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

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Chapter 12

General discussion

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Schistosoma antigens have long been a topic of intensive research. An important reason for this undoubtedly is the interesting life-cycle involving many host-parasite interactions. In particular in the final hosts, *e.g.* humans and cattle, these interactions often may be dangerous for the parasite, as they take place in the blood, the schistosome habitat. The blood is equipped with a number of physiological defense mechanisms (especially immunological and hemostatic ones) against injury and infection, which have forced the parasite to develop its own efficient defense system. It is in the interest of man, as a final host, to overcome these parasite evasion mechanisms by manipulation and education of his own defense systems to recognize and destroy the parasite. For this reason most of the research has been focused on tegumental surface antigens since they are the major targets for the host immune response. However, another source of large amounts of parasite antigens presented to the host is the schistosome gut, a very active organ for the digestion of host blood cells and nutrients, as well as for excretion and regurgitation of undigested compounds.

In the early report of Berggren and Weller (1967) a negatively charged antigen was described which generally is assumed to be identical with CAA, although localization studies had not yet been performed [6]. CCA was first described independently by Carlier *et al.* (1975) [16] and Deelder *et al.* (1976) [26], but not until 20 years after the first description of the antigens, reliable, accurate and reproducible assay-systems for the detection of CAA and CCA were developed and widely applied [23-25,71]. Essential to the success of these diagnostic techniques was the development and application of monoclonal antibodies. Moreover, the use of these CAA- and CCA-specific McAbs has facilitated structural analysis of the antigens, as well as studies on the clearance in experimental animal infection and on the immunopathological involvement in schistosome infections in experimental animals and humans.

Considering circulating antigens as diagnostic markers, a number of other antigens have also been described as potentially useful targets for immunodetection and immunodiagnosis of schistosomiasis. Hayunga *et al.* (1986) developed an assay for detection of cercarial antigen in serum, which could

determine a 100-worm infection in mice, as early as 1 week after infection [39]. Application of this assay would facilitate an early and accurate diagnosis, *e.g.* of infected travellers. However, only a very limited field study using this assay has been performed, which showed that chronic schistosomiasis infections also could be detected [40].

Strand and co-workers described that a schistosome egg antigen containing carbohydrate epitopes, which were cross-reactive with antigens of various life-cycle stages, could be detected in serum of patients using a McAb [37,74]. After praziquantel treatment of the patients antigen levels were shown to decrease [37]. A McAb-based inhibition passive hemagglutination assay for detection of a polysaccharide egg antigen in urine has been used by Ripert *et al.* in several epidemiological studies [47,55,56].

Recently, a highly sensitive and specific ELISA for the detection of circulating soluble egg antigen was developed within our laboratory [53]. In this assay two different McAbs were utilized in a mixture both for coating and detection. The correlation with egg output and serum CAA was very high. The McAbs were shown to be highly reactive with two different repetitive carbohydrate epitopes of soluble egg antigen and also showed a strong reactivity with antigens present on the cercarial and schistosomular surface, as well as recognizing antigens in the parenchyma (flame cells, excretory system) of adult worms [8,52].

An attempt to define and apply circulating antigens other than those described above but still potentially relevant for immunodiagnosis using McAbs present in our laboratory was unsuccessful. Remarkably, it was found that a major portion of the selected McAbs, showing diverse reaction patterns in fluorescence assays and in other tests, was reactive with a non-repetitive epitope on CCA [68], which suggests that the antigen contains a large number of different epitopes.

Over many years, a large number of anti-CAA and anti-CCA McAbs (n=25 and n=55, respectively) has been generated, the various reaction patterns of which are described in Chapter 3. These McAbs displayed a strong isotype restriction with only IgM, IgG3, and IgG1 McAbs. In all, 60% of the McAbs against CAA were of the IgG1, and 80% of the anti-CCA McAbs were of the IgM isotype. In the mouse, a carbohydrate antigen-driven isotype restriction in the form of IgM and IgG3 is well-known [14,49,54], but anti-protein antibodies (needing T-cell help) are generally IgG1 [2,14,50], which isotype corresponds to the human IgG4 [1,30,44]. In this light, the IgG1 preference for McAbs against the carbohydrate antigen CAA is remarkable.

