

An International symposium

Diversity among schistosomes: perspectives for control

The meeting was held at the Department of Parasitology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, P.R. China.

October 16 - October 22, 2000.

Below are the summaries of the talks given at the meeting.

A report of the meeting is due to appear in *Trends in Parasitology* early 2001.

A mirror site in China is proposed for this and other related schistosomiasis information.

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Introduction: Bridges between schistosomes in the East and the West

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Schistosomes afflict humans living within the reaches of some of the largest rivers: along the Chiang-jiang in China, the earliest infections are known from 167 BC (1), along the Nile in Egypt from 1250 BC (2). Schistosomes are associated with the millennial agricultural practices along these rivers. These practices of water management have been very stable over thousands of years and schistosomes can be assumed to have also been stable parasites of humans, whichever was the local balance between pressure of infection and development of resistance in the human population. One example for an outcome of local epidemiological co-evolution comes from Wuhan: A German trade boat traveled through Wuhan in 1934. On 3rd July, 14 members of the crew had a bath in flood waters around Hankou and all of them developed 40.8 degrees fever by 9th August (3). This not only tells us how

badly acute schistosomiasis can hit in some instances, but also how strong a resistance to infection the Chinese population of that area must have had, since they survived (although not healthy) in the presence of year-long exposures to infection. Only very recently was the human-parasite co-evolution dramatically disturbed: water management started to be introduced to control the natural events of flood and draught. The construction of irrigation schemes, at least in Africa during the past decades, has led to a resurgence of schistosomiasis transmission. It is particularly through these experiences in Africa that we have learned the lesson "schistosomiasis is a disease closely linked to agricultural development". Presumably all of us in this symposium will agree with that statement. Yet, what does this statement mean in reality?

For Africa, I believe that the best known example is the Assuan High Dam in Egypt. It was built in 1960 to equilibrate floods and droughts of the river Nile, to generate a continuous water supply for Egypt and to generate electricity. An accepted side effect was technical prestige gained by this enormous construction, the unwanted side effect was the increase of schistosomiasis. Other example: before Lake Volta in Ghana, so far the largest man made lake, was constructed in the 70's (this time predominantly for electricity production), the spread of schistosomiasis was predicted (4). And it happened. Many other irrigation dams and their irrigation systems followed the same pattern. The reason is that water management for agricultural development creates snail habitats in Africa.

For China, agricultural development has other consequences. The scope of water management in this country is not in the first instance for ensure irrigation (as in Africa), but rather to prevent flooding. Enormous dikes have been built along Chinese rivers, an achievement which is not much known outside China. I have learnt - in particular during several visits in endemic areas of Hubei - that water management here has not only developed agricultural productivity, but simultaneously destroys snail habitats.

Thus, water management to increase agricultural production has had so far largely opposite effects in Africa and in China. For the future, the hope is to break the vicious cycle in Africa through improved schistosomiasis control; but there is also concern in China that the Three Gorges Dam may exactly initiate this cycle (5). In this context, a key to schistosomiasis control relates to the biology of vector snails. I feel that even in the present time of molecular sciences and sophisticated techniques, snail and parasite ecology still offer great potential for schistosomiasis control.

I had the privilege not only to see both the African and Chinese schistosomiasis situation through the eyes of my partners and friends, but also to look upon the parasites from some distance of a continent where human schistosomes cannot survive (due to the absence of an adequate vector snail). From this more distant view, I like to draw attention to some aspects of schistosomiasis control, which may need close attention, since scientists tend to extrapolate from one schistosome species to another. Some extrapolations are adequate, others may lead to a trap. Issues to schistosomiasis control which we should closely investigate in order to avoid false extrapolations, may include:

1. Vaccination: This is uniformly considered as a "need" for schistosomiasis control and the list of core candidate antigens favored by researchers in "the West" and "the East" has large overlaps. It is certainly adequate to test vaccine candidate antigens from one schistosome species also in another. Yet one of the top candidates, glutathion-S-transferase, turned out to be present in different isotypes with different potentials of protection in *S. japonicum* as compared to *S. mansoni*. Thus, extrapolation from one species to the other was "dangerous".
2. Serodiagnosis: This is based on antibody detection which is the only routinely working immunological approach. Crude worm and egg antigens are used in "East" and "West". However, search for defined diagnostic molecules continues and, here again, at least one of the candidate antigens from *S. mansoni*, CEF6, is not present in *S. japonicum*, and an analogous situation seems to occur with some *S. japonicum*-antigens. Extrapolation is also "dangerous".
3. Serodiagnosis based on antigen detection: This is a highly desirable goal everywhere. It is common to *S. japonicum* and African schistosomes that no assay appears to be routinely available and

introduced in endemic areas. However, the diagnostic value of candidate antigens most frequently considered for African schistosomes, CAA and CCA, is different for *S. japonicum*. Again, extrapolations among schistosome species must be careful.

In spite of such differences between schistosomes, we can learn from the other side of the globe. Two examples:

1. Praziquantel was developed in the "West" and resistance to this drug by *S. mansoni* is now a matter of concern in Africa. However, resistance seems not to have been reported so far from *S. haematobium* nor *S. japonicum*. Conversely, arthemeter was discovered in the "East" as an alternative drug and has now proven effective also in Africa (6). Both drugs are central to schistosomiasis control irrespective of the country.

2. Haemagglutination (IHA) as a diagnostic tool is relatively old, sometimes considered even as old-fashioned. Yet it is - in my opinion - possibly the most adequate serodiagnostic technique for poor societies in schistosomiasis endemic areas. I dare to speculate that IHA "survived" longer in China due to the relative isolation of your country until some 20 years ago. At that time, ELISA, radioactive and fluorescent techniques were developed as "more advanced", were favored in "the West", were delivered to Africa and - in my speculation - were introduced as being more "trendy" than IHA. Yet these techniques are not sustainable in many endemic areas. In contrast, I believe that Chinese IHA techniques would prove quite efficient in an African context and might favourably perform in comparison to technically more "advanced" tools.

The approach for schistosomiasis control which fascinated me most, was one developed by Prof. Wei Dexiang. Prof. Wei initiated me some 16 years ago into field work for schistosomiasis control. He was one of my great teachers helping me to find "my track". Later, I was guided to share my scientific road with competent and dear colleagues, luckily enough even for many years until now. Let me express my deep thanks for their collaboration and great joy to meet again during this Symposium. Also, I gratefully acknowledge the financial support from the Ministry of Science and Art, Baden Wuerttemberg, and from the German Academic Exchange Council who, respectively, made collaboration with my Chinese partners sustainable and offered the possibility for several of us foreigners to meet now our Chinese colleagues.

I wish that this Symposium will build bridges of understanding and cooperation, that we can learn from each other ways to follow and pitfalls to avoid. I wish that this symposium will create benefit for those people who need control of schistosome infections, wherever their endemic area may be located.

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3. M lens P. "Yangtse-Fieber"-Erkrankungen (*Schistosomiasis japonica*) an Bord eines deutschen Handelsschiffes. *Archiv fuer Schiffs- und Tropenhygiene* (1937), 308-317.
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6. Utzinger J., N oran E:K., N ri A., Lengeler C., Xiao S.H., Tanner M. Oral artemeter for prevention of *Schistosoma mansoni* infection: randomized control trial. *The Lancet* (2000), 355, 1320-1325

1. The Influence of MNNG on the Proliferation of Cultured Cells from Adult *Schistosoma*

japonicum

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AIM To study the function of MNNG on the proliferation of cultured cells from adult *Schistosoma japonicum*. **METHOD** After the cells collected from the adult schistosome were cultured in medium RPMI 1640 containing 20% calf serum and a moderate amount of antibiotics for five days, they were divided into two groups. The cells in the first group were treated by MNNG with concentrations of 0, 1, 2, 3, 4, 6 and 9 μ g/ml for 0, 24, 36 and 48 hours, respectively. The cells in the second group were designed to control group. Afterwards all cells were moved into the original medium. Three weeks later, the rate of calf serum in the medium was adjusted into to 5%. The proliferation and growth of the treated cells were observed by microscope everyday. **RESULT** Most of the cells treated by MNNG with concentrations of 6 and 9 μ g/ml fell off from the wall of cultured flasks after 3 days, and the remained cells cultured in the medium containing 5% calf serum declined gradually. All the cells treated by MNNG of 1, 2, 3 and 4 μ g/ml grew well in the medium. The volume of the cells was larger and more cells in division were observed in the first group than in the control. Especially in the cells treated by MNNG of the concentration of 3 μ g/ml for 48 hours. **CONCLUSION** MNNG with the moderate concentration can induce the proliferation and division of cultured cells from adult *Schistosoma japonicum*.

2. The function of EGF on the proliferation of cultured *Schistosoma japonicum* cells treated with or without MNNG

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AIM To study the effects of EGF on the growth and proliferation of cultured cells from adult *Schistosoma japonicum*. **METHOD** After the cells collected from the adult *Schistosoma japonicum* were cultured in medium RPMI-1640 containing 20% calf serum and a moderate amount of antibiotics for 3 days, they were divided into two groups. The cells in the first group were treated directly by EGF with concentrations of 0, 0.5, 1, 2, 4, 8, 12, 16, 20, 24 and 28ng/ml, respectively. The cells in the second group were designed to treat by MNNG of 3 μ g/ml for 48 hours before they cultured in the medium with EGF of 0, 1, 8, 12, 16 and 20ng/ml, respectively. The growth and proliferation of cultured cells were observed by microscope everyday. **RESULT** Some of the cultured cells in the medium with different concentration of EGF fell off and degenerated in both groups after two weeks. Moreover, along with the increase of the EGF concentration in the medium, the abscission and degeneration of the cells became serious. Cultured cells in the first group fell off or degenerated earlier than those in the second group. **CONCLUSION** EGF cannot induce the proliferation of the cultured cells from adult *Schistosoma japonicum*. On the contrary, it can accelerate the degeneration of the cultured cells, especially for the cells not treated by MNNG.

3. New proteins from schistosomes - database searching, structural predictions, and the implications of sequence polymorphisms.

