RECENT ADVANCES IN BASIC AND APPLIED SCIENCE FOR THE CONTROL OF TAENIASIS/CYSTICERCOSIS IN ASIA

A Ito[1], T Wandra[2], R Subahar[1,3], A Hamid[4], H Yamasaki[1], Y Sako[1], W Mamuti[1], M Okamoto[5], K Nakaya[6], M Nakao[1], Y Ishikawa[1], T Suroso[2], PS Craig[7] and SS Margono[3]

[1]Department of Parasitology and [6]Animal Laboratory for Medical Research, Asahikawa Medical College, Asahikawa, Japan; [2]Directorate General, Communicable Disease Control and Environmental Health, Ministry of Health and Social Welfare, Jakarta, Indonesia; [3]Department of Parasitology and [4]Department of Neurology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia; [5]Department of Laboratory Animal Science, School of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori, Japan; [7]Division of Biological Sciences, University of Salford, Manchester, England

Abstract. Detection of seven specific bands by immunoblot (IB) using glycoproteins (GPs) purified by lentil-lectin affinity chromatography has been the gold-standard for neurocysticercosis (NCC) serodiagnosis since 1989. However, due to the presence of contaminants, it was impossible to apply the GPs to ELISA. Our group at Asahikawa Medical College (AMC) succeeded in purifying the GPs by preparative isoelectric focusing; these higher quality GPs were suitable for ELISA. Based on the results of both IB and ELISA testing, developed at AMC for a field survey in Irian Jaya, it became evident that that area had pandemic NCC. We found many NCC patients, pigs full of cysts, and one dog infected with two cysts: these findings were based on serology. Recently, we conducted another survey to detect of the worm carriers of *T. solium*. Three of the 38 local people were positive by copro-antigen specific to *Taenia* species; these three patients expelled segments of *Taenia* spp and these were confirmed as those of *T. solium* by mitochondrial DNA analysis. When viable eggs of any taeniid species could be obtained, they can be developed into metacestodes in NOD-*scid* mice; it then becomes possible to analyze morphological dynamics, metacestode antigenicity, the efficacy of new metacestocidal drugs, and mitochondrial DNA. Mitochondrial DNA analysis of the specimens obtained in Irian Jaya was compared with that of other isolates worldwide. *T. solium* is now divided into two genotypes: the Asian type, and the Africa-American type. Some aspects of the pathological differences between the Asian and Africa-American types and the antigenic components of these two types are discussed.

INTRODUCTION

Neurocysticercosis (NCC), caused by the larval stage of the pork tapeworm,*Taenia solium*, is one of the most serious cestode zoonoses and emerging diseases worldwide. In this short review article, some of the recent advances in the basic and applied science of taeniasis and cysticercosis are introduced. This review will consider: the serodiagnosis of NCC; the molecular diagnosis of NCC; the application of these diagnostic methods to an epidemiological survey of cysticercosis/taeniasis in West Papua (Irian Jaya), Indonesia; laboratory techniques for the preparation of parasite material suitable for these diagnostic methods; the prospects for the diagnostic use of animal models.

Correspondence: Akira Ito, Department of Parasitology, Asahikawa Medical College, Midorigaoka-Higashi 2-1-1-1, Asahikawa 078-8510, Japan.
Tel: ++81-166-68-2420; Fax: ++81-166-68-2429
E-mail: akiraito@asahikawa-med.ac.jp

RECENT ADVANCES

Serodiagnosis (native and recombinant antigens) in humans and pigs

A summary of essential information regarding contemporary serodiagnosis by immunoblot was by Gottstein *et al* (1986). This was followed by the characterization of the glycoproteins (GPs) of taeniid cestodes by Parkhouse and Harrison (1987). Tsang and others (1989) established a method for the purification of highly specific GPs (7 bands from 8-10kDa up to 50kDa) by lentil-lectin affinity chromatography; Tsang's serodiagnosis by immunoblot has been the gold standard ever since. However, as the purified GPs have some back ground noise (artefacts of higher molecular weights), it was impossible to apply them to ELISA. Ito *et al* (1998) succeeded in purifying these antigens, making them virtually free of the background noise that interfered with ELISA, by preparative isoelectric focusing (PIEF). This was the first ELISA system, which reliably differentiated cysticercosis from other diseases, especially the highly cross-reactive alveolar echinococcosis. The GPs purified by Asahikawa Medical College (AMC) group could be used for the

detection of pigs infected with *T. solium.* Groups in Scotland (Garcia *et al*, 1998) and Belgium (Brandt *et al*, 1992) developed ELISA to detect infected pigs using monoclonal antibodies against some components of the metacestode of *T. saginata* that were shared with those of *T. solium*. However, there remained some cross-reactivity with *T. hydatigena* in pigs. There appears to be no such cross-reaction with sera from pigs infected with *T. hydatigena* when GPs purified by PIEF are used (Sato *et al*, unpublished).