The major theme of this thesis are the results on the use of the CAA- and CCA-specific McAbs for the purification and subsequent molecular analysis of the antigens themselves. A purification procedure was optimized and immunopurified preparations of CAA and CCA were obtained in sufficient

amounts for molecular characterization. In a collaborative study, it was found that CAA contains a novel and unique polysaccharide structure consisting of multiple repeats of a →6)[GlcA-β(1→3)]GalNAc-β(1→ disaccharide unit [7]. This structure may explain the nearly absolute specificity of the CAA-based assays for the diagnosis of schistosomiasis. CCA-detection, especially in urine, was described to be less specific, necessitating a higher cut-off value in the assays [43,71]. The elucidation of the main carbohydrate structures of this antigen as an *O*-linked polysaccharide having the Lewis x trisaccharide as a repeating unit provided a (partial) explanation for these observations. Glycoproteins or glycolipids carrying a single or multiple Lewis x determinant (→3)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→, Le^x) are frequently found on a variety of human cell-types, including granulocytes [32,60] and carcinoma cells [22,35,59]. Current and preliminary investigations indeed indicate an association between a positive reaction in the urine CCA-ELISA and the presence of leukocytes in the urine of *Schistosoma*-negative individuals (Deelder *et al.*, unpublished results).

Another interesting area of research is the role of these schistosome gut-associated antigens in the host-parasite interaction. It is inconceivable that the parasite would certainly not excrete the antigens in such relatively large amounts in order to facilitate immuno-detection. Since the digestive tract of the parasite is the source of the antigens, it is easy to imagine that they play a role in enzymatic digestive processes [51], but the highly glycosylated nature of the antigens suggests a protective function for the gastrodermis [75]. Indeed, CCA shares some structural characteristics with mucins [17,67], compounds known to provide lubrication and physical protection for epithelial cell surfaces [38,64]. Besides this possible "innate" physical protection, a few specific interactions of gut antigens with the host immune system had been described previously [3,29] and/or were further investigated during the course of the present research.

It has been shown that antigens originating from the schistosome gut caused complement consumption in the absence of specific antibodies, although the activation pathway (classical or alternative) could not be identified [3,70]. Therefore, it could be envisaged, that the antigens play a role in avoiding complement-mediated damage by activation of the complement-system in the lumen rather than directly at the vulnerable surface of the gut. Using immunopurified CAA, and CAA-specific McAbs it could be shown that CAA binds to C1q with characteristics similar to the C1q-C1q-receptor interaction (Chapter 10 and [69]). By acting as a C1q-receptor and binding the C1q present locally, CAA may interfere with the binding of C1q-immune complexes with C1q-receptor-containing cells such as monocytes, neutrophils and platelets [21], thereby preventing activation of these cells. In the same study it was shown that immunopurified CCA binds to isolated C1q-heads, while CAA binds to C1q-stalks (similar to the C1q-receptor). In ELISA's, measuring C3 and/or C4 deposition as a measure for complement activation, the purified antigens did not

activate the complement system, neither by the classical nor by the alternative pathway (unpublished results).

Following characterization of the carbohydrate structure of CAA as a glycosaminoglycan-like polyanion containing GlcA-GalNAc repeating units, it is tempting to speculate on the biological effects (apart from the C1q-interaction described above). A number of polyanions (*e.g.* heparin, chondroitin-sulfate) are known for their anticoagulant activities which probably reside in the glycosaminoglycan-region of the molecule [10-12]. Indeed, in a number of studies anticoagulant activities were found in schistosome antigen preparations and preliminary characterizations of the active component do not exclude CAA as a possible candidate [31,41,65,66]. The mechanism of action, however, remains controversial. One study described the inhibition of the activation of Hageman factor (XII) by schistosome adult worm antigens, without inhibiting the activated factor XII itself [31], while another study showed that the activity of factor XII after activation was specifically inhibited by a similar antigen preparation [65,66]. A third study reported that anticoagulation probably occurred only in the factor XII-independent system, the extrinsic pathway [41]. In addition, Robertson and Cain (1985) isolated a number of glycosaminoglycans from *Schistosoma*, predominantly from the tegumental fraction, suggesting that these components may prevent entrapment of the schistosome by the host's blood-clotting process [57]. However, the observation that galactosaminoglucuronoglycan structures did not influence the major blood clotting parameters may argue against the possible anticoagulation effect of CAA [48].

