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FINAL REPORT

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DEVELOPMENT OF METHODS FOR IMMUNODIAGNOSIS OF HUMAN LIVER FLUKE INFECTION

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EXECUTIVE SUMMARY:

Despite minor problems encountered during the research period indicated in the covering page, practically all objectives of the research project have been successfully achieved. Alternative approaches for the detection of Opisthorchis viverrini infection in the humans have been proposed, developed and tested. Ten full-length papers have been published in a number of refereed scientific journals and three more are still in press. In addition, three papers have been recently submitted. If all were accepted, there will be a total of 16 papers. Furthermore, there are 17 Abstracts that have been published in the program books of various International and National Conference or Congress. Lastly, the fund available has made it possible for research training of 3 doctoral (Ph.D.) and 3 M.Sc. students.

RESEARCH OBJECTIVES:

The main objective of this research project is to develop simple, sensitive and specific assay(s) for the diagnosis of a human liver fluke infection caused by Opisthorchis viverrini. The approach is to characterize, identify and purify relevant antigenic components by appropriate physicochemical and immunological methods using a battery of sera from patients, rabbit polyclonal and mouse monoclonal antibodies. Reasons for the needs to develop these alternative methods have been reviewed in Paper No 1.

RESULTS:

The final accomplishment of the project are presented

in Papers No 12 and 13. Both papers describe the 2 methods that have been developed and results from a limited field testing. Papers No 2 to 11 contain data from detailed studies that are required to develop these 2 methods. Papers No 14 to 16 have been recently submitted for consideration for a publication in various others scientific journals. They describe detailed characterization of the genetic properties of the parasite that pave way for the construction of a specific DNA probe used for the detection of parasite DNA in the stools of patients described in Papers 12 and 13.

In our initial proposal, we mentioned the possibility of a production of relevant antigens by recombinant E.coli carrying gene fragment of O.viverrini. Despite a number of attempts in various laboratories under the guide of well-qualified investigators, both abroad and within our own faculty, these attempts were not successful. We were able to insert a specific O.viverrini gene fragment into the E. coli but were not able to make these recombinant bacteria to express the products. Despite these unsuccessful attempts, information obtained was nevertheless important and provided a basis for the construction of a specific DNA probe described in papers 11 to 16.

IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER:

The results achieved in this research project provide 3 alternative approaches for the diagnosis of opisthorchiasis, namely, detection of serum antibody,

detection of soluble antigen in feces and DNA released from eggs expelled with the feces. Although these proposed methods have been tested and compared in a limited field trial, the reliability including particularly sensitivity and specificity has to be tested in a larger field trial which has yet to be started. We are in the process of planning and looking for a future support on this part.

PROJECT OUTPUT:

Lists of publications, meetings attended and graduate students' training are enclosed herewith. As mentioned in the summary on page 2, there will be a total of at least 13 papers published in refereed scientific journals. Three more are in the process and if all are accepted, this will make a total of 16 papers. In additions, there were approximately 17 abstracts of work presented at various local and international scientific meetings. The project also provided opportunity for 3 Ph.D and 3 M.Sc. students to carry on their thesis research.

FORMAL PUBLICATION (Full papers)

1. Sirisinha S: Immunodiagnosis of human liver fluke infections. Asian Pacific J Allergy Immunol 4:81-88, 1986.
2. Sirisinha S, Wongratanacheewin S: Immunization of hamsters against Opisthorchis viverrini infection. Southeast Asian J Trop Med Pub Hlth 17:567-573, 1986.
3. Wongratanacheewin S, Chawengkirttikul R, Bunnag D, Sirisinha S: Analysis of Opisthorchis viverrini antigens by immunoprecipitation and polyacrylamide gel electrophoresis. Parasitology 96:119-128, 1988.
4. Wongratanacheewin S, Bunnag D, Vaeusorn N, Sirisinha S: Characterization of humoral immune response in the serum and bile of patients with opisthorchiasis and its application in immunodiagnosis. Am J Trop Med Hyg 38:356-362, 1988.
5. Wongratanacheewin S, Sirisinha S: Analysis of Opisthorchis viverrini antigens: physicochemical characterization and antigen localization. Southeast Asian J Trop Med Pub Hlth 18:511-520, 1987.
6. Wongratanacheewin S, Charupatana C, Bunnag D, Sirisinha S: Effect of praziquantel treatment on antibody levels and lymphoproliferative responses in patients with opisthorchiasis. Southeast Asian J Trop Med Pub Hlth 19:109-116, 1988.
7. Chawengkirttikul R, Sirisinha S: Antibodies in serum and bile of hamsters experimentally infected with Opisthorchis viverrini. Int J Parasitol 18:721-727, 1988.
8. Billings PB, Utsakhit N, Sirisinha S: Monoclonal antibodies against antigens of the human liver fluke (Opisthorchis viverrini). Parasite Immunol 12:545-557, 1990.
9. Sirisinha S, Sahassananda D, Bunnag D, Rim HJ: Immunological analysis of Opisthorchis and Clonorchis antigens. J. Helminthol 64:133-138, 1990.

