Jpn. J. Vet. Res., **40**, 113−130 (1992)

IMMUNE RESPONSE TO *ECHINOCOCCUS MULTILOCULARIS* INFECTION IN THE MOUSE MODEL: A REVIEW

Matthew C. PLAYFORD and Masao KAMIYA

(Accepted for publication: July 20, 1992)

ABSTRACT

Echinococcus multilocularis is a cestode helminth which, along with *E. granulosus*, *E. oligarthus* and *E. vogeli* is a causative agent of hydatid disease in man. In the intermediate host (including man), cysts formed by the metacestode (larval stage) develop in the internal organs, causing functional impairment which often leads to the death of the host. In this review larval *E. multilocularis* infection in mice, the most popular experimental intermediate host, is examined, and the immune response to the organism is described in detail. Evidence is presented which suggests that cell-mediated immunity (CMI) plays a large role in suppression of larval growth. Congenitally athymic nude mice, and mice treated to remove thymocytes had high susceptibility to infection, while mice strains assessed as having high helper T lymphocyte function showed resistance to infection. The degree of antibody response shown by the host does not correlate with the susceptibility to *E. multilocularis*. Infection with *E. multilocularis* is accompanied by immunosuppression, manifested by inhibition of effector cell chemotaxis and receptor expression, suppressor macrophage and lymphocyte activity, decline in helper T-lymphocyte activity and immune-complex deposition.

Key words: *Echinococcus multilocularis*, mouse, immune response, lymphocyte, cell-mediated immunity, immunosuppression, macrophage.

INTRODUCTION

Echinococcus multilocularis, *E. granulosus*, *E. oligarthus* and *E. vogeli* are cestode helminths of the Family Taeniidae[76]. These organisms are maintained naturally in predator/prey life cycles, with dogs, foxes, wolves and other canids occupying the role of definitive host. Intermediate hosts of *E. granulosus* in nature include a wide range of wild and domestic herbivores, while *E. multilocularis* has been isolated from various species of wild rodents, insectivores and lagomorphs[66], as well as pigs[71] and

Dept. of Veterinary Parasitology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, JAPAN

horses[54]. All *Echinococcus* species will infect humans, causing a chronic debilitating disease that is frequently fatal. *E. multilocularis* is widely distributed throughout the Northern Hemisphere, particularly in the holarctic and Alpine regions. Recent reviews on the immunology of *Echinococcus* infections have been provided by Heath[24], Lightowlers[48] and Gottstein[21]. This paper will examine immune responses to experimental *E. multilocularis* infection in the laboratory mouse, in particular focussing on secondary echinococcosis.

The mouse is a popular experimental host for *Echinococcus* research [9,46,52,85,86]. This stems from the availability and low cost of genetically consistent lines of inbred mice, the relatively fast growth of *Echinococcus* cysts in mice, the opportunity to use immunologically deficient animals such as the Natural Killer (NK) cell deficient beige mouse[59], and athymic nude mouse[36], and the ready availability of reagents to use with the mouse[42]. The normal development of the *Echinococcus* larval cyst mass (LCM) in rodent hosts is accompanied by a host immune reaction that has been described using macroscopic[1,51], histopathological[57], electron microscopic[32,39], serological[1,26,49,60], immunocytochemical[63] and cellular immunological observations[12,18,65]. The resultant picture shows that although there is a rapid antibody response to *Echinococcus* infection, and antibodies[41] and complement[33] are capable of killing larval protoscoleces *in vitro*, they alone are not effective in controlling the growth of hydatid cysts[49,61]. Cell-mediated immunity (CMI) on the other hand, involving granulocytes[7,18,78], macrophages[12] and lymphocytes[2,11,13], has been shown *in vitro* and *in vivo* to have a modifying effect on the viability of larval tissue and/or growth of the LCM.

Immunosuppression during the course of *Echinococcus* infection has been investigated by several researchers[2,9,69], some of whom have drawn a relationship between this and the rapid growth of the LCM in the period approximately six weeks after infection[7]. The mechanism for immunosuppression has been variously linked to increased suppressor T lymphocytes in the spleen[42], suppressor macrophage activity[63], decreased helper T cell function[2,13], saturation of effector cell receptors by immune complexes[78], and inhibition of neutrophil and macrophage chemotaxis in infected animals[7].

FEATURES OF *E. MULTILOCULARIS* LARVAL DEVELOPMENT IN THE MOUSE

Secondary Echinococcosis has become a standard method of propagating *E. multilocularis* in laboratory animals worldwide[11,44,77,87]. Briefly, parasite tissue is excised from the LCM of an infected animal and washed in saline with or without antibiotics. A suspension containing larval germinal epithelium and acephalic vesicular cysts along with protoscoleces (the mature larval stage) is injected into the peritoneal cavity of the recipient. Gottstein et al.[23] have recently proved that it is the vesicular cysts and not the protoscoleces which develop into a new LCM in the recipient animal.