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Genome and expressed sequence tag projects, and empirical searches for protective antigens against schistosomes, are revealing protein types which are not only already well known in vertebrates, but also types for which no detectable homologues exist. In either case it may be important to understand a new protein's structure and function in order best to design new vaccines or drugs. The talk will describe the means by which homologues of new proteins can be found, and how structural predictions can be made from a protein's sequence whether it falls into a well-known class or an entirely new type, and the limitations of such predictions. Much of this can now be carried out via internet sites, and the sites and public domain programs available will be described and demonstrated. A particularly important aspect is the effects of polymorphisms in the sequences of a particular protein, both within and between species of schistosome in terms of vaccine design. This will be illustrated using the Sj-FABPc protein of *Schistosoma japonicum*, which is similar to an *S. mansoni* antigen previously vaunted as a multivalent vaccine against trematodes. The process of structural predictions and testing will be illustrated, as will the mapping of polymorphic sites onto a predicted structure and the implications of sequence polymorphisms for the efficacy and longterm efficacy of a vaccine based on a single recombinant protein.

Selected key www sites:

ExpASY main site for access to tools and databases - <http://www.expasy.ch/>

Proteomics tools - <http://expasy.cbr.nrc.ca/tools/>

Structural modelling program - <http://www.expasy.ch/swissmod/SWISS-MODEL.html>

Freeware structural viewer - <http://expasy.cbr.nrc.ca/spdbv/>

Secondary structure prediction - <http://cubic.bioc.columbia.edu/predictprotein/>

Profile scanning - <http://expasy.cbr.nrc.ca/tools/scnpsit1.html>

Entrez browser - <http://www3.ncbi.nlm.nih.gov/Entrez/index.html>

Schistosomegenome network home page - http://www.nhm.ac.uk/hosted_sites/schisto/index.html

4. Acid-rich organelles within schistosomula (*S.mansoni*), as revealed by LysoTracker Red .

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LysoTracker Red is a fluorescent dye used to label lysosomes in mammalian cells. In freshly transformed schistosomula (but not cercariae) LysoTracker Red labels large organelles which also stain with several other dyes. These other dyes include methylene blue, fluorescent amiloride and the styryl dye FM 143. The function of these organelles is unknown but several lines of evidence suggest that they have a redox function as well as being important in the transformation of cercariae to schistosomula.. They may be also important in protecting the parasite from reactive oxygen intermediates.

5. Presence of heme degrading enzymes in adult worms of *Schistosoma japonicum*

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Schistosome digests host erythrocyte hemoglobin to produce globin as the main source of nutriment, heme was considered to be released as the by-product of the catabolism of hemoglobin in the parasite, heme or heme analogs of high concentration are potential toxic for the parasite itself as well as the host. Heme oxygenase (HO) and biliverdin reductase (BR), the enzymes of heme degradation, have been detected in almost all species of eukaryotes. The presences of HO and BR have been proved in some protozoan parasites like Plasmodia and Leishmania, the presence of HO in Filarial *Setaria digitata* has been also reported recently. In present study, the extract of the adult worm of *schistosome japonicum* was investigated by enzymatic assays of HO and BR using commercial substrates hemin and biliverdin. The specific activities of HO and BR were 60.1 nmol bilirubin /mg/min and 52.2 nmol bilirubin/mg/min respectively, the activities of both enzymes were optimal at pH 8.7 and the rate of reactions came to the peak at 15min of incubation for HO and BR enzymatic reactions. The findings of present study suggest the presence of specific activity of heme catabolic enzymes in *S.japonicum* for the first time. At present, it is difficult to assign any other vital functions to HO of schistosome, however, it is inferable that the presence of heme degrading enzyme system and its catabolite(s) may be involved detoxification of the parasite in host.

6. Antibodies against schistosome proteolytic enzymes induced in mice by DNA-vaccination

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We aim to induce protection in schistosome-infected mice using DNA-based vaccine technology. Full length cDNAs coding for proteolytic enzymes of *Schistosoma* sp. (cathepsin B, aspartic proteinase, Sm32) were cloned in mammalian expression vectors by RT-PCR analysis using specific primers for these enzymes. The expression was driven by the CMV promoter/enhancer in both vectors used (pRK7 and pCDNA3.1). Expression of these antigens was investigated first in vitro in transfected mammalian cell lines (COS-7 and BHK-21 cells) and later in vivo in mice immunized by intramuscular injection with DNA constructs coding for the above-mentioned enzymes. The cells synthesized the respective schistosome antigens as shown by the presence of the specific mRNA signal in slotblot. DNA-vaccinated mice using intra-ear immunization developed antibodies against the respective proteins as shown in Western blots with adult schistosome homogenate. We conclude that DNA-vaccination induced the synthesis of, and a specific antibody response against, the selected schistosome antigens. Future experiments will test the potential of these DNA constructs to induce partial resistance against infection in mice. Together with Prof. Y.-L. Li (Wuhan) we aim to test the DNA vaccines in domestic livestock in order to interrupt the zoonotic cycle of *S. japonicum*.

7. Construction and characterization of the cDNA library from *Schistosoma japonicum* cercariae

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The cDNA library constructed from *Schistosoma japonicum* cercariae was firstly reported. Total RNA extracted from 500,000 *S.japonicum* cercaria was used to synthesize the double-stranded cDNA and cloned into the lTriplEx2 vector using "SMART cDNA Construction Kit". The primary titer of the constructed cDNA library is 1.8×10^7 pfu and the titer of amplified library is 2.5×10^{10} pfu/ml. The average size of inserts is 1.075 kb. The recombinant efficiency is 94.4%. The full length cDNA of *S. japonicum* TPI and JF-2 genes were successfully amplified from the library. All the data showed that a representative cDNA library of *S. japonicum* cercariae has been constructed. The cDNA library constructed from *Schistosoma japonicum* cercariae was firstly reported. Total RNA extracted from 500,000 *S.japonicum* cercaria was used to synthesize the double-stranded cDNA and cloned into the lTriplEx2 vector using "SMART cDNA Construction Kit". The primary titer of the constructed cDNA library is 1.8×10^7 pfu and the titer of amplified library is 2.5×10^{10} pfu/ml. The average size of inserts is 1.075 kb. The recombinant efficiency is 94.4%. The full length cDNA of *S. japonicum* TPI and JF-2 genes were successfully amplified from the library. All the data showed that a representative cDNA library of *S. japonicum* cercariae has been constructed.

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8. Studies on the differences of *Schistosoma* species and strains by using random amplified of polymorphic DNA markers

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The usefulness of random amplified polymorphic DNA markers (RAPD) was assayed in order to discriminate among species, strains and sexes within the genus *Schistosoma*. Six arbitrary decamer oligonucleotides were used as primers to amplify total DNA by the polymerase chain reaction (PCR). Highly variable product patterns were observed between *S.mansoni* and *S.japonicum* by all primers. Some of the tested primers, for example B5, A7, produced patterns included bands that were polymorphism between strains of mainland and Taiwan in China, other primers, for example B6, produced apparently identical produced patterns. Minor differences were observed between male and female adult, fragment of 718 bp was reported as six markers. Data were analyzed by Nei's genetic distance (D), and genetic similarity (S). Nei's genetic distance (D) between two species was more than 0.90; Nei's genetic distance (D) between mainland and Taiwan strains of *S.j* was more than 0.20; 6 strains from marshland-lake regions, they were ranged from 0.000 to 0.029, between marshland-lake regions and mountainous-hilly regions ranged from 0.060 to 0.067. A phylogenetic tree was outlined using SAS version 6.12 computer program. The results already indicated clearly that RAPD markers constitute a powerful tool for the analysis of genetic variability. Mainland and Taiwan in China *S.j*

strains have taken place intra-specific variation. The results indicated that the random amplification of polymorphism DNA (RAPD) may be an extremely useful approach to identification of schistosoma strains, species and sexes, and the results also indicated that schistosoma in China had occurred genetic diversity among different geographical strains.

9. Protection against schistosomiasis by immunization with plasmid DNA encoding 10.6KDa membrane protein of *Schistosoma japonicum*

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Objective To screening new schistosome vaccine candidate, **Methods** *S. japonicum* adult cDNA library was screened using the mAb against membrane antigen of *S. japonicum* schistosomula, and also using sera from immune rabbits vaccinated with irradiated cercariae. Three different pieces of *S. japonicum* cDNA gene fragments were cloned into pGEM-T vector. The sequences of the inserts were determined using an automated DNA sequencer. One of the unknown gene (SjB8) was picked up and its ORF sequence (291 bp) was subcloned into pBK-CMV eukaryotic expression vector. pBK/SjGST recombinants was successfully constructed by the same methods. The recombinant plasmids were identified by restrictive enzymes and PCR amplification. Then, Large-scale preparation of pBK/SjB8 and pBK/SjGST were purified with PEG 8000, adjusted to 1 g / l using normal saline as solvent, injected into BALB/c mice muscle. Three weeks later, the expressed results were observed using histochemistry technique. After that, BALB/c mice were vaccinated with single dosage of 100 g /mice using pBK/SjB8 and pBK/SjGST plasmid DNA, respectively. Two group mice and a normal group mice were challenged with 40 ± 1 *S. japonicum* cercariae after 3 weeks vaccination. All mice were sacrificed at 6th week after challenge. **Results** The results demonstrated that ORF of SjB8 gene was subcloned into the plasmid pBK-CMV and the antigen and antibody reactions were seen in the mice muscular tissue. Compared with the control group, the worm reduction rates of pBK/SjB8 and pBK/SjGST plasmid DNA were 28.9% and 30.5%, respectively ($P > 0.05$). Compared with the control group, the egg reduction rates of pBK/SjB8 and pBK/SjGST were 25.1% and 34.6%, ($P < 0.05$) respectively. **Conclusions** a gene of new schistosome vaccine candidate (SjB8) was cloned into pBK-CMV eukaryotic expression vector and could express schistosome protein. The pBK/SjB8 recombinants were used as DNA vaccine and showed its protective function.

