Pergamon

International Journal for Parasitology, Vol. 27, No. 10, pp. 1203–1211, 1997

Printed in Great Britain
PII: S0020-7519(97)00118-5
0020-7519/97 $17.00+0.00

Basic and Applied Immunology in Cestode Infections: from Hymenolepis to Taenia and Echinococcus

AKIRA ITO

Department of Parasitology, Gifu University School of Medicine, Gifu 500, Japan

Abstract—Ito A. 1997. Basic and applied immunology in cestode infections: from Hymenolepis to Taenia and Echinococcus. *International Journal for Parasitology* 27: 1203–1211. In larval cestode infections, it is well established that the intermediate mammalian host infected with egg-derived metacestodes in the tissue becomes completely immune to reinfection with eggs, whereas autoinfection has been conceived to occur in *Hymenolepis nana*/mouse (and human) and *Taenia solium*/human systems when these hosts are initially infected with metacestode-derived adult tapeworms in the lumen. In this review paper, *the first topic* is immunobiology of *H. nana*/mouse system on the reinfection immunity in order to get critical information as to how the initially ingested parasite (eggs or metacestodes) can develop into adult worms and how autoinfection does or does not occur in immunocompetent mice, since *H. nana* can complete its whole life cycle in the mouse intestinal tissue and lumen. When mice are infected with eggs (=oncospheres) of *H. nana*, they become immune to challenge infections with eggs within a few days (early response) and with cysticercoids within two weeks (late response). The initially established adult worms are expelled later (worm expulsion response). When mice are infected with cysticercoids, either derived from beetles or mice, they become immune to challenge infection with cysticercoids but not with eggs. Therefore, autoinfection occurs in the intestinal tissue for the establishment of cysticercoids in the tissue but never occurs in the intestinal lumen for the establishment of adult worms in immunocompetent mice. *The second topic* is vaccination trial against challenge infection with eggs of Asian Taenia in pigs. Pigs vaccinated with frozen oncospheres of Asian Taenia from Taiwan or Korea or *T. saginata* showed very strong resistance, whereas pigs vaccinated with those of *T. solium* showed partial resistance only. It is suggested that Asian Taenia is much closer to *T. saginata* than *T. solium* from the immunobiological viewpoint. *The third topic* is immunodiagnosis of echinococcosis and cysticercosis. Immunoblot analysis has revealed that Em18 (18 kDa component of crude antigens of *Echinococcus multilocularis* protoscolex) and glycoproteins of *T. solium* cysticerci are highly specific or unique to alveolar echinococcosis and cysticercosis, respectively. *The fourth topic* is discussion on miscellaneous prospects including laboratory animal models for echinococcosis and cysticercosis. © 1997 Australian Society for Parasitology. Published by Elsevier Science Ltd.

Key words: Echinococcus; Taenia; Hymenolepis; immunobiology; immunodiagnosis.

INTRODUCTION

"The mechanism of parasite survival in the immunized host" has been the most interesting unsolved problem in immunoparasitological research. Immunobiology of *Hymenolepis nana* and *Taenia taeniaeformis* has well been analysed, since the natural intermediate hosts for these cestodes are mice and rats and previously infected mice and rats show complete resistance to egg challenge. My original work in parasitology (Ito, 1975) was to analyse antibody responses in reinfection immunity in *H. nana*/mouse system under Prof. Ken-ichi Okamoto who revealed the importance of the thymus in this host–parasite system (Okamoto, 1968; Okamoto & Koizumi, 1972). In *H. nana*/mouse system, mice previously infected with even a single oncosphere (Ito & Yamamoto, 1976) became completely immune to challenge infection within a few days. So, I was interested in the mechanism of the parasite's survival in the immunized host as well as the mechanism of immunity to reinfection. At that time, Glasgow group (Hopkins *et al.*, 1972; Befus, 1975; reviewed by Hopkins, 1980) showed the immunogenicity of adult tapeworms of *H. diminuta* and gave me a great influence.

In this paper, I would like to introduce my recent work using these cestodes in mice and rats with some applied work for cysticercosis and echinococcosis. As

most papers published by my group from 1975 until 1983 have been reviewed by Ito & Smyth (1987), Williams (1982) and Rickard (1983), a major part of this article is focused on my recent work during the last decade with brief summary of classic work, if necessary. There are some more up-to-date reviews on cestode infections by Lightowlers *et al.* (1993) and Craig *et al.* (1996).

IMMUNOBIOLOGY OF HYMENOLEPIDID INFECTIONS

Immunobiology of Hymenolepis nana*/mouse system*

Hymenolepis spp. require beetles and rodents for completion of the life cycle. However, *H. nana* is unique, since it can complete its whole life cycle in the small intestine of a single mouse host. Oncospheres differentiate into metacestodes (cysticercoids) in the mouse or rat intestinal tissue within 4 days or in beetles approximately 2 weeks under 20°C. Metacestodes developed either in mice or beetles develop into mature adult worms in the mouse intestinal lumen within 10 days. *H. nana* (adapted to mice) does not develop into adult worms in rats (Ito, 1983; Ito & Kamiyama, 1984).