Finally, Chiang and Caulfield (1989) described that polyanions like suramin, heparin, and dextran sulfate caused displacement of human lipoproteins from the schistosomulum tegument [18,19]. Opposed to the other above described phenomena, this possible effect of the polyanion CAA might prove to be a disadvantage for the parasite, because the surface-bound lipoproteins are thought to mask parasite tegument antigens, which otherwise are recognized by host antibodies [18,19].

The major *O*-linked polysaccharide chains on CCA are composed of multiple repeating Le^x units (Chapter 7 and [67]). In a pool of glycoproteins isolated from adult schistosome worm-pairs, Srivatsan *et al.* (1992) have recently demonstrated a similar structure with at least four repeating units of the Le^x determinant attached to N-linked carbohydrate chains [62]. Due to the antigen preparation procedure the source of the antigen could not be located, while CCA clearly originates from the gut of the parasite [27]. Besides this, we found that CCA consists of about 80% carbohydrates, and electrophoresis resulted in an antigen smear of 70 - 200 kDa [28,68]. The anti-Le^x McAb used by Srivatsan *et al.* reacted clearly with two bands (one band at 200 kDa and another (broad) band at about 90 kDa) on a Western blot of isolated glycoproteins [62]. In our hands, treatment of CCA with α-fucosidase did not result in a reduction of

McAb-binding (unpublished results), while Srivatsan *et al.*, on the other hand, found a 60% reduction in binding of the antigens to an affinity-column prepared with antibodies from an infected hamster, indicating that only part of their antigens was not susceptible to α-fucosidase. Besides these differences, there are also structural similarities since anti-Le^x McAbs bound to both antigen preparations. It is likely from these results that the schistosome worms are able to synthesize a series of different antigens containing the Le^x or poly-Le^x epitopes, both on *N*-linked and *O*-linked carbohydrate chains.

The finding that the major polysaccharide structures on CCA contained Le^x as a repeating unit produces some interesting hypotheses. Le^x- and, to a much larger extent, sialylated Le^x-containing glycoproteins, play an important role in granulocyte and monocyte adhesion to endothelial cells and platelets [34,45,46,61,63,73,76], thereby recruiting granulocytes to sites of inflammation [13,61]. In this context, it could be hypothesized that the excretion of relatively large amounts of a poly-Le^x containing component by the parasite induces local anti-inflammatory and anti-thrombogenic effects [61,62]. Inflammatory reactions as well as blood coagulation are host protection mechanisms, which potentially are very harmful to the schistosome, and the interference of CCA with these mechanisms may thus be one of the important parasite's survival strategies.

Circulating human granulocytes are rich in Le^x and carry in relatively high abundance branched *N*-linked polysaccharides having Le^x repeating units [60]. Deelder *et al.* (1989) [29] demonstrated that the predominant IgM response in humans, as measured by immunofluorescence assay, against *Schistosoma mansoni* gut-associated antigens was actually directed against CCA. In addition, a mild to moderate neutropenia in chronic schistosomiasis patients has been observed [9], which might be caused by an inhibitory factor in the sera of these patients delaying the maturation of neutrophils in the bone marrow and spleen [9,58]. From these three observations, we proposed the hypothesis that excretion of CCA evokes high titres of anti-poly-Le^x antibodies, which are also directed against identical host carbohydrate structures on *e.g.* neutrophils, thereby causing complement mediated antibody-dependent lysis of these cells. In this context, Ko *et al.* (1990) [42] found that a murine protective IgM McAb, raised against *S. mansoni* eggs, recognized the Le^x determinant (also called SSEA-1, stage-specific embryonic antigen-1), and showed binding to the surface of live schistosomula. In the present study (Chapter 9) it is shown that McAbs, as well as affinity-purified human IgM antibodies directed against CCA, recognized human granulocytes, and, in the presence of complement, caused lysis of the cells.

