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

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Summary

The research reported in this thesis has been performed within the scope of ongoing research to improve methods for diagnosis of human schistosomiasis and to study the immunological interaction of the parasite *Schistosoma* with the host. Placed within a larger framework, the results could improve the understanding of the host–parasite relationship in general and assist in the development of better diagnostics or therapeutic and intervention strategies. Ever since the first description of schistosome circulating antigens, their potential value for an immunodiagnostic test for schistosomiasis has been realized. Although other antigens were described later, the two antigens which have been most extensively studied are two glycoconjugates originating from the gut of the parasite: circulating anodic antigen (CAA) and circulating cathodic antigen (CCA). The origin, localization, and a number of characteristics of these two gut–associated antigens as well as a number of others have been described in **Chapter 1** of this thesis. This literature overview also briefly deals with host–parasite immunological interactions and with the putative physiological role of CAA and CCA in the parasite itself.

The immunologically and structurally dominant elements of CAA and CCA involve carbohydrate structures, the characterization of which constitutes a major part of this thesis. For this reason, in **Chapter 2** a short general overview is given on glycoconjugates with respect to their structure and techniques used for structural analysis. Examples given are primarily from the field of parasitology research and, more specifically, schistosomiasis research. The amounts of purified antigenic material which can be isolated from parasites is usually limited, and, therefore, immunochemical methods (*e.g.* employing antibodies, or lectins) are frequently used in the analysis of carbohydrates of parasite antigens. Most of the findings which have been described for glycoconjugates of *Schistosoma* are reviewed in the second part of **Chapter 2**.

To improve the detection of CAA and CCA, a large panel of monoclonal antibodies (McAbs) directed against CAA and CCA, as well as against various other *Schistosoma* antigens, were developed over many years. An analysis of

these anti-CAA and anti-CCA McAbs is presented in **Chapter 3**. A remarkable isotype restriction is observed because the McAbs only showed IgM, IgG3 and IgG1 isotypes (anti-CAA 60% IgG1, anti-CCA 80% IgM). It is also observed, using different techniques, that the anti-CCA McAbs recognized a much more heterogeneous pattern of antigens than the anti-CAA McAbs.

To detect other gut-associated antigens as potential candidates for immunodiagnostical use, a study is undertaken to analyze McAbs reactive with *Schistosoma mansoni* gut-associated antigens other than CAA, CCA, or already well-studied gut proteases. The outcome of this study, described in **Chapter 4**, corroborated the important role for CCA in immunorecognition in experimental or human infections, as well as a number of well-known, but still interesting antigen-directed isotype restrictions, *e.g.* significantly more IgM McAbs than IgG McAbs recognized common carbohydrate epitopes present on gut and egg-shell antigens.

The application of the FITC-anti-FITC system as a technical improvement and alternative for the ultrastructural localization of antigens is described in **Chapter 5**. Two examples of specific applications of the system for detection of antigens in sections of *S. mansoni* adult worms are given: 1. detection of CCA by an FITC-labeled anti-CCA McAb followed by a gold-labeled anti-FITC McAb; and 2. detection of various antigens by human IgM antibodies pooled from patients infected with *Schistosoma*, followed by an FITC-labeled anti-human IgM-antiserum, and a gold-labeled anti-FITC McAb.

Diagnosis of schistosomiasis within our laboratory makes use of the serological detection of human IgM antibodies against gut-associated antigens by an immunofluorescence assay (IFA) on sections of adult worms. By inhibition studies, it has earlier been shown that these IgM antibodies were predominantly directed against CCA. To more specifically study the IgM response against CCA as well as to standardize and simplify the IFA for larger scale use, three ELISA-based systems are developed with the use of immunopurified CCA (**Chapter 6**). In all three assays, intra- and inter-assay variation is lower than 5% while in neither of the assays false negatives are found. In two of the three assays (direct CCA- and indirect CCA-coat) one false positive is found. For these reasons, as well as for practical and economic advantages, the antibody-capture assay is finally selected.

The application of CAA- and CCA-specific McAbs facilitated the immunopurification of sufficiently large amounts of CAA and CCA for detailed structural analysis. The primary structures of the carbohydrate parts, which are immunodiagnostically and immunologically most relevant, are described in **Chapters 7** and **8**. The major carbohydrate structures of CCA are O-linked (via Thr) polysaccharides containing the Lewis x trisaccharide (→3)Galβ(1→4)-

[Fucα(1→3)]GlcNAcβ(1→) as the repeating unit and GalNAc as the reducing terminal monosaccharide. The minor carbohydrate fraction comprised disaccharides to hexasaccharides, having the Galβ(1→3)GalNAc-OL core in common. The major carbohydrate chains of CAA are Thr-linked polysaccharides consisting of disaccharide →6)[GlcAβ(1→3)]GalNAcβ(1→ repeating units, probably connected to the protein via an as yet unknown core saccharide structure with GlcNAc at the reducing end. The detection in CAA of small amounts (<5%) of the CCA-specific O-linked polysaccharides with Lewis x as repeating unit, probably accounts for the generally observed cross-reactivity of anti-CCA McAbs with intact CAA.

Because the carbohydrate structure of CCA resembles a major granulocyte surface antigen, and in schistosomiasis patients, a significant IgM response against CCA usually is found, as well as, in some studies, decreased neutrophil counts, the possible role of anti-CCA antibodies in granulocytotoxicity is investigated (**Chapter 9**). Indeed, it is shown, that anti-CCA McAbs in the presence of complement mediated the lysis of granulocytes, and an identical effect is observed for purified anti-CCA IgM antibodies in sera from schistosomiasis patients.

Using purified antigens and specific McAbs, CAA but not CCA is found to interact with the first complement component C1q (**Chapter 10**). Applying purified C1q fragments in ELISA, it is proven that the interaction of C1q with CAA occurs via the collagen-like stalks of C1q. This interaction resembles the binding of C1q to its cellular C1q-receptors on such cells as monocytes, neutrophils, and platelets. This binding normally induces activation of these cells followed by antibody-independent cytotoxicity. Interference of CAA with this C1q-C1q-receptor interaction may decrease this cellular cytotoxicity mechanism against the parasite.

Finally, to contribute to the study of the physiological role of CAA and CCA, the *in vivo* and *in vitro* excretion patterns of CAA and CCA by newly transformed and developing schistosomula as well as by 7-week old adult worms are investigated and discussed with respect to clearance mechanisms (**Chapter 11**). It is observed that more CAA than CCA is produced *in vitro* during the first days of development, but that after about one week this ratio is already reversed. Adult worms, isolated from infected hamsters and incubated *in vitro* produce about 2 times more CCA than CAA. In sera of infected mice, 10 to 100 times more CAA than CCA is detected, while in the urine this is the opposite, which indicates that the clearance mechanisms of the two antigens are very different.

This thesis concludes with a general discussion in **Chapter 12**.