Other workers have transplanted solid portions of the LCM into the recipient animal[1,20,53]. Propagation of *E. granulosus* is said to require the transfer of protoscoleces[75]. A certain portion of the injected dose of larval tissue survives in the recipient's peritoneal cavity, forming a new LCM. This method has also been used to test the viability of larval tissue excised from lesions in human patients[67] and from pigs[37], and for testing the effect of anthelminthics[72] and immunosuppressive drugs[45]. In some strains of mice, growth of the LCM within the peritoneal cavity or in the subcutaneous space has been described as having a restrictive stage (first 6 to 7 weeks) and a progressive stage (7 to 14 weeks)[3,18,45]. This is due to the LCM displaying slow growth in the first 6 to 7 weeks, and rapid growth thereafter.

Yamashita et al.[84] found that the growth of *E. multilocularis* varied between and even within strains of laboratory mice. Certain mice were designated as suitable hosts (Type 1) with rapid larval development and low host tissue reaction, while others (Type 2) had slow larval development and extensive host tissue reactions. In one strain of mice (KK), the males were type 1 and the females type 2, showing marked resistance to multilocular echinococcosis in the females only. Experimental echinococcosis in species other than mice has been attempted with varying results. Ohbayashi et al.[58] examined naturally occurring and experimental echinococcosis in 25 species of rodents and one species of rabbit, while Yagi, 1991 (pers comm.) used the vole to study development of the parasite in its natural intermediate host. Ohbayashi[57] found that brood capsule (the intial larval stage) formation in the liver after oral inoculation with eggs varied according to the experimental host. Cotton rats were the most permissive host, with brood capsules forming after only 20 days. DBA, CF#1 and C57BL/6 mice took 49, 90 and 150 days respectively for brood capsules, and 60–90, 150 and 210 days respectively for protoscoleces to establish. The longer time taken until brood capsule formation was associated with a greater host tissue reaction. Kamiya et al.[34] found brood capsule and protoscolex formation in the nude mouse to occur in 2 and 4 weeks P.I. respectively.

Other methods of studying experimental echinococcosis in laboratory rodents include oral inoculation of eggs from adult cestodes[84], and subcutaneous[3,40,86], intracerebral[75] and intrahepatic[84] secondary infection. The method of oral inoculation with eggs has advantages in that primary lesions mimic those of a natural infection[44] and are usually located in the liver[57]. However, the technique is hazardous in that eggs from the adult worm are infective to humans[83]. Moreover, until the recent development of techniques to grow adult Echinococcus to maturity in laboratory rodents[38], it necessitated keeping the definitive host (usually a dog) and harvesting its feces for eggs.

Yamashita & Ohbayashi[84] found susceptibility of mice to intrahepatic injection to be low, resulting in a total of only 18 cases positive out of 50 inoculated, and concluded that "the susceptibility of oral, peritoneal and intrahepatic cases tends to

decline in order." Besides this, the rate of scolex formation in intrahepatic inoculation was very low. Larval tissue inoculated into the peritoneal cavity frequently spreads to the liver[82], abdominal organs, diaphragm and occasionally to the pleural cavity. Metastasis occurs rarely in the laboratory mouse, but has been described in the vole and cotton rat[58], and Mongolian gerbil[20].

The Mongolian gerbil (*Meriones unguiculatus*) has been used as an experimental host by many researchers[20,43,72]. Delabre et al.[16] compared the growth of *E. multilocularis* larval tissue in the OF1 mouse with that in gerbils, using electron microscopic studies. They reported that the tegumental cytoplasmic syncytium (the outer portion of the germinal layer) in the OF1 mouse was thinner and degenerated compared to that in the gerbil, and that while the brood capsules of the gerbil cysts were fertile, producing many protoscoleces, those in the OF1 mouse produced none.

HOST FACTORS INVOLVED IN THE RESPONSE TO *E. MULTILOCULARIS* INFECTION IN MICE

1. Age

Age-resistance to *E. granulosus* in white mice was demonstrated by Schwabe et al.[74]. Mice 15–48 days of age were highly susceptible to protoscoleces injected intraperitoneally, while mice 71 days of age or older suppressed the growth of larval cysts. Kamiya[31], showed that AKR mice 29 days of age or younger were highly susceptible to oral inoculation with *E. multilocularis* eggs, while those 48 days old were relatively resistant. Older mice (83–148 days) had intermediate susceptibility.