Recently, another interesting effect of carbohydrate structures similar or identical to CCA (*i.c.* fucosylated (Le^x and Le^y) oligosaccharides) on the host immune system was described by Harn and co-workers [36,72]. These authors showed that Le^x and Le^y carbohydrates (conjugated to human serum albumin) induced

proliferation of human peripheral blood mononuclear cells (including B cells) [36], as well as interleukin 10 production by isolated B cells ($B220^+$) of infected but not of non-infected mice [72]. Moreover, they were able to show the presence of antibodies in the cerebrospinal fluid of schistosomiasis patients with cerebral disorders against these fucosylated oligosaccharides [33]. Based on these very new phenomena, the authors suggested that Le^x- and/or Le^y-containing oligosaccharides played a role in the immunoregulation of the helper T cell response in schistosomiasis and maybe other chronic infectious diseases [72]. In addition to the above mentioned highly local (anti-inflammatory and anti-thrombogenic) or indirect (induction of anti-Le^x antibodies) effects, this hypothesis describes a more general effect of excreted CCA.

Appriou *et al.* (1989) described an IgM McAb recognizing a carbohydrate epitope of a schistose gut-associated antigen [3]. The structural characteristics of this antigen, its localization, and presence in different developmental stages, including eggs, indicated a similarity with CCA [4]. Indeed, comparisons between the 'Appriou-McAb' and the anti-CAA and anti-CCA McAbs, which were performed in our laboratory, confirmed the presence of common epitopes with CCA and not with CAA. Some anti-CCA McAbs, predominantly those which also recognized an egg antigen, showed inhibition of the 'Appriou-McAb' in an immunofluorescence assay (Deelder *et al.*, unpublished results). The 'Appriou-McAb' displayed an inhibitory effect on immunity by passive transfer experiments in mice, both in a secondary and in a primary infection indicating interference with innate immune systems [3]. These investigators hypothesized that inhibition was due to a masking by the antibodies of the complement-activating surface structures on schistosomula. However, IgM deposition on the schistosomulum-surface would also activate the complement system (via the classical route). If the McAb described by Appriou *et al.* would also recognize the main carbohydrate chains of CCA which have a poly-Le^x structure, a weakening of the immune system by complement-dependent lysis of granulocytes, which carry multiple Le^x epitopes on their surface, might also be hypothesized as a possible inhibition mechanism (see also Chs. 7, 9, and above). In contrast to the results of the passive immunization described above, we could not demonstrate an inhibitory effect on immunity, using several different anti-CCA McAbs in passive transfer experiments (unpublished data). Moreover, Ko *et al.* (1990) described a McAb which recognized the Le^x (SSEA-1) epitope but which showed a host-protective effect after passive immunization of mice [42].

The kinetics of CAA and CCA relative to the physiology of the schistosome was investigated by determining the excretion patterns of *in vitro* and *in vivo* developing schistosomula and adult worms (Chapter 11). The observation that the feeding of red blood cells had no effect on the *in vitro* production of the antigens revealed no direct enzymatic role in digestion, and agreed with nature of the antigens themselves (*i.e.* mucin-like molecules). In the growing parasite, CAA

and CCA appeared to be continuously excreted at a low level but showed a steep increase after about 10–15 days which coincides with the rapid development of the gut at that stage [5,15,20]. This supports the potential gut-protective role already indicated above.

In summary, the research described in this thesis focused mainly on two dominant *Schistosoma* gut-associated antigens, CAA and CCA, which are major targets in highly specific and sensitive McAb-based immunodiagnostic assays for schistosomiasis. After purification of the antigens by specific McAbs, the molecular structures of the immunologically dominant carbohydrate parts were elucidated, pointing to distinctive biological functions. Finally, it could thus be shown that CAA and CCA are indeed involved in specifically manipulating parts of the host defense system.

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