The research that we did using this host-parasite relationship, reviewed by Ito & Smyth (1987), has revealed that (1) an oral infection with even a single oncosphere derived from ingestion of a few eggs evokes complete immunity to reinfection in mice (Ito & Yamamoto, 1976), (2) all developmental stages of this parasite are recognized as antigens by the infected host (Ito & Onitake, 1987; Ito *et al.*, 1988a), (3) mice infected with a single oncosphere become completely immune to reinfection with eggs and with metacestodes (the immune response to egg challenge is called "early response" and expressed within a few days, whereas that to metacestode challenge is called "late response" and expressed within two weeks in most strains of mice (Ito, 1982; Ito & Kamiyama, 1984), (4) autoinfection usually does not occur in most strains of immunocompetent mice (see Section **3** in this review; Ito, 1982; Ito *et al.*, 1988b), (5) immunogenicity of metacestodes recovered from mice, rats or beetles has no difference (Ito & Onitake, 1986), and (6) adult worms are expelled by mechanisms other than the early and late responses against reinfection (worm expulsion response) (Vorst *et al.*, 1988; Watanabe *et al.*, 1994).

Although mice infected with *H. nana* show different immune responses against different stages of this parasite and passive transfer of immunity to egg challenge, (the early response) can be demonstrated with sera (Ito, 1977) or cells (Asano *et al.*, 1986; Asano *et al.*, 1991), it is impossible so far to vaccinate mice in this system. Although there was no critical difference in immunogenicity of metacestodes derived from beetles and mice (Ito & Onitake, 1986), we have to keep in mind that oncospheres of this parasite can develop into metacestodes in mammalian intestinal tissue and in beetles. There is no other parasite which can develop into metacestode stage either in invertebrate (mainly beetles) or in vertebrate (mainly mice, humans) (Smyth, 1969). It may be interesting to look for some other cestodes which may show such a diversity in the host specificity as *H. nana*.

Immunobiology of H. nana*/rat system*

As mentioned above, *H. nana* (at least adapted to mice) does not develop into adult worms in the rat host (Ito & Kamiyama, 1984). Rats treated with antilymphocyte serum or congenitally athymic nude rats show basically similar resistance to the lumen phase of *H. nana*. However, when rats orally inoculated with eggs or cysticercoids (either beetle- or mouse- or rat-derived) are treated with corticosteroid during the expected lumen phase of this parasite, i.e. from day 4 of egg inoculation or from day 0 of cysticercoid inoculation, cysticercoids derived from eggs in rats or inoculated orally develop into mature adult worms. Cessation of treatment with corticosteroid induces rapid expulsion of the growing adult worms from the rats. Therefore, the effect of cortisone is not simply ascribed to its immunosuppresion (Ito & Kamiyama, 1984). We do not know the critical mechanism of the induction of adult cestodes in such unnatural host animals treated with corticosteroid. Corticosteroid may affect not only the rat's physiological condition, thereby making the host suitable for the development of *H. nana* into adult worms, but it might also affect the worm directly (Moss, 1972).

Immunobiology of other Hymenolepis spp. infection

H. microstoma and *H. diminuta* obligatory require insects and rodents (mice and rats) for completion of their life cycles, respectively. These cestode species are intestinal parasites in rodents and have been studied to be good models for mucosal immunity (Hopkins, 1980; Vorst *et al.*, 1988). Using the two (tissue and lumen) stages of *H. nana* and the lumen stage of these two species, we have found that there exists stage specific cross immunity (Ito *et al.*, 1988c). In *H. microstoma*/mouse system, mice kept with infected mice showed antibody response against egg antigens. It was due to the fact that mice could be sensitized with eggs in the feces, since mice orally inoculated with eggs

showed similar antibody responses (Ito *et al.*, 1989). Furthermore, when mice were orally inoculated with shell-free eggs, oncospheres could invade the host intestinal tissue (Onitake *et al.*, 1990). It is suggested that any mammals might have chances to be sensitized with cestode eggs or oncospheres through environmental contamination (Ito, 1992).

IMMUNOBIOLOGY OF TAENIID INFECTIONS

From Taenia taeniaeformis *to Asian Taenia*

Taenia taeniaeformis requires rodents (usually rats and mice) and cats for completion of its life cycle. *T. taeniaeformis* infections in rats and mice are of special interest to the experimental immunoparasitologists, since both active and passive immunity can be readily demonstrated in these rodents (Williams, 1982; Rickard, 1983; Lightowlers *et al.*, 1993).

In *T. taeniaeformis* infection in rats, we found that an oral infection with even a single live oncosphere evokes complete immunity to reinfection as similar as in *H. nana* infection in mice, whereas vaccination requires at least 500 killed oncospheres (Ito & Hashimoto, 1993). So, it is evident that a single oncosphere invading the host tissue is sufficient to evoke complete protection in general. The discrepancy between infection and immunization may be explained by either that (a) killed oncosphere has not so much host protective antigens as the invading oncosphere can release in the tissue (quantity of host-protective oncosphere antigens) or that (b) the host protective antigens may be shared between oncosphere and post-oncosphere stages (quality of host-protective antigens). Anyway, the host protective antigens of the oncosphere have been studied and recombinant antigens have been produced (Johnson *et al.*, 1989; Ito *et al.*, 1991). In *T. ovis* infection in sheep, recombinant vaccine has been developed as commercial vaccine by Australian and New Zealand group (Johnson *et al.*, 1989).

The main work using *T. taeniaeformis* was to vaccinate rats with different materials including intact eggs and recombinant antigens of this parasite (Ito *et al.*, 1994a) and heterologous species (Ito *et al.*, 1994b). The results so far obtained strongly suggest that a single dose of frozen eggs or frozen oncospheres with or without adjuvant is highly effective for vaccination of rats (Ito & Hashimoto, 1993). Those of heterologous species are less effective (Ito *et al.*, 1994b). Based on these results, we have been vaccinating pigs with frozen oncospheres of *T. saginata, T. solium*, Asian Taenia from Taiwan and Korea in order to get some additions to the taxonomic problem of Asian Taenia (Fan *et al.*, 1997). Pigs vaccinated with frozen oncospheres of Asian Taenia, either from Taiwan or Korea, or *T. saginata* showed very strong resistance to challenge infection with eggs of Asian Taenia from Taiwan, whereas pigs vaccinated with those of *T. solium* showed partial resistance only. It is suggested that Asian Taenia is much closer to *T. saginata* than *T. solium* from the immunobiological view point.