10. Amornpant S, Sarasombath S, Sirisinha S: Production and characterization of monoclonal antibodies against excretory-secretory antigen of the liver fluke (Opisthorchis viverrini). Intern J Parasitol 21:421-428, 1991.
11. Sermswan R, Mongkolsuk S, Sirisinha S: Characterization of the Opisthorchis viverrini genome. J Helminthol 65:51-54, 1991.
12. Sirisinha S, Chawengkirttikul R, Sermswan R, Amornpant S, Mongkolsuk S, Panyim S: Detection of Opisthorchis viverrini by monoclonal antibody-based ELISA and DNA hybridization. Am J Trop Med Hyg 44:140-145, 1991.
13. Sirisinha S, Chawengkirttikul R, Sermswan R: Immuno diagnosis of opisthorchiasis Proceedings of the 33rd SEAMEO-TROPMED Regional Seminar on Emerging Problems in Food-Borne Parasitic Zoonosis: Impact on Agriculture and Public Health, Chiang Mai Thailand. Thai Watana Panich Press Co., Ltd. Bangkok, 1991. pp 179-183.
14. Sermswan R, Mongkolsuk S, Panyim S, Sirisinha S: Isolation and characterization of Opisthorchis viverrini specific DNA probe. Mol Cell Probes 5:399-407, 1991.
15. Korbsrisate S, Mongkolsuk S, Haynes JR, England D, Sirisinha S: Complete nucleotide sequence of small subunit ribosomal RNA gene from Opisthorchis viverrini. Gene 105:259-261, 1991.
16. Korbsrisate S, Mongkolsuk S, Haynes JR, England D, Sirisinha S: Cloning and characterization of ribosomal RNA gene of Opisthorchis viverrini. Parasitology 104:323-329, 1992.

ORAL/POSTER PRESENTATIONS

1. Immunodiagnosis of human liver fluke infection caused by Opisthorchi viverrini. Medical Microbiology Towards 2000, Hong Kong, 29 May - 1 June, 1987.
2. Approaches to develop immunodiagnostic methods for liver fluke infection caused by Opisthorchis viverrini. Conference of USAID Scienic Research Award Grantees, Nakorn Pratom, Thailand 24 - 26 July 1987.
3. Diagnosis of opisthorchiasis. Symposium organized by Ministry of Public Health, Petchburi, Thailand, 6 August 1987.
4. Biotechnology research in the study of opisthorchiidae in Thailand. Thailand-U.S. SEAMEO-TROPED Conference on Applications of Biotechnology on the Study of Animal Parasites and Their Vectors. Bangkok, Thailand, 23-25 November, 1987.
5. Development of methods for immunodiagnosis of human liver fluke infection. PSIC Conference on Biotechnology for Health and Agriculture. Washington, D.C., USA, 6-9 June 1988.
6. The characterization of genome and repeated DNA of O.viverrini. Same as No.5
7. Analysis of Opisthorchis viverrini antigens with emphasis on development of immuno-diagnostic assay. XIIth International Congress for Tropical Medicine and Malaria. Amsterdam, Netherlands, 18-23 September, 1988
8. Immunology of cholangiocarcinoma. Conference on Development of Early Diagnosis and Management of Cholangiocarcinoma Associated with Opisthorchiasis. Bangkok, Thailand. 9-11 January 1989.
9. Control of opisthorchiasis in Thailand. Annual Meeting of Medical Technology Society of Thailand. Khon Kaen, Thailand, 18-20 January, 1989.
10. Development of methods for diagnosis of human liver fluke infection. 30th Year Cerebration of the Faculty of Science, Mahidol University. Bangkok, Thailand, 12-14 January 1989.

11. Cloning of a repetitive DNA element from Opisthorchis viverrini. Second SSM International Congress for Microbiology, Singapore, 31 October - 3 November, 1989.
12. Monoclonal antibodies specific for human liver fluke. 7th International Congress of Immunology, Berlin, Germany. 30 July- 5 August, 1989.
13. Alternative approaches for the diagnosis of liver fluke infection caused by Opisthorchis viverrini. Workshop on AID/SCI Funded Research in Immunology in Thailand, Chiang Mai, Thailand, 4-6 December, 1989.
14. Potential of Opisthorchis viverrini monoclonal antibodies in the diagnosis of opisthorchiasis. Annual Meeting of The Allergy and Immunology Society of Thailand. 30 March, 1990.
15. Monoclonal antibodies and DNA probes for the diagnosis of opisthorchiasis. VIIth International Congress of Parasitology. Paris, France 20-24 August, 1990.
16. Immunodiagnosis of opisthorchiasis. 33rd SEAMEO-TROPED Seminar on Zoonosis: Impact on Agriculture and Public Health. Chiang Mai, Thailand. 14-17 November, 1990.
17. Overview of immunology of parasitic infection and alternative approaches to detect liver fluke infection. ASEAN Conference in Medical Laboratory Technology, Bangkok, Thailand, 1-5 April, 1991.

GRADUATE STUDENTS' THESES

1. Surasak Wongratanacheewin "Characterization of humoral immune response in the serum and bile of patients with opisthorchiasis and its application in immunodiagnosis" (Ph.D. 1987)
2. Rasana Sermswan "Construction of specific DNA probes for Opisthorchis viverrini detection and an attempt to clone genes coding for diagnostic epitopes" (Ph.D. 1990)
3. Sunee Korbsrisate "Cloning and characteriza-tion of ribosomal RNA genes of Opisthorchis viverrini" (Ph.D., approximately Mid 1991)
4. Nantana Utsakhit "Production and characterri-zation of monoclonal antibodies against potentially diagnostic antigens from the liver fluke (Opisthorchis viverrini)" (M.Sc. 1989)
5. Sorujisiri Amornpant "Production and character-ization of monoclonal antibodies against metabolic products (excretory secretory antigens) of the liver fluke (Opisthorchis viverrini)" (M.Sc. 1989)
6. Teeraporn Bureerug "Attempts to identify tumor-associated antigen(s) in cholangiocarci-noma" (M.Sc. 1990)