Supported by National Nature Science Foundation of China(No.39570646) and Nature Science Foundation of Anhui Province (No.00022031)

10. Cloning and Expression of 14-3-3 Signalling Protein of *Schistosoma japonicum*

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14-3-3 protein is a family of conserved regulatory molecules expressed in all eukaryotic cells including plants, protozoa, and mammalian animals. A striking feature of the 14-3-3 proteins is their ability to bind a multitude of functionally diverse signaling proteins, regulate the activities of enzymes including protein kinase C, activate tyrosine and tryptophan hydroxylase, and stimulate Ca^{2+} -

dependent exocytosis. This plethora of interacting proteins allows 14-3-3 to play important roles in a wide range of vital regulatory processes, such as mitogenic signal transduction, apoptotic cell death, and cell cycle control. The present work is a novel approach to study of the potential role of Sj14-3-3 in immuno-prevention and immunodiagnosis of schistosomiasis by its double blockage of signal transduction and immunoreaction. *S.japonicum* (Chinese strain) adult worms were harvested from infected rabbits by hepatoportal perfusion. Schistosome total RNA was isolated and the first strand of cDNA was synthesized. A pair of primers were generated for amplification of Sj14-3-3 gene based on the open reading frame of GenBank database: P1: EcoR I forward: 5'-TAGGAATTCATGAGGGATTCGTTC-3' and P2: Xho I reverse:5'-TAGCTCGAGTCAGCCATCATTTC-3'. PCR products were purified and ligated to the TA cloning vector pGEM-T (Promega) with T₄ DNA ligase. Transformation was performed with *E.coli* XL-1blue strain by DMSO. The recombinant plasmids were verified by restriction digestion followed by agarose electrophoresis. Sj14-3-3 gene was sequenced by automated DNA sequencer(ABI 377). pGEM-T/Sj14-3-3 was digested with EcoR I and Xho I and the target fragments, with an open reading frame of 765 bp were inserted to plasmid pBK-CMV for prokaryotic expression. The β -galactosidase-Sj14-3-3 fusion protein was induced by IPTG and confirmed by SDS-PAGE followed by Western blot. The recombinant fusion was identified as a predominant band corresponding to a mw. of 30 kDa. The effect of pBK-CMV/Sj14-3-3 DNA vaccine on the challenge of *S. japonicum* cercaria is under investigation. Monoclonal antibody to recombinant Sj14-3-3 is in preparation.

11. Nitric Oxide Induces Apoptosis in *Toxoplasma gondii* Tachyzoite via Calcium Signal Transduction Pathway

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Objective To explore whether NO is capable of inducing apoptosis in *Toxoplasma gondii* tachyzoite and its calcium signal transduction mechanism. **Methods** Apoptosis induced by NO in *Toxoplasma gondii* tachyzoite was investigated by TUNEL method, electron microscopy and agarose gel electrophoresis. Cytoplasmic free calcium concentration in toxoplasmatic tachyzoite was determined by Fura-2 fluorescein load technique. **Results** NO donor, sodium nitroprusside (SNP) induced apoptosis in toxoplasmatic tachyzoite in a time- and dose- dependent manner by TUNEL. N-acetylcystein, a NO scavenger, greatly inhibited SNP-induced tachyzoite apoptosis, but potassium ferricyanide, an analogue of SNP but devoid of NO, alone can't induce tachyzoite apoptosis. SNP-treated Toxoplasmatic tachyzoite have shown characteristic morphological features of apoptosis, including aggregation of chromatin at the nuclear membrane, condensation of nucleus and apoptotic body by electron microscopy and characteristic biochemical features, including DNA fragmentation and the appearance of the "DNA ladder" by agarose gel electrophoresis. The cytoplasmic free $[Ca^{2+}]_i$ in resting tachyzoite were 108.49 ± 4.87 nmol/L (n=15). $[Ca^{2+}]_i$ in the process of SNP-induced Toxoplasmatic tachyzoite apoptosis was significantly higher than that in control groups in time- and dose- dependent manner, which was greatly inhibited by N-acetylcystein, but $K_3Fe(CN)_6$ alone can not induce increase in $[Ca^{2+}]_i$. Chelation of extracellular calcium with EGTA entirely inhibited the increase in and voltage-dependent calcium channel blockers, verapamil only partially inhibited the increase in $[Ca^{2+}]_i$ induced by SNP. However, EGTA and verapamil inhibited SNP-induced apoptosis in *T.gondii* tachyzoite. **Conclusion** It has been shown that NO donor, SNP play a role in antitoxoplasmatic tachyzoite infection through inducing tachyzoite apoptosis according to characteristic morphological and biochemical features and SNP-induced apoptosis in *T.gondii* tachzoite is mediated by elevating cytoplasmic free calcium concentration, which is mainly resulted from the entry of extracellular

calcium. These findings indicate potential chemotherapeutic importance of NO.

12. Gene Discovery through Expressed Sequence Tag Sequencing in *Schistosoma japonicum*

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Partial cDNA sequencing to generate expressed sequence tags (ESTs) is being used at present for the fast and efficient obtainment of a detailed profile of genes expressed in various tissues, cell types or development stages. Genome projects have taken advantage of EST studies because ESTs represent a particular type of sequence-tagged sites useful for the physical mapping of genomes. ESTs can serve the same purpose as sequence-tagged sites, with the additional bonus of pointing directly to expressed genes. One of the most interesting applications of the EST database (dbEST) is gene discovery. A significant development with important implications in this field has been the enormous growth of the dbEST. Novel genes can be found by querying the dbEST with a DNA or protein sequence. In 1994, the Special Programme for Research and Training in Tropical Diseases of the World Health Organization launched an initiative to analyze the genome of *Schistosoma*. The network was established, with the aims of (i) gaining significant knowledge on the molecular biology of *Schistosoma*; (ii) identifying new genes and their products which could be used to design new drugs, to speed up vaccine development, and to improve diagnosis. (iii) Sharing material and expertise and providing an information system that is accessible globally to researchers in the field.

Schistosoma japonicum (S.j) is one of three schistosoma species responsible for schistosomiasis that is a serious parasitic disease in the mainland of China. Vaccine or new drugs development are considered to be necessary for schistosomiasis control in China. Therefore, generation, identification and evaluation of ESTs from S.j have significant values. In 1998, we began to establish the large scale sequencing for the expressed genes of Chinese strain S.j. Here we report the results of recent efforts to characterize ESTs from the cDNA library of S.j adult worm. 331 ESTs were generated by PCR directed sequencing, and then ESTs were searched for significant similarities to sequences deposited in schistosoma database of EMBL using the basic local alignment search tool program for nt (Blastn). Of them, only 18 ESTs (5.44%) were previously known S.j, which were considered to be identified genes; 25 ESTs (7.55%) were similar to database sequence of *Schistosoma mansoni* or other organisms, which were named as putative identification for the gene; 249 ESTs (75.22%) had no significant matches with sequences of S.j or other schistosome, which were considered to be no identified genes; 39 ESTs (11.78%) were redundant sequences. Among them, 304 ESTs sequence data have been deposited in dbEST with the following GenBank accession Nos: AI725355-AI725361, AI740189-AI740204, AI816553-AI816554, AW160107-AW160132, AW231224-AW231255, AW282251-AW282275, AW329868-AW329884, AW736722-AW736758, AW738819-AW738828, BE123822-BE123844, BE128954-BE128960, BE187669-BE187701, BE217367-BE217399.

The project was supported by the grant named "211" project from Guangdong Government, China. ID number is 98169.

13. Immunological and biochemical characteristics of cercarial elastase from *Schistosoma mansoni*, *S. haematobium*, *S. japonicum* and *Trichobilharzia ocellata*

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Cercarial secretions (CSs) from different species of *Schistosoma* and from *Trichobilharzia ocellata* showed a 30 kDa digestion band in gelatin gel electrophoresis. This band corresponds to the cercarial elastase (CE). Using a colorimetric substrate, the specific activity of serine protease was always 2-3 folds higher in CSs from *T. ocellata* compared to *S. mansoni*. The enzyme showed optimal activity at pH 10, is Ca⁺⁺-dependent (inhibition with EDTA), trypsin like (inhibition with anti-pain), and is a serine proteinase (inhibition with PMSF). Also minute concentration (8 nM) of aprotinin, a potent inhibitor for serine proteases, abolished 90 % of the activity in CSs of both parasites. IgG measurement by ELISA showed a high degree of cross-reaction among CSs from different species with both Chinese and African infection sera. In western blots, using infection sera from humans, heavily infected mice as well as rabbits, the reactivity towards the 30 kDa protein fraction was, however, absent. This suggests the absence of an antibody response against the enzyme following natural infection. Moreover, when sections from infected snails (*Biomphalaria* and *Bulinus*) were analyzed by immunofluorescence using the same infection sera, only the tegument of the developing cercariae was recognized, but the acetabular glands were not reactive. In contrast, when sections were tested with antisera against CE from either *S. mansoni* or *S. haematobium*, reactivity was exclusively concentrated in the preacetabular glands of the cercariae. Sections from *S. japonicum*-infected snails (*Oncomelania*) did not cross-react with these antisera. In conclusion, although CE is essential in the penetration process of schistosomes, it does not apparently induce an antibody response following natural infection. MB was supported by a scholarship from the German Academic Research Council (DAAD), who also supports the collaboration between RR, YLL and AR.

14. Hybridoma cell agglutination as a novel test to detect circulating antigen: diagnosis of infections with *Schistosoma japonicum*

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Serodiagnostic techniques used most widely to detect parasitic infections include enzyme-linked immunol assays, immunofluorescent and haemagglutination tests. The detection of circulating parasite antigen(s) opens the possibility to differentiate between actual and past infections. We used a monoclonal antibody (H226) against schistosome 31/32 kD antigens (Ruppel et al., 1987) to develop a sandwich-ELISA, which is able to detect circulating schistosome antigen in sera of patients infected with *Schistosoma japonicum*, *S. mansoni*, *S. haematobium* or *S. intercalatum* (Li et al. 1996). This test has shown that the epitope corresponding to H226 circulates in patient serum and is highly specific for schistosomes. Using the hybridoma cells, which secrete H226, we have now developed a novel serodiagnostic test, which is based on the agglutination of hybridoma cells. In the presence of specific antigen, agglutination of the fixed and stained cells occurs and can be visualized in analogy to traditional erythrocyte agglutination. The circulating antigen, which can be detected by the test, appeared to be relational with the stages and dosages of infection in the mice. The sensitivity of this

test was high with acute schistosomiasis japonica (97%, n = 32) and moderate with chronic cases (75%, n = 57). No positive reactions were obtained with healthy persons (n = 78) or patients infected with other parasites (*Clonorchis sinensis*, n = 20; *Paragonimus westermani*, n = 20; *Plasmodium vivax*, n = 10) or suffering from lupus erythematoides (n = 5) or mononucleosis (n = 10). The test procedure may become useful to diagnose also other infections.