The results mentioned above are concerned with immune responses to cestode infections mainly in laboratory animals, whereas topics described below are mainly serodiagnosis in humans.

IMMUNODIAGNOSIS OF ECHINOCOCCOSIS AND CYSTICERCOSIS

Echinococcosis in humans

Echinococcosis, either alveolar (AE) or cystic (CE), is one of the most serious helminthic zoonoses. AE is caused by the larval stage of *Echinococcus multilocularis*, whereas CE is by *E. granulosus*. Due to the geographical distribution of AE and CE, both AE and CE are very common in some parts of the northern hemisphere (Schantz *et al.*, 1991; Craig *et al.*, 1991, 1992). A complete cure may only be expected by radical surgical resection of the lesions. As the clinical manifestations including morbidity and mortality critically differ between AE and CE, the establishment of improved methods for early detection of AE patients is a critical need, since in many cases, patients are diagnosed after AE has advanced to the point that lesions are nonresectable and the outcome is fatal. In the present paper, I introduce my recent international collaboration work on the establishment of simple means using Em18 (previously undescribed epitope of low molecular weight of 18.5 kDa) for differential serodiagnosis of AE from other parasitic diseases including CE and cysticercosis (Ito *et al.*, 1993a, 1993b, 1995).

Em18 and Em16 Immunoblot analysis: Using serum samples from patients related with AE, CE, double infection of AE + CE, cysticercosis and other parasitic diseases, antibody responses against crude antigens of protoscolex of *E. multilocularis* have been analyzed by immunoblotting. It has been found that Em18, one of the two previously undescribed epitopes, is specifically recognized by AE sera. Serum samples showing antibody response against Em18 are exclusively from AE (Ito *et al.*, 1993a). The predominant IgG subclass recognizing Em18 is IgG4 or IgG1 or IgG4 + IgG1 but never IgG2 (Wen & Craig, 1994; Wen *et al.*, 1995; Ito *et al.*, 1995). Em18 is expected to be highly useful for differentiation of active AE from inactive AE, since there was a good correlation

between the antibody response against Em18 and the presence of active lesions (Ito *et al.*, 1995; Wen *et al.*, 1995; Ma *et al.*, 1997).

Em2^{plus}-ELISA versus PP-Em18/16-ELISA: A new ELISA system using partially purified Em18/Em16 enriched fraction (PP-Em18/16-ELISA) has been evaluated for serodiagnosis of AE (Ito *et al.*, 1997a) compared with Em2^{plus}-ELISA (Gottstein *et al.*, 1993). A total of 194 serum samples were examined: 127 sera from AE (79) and CE (48) in China where both AE and CE are endemic, 21 sera from CE in Australia where CE only exists, 35 sera from other parasitic diseases including cysticercosis, paragonimiasis and sparganosis in Korea where no indigenous AE nor CE exists and 11 normal sera. Antibody levels by PP-Em18/16-ELISA were much higher in AE than in CE and it was also true for Em2^{plus}-ELISA. Some of CE from China showed exceptionally higher levels of antibody in comparison with those of CE from Australia. It is suggested that these strongly positive cases of CE from China may have been exposed to both species of *Echinococcus*. Although most of sera from paragonimiasis showed high antibody levels by Em2^{plus}-ELISA, they were negative by PP-Em18/16-ELISA. Therefore, PP-Em18/16-ELISA is expected to be more reliable for differentiation of AE from CE, cysticercosis and others (Ito *et al.*, 1997a).

Echinococcosis in wild and domestic animals

Major work is concerned with detection of antibody responses in the intermediate host animals. Antibody responses in rodents naturally infected with *E. multilocularis* have been analyzed by immunoblot. Serum samples from the suitable intermediate host, *Clethrionomys rufocanus bedfordiae*, naturally infected with *E. multilocularis* showed similar antibody responses as in AE patients (Ito *et al.*, 1994c), whereas those from *Rattus norvegicus*, naturally infected with this parasite showed almost none (Ito *et al.*, 1996). The latter rodents, which have been conceived to be resistant to this parasite infection, were simultaneously infected with *T. taeniaeformis* but showed no antibody response against *T. taeniaeformis* either. Therefore, we speculate that Norway rats may only be infected with *E. multilocularis* under some immunosuppressed condition or unresponsiveness to these cestodes at least (Okamoto *et al.*, 1992).

It is stressed that Em18 is highly specific to *E. multilocularis*, and antibody response against Em18 is reasonably reliable for differentiation of AE from other helminthic infections by immunoblot and ELISA in humans (Wen & Craig, 1994; Wen *et al.*, 1995; Ito *et al.*, 1997a; Ma *et al.*, 1997) and expected to be useful for detection of domestic animals contaminated with *E. multilocularis* in the endemic area. We also are interested in immunodiagnosis of *E. granulosus* infections. Although almost all components of the crude antigens of *E. multilocularis* protoscolex other than Em18 are cross reactive with sera from CE, these antigens appear to be genus specific and more sensitive for detection of CE than cyst fluid antigens of *E. granulosus* except antigen B (Ito *et al.*, unpublished).

