40 kDa	[68]
immunoelectrophoretic motility: cathodic		[25,27,46]
resistant to	120 min boiling	[24]
	10% Trichloroacetic acid	[25]
	protease	[25]
	ribonuclease, neuraminidase, amylase	[25]
destroyed by periodate		[25]
immunogenic properties		
specific antibodies demonstrated 3 – 4 weeks after infection		[46,47]
IgM levels higher than IgG levels		[46]
biological properties		
Schistosoma genus-specific antigen, demonstrated in *Schistosoma mansoni*, *S. haematobium*, *S. japonicum*, *S. intercalatum*, (*S. curassoni*, *S. bovis*, *S. mattheei*[b])		[25,46,73,101]
detected in host's serum, urine, or milk		[25,39,44,111]
localization		
in the adult worm and the developing schistosomulum:		
	primarily localized in epithelial cells of the gut	[27,46]
	in gut lumen in lysosome-like bodies	[41]
	intracellularly in Golgi apparatus and cytoplasmic vesicles	[42]
	male tegument	[8]
in eggs:	contradictory results	[44,46]
in miracidia:	not found	[41]
in cercariae:	in cytoplasm and surface coat of gut epithelium	[41]
also found in the host in:		
	glomerulus of kidney	[41]
	Kupffer cells	[43,46]
	macrophages in spleen	[43]

[a] percentages given by the authors; the percentage amino acids was probably obtained by subtracting from 100% the total weight of sugars (63%) found by methylation analysis

[b] Deelder, *et al.*, unpublished observations

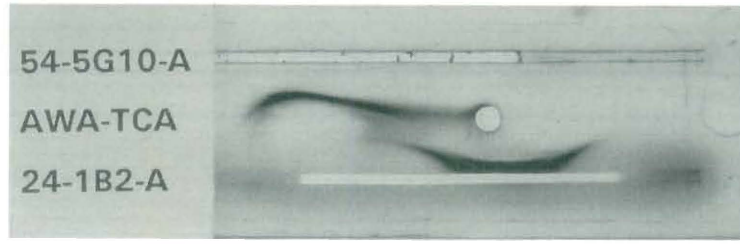

Figure 4. Immunoelectropherogram of an anti-CAA (upper lane) and an anti-CCA (lower lane) McAb against AWA-TCA.

In the urine of schistosomiasis patients, Carlier *et al.* (1975) [24] have found a second circulating antigen, 'M antigen', which in immunoelectrophoresis appeared neutral or slightly cathodic (Fig. 4) [25,27]. The latter phenomenon clearly distinguished this antigen from CAA. Further studies showed similarities with respect to stability and solubility, but distinct differences with respect to monosaccharide composition [25,27]. Apart from its presence in urine, M antigen was also demonstrated in patient sera, milk of infected mothers, as well as in sera and urine of animals infected with *Schistosoma mansoni* [25,111]. Independently, Deelder *et al.* (1976) demonstrated a circulating cathodic antigen (CCA) present in serum and urine of infected hamsters [44]. This antigen was localized in epithelial cells of the schistosome gut and detected in excretory and secretory products. It is now generally accepted that M antigen and CCA are identical [46,54][1]. A summary of the characteristics of CCA is given in Table 2.

Apart from these antigens detectable in the circulation of the host, a very early report by Okabe and Tanaka (1958) [91] described an antigen in the urine of patients or experimental animals with schistosomiasis japonica. This antigen appeared to be a heat-labile, easily degradable protein, but it has not been investigated whether the antigen was indeed *Schistosoma*-specific or whether it was *e.g.* a host-derived inflammatory (acute-phase) protein [92].

Ripert *et al.* (1988) described a circulating gut-associated antigen, excreted in the urine of schistosomiasis patients, which was predominantly present in eggs [103]. This antigen showed characteristics similar to those of CCA, such as being excreted in high concentrations in the urine, stability towards protein denaturing agents, sensitivity to periodate, and being localized in the schistosome gut [5,6]. However, CCA is not present in large amounts in the

[1] Like CAA, the acronym CCA is now widely used instead of M antigen, and throughout this thesis we also will use CCA.

eggs, whereas the antigen described by Ripert and Appriou is predominantly an egg antigen [6]. The McAb recognizing this antigen has been investigated in our laboratory for cross-reactions with anti-CAA and anti-CCA McAbs. The recognition and binding patterns in several immunochemical techniques indicated an antigen specificity different from our anti-CAA or anti-CCA McAbs. However, common epitopes might be present on CCA as a few anti-CCA McAbs (also recognizing an egg antigen) showed inhibition of the McAb of Ripert using immunofluorescence assay on adult worm sections (Deelder *et al.*, unpublished results).