Acknowledgements.

Prof. Michael Kirschfink (Dept. of Immunology at Heidelberg University) kindly provided the sera from healthy Germans and lupus erythematoides patients and helped with valuable discussions. This work was supported by the Ministry of Science and Art Baden-Württemberg (Germany), by the German Academic Exchange Council (DAAD), by Science & Technology Committee of Hubei Province (P.R. China) and by the Ministry of Health of Hubei province (P.R. China). We gratefully acknowledge these sources of support.

15. Studies on the dipstick of schistosomiasis japonica

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In this study, Studies on the fast diagnose test paper of Schistosomiasis japonica, the Schistosoma japonicum soluble egg antigen (SEA) the S.japonicum adult worm membrane antigen WMAg, cercaria antigen (CeAg), recombinant antigens and the anti-SEA, anti-SEA140kDa, anti-SWAg, anti-GAA, anti-CCA, anti-id-McAb were extracted, purified and screened by the techniques of the Modern Molecular Biology, the immunology and the genic engineering. These purified proteins were used in the studies of the Valkiris' dot-immunogold filtration assay (DIGFA) and the dot-immunochromatographic assay (DICA) after immunogold mark. The results showed that this is a useful method for the quickly serological diagnosis of schistosomiasis japonica, which is named as Colloidal Gold Sj-DIPSTICK. This method was employed for detecting 325 cases with schistosomiasis (28 cases with acute schistosomiasis and 297 cases with chronic schistosomiasis were verified by the miracidium hatching test), 153 cases with paragonimiasis, 79 cases with clonorchiasis, 157 cases with cysticercosis, 184 cases with trichinelliasis, 165 cases with hepatitis, 481 cases with pulmonary tuberculosis, 876 cases from the special clinic of our department and 518 healthy persons. And it was compared with some regular detective methods. Both suggested that the Colloidal Gold Sj-DIPSTICK is more qualified. The reasons are:

1. Simple: The method needs no apparatus, is suitable in the primary hospital and the field surveys.
2. Quick: The reaction can be performed in 2-5 minutes.
3. High sensitivity: The positive detective rate is as high as 97.31-100%.
4. High specificity: The cross-reactions were less than 5% (0.62-4.89%). The rates were 1.96% with paragonimiasis, 1.33% with clonorchiasis, 0.64% with cysticercosis, 4.89% with trichinelliasis, 1.21% with hepatitis and 0.62% with pulmonary tuberculosis, respectively.
5. Low false positive rates: The false positive rates were 0.19% with the healthy persons and 1.25% with the cases from the special clinic of our department.

16. *Schistosoma mansoni* and *S. japonicum*: transformation of cercariae to schistosomula and their interaction with complement

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Cercariae and schistosomula (Sla) are major targets of the host immune attack against schistosomes. To study the immune interaction with these larvae, "mechanically" in vitro transformed Sla are frequently prepared by the "syringe passage" (developed for *Schistosoma mansoni* by Colley & Wickel). We investigated possible differences in the transformation of *S. mansoni* and *S. japonicum*. Cercariae were transformed to Sla by sedimentation on ice, transfer into medium (+ 5% of inactivated calf serum) at room temperature and different further treatments: (i) Simple pipetting with pasteur pipettes for 20 times transformed cercariae of *S. mansoni* (average 67%), but not of *S. japonicum* (<5%). (ii) Pressure through a needle (G21; Colley & Wickel) perfectly transformed cercariae after 20 strokes into viable Sla in the case of *S. mansoni* (95-100%), but not *S. japonicum* (<50%). For *S. japonicum*, the thinnest needle (G27) was required for at least 70% transformation. However, this procedure also killed some 10% of *S. japonicum*, but only <5% of *S. mansoni*. Thus, mechanical transformation is easier with *S. mansoni* than *S. japonicum*. (iii) Short incubation (3h) in fresh human serum (50%) transformed at least 90% of cercariae of *S. mansoni*, but only about 10% of *S. japonicum* into Sla. Heat-inactivated human serum had nearly no effect. Prolonged incubation (24 h) with fresh, but not inactivated serum resulted in more than 80% cytotoxicity against *S. japonicum* cercariae (these did not transform to Sla) and Sla of *S. mansoni*. Thus, the host complement (C) appears to promote transformation of *S. mansoni*, but not of *S. japonicum*. Sla are known to be able to activate C by the alternative and classical pathways. However, a third pathway was recently discovered which is initiated by MBL (mannose-binding lectin), a molecule with functional characteristics of IgM, IgG, and C1q. The MBL-pathway has not been investigated with respect to a possible interaction with schistosomes. By immunofluorescent assays, we detected MBL on the surface of Sla of *S. mansoni* after incubation with normal fresh human serum, but neither with heat-inactivated nor MBL-deficient serum. C4 (activated by the classical and MBL pathways) bound to Sla in C1q-deficient serum (which prevents classical, but not MBL-pathway activation), but not in MBL-deficient serum. Incubation with normal serum deficient in factor B (required for alternative activation), greatly reduced, but not completely prevented binding of C3c. However, deposition of MBL on Sla was not apparently affected by the absence of factor B. This is the first evidence that Sla are able to activate the MBL pathway of C.

We thank the Medical Faculty of Heidelberg University, the German Academic Exchange Council (DAAD) and the Ministry of Science and Art.

17. Immune-reaction of African and Chinese schistosomiasis patient sera in line-blot with recombinant Sj22

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A tegument-associated protein of 22 kD is expressed on *Schistosoma mansoni* larvae and adult worms. We have cloned this molecule from a *S. japonicum* cDNA library as recombinant His-tag-protein (rSj22) and purified it to near homogeneity. To be tested as potentially serodiagnostic antigen, rSj22

was dissolved in appropriate buffer and applied to nitrocellulose in a manner analogous to writing with ink on paper. We termed this novel technique as "line blot" (Beck, unpublished). The nitrocellulose strips are then ready to be processed in a way analogous to western blots with some modifications. We tested sera from schistosomiasis patients infected with *S. mansoni* (N =120 from Mali) or *S. haematobium* (n = 20 from Nigeria). All of these patients had a chronic infection. Over 95% of these African sera gave a positive result in Sj22- line blot. Patients with *S. japonicum* (n = 60 from the Hubei Province) were classified as acute, chronic or late stage schistosomiasis. The vast majority of chronic cases gave a positive reaction. However, among acute and late cases a substantial part of sera did not react. All healthy control sera were negative. Positive reactions with Sj22-line-blot were also obtained with sera from mice obtained 6 weeks after infection with 150 - 200 cercariae, but not with naive mice. We conclude: (i) rSj22 is valuable for diagnosis of infections with all human schistosomes; (ii) with *S. japonicum*, a differential response is observed with different stages of the disease.

18. Induction of immunoregulation on granulomatous formation in mice infected with *Schistosoma japonicum* by fractional protein SjR47

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It was first reported that SjR47 combined with erythrocytes of the rabbits infected with *Schistosoma japonicum* could be detected using anti-SEA PcAb. The results showed that SjR47 existed on erythrocytes of infected rabbits via elution using a special eluent, and the SjR47 were successfully separated and purified. The further study indicated that SjR47 were egg original. Immunological characteristics of fractional antigens were determined by serological method and the results showed that SjR47 had significant antigenicity and immunogenicity. The effects of anti-pathology immunity were induced by the SjR47 antigens in mice, the results showed that the reduction rates of total eggs in liver of immunized mice were 72.36% in experiment 1, and the reduction rates of total eggs and mature eggs were 57.25% and 74.70% respectively in experiment 2. The egg granulomatous formations in immunized mice were all diminished in two experiments. We further studied on the mechanisms of protective immunoregulation of egg granulomatous formation in mice immunized with the fractional antigens, the results showed that the specific antibody and subclass levels of sera from immunized mice were significantly higher than those of infection control, especially the IgG₁, in 0-12wk after challenge infection; the levels of splenic CD4⁺ and CD8⁺ T cells in immunized mice were high in early stage (4wk) post infection and then decreased gradually, the ratio of CD4⁺/CD8⁺ T cells was also decreased gradually, the results indicated that the cellular immune response may be inhibited in immunized mice; Serum TNF- α levels of immunized mice were found significantly higher than those of infection control in early stage (before worm maturing) post infection, and suggesting that the TNF- α effects of mediating granuloma formation and inducing parasite egg-laying were diminished. These results indicated that the protective immunity of anti-pathology in mice immunized with SjR47 antigens appeared predominantly in early stage after infection and closely related to cellular (cytokine) and humoral immunity.

These findings suggest SjR47 can induce obvious protective immunity and of markedly theoretical and applicable value. It is worthy to be further studied.

19. Rapidly Detecting *Schistosoma japonicum* Specific Antibody in Sera of Chinese Patients

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Objective To find a fast, simple, high specific and high sensitive detective method of *Schistosoma japonicum* specific antibody in sera of Chinese patients. **Method** The antibody was detected by a new method which combined high specificity and high sensitivity of Western blot, fastness of DIFA (Dot immunofiltration assay) and simplicity of IGGA (immune glutinous gold assay). 1) The fiber membrane was made: the membrane was covered *Schistosoma japonicum* antigens by SDS-PAGE; 2) the box was made: the box included the fiber membrane and absorbed water material; 3) detective the specific antibody: the sera of *Schistosoma japonicum* patients and antibody of anti-human IgG linked glutinous gold was dropped in the box; 4) observation result: there were the golden band was positive and there were no the golden band was negative. **Results** Reaction time was quite enough in 5 minutes on-the-spot; detective steps only need one; rate of specificity and sensitivity all were 100%. **Conclusion** This was a fast, simple, high specific and high sensitive detective new method of *Schistosoma japonicum* specific antibody in sera, and it may be used to detect *Schistosoma japonicum* patient's on-the-spot.