Cysticercosis in humans and pigs

In *Taenia solium* infection, humans (the only definitive host) may suffer from cysticercosis due to the accidental uptake of eggs released from himself (or herself) (autoinfection) or from other people who have adult worms. There is a report on the recent outbreak of neurocysticercosis in New York. Jewish people, who had no custom to eat pork or never been to any endemic countries such as Mexico or other Latin America or Southeast Asia or Africa, had neurocysticercosis in New York due to the environmental contamination with eggs from the immigrants or refugees from such countries (Schantz *et al.*, 1992). Due to the increase in the populations of immigrants, refugees, tourists and the complexity in religions in almost all over the world, we expect that cysticercosis is becoming one of the complex and serious food-borne parasitic zoonoses or the emerging parasitic diseases (Simanjuntak *et al.*, 1997).

Cysticercosis is one of the most serious zoonotic parasitic diseases due to accidental infection with eggs of *T. solium*. There are two major strategies for the detection of this zoonosis similar as for echinococcosis. One is antibody detection in the intermediate host including humans and pigs. It is very important for public health and economy in the live stock. The other is detection of coproantigens in the definitive host, human (Allan *et al.*, 1990, 1992, 1993). As the definitive host so far known is only human, detection and treatment of such worm carriers are most important for the control and prevention of taeniasis/cysticercosis (Craig *et al.*, 1996).

Serological study has been carried out as an international collaboration project with Korean, Mexican, American and Japanese groups. Most recently, using isoelectric focusing, we have found some antigenic components from metacestodes of *T. solium* unique and highly useful for serodiagnosis of cysticercosis in humans and pigs (Ito *et al.*, unpublished). It remains to be determined whether these antigens are the same as those reported by other groups (Cho *et al.*, 1988; Tsang *et al.*, 1989).

Cysticercosis due to inadequate treatment of taeniasis patients (?)

Autoinfection with eggs produced by the first generation adult tapeworms in the definitive host's intestine is common in *H. nana*/mouse or human and *T. solium*/human systems. However, as mentioned above (**1-a**), autoinfection with secondary massive adult burdens of *H. nana* only occurs in some mice, either (1) with immunodeficiency or immunosuppression or (2) with the delay in onset of the late response against the lumenal stage exclusively in very few strains of mice (Ito, 1982). The latter should have some genetic background. In immunocompetent mice, when they are inoculated with eggs, they become completely immune to reinfection with eggs within a few days (early response), whereas when they are inoculated with cysticercoids, derived from either beetles or mice, they become immune to reinfection with cysticercoids within 10 days (before the cysticercoid-derived adult tapeworms mature) (late response) but not at all with eggs until they are sensitized with eggs. Therefore, in any immunocompetent mice, eggs produced by the first generation adult worms (derived from orally inoculated cysticercoids) can autoinfect the host and develop into cysticercoids in the intestinal tissue but never develop into adult worms afterwards. Based on the knowledge from immunobiology of *H. nana/mouse system*, it is expected that cysticercosis may occur in any people who have never been exposed to eggs of *T. solium*.

In many textbooks, there is a description on *T. solium*/human system that we have to take care for treatment of adult worm carriers. If eggs in the worm(s) are released through the inadequate treatment, cysticercosis may be induced in the taeniasis patients due to accidental release of eggs. As we mentioned (Ito & Smyth, 1987), taeniid tapeworms have already matured before gravid segments are found by the worm carrier himself (or herself) or clinician. There is a report that the number of taeniid eggs in gravid segments kept in the host intestine is extremely more than that expelled from the host (Coman & Rickard, 1975). It means that some of eggs have been released from the segments in the worm carrier's intestine. If cysticercosis due to autoinfection by the invasion of oncospheres could occur, it should have occurred before the worm carrier or clinician recognizes the tapeworms. Worm carriers may have been infected with oncospheres derived from continually released eggs until the host becomes immune to reinfection with eggs (early response). Cysticercosis due to autoinfection may be available only when oncospheres could invade the intestinal tissue of the worm carriers themselves until the early response blocks additional oncospheres' invasion but not after treatment unless the worm carriers are immunodeficient or immunosuppressed.

Immunodiagnosis of taeniid cestode infections in the definitive host

Echinococcosis is transmitted to humans from carnivorous animals, mainly from dogs (*E. granulosus*) or foxes (*E. multilocularis*), whereas cysticercosis is from human (worm carrier) to human through eggs released from worm carriers. How to detect infected definitive hosts is the key for the control of these metacestodiases from a public health point of view. As eggs of these species are biohazardous to the person who has to handle them, many groups have been trying to develop safer system to detect either coproantigens (Allan *et al.*, 1990, 1992, 1993; Sakai *et al.*, 1995; Sakashita *et al.*, 1995; Nonaka *et al.*, 1996) or species specific DNA from eggs in faeces by PCR (Mathis *et al.*, 1996). Salford group has established methods for detection of coproantigens of *E. granulosus* in dogs and *T. solium* in humans (Craig *et al.*, 1996).

The most reliable method should be to detect antigens with specificity for species and juvenile stage in order to detect the early stage of infection or those shared by juveniles and adult worms in order to detect some stages of ongoing infection. Most recently, Nonaka *et al.* (1996) the Hokkaido group in Japan has established an ELISA system using monoclonal antibody specific to juveniles of *E. multilocularis* to detect coproantigens of this parasite from just two days of oral inoculation with protoscoleces.