Several groups have produced recombinant antigens (Chung *et al*, 1999; Sako *et al*, 2000) and synthetic peptides (Greene *et al*, 2000) suitable for the serodiagnosis of NCC in humans. However, the sensitivity of detection of seropositive samples using these antigens is not as high as that of the GPs purified from the native antigens. Another method of laboratory serodiagnosis (although not for seroepidemiological work) is the application of GPs purified from metacestodes of *T. crassiceps*. However, we can use recombinant antigens of *T. solium* and it is better to focus on these for the diagnosis of *T. solium* cysticercosis, especially as trials of the application of closely related taeniid cestodes are likely to be frustrated by shared antigenic components that will make it difficulf to differentiate NCC (Ganble and Murrell, 1998; Ito, 2002).

DNA, pathology, antigenic polymorphism of *T. solium*

As we mentioned at the Joint International Tropical Medicine Meeting 2001 (JITMM 2001) (Okamoto *et al*, 2001), there are two types of DNA sequence of *T. solium*: the Asian type and the Africa-American type (Nakao *et al*, submitted). So far we know, there are two types of pathogenicity of NCC. One is the Asian type, with subcutaneous cysticercosis; the other is the Africa-American type, without subcutaneous cysticercosis. There is a crucial difference between the immunoblot results of cyst fluid of *T. solium* prepared from Asia (China and Irian Jaya) and those from Africa-America (Ecuador and Mozambique) (Ito *et al*, 2002). It is therefore necessary to be aware of the origin of materials for serology and DNA analysis.

Epidemiological survey of NCC in Irian Jaya, Indonesia

The NCC outbreaks in West Papua (Irian Jaya) in the early 1970s have been thouroughly documented (Tumada and Margono 1973; Gajdusek, 1978; Simanjuntak *et al*, 1997). Seroepidemiological surveys, using the GPs produced by the AMC group, has been conducted in Irian Jaya (Wandra *et al*, 2000). It is now clear that serology by immunoblot and ELISA is available for the detection of infected pigs (Ito *et al*, 1999; Subahar *et al*, 2001) and dogs (Ito *et al*, unpublished). We have detected, by serology, one dog with two cerebral *T. solium* cysts (DNA confirmation). Our most recent field survey was conducted in order to detect the worm carriers of *T. solium* (local people) in Irian Jaya. In 2001, we found three local people who were copro-antigen positive (of a sample of 38 persons). They all had adult *T. solium*, the segments of which were confirmed to be those of *T. solium* by mitochondrial DNA analysis (Okamoto *et al*, unpublished). We are currently conducting a similar survey in order to detect worm carriers while simultaneously checking for NCC in these carriers by serology. Such a strategy for the detection of NCC cases in humans and animals (pigs and dogs) is likely to be applied in other countries where NCC is endemic (Singh *et al*, 2002).

A laboratory animal models for cysticercosis

We found that severe combined immunodeficient (scid) mice were highly susceptible to *in vitro* hatched oncospheres of *T. solium* and *T. saginata asiatica* (*T. asiatica*), which developed into mature metacestodes (Ito *et al*, 1997a, b; Ito and Ito, 1999). Recently, we found that NOD-*scid* (non-obese diabetic), severe combined immunodeficient mice showed a much greater susceptibility to experimental infection with not only these two cestodes but also with *T. saginata* (Ito *et al*, 2001).

What do mouse models contribute to the understanding of human taeniid infections?

It is very easy to differentiate taeniid cestode segments expelled from patients by mitochondrial DNA analysis or PCR (Bowles and McManus, 1994; Yamasaki *et al*, in preparation). When viable eggs of these three human taeniid cestodes are available, NOD-scid mice can be infected by *in vitro* hatched oncospheres; the detailed development of metacestodes can then be analysed. For example, the oncospheres of *T. saginata asiatica* grow to 2-3mm in diameter in the liver of pigs (Fan, 1988), whereas they grow to 10mm or more in NOD-*scid* mice within 3-4 months of infection, when their size is similar to that of *T. saginata* (Ito *et al*, unpublished). A mouse model system might be expected to: (a) serve as an evaluation system for new drugs, replacing the use of domestic animals (Gonzalez *et al*, 1998); (b) allow the comparison of the antigenic components of the three human *Taenia* (*T. solium*, *T. saginata*, *T. saginata asiatica*); (c) assist in the detection of circulating antigens for the monitoring of ongoing infections especially in humans - pigs are usually killed within 6 months of birth and therefore do not need for checking ongoing infection.

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