20. The surgical treatment of portal hypertension - Theory and practice of pericardial devascularization with splenectomy

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Objective: To review our experiences and evaluate the efficacy of pericardial devascularization with splenectomy for treatment of portal hypertension. **Methods:** from May 1972 to October 1999, 508 patients with portal hypertension were treated with this operation. 319 of them were posthepatic cirrhosis, 141 were last stage Schistosomiasis or accompanied the virus chronic hepatitis. **Results:** The bleeding control rate was 96.9%, overall operative mortality rate was 4.5%. The major causes of death were upper gastrointestinal bleeding, intraabdominal hemorrhage, hepatic failure and hepatorenal syndrome, postoperative infection and thrombosis of portal vein system. The mean follow-up time was 3.8 years. Actual survival rate was 94.1% at 5 years, and 70.7% at 10 years. Recurrent bleeding rate was 6.2% at 5 years, and 13.3% at 10 years. **Conclusion:** The pericardial devascularization with splenectomy was the first choice for treatment of portal hypertension, especially for posthepatic cirrhotic patients. Our experiences were: (1) Whole and thorough porto-azygos disconnection of pericardial area is the key of this operation; (2) The operation should be performed in according to the pericardial anatomy with correct surgical techniques; (3) The operation candidates and opportunity have to be selected rationally; (4) It is necessary to emphasize the pericardial management to prevent postoperative complications.

21. The relationship between schistosomiasis japonica and the infection with HBV

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Aim: To study the relationship between schistosomiasis japonica and the infection with HBV.

Method: 216 patients with schistosomiasis japonica were studied, 190 of them with chronic schistosomiasis japonica and 26 patients in late stage were confirmed by physical examination, the rectoscopy and ultrasonoscope. All patients were tested with the diagnostic kit of HbsAg. **Results:** 87 (40.3%) cases were in cirrhosis and 33 (15.3%) cases showed hepatitis B antigenemia among 216 cases. 23 of 87 (26.4%) cases with cirrhosis were positive in HbsAg test. 10 of 129 (7.75%) cases without cirrhosis were positive in HbsAg. 13 of 26 (50%) cases with late schistosomiasis japonica were positive in HbsAg test. The rate of HBV infection is higher in the patients with schistosomiasis japonica than that in the population without schistosoma japonicum infection (15.3%: 8.5%) and the rate of HBV infection is higher in the patients with cirrhosis than that without cirrhosis. It is highest in the patients with later schistosomiasis. **Discussion:** The higher infection rate of HBV in the patients with schistosomiasis japonica may be involved in the immunity of the host, generally, the patients with schistosomiasis have an impaired immune response. The current studies suggest that a “pure” Schistosoma japonicum infection is not involved in cirrhosis and the coexisting hepatitis B may be a major cause of cirrhosis in the patients with schistosomiasis. Also it is possible that the infection of HBV accelerates the development of schistosomiasis from the earlier stage to late stage.

22. Studies on Human Cellular Immune Responses in an Endemic Area for Schistosomiasis Japonica

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Chemotherapy remains the cornerstone of intervention, but reinfection necessitates frequent retreatment. There is thus a high priority need for the development of effective anti-schistosomiasis vaccines. Identifying the immunological mechanisms responsible for host resistance against schistosome is a fundamental step in rational vaccine design.

In rat, primate and human schistosomiasis, it is widely agreed that the mechanism of antibody-dependent, cell-mediated cytotoxicity (ADCC) plays a major role in the expression of acquired resistance. In all animal models and in humans, elevated immunoglobulin E (IgE) concentrations and blood and tissue eosinophilia are hall-marks of schistosomiasis. The protective role of IgE antibody was observed initially in rats by passive transfer experiments *in vivo*, and confirmed by several immunoepidemiological studies in human infection.

A major conceptual revolution in immunology by dividing mouse T helper cells into two populations with contrasting and cross-regulating cytokine profiles, deeply influenced our understanding of the immunity to schistosomes in human populations and in experimental models. It is universally accepted that in humans, primates and rats, protective immunity to schistosomes is associated with Th2 responses (IL-4, IL-5, IgE, IgG4 and eosinophils). IgE responses and eosinophils in schistosomiasis and their role in protective immunity have been regarded as part of a Th2-dependent regulatory circuit.

Protective immunity induced by immunisation with irradiated schistosomes is dependent on Th1-type responses, with interferon-gamma having a central role. By contrast, non-attenuated infections do not seem to induce significant levels of specific immunity, and the onset of egg production results in a switch to Th2 cytokine production. The skewing of Th1 and Th2 cytokine responses may in fact be a strategy evolved by the parasite to limit an effective host immune response. In fact, the differences observed in different hosts and even sometimes in the same host, indicate that the complex interaction between host and parasite cannot simply be restricted to the context of Th1 and Th2 cells and the

effects of their products. It is likely that expression of immunity against schistosomes is the result of the combination and appropriate balance of cytokines and effector cells, leading in some instances to a successful immune response. The most recent studies of Jankovic et al, using B-cell-deficient and IFN-gamma knockout mice, have strongly reinforced the belief that effective vaccination against schistosomes depends on the simultaneous induction of both humoral and cell-mediated immunity. Therefore, it is necessary to find the candidate antigens expressing epitopes which can stimulate specific T cells and/or B cells for a cocktail vaccine.

The present study was designed to explore the characteristics of human cellular immune response and the association between cellular immunity and humoral immunity in the endemic area with schistosomiasis japonica and to find more B-cell and T-cell epitopes from some recombinant antigens to participate in the vaccine candidates. A group of 129 individuals aged 14-41 years old in Nanshan Island of Poyang Lake were selected. The levels of IL-4, IL-5, IL-10 and IFN- γ stimulated by SEA and SWAP were detected in the supernatant of whole blood culture by Sandwich ELISA. Of the 129 individuals, 93 ones who were confirmed to be fully cleared the infection by praziquantel were tested again 45 days after chemotherapy and were observed the relationship between reinfection, exposure and cytokine levels. The correlation between the cytokine levels and age, infection intensity was analyzed and the cytokine levels before treatment were compared with those after treatment. At the same time, a panel of recombinant antigens (rSj14, rSj22.6, rSj23, rSj26, rSj28, rSj62, rSj97, TPI) were tested to observe their potential capacity to induce specific cytokine levels in vitro.

The main results of the study are as follows:

1. The results showed that age was a significant determination of SEA-specific IL-4, IL-5, IL-10 and IFN- γ and SWAP-specific IL-10 levels and there was a significant inverse correlation between age and these cytokine levels by Spearman Rank Correlation analysis. It may be suggested that age-dependent is an epidemiological character of human cellular immune responses, which is consistent with the age-dependent acquired resistance and isotypic antibodies distribution.
2. There were non-responders whose cytokine, especially IFN-gamma levels were lower than 15.6ng/l, when stimulated by SWAP or SEA. The proportion of non-responders in egg-positive group (EPG>0) was higher than egg-negative group (EPG=0). The mean level of each stimulated cytokine was significantly higher in EPG=0 group than in EPG>0 group, there was a significant difference between the two groups ($P < 0.05$). This suggests that the cell-mediated immune response of the human population in endemic area with schistosomiasis japonica was down-regulated in general rather than to control selectively Th1 or Th2 subset, especially in egg positive individuals.
3. In EPG>0 group, the non-responders were 59.1%-88.6% of total subjects before treatment and there were still some non-responders after chemotherapy, but the percentage of non-responders in EPG>0 group reduced very significantly. Comparison of the mean cytokine levels before and 45 days after praziquantel treatment of 44 subjects with original egg positive indicates that after chemotherapy all cytokine levels significantly increased, except the level of IL-4 stimulated by SEA. It may be due to the restoration of the down-regulated immune response after sterilizing the parasites or to large amount of adult worm antigen release because of chemotherapy, resulting in the increase of corresponding cytokine level stimulated by the antigens in vitro.
4. The levels of IL-4 and IL-5 stimulated by all the recombinant antigens were not detectable, while the control antigens PHA, SKSD and PPD could induce IL-4 and IL-5 responses. But all the recombinant antigens could stimulate IL-10 and IFN- γ responses. This suggests that Th2 cytokines can not be induced by the recombinant antigens, which may be due to the simple structure of the small peptides without the predominant Th2-epitopes. This may explain the limited success of most subunit vaccine protocols designed to preferentially induce either Th1 or Th2 responses.
5. Multiple and non-conditional logistic regression analysis showed that individuals with the higher levels of SEA-specific IFN-gamma were 6.5 times less likely to become reinfected after chemotherapy than those with the lower levels. This suggests IFN-gamma may associated with acquired immunity.

23. Schistosomiasis in Egypt: An overview and synopsis on recent changes

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Schistosomiasis remains the most important parasitic disease causing a major public health problem in Egypt. The disease, caused by infection with snail transmitted blood trematodes, is known to be endemic in the country for centuries. Calcified *Schistosoma haematobium* eggs have been found in tissues of Egyptian mummies from as early as the 20th dynasty (1184-1087 BC). Hematuria, possibly due to schistosomal infection, is recorded in several papyri belongs to the 16th century BC. Rural populations due to agriculture practices are at risk of acquiring infection with *S. haematobium* and/or *S. mansoni* species. Currently, *S. haematobium* is prevalent along the Nile valley and the Nile Delta region while *S. mansoni* overlaps only in the Nile Delta and can hardly be found in upper Egypt. In 1913, for the first time the health administration gave official attention to schistosomiasis as a public health problem. In 1935, nation wide spot surveys by Scott estimated the prevalence rates of *S. haematobium* and *S. mansoni* to be 48% and 32%, respectively. In 1955, the Ministry of Health (MoH) repeated these surveys and reported comparable over all rate (38%) for *S. haematobium* infection, but significantly lower rate (9%) of *S. mansoni*. Another survey conducted in 1976 revealed prevalence rates of 20%, 30.1% and 8% for *S. haematobium*, *S. mansoni* and concurrent infection with both species, respectively in lower Egypt; 26.7% and 7.2-4.1% for *S. haematobium* in middle and upper Egypt, respectively. Despite efforts to control schistosomiasis, in 1980, MoH estimated 1.8-29.8% and 12.8-17.3% for *S. mansoni* and *S. haematobium*, respectively in lower Egypt; 27.1-33.6% and 17-37% for *S. haematobium* in middle and upper Egypt, respectively. All mentioned rates are generated based on single urine and stool examinations using relatively insensitive techniques, indicating that these prevalence rates are probably understated. The introduction in 1988 of praziquantel formulated in Egypt (Distocide), coincided with a country wide advertising campaign on TV, explaining the life cycle of schistosoma, the danger of infection, and the availability of free diagnosis and treatment using a safe oral drug. Such efforts resulted in significant decrease in endemicity of schistosomiasis. To date, MoH is moving towards a new strategy of selective diagnosis and mass treatment.