PROBLEMS IN IMMUNOBIOLOGY AND IMMUNODIAGNOSIS OF ECHINOCOCCOSIS AND CYSTICERCOSIS

As these cestodes appear to change their antigenicty through differentiation and development not only in the intermediate but also in the definitive host (Ito & Onitake, 1987; Nonaka *et al.*, 1996), it may be most important how to detect the early stage of infection in echinococcosis and cysticercosis with high specificity and sensitivity. Therefore, it is interesting and important to analyze antibody response against Em18 in laboratory animals experimentally infected with eggs of *E. multilocularis*. It is expected that Em18 may be some component shared with early stage and protoscolex of *E. multilocularis*. Most recently, Sarciron *et al.* (1997) has found that alkaline phosphatase of *E. multilocularis* is another putative candidate marker and recommended to compare with Em18. In contrast, there are no good candidate antigens criti-

cally specific to CE. Almost all candidate antigens from cyst fluid of *E. granulosus* including antigen 5 or antigen B reported so far appear to be basically shared with AE and cysticercosis (Lightowlers & Gottstein, 1995; Craig *et al.*, 1996). Our recent work using crude antigens of protoscoleces of *E. multilocularis* has implied that antigens of protoscoleces are more sensitive for detection of CE cases (Ito *et al.*, unpublished). It is not difficult to prepare antigenic materials for immunodiagnosis of AE or CE, since preparation of protoscoleces of *E. multilocularis* is very easy in rats instead of mice or jirds. It is easy to obtain 30–40 ml packed protoscoleces from a rat (Ito *et al.*, unpublished).

In cysticercosis, there are many reports stressing the importance of glycoproteins (GPs) in cyst fluid of various species in taeniidae (Parkhouse & Harrison, 1987; Kamanga-Sollo *et al.*, 1987; Cho *et al.*, 1988; Tsang *et al.*, 1989). Some GPs, at least, are highly specific to cysticercosis (Tsang *et al.*, 1989). It is most important to evaluate the usefulness of such specific antigens for the early detection of the infection with image analysis. The ability to detect antibody response against the early stage of these cestodes or to detect circulating antigens will be more reliable if we can compare the sensitivity of these immunodiagnostic means with ultrasonographic (US) or CT image analysis etc., since the best way for differential diagnosis based on experience is combination of US to detect the lesions and serodiagnosis (Wen *et al.*, 1995). It is expected that *T. taeniaeformis*/rat as well as *T. solium*/pig system will be highly informative for such a study.

Another problem is how to prepare purified specific antigenic component(s) useful for differential serodiagnosis. In order to overcome this problem, the best way may be application of recombinant DNA technology for production of Em18, since Em18 is a very minor component with high immunogenicity. An alternative idea is to establish some experimental animal models for cysticercosis, either bovis or cellulosae or others. The target parasite with public health and economic importance is *T. solium*. We have just established experimental animal models for *T. solium* and Asian Taenia (Ito *et al.*, 1997b, 1997c) and *T. saginata* (Ito *et al.*, unpublished). *In vitro* hatched oncospheres of these human Taenia develop into fully mature metacestodes under the skin or the peritoneal cavity of female mice with severe combined immunodeficiency (scid). This scid mouse model can be highly useful for preparation of a standardized larval stage to produce antigens for serodiagnosis. We may compare cyst fluid antigens of various taeniid cestodes including *E. granulosus* as well as *E. multilocularis* not recovered from different host species but recovered from genetically homogeneous mice, since eggs of *E. granulosus* may infect mice either orally (Williams & Colli, 1970; Colli & Schantz, 1975) or by subcutaneous inoculation with eggs (Dempster *et al.*, 1991). Further evaluation of candidate antigens for serodiagnosis in echinococcosis or cysticercosis should be done by immunoblot or immunoprecipitation in order to establish ELISA or other simpler methods using purified protein antigen(s) with better resolution or by monoclonal antibody against specific GPs as shown by Nonaka *et al.* (1996). Purification of putative antigens, which is highly reliable for differential serodiagnosis of echinococcosis, cysticercosis and taeniasis at the early stage, is the key point to obtaining better resolution in the future.

Echinococcosis and cysticercosis are included in the list of "Emerging and other communicable diseases: Strategic plan 1996–2000 (WHO/EMC/96.1)". As these metacestodiases are spreading world wide, we should launch international collaboration projects for epidemiological survey in both intermediate and definitive hosts and laboratory work to evaluate and standardize the immunodiagnosis and DNA analysis for differentiation of strains of Echinococcus and species of Taenia, either human or domestic animal in origin (Schantz *et al.*, 1991, 1992; Craig *et al.*, 1992; Gottstein, 1992; Bowles & McManus, 1993; Allan *et al.*, 1993; McManus & Bowles, 1994; Wen *et al.*, 1995). Image analysis, immunodiagnosis and DNA analysis will all become more important for epidemiology of echinococcosis and cysticercosis. The WHO informal echinococcosis working group may have important contributions for such international collaboration projects.

Acknowledgements—The work mentioned above was supported in part by the Nissan Science Foundation, the Uehara Memorial Foundation, the Ohyama Health Foundation, the Mochida Memorial Foundation, the Ichiro Kanehara Foundation, and a grant-in-aid for international collaboration project on echinococcosis and cysticercosis (63480168, 06044089, 07044243, 09044279) to A. Ito.

REFERENCES

Allan J. C., Avila G., Garcia N., Flisser A. & Craig P. S. 1990. Immunodiagnosis of taeniasis by coproantigen detection. *Parasitology* **101:** 473–477.

Allan J. C., Craig P. S., Noval J. G., Mencos F., Liu D., Wang Y., Wen H., Zhou P., Stringer R., Rogan M. & Zeyhle E. 1992. Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. *Parasitology* **104:** 347–355.

Allan J. C., Mencos F., Garcia-Noval J., Sarti E., Flisser A., Wang Y., Liu D. & Craig P. S. 1993. Dipstick dot ELISA for the detection of *Taenia* coproantigens in humans. *Parasitology* **107:** 79–85.

Asano K., Shinoda M., Nakamura F. & Okamoto K. 1986.

