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

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our little systems have their day
they have their day and cease to be
they are but broken lights of Thee
but Thou O Lord art more than they

Alfred Tennyson

aan mijn vader en moeder
aan Betty
aan Rieke, Paul en Jaap

Cover illustration: gut-associated antigens of *Schistosoma mansoni*, visualized by immunofluorescence microscopy (McAb 99-1G3-A) on a section of adult male worms fixed in Rossman's fixative

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Contents

Preface

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Human schistosomiasis (bilharzia) is one of the major parasitic diseases in the world, affecting 200 million people predominantly in third world countries. In areas where the disease is highly prevalent it causes important health problems, and it also has socially-economic effects on the population. As the parasite is transmitted through surface water, increasing irrigation measures may also contribute to expansion of the disease. Laboratory research on schistosomiasis is mainly directed at the (immunological) interaction of the parasite and the host and the improvement of diagnostic methods. Besides having fundamental scientific interest and/or using *Schistosoma* infection as an immunological model, research efforts may also lead to improved therapeutic strategies or, finally, to the development of an effective vaccine.

Schistosomiasis is caused by the presence of the blood-fluke *Schistosoma* (Trematoda) in the blood-vessels of mammalian hosts. The current method for diagnosis of schistosomiasis in developing countries is the parasitological examination of urine and faeces for the presence of *Schistosoma* eggs. An alternative method which is now increasingly used is based on the detection of *Schistosoma* antigens in the circulatory system or the urine of the host. The gut of the parasite is an important source of these antigens since many gut-associated antigens are excreted into the circulation of the host following digestion of food (*e.g.* blood cells, proteins) by the parasite. Two major gut–associated antigens which have been thoroughly studied with regard to diagnostic detectability, are the circulating anodic antigen (CAA) and the circulating cathodic antigen (CCA).

Chapter 1 of this thesis includes a general introduction to *Schistosoma mansoni* gut-associated antigens with particular emphasis on CAA and CCA, and deals also shortly with host-parasite interactions. In addition, a literature overview is presented dealing with localization of the antigens in the parasite, detection in the host circulation, as well as development of the host immune response. As important components of these antigens are carbohydrate structures, 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 mostly from the field of parasitology and schistosomiasis research.

To improve the detection of CAA and CCA a large panel of monoclonal antibodies (McAbs) not only directed against CAA and CCA, but also against various other *Schistosoma* antigens was developed. An analysis of anti-CAA and anti-CCA McAbs generated during the ongoing research of many years is

presented in **Chapter 3**. To detect other gut-associated antigens as potential candidates for immunodiagnosis, a study was undertaken to analyze McAbs reactive with *Schistosoma mansoni* gut-associated antigens other than CAA, CCA, or well-studied gut proteases. The outcome of this study, described in **Chapter 4**, corroborated the important role of CCA. **Chapter 5** describes a technical improvement in the detection or ultrastructural localization of antigens using an anti-FITC McAb. As an example the detection of CCA in *S. mansoni* adult worms is used. In a routinely used serodiagnostic assay (immunofluorescence on sections of adult worms) human IgM antibodies are measured which are directed against gut-associated antigens (predominantly against CCA). To more specifically study the IgM response against CCA and to simplify this detection an ELISA system was developed with the use of immunopurified CCA, as described in **Chapter 6**.

The immunopurification of sufficiently large amounts of CAA and CCA allowed for their structural analysis. The elucidation of the primary structures of the carbohydrate parts, most relevant immunodiagnostically and immunologically, is described in **Chapters 7** and **8**. Because the carbohydrate structure of CCA was found to resemble a major granulocyte surface antigen, the possible role of anti-CCA antibodies in granulocytotoxicity was investigated in **Chapter 9**. In **Chapter 10** a possible function for CAA is described, as it was shown to interact with the first complement component C1q, which may result in an interference with cellular cytotoxicity mechanisms against the parasite. Finally, to contribute to the study of the possible 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 were investigated and discussed with respect to clearance mechanisms in **Chapter 11**. This thesis concludes with a short general discussion in **Chapter 12**.