24. Field studies on snail transmitting schistosomiasis in Egypt: environmental factors and effect of selected molluscicides

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Seasonal variation of *Biomphalaria alexandrina* and *Bulinus truncatus* populations and their infection

rates with schistosome and other trematode cercariae were studied in four water courses located in Giza and Faiyoum Governorates. The abundance of both species varied from year to year and according to the type of habitat. The prevalence of *Schistosoma mansoni* in *Biomphalaria* snails was 0.29%, while the prevalence of *S. haematobium* in *Bulinus* snails was 1.36%. Seasonal variations of age structure of the two vector snails were monitored throughout the survey period. The infection rates with schistosome and other trematodes among *Bulinus* and *Biomphalaria* snails increased with the increase in their size. The antagonistic interaction between schistosome and non-human cercariae, specially echinostome, will be discussed.

A survey of *B. alexandrina* and *B. truncatus* was also executed at five watercourses in Qalyoub region. An attempt was made to correlate snail distribution with the concentrations of four metals of ecotoxicological importance (i. e. copper, zinc, lead and manganese). The results showed that there was a correlation between heavy metals concentrations, particularly copper, and the distribution of the vector snails.

In recent years, several molluscicides of plant origin have been studied as source for biological reagents to control snail-transmitted parasitic diseases. Previous studies showed that an Egyptian weed, *Solanum nigrum* L has molluscicidal activities. Now we present data on the optimum time for plant collection and the characteristics of the collected plant specimens. Our results revealed that the toxicity of *Solanum nigrum* increased during warm seasons but decreased dramatically during winter. Toxicity of *S. nigrum* fruits gradually decreased with increase in fruit size. *S. nigrum* (black fruits) was more toxic than the other two types, *S. nigrum* v. *vellosum* (yellow fruits) and *S. nigrum* v. *judaicum* (red fruits).

25. Study of plant molluscicide from *Jatropha curcas* seed (JCS) in laboratory

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At present, due to *Schistosoma japonicum* are still endemic in china, especially *Oncomelania hupensis*, Intermediate host is widespread in flood plain and lake region, which lead to snail control very difficult. Currently, the control of Schistosomiasis mainly depends on mass chemotherapy to the people and large-scale environment modification to the snail habitats in China. Meanwhile, chemical molluscicides are also used to control snail that distribute in high risk area. But chemical molluscicides have the disadvantage of much more expensive and pollution the environment than the plant molluscicides. There are a lot of plant molluscicide has a function of killing the snail in the world. Among of these, the wild plant of *jatropha curcas* (Euphorbiaceae) show has a promise function of molluscicide to *B. Glabrata* and *O. Hupensis* that was reported by Dr. A. Ruppel (Heidelberg university) and Profesor Li yonglong (Tongji medical university). For further understand the molluscicidal activity of JCS that was collected from Yunnan province in China, The project of development study on *jatropha curcas* seed have been carried out in our institute..

The result indicated that there are No.2 and No.6 of JCS showed a high effect to the snail. Under the concentration of 10mg/L of No. 2 and 6 .25mg/L of No. 6, the snail exposed the solution for 72 hours, that could be reached the 100% mortality of the snail by immersing method. The spray method was used with the concentration of 6g/m² of No. 2 and No. 6 JCS in field trial, after spraying the molluscicide for 5 days, the mortality of the snail was also 100%. In addition, acute toxicity to fish on LC₅₀ of No. 2 JCS was 696.31 mg/L. The result showed that JCS is a promise plant molluscicide.

26. Strategies of schistosomiasis control in Wuhan Xu Mingxing, Zhou Dunjin, Geng Hui, Yao Qun,

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Wuhan locates in the middle reaches of the Changjiang River, surrounded by vast lakes and canals which leading to the river, apparent seasonal fluctuation of water level offers a suitable environment for snails breeding. Thus schistosomiasis epidemic in Wuhan areas is typical marsy type.

Establishment of professional organizations for control of schistosomiasis in the endemic areas

The professional organizations have been established in Wuhan since 1950'. There are 3 levels professional organization in Wuhan. 1) The Office of Schistosomiasis Control and the Institute of Schistosomiasis Control of Wuhan are the first level, former is an executive department in charge of making policy, corresponding control strategy and approaches, the later is responsible for professional and technical directions to the basic organizations in the county. 2) The Schistosomiasis Control Office and the Station of Schistosomiasis Control in each endemic county are the second level organizations. The major duty and main task of Schistoamiasis Control Office in the county are collected funds from local government and individuals and supervises the Station of Schistosomiasis Control in the local county to carry out routine work including of surveying snails distribution, using molluscicides to eliminate snails, preventing new infected to people and so on. 3) In the hospital of each town, there is often a few of experienced doctors take the responsibilities for controlling and treating schistosomiasis and eliminated snails in the local area. In the endemic areas, each village even has a health worker offering responsible for basic service.

The strategies for control of Schistosomiasis history and current schistosomiasis control strategies

During 40 years the control strategies went through two stages. Before 1980's, killing the snails (*Oncomelania hupensis*) was a main method to interrupt the parasite's life cycle and prevent against human infection, after 1980's the control of schistosomiasis focused on the concept of morbidity control by application wide-scope chemotherapy and healthy education to individuals in the endemic areas.

Achievements of schistosomiasis control Through 40 years endeavor the considerable success has been achieved. By the end of 1999, 85.8% of the total snail-ridden area have been eliminated, 98.03% of the patients with schistosomiasis have been cured. Today the disease has been effectively controlled in 73.83% of the epidemic regions.

Problems in schistosomiasis control The results of the survey in 1999 showed that the total number of schistosomiasis patient was about 5000 and at present snail-ridden area in wuhan was 137 million m² but 95% snail-ridden area was distributed over the Changjiang River and its two branches Fuhe River and Dongjing River, which is very difficult to elimination because water level of the Changjiang River can not be controlled due to the seasonal fluctuation, for examples, the incidences of schistosomiasis are increasing as a result of 1998 year catastrophic flood, which inadvertently provide an ideal breeding sites for the snails vectors. Although the prevalence of infection has decreased to 2.02% and 85.8% of the snail-ridden area has been eliminated, but once the control projects stop, the snail-ridden area will spread quickly and the prevalence of infection will increase immediately. So schistosomiasis control will be a long-term task.

27. Impact of environmental change and schistosomiasis transmission in the middle reaches of the Yangtze river following the three gorges dam project

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AIM: To investigate major ecological environmental factors that might affect the transmission of *Schistosoma japonica* in Jiang Han Plain of Hubei province following the Three Gorges Dam project on the Yangtze River in China, and to provide the relevant countermeasures of schistosomiasis control.

METHODS: Data on the water level of the Yangtze River, riparian water table, annual rainfall and yearly evaporation were collected by means of field surveys and observation. Meanwhile, the snail distribution and schistosomiasis prevalence of human were investigated and analyzed with statistics method. **RESULTS:** After construction of the dam, the middle water level of the Yangtze River will last a longer period in the flood season, the fluctuation of riparian water table will follow the water level of the Yangtze River up and down. The prevalence of the people and snail density differed significantly between years which were correlate with high, middle and low typical water levels in the Yangtze, and it has a liner regression relationship between the prevalence of the people and the density of the snail with the depth of the water level, annual rainfall, yearly evaporation and ground altitude in the survey area. It has been confirmed the range of the water table is affected within 15 km along with the Yangtze River in flood season and 7 km in the low-water season. The endemic area of Hubei province has 52 cities or counties, of which 25 cities are along the Yangtze River. The snail area and infected people in 25 cities were 61.1% and 63.7% of whole province respectively in 1996.

CONCLUSION: The impact of schistosomiasis transmission in Jiang Han Plain following the Three Gorges Dam project is 1) on the positive aspect, the enlargement of endemic area and the rise of prevalence of schistosomiasis due to flood disaster would be controlled or reduced after three gorges dam project completed. 2) on the negative aspect, because of the change of the water level in the Yangtze River, the riparian water table would be supplied by the side of the river, which will lead to increase the area of low land in Jian Han Plain. This situation might enlarge the snail habitats and the range of schistosomiasis transmission in this area after three gorges dam project completed.

* Supported by UNDP/WORLD BANK/WHO Special Program for Research and Training in Tropical Diseases (TDR) and Ministry of Health in China.

28. Diagnosis, treatment and follow-up of acute infections with *Schistosoma mansoni* and *S. haematobium* in the absence of reinfection

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Patients from endemic areas are distinctly different from those of non-endemic areas with respect to diagnosis, treatment and follow-up of schistosomiasis. As a rule, the former are chronically exposed from early childhood and usually continue to be exposed after treatment, which they often receive repeatedly over a life time. Parasite burden is high and patients often suffer from irreversible sequelae of long standing infestation. The latter are most often only lightly infected since exposure is short term. Significant pathological manifestations are therefore rare even though exceptions are well known (a video of pathological findings of the urinary bladder on cystoscopy will be demonstrated).