Hymenolepis nana: passive transfer of mouse immunity by T-cell subset of phenotype Lyt-1. *Experimental Parasitology* **61:** 373–378.

Asano K., Muramatsu K. & Okamoto K. 1991. *Hymenolepis nana*: adoptive transfer of protective immunity and delayed type hypersensitivity response with mesenteric lymph node cells in mice. *Experimental Parasitology* **72:** 24–32.

Befus A. D. 1975. Secondary infections of *Hymenolepis diminuta* in mice: effects of varying worm burdens in primary and secondary infections. *Parasitology* **61:** 61–75.

Bowles J. & McManus D. P. 1993. Molecular variation in Echinococcus. *Acta Tropica* **53:** 291–305.

Cho S. Y., Kim S. I., Kang S. Y. & Kong Y. 1988. Biochemical properties of a purified protein in cystic fluid of *Taenia solium* metacestodes. *Korean Journal of Parasitology* **26:** 87–94.

Colli C. & Schantz P. M. 1975. The effect of egg dose, and host age and sex on susceptibility of CF1 albino mice and jirds (*Meriones unguiculatrus*) to primary infection by *Echinococcus granulosus*. *Revista do Insttituto de Medicina Tropical de Sao Paulo* **17:** 59–68.

Coman B. J. & Rickard M. D. 1975. The location of *Taenia pisiformis, Taenia ovis* and *Taenia hydatigena* in the gut of the dog and its effect on net environmental contamination with ova. *Zeitschrift fur Parasitenkunde* **47:** 237–248.

Craig P. S., Deshan L. & Zhaoxun D. 1991. Hydatid disease in China. *Parasitology Today* **7:** 46–50.

Craig P. S., Rogan M. T. & Allan J. C. 1996. Detection, screening and community epidemiology of taeniid cestode zoonoses: cystic echinococcosis, alveolar echinococcosis and neurocysticercosis. *Advances in Parasitology* **38:** 169–250.

Craig P. S., Deshan L., Macpherson C. N. L., Dazhong S., Reynolds D., Barnish G., Gottstein B. & Zhirong W. 1992. A large focus of human alveolar echinococcosis in central China. *Lancet* **340:** 826–831.

Dempster R. P., Berridge M. V., Harrison G. B. L. & Heath D. D. 1991. *Echinococcus granulosus*: development of an intermediate host mouse model for use in vaccination studies. *International Journal for Parasitology* **21:** 549–554.

Fan P. C., Chung W. C., Eom K. S. & Ito A. 1997. Vaccination trials against Taiwan Taenia eggs in pigs injected with frozen oncospheres of Taiwan Taenia, Korean Taenia, *T. saginata* or *T. solium*. *Parasitology* **114:** 541–544.

Gottstein B. 1992. Molecular and immunological diagnosis of echinococcosis. *Clinical Microbiology Reviews* **5:** 248–261.

Gottstein B., Jacquier P., Bresson-hadni S. & Eckert J. 1993. Improved primary immunodiagnosis of alveolar echinococcosis in humans by an enzyme-linked immunosorbent assay using $Em2^{Plus}$ antigen. *Journal of Clinical Microbiology* **31:** 373–376.

Hopkins C. A. 1980. Immunity and *Hymenolepis diminuta*. In: *Biology of the Tapeworm* Hymenolepis diminuta (Edited by Arai H. P.), pp. 551–614. Academic Press, New York.

Hopkins C. A., Subramanian G. & Stallard H. 1972. The development of *Hymenolepis diminuta* in primary and secondary infections in mice. *Parasitology* **64:** 401–412.

Ito A. 1975. *In vitro* oncospheral agglutination given by immune sera from mice infected, and rabbits injected, with eggs of *Hymenolepis nana*. *Parasitology* **71:** 465–473.

Ito A. 1977. The mode of passive protection against *Hymenolepis nana* induced by serum transfer. *International Journal for Parasitology* **7:** 67–71.

Ito A. 1982. *Hymenolepis nana*: Immunogenicity of a lumen phase of the direct cycle and failure of autoinfection in BALB/c mice. *Experimental Parasitology* **54:** 113–120.

Ito A. 1983. *Hymenolepis nana*: Maturation in an immunosuppressed unnatural rat host. *Experimental Parasitology* **56:** 318–326.

Ito A. 1992. Cysticercosis in Asian–Pacific Regions. *Parasitology Today* **8:** 181–182.

Ito A. & Hashimoto A. 1993. Vaccination with hatched but non-activated, non-viable oncospheres of *Taenia taeniaeformis* in rats. *Journal of Helminthology* **67:** 165–168.

Ito A. & Kamiyama T. 1984. *Hymenolepis nana*: Worm recovery from congenitally athymic nude and phenotypically normal rats and mice. *Experimental Parasitology* **58:** 132–137.

Ito A. & Onitake K. 1986. Complete resistance to challenges with *Hymenolepis nana* cysticercoids derived from mouse, rat and beetle in mice. *International Journal for Parasitology* **16:** 623–628.

Ito A. & Onitake K. 1987. Changes in surface antigens of *Hymenolepis nana* during differentiation and maturation in mice. *Journal of Helminthology* **61:** 129–136.

Ito A. & Smyth J. D. 1987. Adult cestodes. In: *Immune Responses to Parasite Infections: Immunology, Immunopathology, and Immunoprophylaxis* (Edited by Soulsby E.J.L.), Vol. 2, pp. 115–163, CRC Press, Boca Raton.

Ito A. & Yamamoto M. 1976. The mode of active protection against *Hymenolepis nana* reinfection in mice inoculated with different doses of shell-free eggs. *Japanese Journal of Parasitology* **25:** 247–253.