Very few functional studies have been performed on the gut-associated antigens. While it is clear that *e.g.* the gut-associated proteases cathepsin B and hemoglobinase are involved in digestive processes, different functions for CAA and CCA have been suggested, but none of these could be experimentally supported. The localization of CCA in the gut epithelium of the adult worm, as well as structural characterization data (molecular heterogeneity, glycoprotein composition, and the presence of O-glycosidic linkages) support the hypothesis that the antigen is a mucin or mucus glycoprotein-like component [27], which protects the gastrodermis of the worm against proteolytic secretions. The observation that the half-life in the schistosome gut of GlcNAc-containing polysaccharides was significantly longer than the half-life of gut secretory proteins also suggests that these carbohydrate structures might function as a protective surface coat [131]. Deelder *et al.* (1989) [48] postulated that CCA and the formation of anti-CCA antibodies may play a role in the parasite's evasion of the immune response of the host. The reasons for this were that many anti-CCA McAbs recognize epitopes of the schistosome egg, and carbohydrate egg antigens are supposed to elicit 'blocking' IgM antibodies which may interfere with the binding of protective IgG antibodies directed against surface antigens of the schistosomulum [19,20,50]. The above described IgM McAb of Appriou *et al.* (1986) directed against a gut-associated antigen similar but not identical to CCA [6], showed an inhibitory effect on immunity after passive transfer in mice [5]. The authors suggested that the McAb blocking activity might interfere with non-specific immune mechanisms. Using several anti-CCA McAbs in passive transfer experiments, however, we could not demonstrate an inhibitory effect on immunity (unpublished observations). Van Egmond *et al.* (1981) [124] showed that purified CCA preparations, as well as a preparation of excretory and secretory antigens, are capable of inducing complement activation. The exact mechanism of this complement activation could not be found.

Likewise, the role of CAA in the physiology of the schistosome remains speculative; perhaps it protects the gastrodermis against digestive enzymes, low pH, or host materials such as antibodies or complement [83,86]. Once in the circulation of the host, this highly negatively charged compound could interfere with the normal surface interactions of the host cells, possibly altering immune

responses or blood clotting processes [86]. A preparation of excretory and secretory antigens containing CAA in relatively large quantities has been shown to interfere with hemostatic processes [72]. Tsang *et al.* (1977) [121,122] described an anticoagulatory activity in a whole worm homogenate of *Schistosoma mansoni*, which is not destroyed by heating the antigen to 100°C for 10 min, suggesting that CAA might be involved. Robertson and Cain (1985) have described the analysis of glycosaminoglycans from *Schistosoma mansoni* and suggested that these structures (including CAA which has a glycosaminoglycan-like monosaccharide composition) might be involved in the prevention of entrapment of the parasites by the host's blood-clotting process [104].

Both CAA and CCA have been described to be involved in pathology as CAA- and CCA-containing immune complexes have been found to be deposited in the kidney and in the liver [26,52,64,65]. It has been reported that schistosomal-specific nephropathy exists and can lead to end-stage renal disease, with CAA and CCA as major responsible antigens [117,118]. This schistosomal-specific nephropathy, however, does not show any remission after anti-schistosomal treatment [119], suggesting that also other mechanisms are involved in the induction of pathology, as has already been described earlier [126]. In schistosomiasis haematobium an association between the disease and bladder cancer has been described [70,112,120], but it is unlikely that antigens or immune complexes play a role herein. Other mechanisms have been suggested such as urothelium chromosomal damage caused by *S. haematobium* infection [106].

In conclusion, the gut-associated antigens of *Schistosoma* constitute an immunologically and physiologically important group of antigens. Analysis of two major antigens, CAA and CCA, which are the targets in two recently developed immunodiagnostic assays for schistosomiasis, indicated that the important epitopes are carbohydrates. Therefore, a major theme of this thesis will be the molecular characterization of these carbohydrate structures. Based on the results, the role or effect of these antigens will be investigated in relation to the immune system of the host.

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