Diagnosing schistosomiasis relies on serological methods and on direct diagnostic techniques (demonstration of eggs on microscopy; measurement of circulating antibodies or antigens) since clinical features are non-specific. Sensitivity of egg detection in stool or urine is a problem in lightly infected patients from nonendemic areas. Diagnosis in these patients relies therefore mostly on the history of exposure and the demonstration of specific antibodies. Eosinophilia is not sufficiently sensitive for screening purposes. To demonstrate specific antibodies, immunofluorescence antibody tests (IFAT) using gut associated and membrane bound antigens and enzyme linked immunosorbent assays (ELISA) using adult antigen extracts, crude soluble egg antigens and recombinant antigens are

employed. The former tend to become positive first, the latter after significant amounts of eggs have been deposited. Sensitivity and specificity are satisfactory particularly when sequential testing with a screening test and a confirmatory test is done. However, in patients from endemic areas, sensitivity of available tests may be low. Treatment of schistosomiasis is well established and standard WHO recommended praziquantel treatment schedules result in eradication in at least 85% with a reduction in parasite load of > 99% in the remaining 15%. In patients of nonendemic areas who are not at risk to reexposure repeated doses of praziquantel might be of advantage to achieve definite eradication of the parasite. Follow up of patients to evaluate treatment is difficult, particularly in those with low parasite burden. Direct demonstration of eggs is not a reliable option in these patients, nor does disappearance of eosinophilia rule out ongoing infection. Antibodies (IFAT, ELISA) do persist over years and do not regularly decline over time in a predictable fashion. Circulating antigens (anodic antigen CAA, cathodic antigen CCA) is an option to demonstrate active or persistent infection after therapy. It is worthwhile to remember, however, that the sensitivity of circulating antigen tests is very low in patients with recent infection.

29. Impact and countermeasures on the acute cases of schistosomiasis transmission by flood of Yangtze river

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Aim: To investigate and study impact on the acute cases on *Schistosoma japonicum* by flood and to make scientific countermeasures. **Methods:** To review the condition of acute cases of schistosomiasis for four years of 1989, 1991, 1996 and 1998 in seven counties that distribute the riverside of the Yangtze River and to analyze and evaluate the effect of health education, early screening and treatment, as well as snail control at the high risk area that performed during flood season in 1998. **Result:** The acute cases of schistosomiasis was dropped 15.34%-67.71% that compared 1998 and 1989, which showed there were no serious epidemic condition in high flood disaster year and also got significant efficiency of schistosomiasis control. **Conclusion:** The transmission of schistosomiasis is obviously impacted by natural factor and especially has a direction relationship with heavy flood, but as long as insistence scientific prevention and treatment, health education, as well as early treatment timely that the acute cases of schistosomiasis could be controlled and reduced impairment clearly.

30. Attenuation of *Schistosoma mansoni* cercaria with a molluscicide and cercaricide derived from the Egyptian weed, *Solanum nigrum* L.

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We have previously showed that water extract of leaves of the Egyptian weed *Solanum nigrum* L.(FLWE) has molluscicidal and cercaricidal effect. This extract was used in an attempt to chemically attenuate cercariae of *Schistosoma mansoni*. Cercariae were exposed to 5, 10, 15 and 20 ppm of the extract for 30 minutes, immediately before mice infection to quantify the effect of varying levels of exposure to FLWE on the activity of cercariae and its capacity to generate adult worms in mice. There

was a dose dependent effect of exposure to FLWE on cercarial penetration. The proportion of non-penetrated larvae increased from 3% in untreated cercariae to 26 % in those treated with a concentration of 20 ppm. The mean numbers of established adult worms declined from about 17 worms/mouse with untreated cercariae to 0.17 worms/mouse following treatment with 20 ppm of FLWE. BALB/c mice exposed to treated cercariae produced IgM and IgG antibody responses that were estimated by ELISA using cercarial and adult worm antigen preparations. IgG antibody levels increased between the 4th and 8th week posts infection in sera from control animals and those infected with cercariae treated with 5 ppm of FLWE. Mice exposed to cercariae after treatment with 10, 15 or 20 ppm of FLWE showed no rise in IgG antibody levels. IgM antibody levels increased between the 2nd and 8th weeks in sera from control animals and those infected with cercariae treated with 5 ppm FLWE. Cercariae treated with 10 and 15 ppm induced low IgM antibody levels against cercarial antigen, while there was no response against adult worm antigen. Mice exposed to cercariae treatment with 20 ppm FLWE showed no rise in IgM anti-cercarial and anti-adult worm antigens. These results suggest that treatment of cercariae with sub-lethal doses of FLWE resulted in a possible attenuation effect. Further studies are needed to determine whether such effect can be used to induce useful level of protection in mice towards further cercarial challenge.

31. Use of Landsat TM satellite surveillance data to measure the impact of the 1998 flood on snail intermediate host dispersal in the lower Yangtze River Basin

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To assess the impact of the 1998 flood on snail distribution in the lower Yangtze River basin, two study areas were selected, one in the Poyang Lake region, and the other along the Yangtze River in Jiangsu province. Using image analysis software, geocoded Landsat TM data were used to create TNDVI maps based on the formula $TNDVI = \sqrt{[(band4 - band3) / (band4 + band3) + 0.5]}$. The images taken in the flood season were recorded as water class and land class. The images taken during springtime were classified based on TNDVI. Composite images were created based on the time difference analysis, combining the flood season images and spring vegetation images to produce a final composite image in which potential snail habitats were identified. When compared with ground survey data collected in 2000, the correspondence rate between potential snail habitats identified by image analysis of 1998 Landsat TM data and ground survey data in spring of 2000 was over 90% in both regions. Results indicate that ecology based methods using Landsat TM image analysis provide a new way to create snail habitat maps and predict snail distribution under specific environmental conditions associated with the extent of the annual flood season.

32. Study of plant molluscicide from *Solanum xanthocarpum* extraction

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Oncomelania hupensis is a unique intermediate host of *Schistosomiasis japonica*, therefore snail control is a one of most important approaches of morbidity and mortality control to *S. japonica*. The snail control it is still adopted the chemical molluscicide with niclosamide at the high risk area of the snail habitats in China. Where are more than 700 million square meters of the snail habitats in Hubei province, in which about 60 % is distributed around the residence area. People and cattle contact infected water frequently by daily work. It is easy infected or reinfected the *S. japonica*. Especially this area is formed network of canal and ditches, where are very hard to change the situation by environmental modification to do the snail control in this area. Hence, except health education and chemotherapy currently, the molluscicide is still a effective measures to control the disease.

The study of plant molluscicide with *Solanum xanthocarpum* extraction was performed by means of biological determination to target snail and non-target living things as well as safety evaluation in accordance with WHO recommended the method and Guidelines of Organization of Economic Cooperation and Development (OECD). It is expected to find out the source of cheap, effective and environmental acceptable non-chemical molluscicide that potency to both of amphibious snail and fresh water snail in the endemic area of schistosomiasis in the world.

For exploring a novel plant molluscicide that has a high potency to target snail and low toxicity to non-target living things. Our institute collaborated with Central China Normal University to screen a lot of plant molluscicidal since 1980's. Recent years, the research is focused on one and two high potency plant molluscicide to perform the isolation, purification and extraction as well as biological effect determination to the target snail. The initial test result showed that the immersion method was used to the amphibious snail of intermediate host of *S. japonica*, adult *Oncomelania hupensis*; fresh water snail of intermediate host of *S. mansoni*, adult *Biomphalaria glabarate* and the intermediate host of *Fascioliasis*, adult *Lymnaea stagnalis* by the extraction of *Solanum xanthocarpum* under the room temperature 25 °C for 48 hours. The LC₅₀ of *O. hupensis* was 0.332 ppm and 95% confidence limits was 0.264-0.418ppm; of *B. gabarate* was 0.858ppm and 95% confidence limits was 0.661-1.114 ppm; of *L. Stagnalis* was 0.747 ppm and 95% confidence limits was 0.575-0.971 ppm. None of three kinds of snail can be seen the water-leaving behaviour in the concentration of 2.16 ppm that was the death rate of above 90% to the snail for 48 hours. The death rate of 100% that for *O. hupensis* was 4.32 ppm and for *B. glabarate* and *L. Stagnalis* was 8.64 ppm for 48 hours. As the time of immersing snail prolongation, the concentration of 100% death rate for the snail is also decreased gradually. It is evidenced that the extraction of *S. xanthocarpum* has a high potency not only to amphibious snail in China and also to the fresh water snail at abroad.

It was reported by Kusano, G et. al. that *S. xanthocarpum* has a nature of soap and some steroidal and alkaloid materials could be extracted from the plant which could enhance the contraction and tension of the atrium of cat and the heart of frog in vitro. However, it is unknown what bioactive molluscicidal properties of *S. xanthocarpum* that could kill the three kinds of snail. The research result would be provided the basic data for further mechanism research and the synthetic for the molluscicidal properties as well as the field application. It is expected a novel weapon for the snail control will be found out.

33. Clinical and pathological aspects of infections caused by bird schistosomes

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Schistosome larval stages of cosmopolitan bird schistosomes are known as the causative agents of

human cercarial dermatitis. Despite the fact that cercariae of these schistosomes possess an ability to penetrate into the skin of various vertebrates, their following development in a nonspecific (mammalian) host is incomplete. Depending on the species, in specific hosts (birds) adults mature and lay eggs either in visceral organs or in nasal cavities and, therefore, various migration routes seem to be utilised in order to reach their specific target organ/tissue. The location of the parasites in nasal cavities represents an unusual event; all known nasal bird schistosome species belong to the genus *Trichobilharzia*.

In nonspecific hosts, partial development of various bird schistosomes (e.g. *Bilharziella polonica*, *T. szidati* and *T. regenti*) includes successful cercaria-schistosomulum transformation, following migration, growth and survival in various organs/tissues; factors resulting in the killing of schistosomes before maturation remain to be solved. The character of migration route of these parasites in mammals is similar to that observed in bird host, e.g. also in mice *T. szidati* and *B. polonica* schistosomula are able to migrate to host lungs, where they survive for a relatively long period.

In case of *T. regenti* it was observed, that location in the bird nasal veins or mucosa follows previous migration through the avian central nervous system. It was observed that the infection of CNS and nasal cavities leads to leg paralysis or orientation/balance disorders and petechiae or hemorrhage, respectively. Our study showed that similar type of neuromotoric symptoms may occur in case when mouse is infected by *T. regenti* cercariae. Although the parasites were unable to reach adulthood in mammals they also develop in the CNS. The histopathological study showed that immature flukes were located between dura mater and arachnoidea in various parts of spinal cord and brain and in white mass of spinal cord in both types of host. When located under meninges, an inflammatory response developed in the tissue surrounding most of the parasites. In white mass of spinal cord the host immune reaction was accompanied by dystrophic and necrotic changes of neurons, perivascular eosinophilic inflammation and cell infiltration around the central canal. In duck nasal mucosa formation of granulomas around the eggs was observed.