Ito A., Asano K. & Okamoto K. 1994. Protective antibodies against *Taenia taeniaeformis* in rats infected with eggs or injected with non-viable oncospheres or recombinant antigens of oncosphere. *Parasite Immunology* **16:** 463–468.

Ito A., Onitake K. & Andreassen J. 1988. Lumen phase specific cross immunity between *Hymenolepis microstoma* and *H. nana* in mice. *International Journal for Parasitology* **18:** 1019–1027.

Ito A., Schantz P. M. & Wilson J. F. 1995. Em18, a new serodiagnostic marker for differentiation of active and inactive cases of alveolar hydatid disease. *American Journal of Tropical Medicine and Hygiene* **52:** 41–44.

Ito A., Wang X. G. & Liu Y. H. 1993. Differential serodiagnosis of alveolar and cystic hydatid disease in the People's Republic of China. *American Journal of Tropical Medicine and Hygiene* **49:** 208–213.

Ito A., Honey R. D., Scanlon T., Lightowlers M. W. & Rickard M. D. 1988. Analysis of antibody responses to *Hymenolepis nana* infection in mice by the enzyme-linked immunosorbent assay and immunoprecipitation. *Parasite Immunology* **10:** 265–277.

Ito A., Lightowlers M. W., Rickard M. D. & Mitchell G. F. 1988. Failure of auto-infection with *Hymenolepis nana* in seven inbred strains of mice initially given beetle-derived cysticercoids. *International Journal for Parasitology* **18:** 321–324.

Ito A., Itoh M., Andreassen J. & Onitake K. 1989. Stage-specific antigens of *Hymenolepis microstoma* recognized in BALB/c mice. *Parasite Immunology* **11:** 453–462.

Ito A., Bøgh H. O., Lightowlers M. W., Mitchell G. F., Takami T., Kamiya M., Onitake K. & Rickard M. D. 1991. Vaccination against *Taenia taeniaeformis* infection in rats using a recombinant protein and preliminary analysis of the induced antibody response. *Molecular and Biochemical Parasitology* **44:** 43–52.

Ito A., Nakao M., Kutsumi H., Lightowlers M. W., Itoh M.

& Sato S. 1993. Serodiagnosis of alveolar hydatid disease by Western blotting. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87:** 170–172.

Ito A., Fan P. C., Chung W. C. & Suzuki M. 1994. Cross protection against *Taenia taeniaeformis* in rats vaccinated with non-viable oncospheres of Asian *Taenia* or *T. saginata*. *Journal of Helminthology* **68:** 83–85.

Ito A., Nakao M., Ito M., Matsuzaki T., Kamiya M. & Kutsumi H. 1994. Antibody responses in the wild vole, *Clethrionomys rufocanus bedfordiae*, naturally infected with *Echinococcus multilocularis* by Western blotting. *Journal of Helminthology* **68:** 267–269.

Ito A., Okamoto M., Kariwa H., Ishiguro T., Hashimoto A. & Nakao M. 1996. Antibody responses against *Echinococcus multilocularis* antigens in naturally infected *Rattus norvegicus*. *Journal of Helminthology* **70:** 355–357.

Ito A., Ma L., Itoh M., Cho S. Y., Kong Y., Kang S. Y., Horii T., Pang X. L., Okamoto M., Yamashita T., Lightowlers M. W., Wang X. G. & Liu Y. H. 1997. Immunodiagnosis of alveolar echinococcosis by ELISA using partially purified Em18/16 enriched fraction. *Clinical and Diagnostic Laboratory Immunology* **4:** 57–59.

Ito A., Chung W. C., Chen C. C., Ito M., Endo S., Okamoto M. & Fan P. C. 1997. Human *Taenia* eggs develop into cysticerci in *scid* mice. *Parasitology* **114:** 85–88.

Ito A., Ito M., Eom K. S., Chung W. C., Chen C. C., Ma L., Endo S. & Fan P. C. 1997c. *In vitro* hatched oncospheres of Asian Taenia from Korea and Taiwan develop into cysticerci in the peritoneal cavity of female *scid* (severe combined immunodeficiency) mice. *International Journal for Parasitology* **27:** 631–633.

Johnson K. S., Harrison G. B. L., Lightowlers M. W., O'Hoy K. L., Cougle W. G., Dempster R. P., Lawrence S. B., Vinton J. G., Health D. D. & Rickard M. D. 1989. High level protection against a helminth parasite induced by a defined recombinant antigen. *Nature* **338:** 585–587.

Kamanga-Sollo E. I., Rhoads M. L. & Murrell K. D. 1987. Evaluation of an antigenic fraction of *Taenia hydatigena* metacestode cyst fluid for immunodiagnosis of bovine cysticercosis. *American Journal of Veterinary Research* **48:** 1206–1210.

Lightowlers M. W. & Gottstein B. 1995. Echinococcosis/Hydatidosis: antigens, immunological and molecular diagnosis. In: *Echinococcus and Hydatid Disese* (Edited by Thompson R. C. A. & Lymbery A. J.), pp. 333–410, CAB International, Wallingford.

Lightowlers M. W., Mitchell G. F. & Rickard M. D. 1993. Cestodes. In: *Immunology and Molecular Biology of Parasitic Infections* (Edited by Warren, K. S.), pp. 438–472. Blackwell Scientific Publications, Oxford.

Ma L., Ito A., Liu Y. H., Wang X. G., Yao Y. Q., Yu D. G. & Chen Y. T. 1997. $Em2^{plus}$-ELISA and Em18-Western blots for the follow-up of alveolar echinococcosis treated with albendazole. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91:** 476–478.

