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Proteases of the human parasite *Schistosoma* and the snail intermediate host *Biomphalaria glabrata*: biochemical, molecular and immunological characterization

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Cercarial secretions from different species of the parasite *Schistosoma* and from *Trichobilharzia ocellata* contain a serine protease, cercarial elastase, which was evidenced as 30 kDa band in gelatin gels. Sera of patients infected with *S. mansoni*, *S. haematobium* or *S. japonicum* contain IgG which react in ELISA with cercarial secretions from all schistosomes and cross-react among the different parasite species. In western blots, however, sera from patients as well as from heavily infected mice or rabbits did not react with a 30 kDa protein. Moreover, when sections from infected snails (*Biomphalaria*, *Bulinus* and *Lymnaea*) were analyzed by immunofluorescence using the same infection sera, only the tegument of the developing cercariae was recognized, but not the acetabular glands. In contrast, when anti-sera against purified cercarial elastase from either *S. mansoni* or *S. haematobium* were tested with sections of infected *Biomphalaria* or *Bulinus*, fluorescence was strong in the preacetabular glands of the cercariae of either species, but undetectable with the tegument. Cross-reactivity of both anti-sera extended to *T. ocellata*-infected *Lymnaea*, but not to *S. japonicum*-infected *Oncomelania*. In conclusion, although immunization with purified cercarial elastase results in antibody production, the enzyme does not induce an apparent antibody response following natural infection.

Serine protease and phenoloxidase activities in lysates from hemocytes of the snail *Biomphalaria glabrata* were also described in this report. Snail hemocytes are supposed to be the immune cells which interact with larval stages of the parasite *S. mansoni*. The activities of both enzymes were shown to have alkaline optimal pH around 9.5. The serine protease was inhibited by EDTA, PMSF, antipain and aprotinin, and the phenoloxidase activity by diethydithiocarbamate. By comparison, the serine protease activity in secretions of *S. mansoni* cercariae also had an alkaline optimum around pH 10.5 and was sensitive to the same inhibitors. Also, serine protease activities from snails and cercariae had the same molecular weight of 28 kilodalton. However, the K_m value of the serine protease(s) and the K_i values of different inhibitors were generally lower for the snail enzyme than for the cercarial enzyme. The serine protease activity varied among individual snails but activity in hemocyte lysates and hemolymph correlated strongly. There was no detectable difference in levels of activity between snails which are susceptible or resistant to schistosome infection.

The elastase from *S. mansoni* was further investigated with the long-term goal to evaluate its potential as a candidate for a DNA-vaccine against schistosomes. In order to induce an antibody response against a construct coding for the elastase of *S. mansoni*, RNA from adult *S. mansoni* worms was reverse transcribed into cDNA. The elastase gene was amplified with specific primers by RT-PCR. For optimal expression of the elastase gene in mammalian cells, we inserted in the sense primer a Kozak consensus sequence (A/GnnATGG) flanking the start codon of the gene. The correct construction was verified by sequencing. The homology of the DNA sequences between the published cercarial enzyme (Newport et al., 1988) and the elastase gene from the adult worms (this report) is 99.4%. For cloning, a mammalian expression vector (pRK7) was used in which expression of the elastase is driven by the CMV promoter/enhancer. The construct in pRK7 was used to transfect COS-7 cells using the calcium-phosphate method. After 48 hours, RNA was extracted from the transfected cells, transferred into a slot-blot, and then hybridized with digoxigenin-labeled DNA probes specific for cercarial elastase. The results showed successful transcription of the elastase in COS-7 cells. Expression of the elastase *in vivo* in muscle tissue of mice was tested by intramuscular injection of the elastase DNA construct into mice. 12 days after injection, RT-PCR analysis was done with RNA extracted from muscle tissue and specific elastase primers. Expression of the enzyme at the transcriptional level in muscle tissue from immunized mice was shown. In contrast, we could not detect transcription of elastase neither in normal muscle tissue nor in muscles from mice injected with the empty pRK7 vector. Thus, the elastase construct was successfully expressed *in vitro* and *in vivo*.

Next the potential of the DNA expression construct to induce an antibody response *in vivo* was investigated. For this purpose, mice were vaccinated with the DNA construct by intra ear (i.e.) immunization. Vaccination with the empty vector was used as control. Sera from individual mice were collected after various vaccination schemes and analyzed in western blots with cercarial secretions as antigen. Sera of mice vaccinated with two i.e. injections, 2 weeks apart with 50 ? g DNA per injection could recognize a 30 kDa band corresponding to the native enzyme. The sera of these mice were also used for immunofluorescence analysis on sections of infected *B. glabrata* snails. The sera which had been reactive in western blots, gave very intense fluorescence reaction inside the heads of the developing cercariae in. Fluorescence was limited to 1 or 2 compartments which correspond to the preacetabular gland region. Neither postacetabular glands nor tegument of the cercariae showed any fluorescence reaction with sera from mice immunized with the elastase DNA construct. In contrast, sera from infected mice gave fluorescence reaction only with the tegument of the developing cercariae but not with any of the cercarial glands. Normal mice sera reacted neither with the tegument nor with the cercarial gland.

DNA-based vaccine technology was also used to induce an antibody response in mice against an adult schistosome asparaginyl endopeptidase (Sm32), which is a gut-associated enzyme of the worms. The cDNA coding for Sm32 was cloned into the same mammalian expression vector (pRK7) and expressed for the first time in transfected mammalian cells as well as in mice immunized with the Sm32-encoding DNA construct. DNA-vaccinated mice developed antibodies which recognized the native protein not only in homogenates of *S. mansoni* worms, but also in the gut of the parasites. We conclude that the DNA-construct induced the expression of, and a specific antibody response against, Sm32 in mice.

In conclusion, this thesis provides an immunological and molecular characterization of schistosome enzymes, elastase and asparaginyl endopeptidase, which can be considered as DNA-vaccine candidates against schistosomiasis.