Despite the fact, that the development of *T. regenti* in a nonspecific host was incomplete, the occurrence of similar neuromotoric symptoms both in bird and mammalian hosts underline the necessity to evaluate the real medical importance of infections caused by bird schistosomes.

Note: The study was supported by the Grant of the Czech Ministry of Health (No. IGA MU CR 4945-3) and Grant Agency of the Charles University (No.GA UK/00-38/1LF).

34. Purification and partial aminoacid sequencing of *Clonorchis sinensis* 15/16kDa proteins Pei Fuquan, Huang Jiankang Cui Huier, Pan Bo, Ran Caiwen, Wu Jun, Fang Yueyi, Liu Meizhen, Liang Wenjia, Zhang Qiming

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MATERIALS AND METHODS

Purification of Cs15/16 by HPLC and the assay of its antigenic activities

The used HPLC system was HP1100 (Huipu, USA) and the column TSK-DEAE-3SW 21.5×150mm, LKB. The antigen purity was identified by analytic SDS-PAGE and the diagnostic activities of all the purified antigens were assayed with indirect routine ELISA. The positive sera used were taken from the patients in the endemic areas.

N-end partial aminoacid sequencing and homology analysis

The analytic apparatus was Proteins/Polypeptides aminoacid sequencer, BECKMAN LF3200. The acquired sequences were homologically compared with the *Clonorchis sinensis* cysteine proteinase (cscp) genes and Cs28 and Cs 31 protein genes in Genbank.

RESULTS AND CONCLUSIONS

Sixteen purified samples (H1~H16) were acquired from CAA (*Clonorchis sinensis* adultworm antigens) by HPLC. Of them, H3, H11 and H13 showed single stained bands on SDS-PAGE (12% gel concentration). Their molecular weights were 15/16 kDa and they demonstrated different degrees of antigenic activities by ELISA. The diagnostic activity of H13, with the sensitivity 90% and the specificity 95% was close to that of CAA. The N-end partial amino acid sequences of these three samples were successfully assayed (see fig.1). Of these sequences, the 14 N-end AAs of H11 were identical to those of H13. We supposed that H11 and H13 could be the samples of the same proteins. The homology analysis showed that all the acquired sequences were not homologous to the above-mentioned genes reported in Genbank.

35. Studies on relationship between oxidative damage of multi-organ tissues and formation of trichinosis

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AIM: The paper observed the different levels and content of XOD, MDA and GSH in the different organ tissues and sera from mice infected by *T. spiralis* in order to probe the oxidative damage of multi-organ tissues and formation of trichinosis. We finally understood the pathogenesis of trichinosis.

METHOD: The experiment was randomly divided into four examined groups and a controlled group. There were six mice in each group. The examined mice fed to capsules with living larva of *T. spiralis*, and that controlled mice fed to normal mice meat before experiment. We collected the intestinal tissues, hepatic tissues, cardiac tissues, brain tissues, lung tissues and kidney tissues as well as blood to centrifugate on 3, 7, 14, 21, 30 day post infection, and then keeping examined materials into the refrigerator. The different levels and content of XOD, MDA and GSH in the above examined materials and sera were determined by those methods introduced in the reference.

RESULT and DISCUSSION : These results showed that the level and content of MDA in intestinal tissues and the levels and content of MDA and XOD in hepatic tissues in infected mice were higher significantly than that in controlled groups from 3-7 days after infection ($p < 0.01$), the levels of GSH were significantly lower than that in control groups at the same time ($p < 0.01$). These results indicated the oxidative damage of intestinal tissues and hepatic tissues had produced in early stage of trichinosis. After 14 days, the level of GSH in intestinal tissues gradually got back normal level, the level of MDA firstly fell off and then went up again. The level of XOD in hepatic tissues also returned gradually, but the levels of MDA and GSH went up constantly and kept higher level. The level of XOD in cardiac tissues obviously rose after 14 days, and reached to peak after 30 days. MDA firstly rose and then fell down. GSH rose significantly ($p < 0.05$) after 30 days, at the same time MDA in brain tissues and sera kept higher level. These results in this stage suggested the oxidative damage was transferred to visceral organic tissues from intestinal and hepatic tissues with change of live cycle of *T. spiralis*, and showed the oxidative damage started to focus to important visceral organs. The levels of GSH and MDA of brain tissues showed reverse change from 14 to 30 days. The level of MDA in sera was steadily maintained at a higher level.

CONCLUSION: These results indicated that *T. spiralis* could induce free radicals to damage some organs (and tissues of mice with trichinosis, and oxidative metabolites were so accumulated that toxemia was formed. The two pathogenic factors might be closely related to the happening and development of trichinosis.

36. Preliminary Screening Potential Protective Antigen from *Schistosoma japonicum* Cercariae

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Sera from rabbits vaccinated multiply with ultraviolet-attenuated cercariae of *Schistosoma japonicum* were used to screen novel effective protective antigen against schistosoma. Comparing soluble cercariae antigen(SCA) with soluble adult worm antigen(SAWA), Western blot analysis suggested that the epitopes recognized on the Mr>97Kda antigen both in SCA and SAWA. The epitopes recognized on Mr 215 Kda, 195 Kda, 170 Kda, 155 Kda, 140 Kda and so on were shown to be cross-reacted with both immunized sera and infected sera whereas immunized sera specifically recognized a SCA 104 KDa and a SCA 60 KDa antigen which showed an enhanced reactivity with immunized rabbit sera in comparison with infected rabbit sera. No SAWA antigen were recognized specially. Thus, SCA104KDa and SCA60KDa were screened preliminarily and might be correlated with protection against *S.japonicum*. Further research is needed for the two potential protective antigen candidates.

37. Differences and similarities in life cycle strategies of human and bird schistosomes

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Experimental work on bird schistosomes was scarce in the past. However, the importance of these parasites is growing at present. This is caused by several facts:

- 1) These parasites belong to important pathogens of birds, mainly in areas where an intimate contact with infected snails occurs.
- 2) Bird schistosomes are frequently responsible for human cercarial dermatitis.
- 3) Infections of laboratory mammals by bird schistosomes may include parasite migration to the lungs and central nervous system.
- 4) Due to some similarities in parasite adaptations, bird schistosomes (genus *Trichobilharzia*) may be considered as a useful model for human schistosomes.

HUMAN SCHISTOSOMES (GENUS <i>SCHISTOSOMA</i>)	BIRD SCHISTOSOMES (GENUS <i>TRICHOBILHARZIA</i>)
Egg laying and release	
Eggs released with the host faeces or urine in the outer environment; eggs already mature with miracidia; hatching in hypo-osmotic conditions (<i>Schistosoma</i> , <i>T. ocellata</i> , <i>T. szidati</i> , <i>T. franki</i> , etc.)	
Eggs in the nasal discharge (<i>S. nasale</i>)	Eggs hatch in the host tissue; iso-osmotic conditions, miracidia move freely in the nasal mucosa (<i>T. regenti</i>)
Many eggs trapped in the tissue, immune response, granulomas	
Infection of intermediate snail hosts	
Usually strict specificity to snail species or even geographical populations (strains); given by specific host-recognition (miraxons), immune evasion of parasites within snails and immunological properties of snails	
Penetration of vertebrate skin	
Specific recognition of vertebrates by cercariae; usually unsaturated fatty acids as stimuli	
Specificity to relevant hosts (<i>S. haematobium</i> ; limited spectrum, <i>S. japonicum</i> =broad spectrum of hosts); penetration into non-host mammals and birds - ???	Low specificity – cercariae penetrate the skin of birds and mammals, the latter are even more attractive
Transformation of parasites to schistosomula	
Loss of resistance to hypo-osmotic water environment, switch to anaerobic metabolism, changes in surface properties (shedding of carbohydrate-rich glycocalyx, antigen exchange, building of a protective double membrane); in case of bird schistosomes – the same changes in birds and mammals	
Migration of parasites within vertebrates	
Skin – blood circulation – lungs – blood network of target organs ??? (<i>S. nasale</i>)	Skin – blood circulation – lungs – blood network of target organs intestinal wall as target tissue (<i>T. szidati</i>) peripheral nerves – CNS – nasal cavity (<i>T. regenti</i>)
Duration of infections and fate of parasites	
Usually years	Usually weeks or few months
Maturation in specific mammalian host	Maturation in specific bird host, failure in mammals

More information on bird schistosomes: <http://www.natur.cuni.cz/~horak>

38. The Studies of Humoral Immunity in Rabbits Vaccinated with Ultraviolet-Attenuated Cercariae of *Schistosoma japonicum*

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Rabbits were vaccinated multiply with ultraviolet-attenuated cercariae of *Schistosoma japonicum*, of which the levels of IgG antibody in serum were observed dynamically. At the same time, sera with vaccinated rabbits transferred passively to mice at different time to detect the resistant. The results showed the levels of IgG antibody was detected 2 weeks after vaccination, peaked by 5 weeks and attenuated rabbit sera conferred significant levels of protection (51%) when transferred before challenge. This suggested that antibody might play an important role in the protective immunity elicited by uv-attenuated *Schistosoma japonicum* cercariae multiply.

Credits and Acknowledgements

Organisation of the meeting and assembly of programme and summaries – Yong-long Li and Andreas Ruppel.

Editing and formatting for this version – Malcolm Kennedy (malcolm.kennedy@bio.gla.ac.uk)

Web page and version – David Johnston.

This was the sixth in a series of annual meetings on the subject and was held 16 to 22 October 2000 at the Tongji Medical College, Huazhong University of Science and Technology, Wuhan, P.R. China. The meeting was organised and chaired by Yonglong Li and Andreas Ruppel. It received support from the Tongji Medical College of Huazhong University of Science and Technology (Y.L.), the German Academic Exchange Service DAAD (travel grants to A.R. for Cairo and Heidelberg), the Ministry of Science and Art Baden-Württemberg (A.R.), the European Community (J.R.K.) and the Royal Society (M.W.K.).