Mathis A., Deplazes P. & Eckert J. 1996. An improved test system for PCR-based specific detection of *Echinococcus multilocularis* eggs. *Journal of Helminthology* **70:** 219–222.

McManus D. P. & Bowles J. 1994. Asian (Taiwan) Taenia: Species or strain?. *Parasitology Today* **10:** 273–275.

Moss G. D. 1972. The effect of cortisone acetate treatment on the growth of *Hymenolepis microstoma* in mice. *Parasitology* **64:** 285–294.

Nonaka N., Iida M., Yagi K., Ito T., Ooi H. K., Oku Y. & Kamiya M. 1996. Time course of coproantigen excretion in *Echinococcus multilocularis* infections in foxes and an alternative definitive host, golden hamsters. *International Journal for Parasitology* **26:** 1271–1278.

Okamoto K. 1968. Effect of neonatal thymectomy on acquired resistance to *Hymenolepis nana* in mice. *Japanese Journal of Parasitology* **17:** 53–59.

Okamoto K. & Koizumi M. 1972. *Hymenolepis nana*: Effect of antithymocyte serum on acquired immunity in mice. *Experimental Parasitology* **32:** 56–61.

Okamoto M., Fujita O., Arikawa J., Kurosawa T., Oku Y. & Kamiya M. 1992. Natural *Echinococcus multilocularis* infection in a norway rat, *Rattus norvegicus*, in southern Hokkaido, Japan. *International Journal for Parasitology* **22:** 681–684.

Onitake K., Sasaki J., Andreassen J. & Ito A. 1990. *Hymenolepis microstoma*: oncospheres invade the intestinal tissue of the definitive host. *Journal of Helminthology* **64:** 168–170.

Parkhouse R. M. E. & Harrison L. J. S. 1987. Cyst fluid and surface associated glycoprotein antigens of *Taenia* sp. metacestodes. *Parasite Immunology* **9:** 263–268.

Rickard M. D. 1983. Immunity. In: *Biology of the Eucestoda* (Edited by Arme C. & Pappas P. W.), Vol. 2, pp. 539–579. Academic Press, London.

Sakai H., Malgor R., Basmadjian I., Gallardo R., Carmona C., Sato H., Oku Y. & Kamiya M. 1995. An enzyme-linked immunosorbent assay (ELISA) for the detection of *Echinococcus granulosus* coproantigens in dogs. *Japanese Journal of Parasitology* **44:** 453–461.

Sakashita M., Sakai H., Kohno H., Ooi H. K., Oku Y., Yagi K., Ito M. & Kamiya M. 1995. Detection *of Echinococcus multilocularis* coproantigens in experimentally infected dogs using murine monoclonal antibody against adult worms. *Japanese Journal of Parasitology* **44:** 413–420.

Sarciron E. M., Bresson-Hadni S., Mercier M., Lawton P., Duranton C., Lenys D., Petavy A. & Vuitton D. A. 1997. Antibodies against *Echinococcus multilocularis* alkaline phosphatase as makers for the specific diagnosis and the serological monitoring of alveolar echinococcosis. *Parasite Immunology* **19:** 61–68.

Schantz P. M., Gottstein B., Ammann R. & Lanier A. 1991. Hydatid and the Arctic. *Parasitology Today* **7:** 35–37.

Schantz P. M., Moore A. C., Munoz J. L., Hartman B. J., Schaefer J. A., Aron A. M., Persaud D., Sarti E., Wilson M. & Flisser A. 1992. Neurocysticercosis in an orthodox Jewish community in New York City. *New England Journal of Medicine* **327:** 692–695.

Simanjuntak G. M., Margono S. S., Okamoto M. & Ito A. 1997. Taeniasis/cysticercosis in Indonesia as an emerging disease. *Parasitology Today* **13:** 321–323.

Smyth J. D. 1969. *The Physiology of Cestodes*. pp. 279. Freeman and Company, San Francisco.

Tsang V. C. W., Brand J. A. & Boyer A. E. 1989. An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigen for diagnosing human cysticercosis (*Taenia solium*). *Journal of Infectious Diseases* **159:** 50–59.

Vorst V., Dhont H., Cesbron J. Y., Capron M., Dessaint J. P. & Capron A. 1988. Influence of an *Hymenolepis diminuta* infection on IgE and IgA bound to mouse intestinal eosinophils. *International Archives of Allergy and Applied Immunology* **87:** 281–285.

Watanabe N., Nawa Y., Okamoto K. & Kobayashi A. 1994. Expulsion of *Hymenolepis nana* from mice with congenital deficiencies of IgE production of mast cell development. *Parasite Immunology* **16:** 137–144.

Wen H. & Craig P. S. 1994. IgG subclass responses in human

cystic and alveolar echinococcosis. *American Journal of Tropical Medicine and Hygiene* **50:** 741–748.

Wen H., Craig P. S., Ito A., Vuitton D. A., Bressan-Hadni S., Allan J. C., Rogan M. T., Paollilo E. & Shambesh M. 1995. Immunoblot evaluation of IgG and IgG-subclass antibody responses for immunodiagnosis of human alveolar echinococcosis. *Annals of Tropical Medicine and Parasitology* **89:** 485–495.

Williams J. F. 1982. Cestode infections. In: *Immunology of Parasitic Infections* (Edited by Cohen S. & Warren K. S.), pp. 676–714. Blackwell Scientific Publication, Oxford.

Williams J. F. & Colli C. W. 1970. Primary cystic infection with *Echinococcus granulosus* and *Taenia hydatigena* in *Meriones unguiculatus*. *Journal of Parasitology* **56:** 509–513.