2. Sex

Sex differences to primary multilocular hydatid infection have been noted in KK and NC strains of mice[82]. Females of KK strain showed a "remarkable" degree of resistance to *E. multilocularis* compared to males, with both infection rate and extent of lesions being less than in identically inoculated males[88]. No sex difference in susceptibility was noted in orally-infected AKR strain mice[31]. However, castration of male AKR mice before oral inoculation with *E. multilocularis* eggs caused a delay in the maturation of protoscoleces compared to sham-castrated mice[35]. Sex-related differences were also noted in nude mice[34].

3. Antibodies

Orihara[60] demonstrated an almost linear relationship between extent of lesions in CF#1 mice orally infected with *E. multilocularis* eggs, and serum titers of antibodies detected by complement fixation test. Mice inoculated subcutaneously with a vaccine containing *E. multilocularis* antigen showed a mild antibody response[22]. Ali-Khan[1] showed that the antibody response was detected earlier when larger inoculating doses of larval material (intra-peritoneal-I.P.) were used in three strains of mice. However, the antibody titer bore no relation to the sensitivity of the mouse strain, with C57BL and DBA mice harboring a parasite mass 9 and 4 times less than the C57L mice, but having approximately similar antibody titres. Liu et al.[49] found that both total mass,

and degree of development of *E. granulosus* cysts in mice affected the antibody titer. However, they detected "no apparent inhibition of the growth of secondary hydatid cysts in mice with high levels of antibodies".

Serum from infected hosts which had been heat inactivated to destroy complement was found to be cytotoxic to *E. multilocularis* protoscoleces *in vitro*, and serum drawn from infected animals and injected into *E. multilocularis* and *E. granulosus* cysts killed the cysts *in vivo*[41]. The authors of this study proposed that circulating anti-*Echinococcus* antibodies can kill the cyst if they contact the inner surface of the germinal membrane in sufficient quantities, but that the cyst membrane may be impermeable to antibodies so that this does not occur naturally in the infected host. Rau and Tanner[65] further surmised that specific antibodies may promote adherence of effector cells *in vivo*. Evidence of a protective role comes from Dottorini, et al.[19], who showed that antibodies to the 0.8M scolex fraction of the immunizing antigen were found to be increased in resistant (without cysts after challenge infection) compared to susceptible (with cysts after challenge infection) BALB/c mice immunized with a variety of fractions from *E. granulosus* scoleces. Further, Dempster et al.[17] obtained greater than 90% protection from intraperitoneal challenge with oncospheres by previously immunizing BALB/cJ mice with sonically disrupted oncospheres subcutaneously. Passive transfer of this protection to naive animals by serum injection demonstrated that it was due to antibody.

Ali-Khan & Siboo[4] observed a replacement of lymphocytes by plasma cells in the draining lymph nodes of subcutaneous larval cysts, and commented that this led to disorganization of the node and immune deviation. Bresson-Hadni et al.[13] discounted the effect of B cells on the progress of *E. multilocularis* infection, stating that "B lymphocytes were rarely observed in the granuloma" surrounding the parasite. However, specific host antibodies IgG1, IgG2a, IgG2b and IgM as well as complement component C3 but not IgA or C-reactive protein were detected on intact cyst membranes from *E. multilocularis*-infected C57BL/6J mice 12 weeks P.I. Antibodies were bound to epitopes on the laminated layer but were not detected on the germinal layer[5].

Antibodies and circulating immune complexes (IC) have been implicated in the immune suppression accompanying *E. multilocularis* infection. This supposition is based on evidence that in the latter stages of infection the levels of serum IC parallels the increase in weight of the LCM[6], leucocytes in the LCM had reduced numbers of Fc and C3d receptors, and that 31 to 72% of immigrant macrophages in the LCM contained immune complexes[79]. Moreover, non-specific binding of Fc fractions of the immunoglobulin of various species to the hydatid cyst was observed by fluorescent labelling[8]. Rickard[68] proposed the theory of 'blocking antibodies' to explain the long-term survival of *Taenia pisformis* cysticerci in rabbits, and it is possible that this phenomenon also acts in *E. multilocularis* infection.

4. Complement

In vitro studies have shown that evaginated or invaginated protoscoleces of *E. multilocularis* will undergo lysis within an hour after contact with fresh normal human, cotton rat, sheep, guinea pig, B. 10. D2/n mouse serum or infected cotton rat serum[40]. This protoscolicidal effect could be readily inhibited by heat inactivation at 56°C, by 0.01M EDTA, or by incubation with Cobra Venom Factor at 37°C. All of these treatments destroy complement. Electron microscopic studies of lesions in *E. multilocularis* protoscoleces after such treatment revealed 'tegumental bubbles'[39], crater-shaped pits on the tegumental surface, and loss of microtriche structure[32]. Kamiya et al.[33] showed that the lytic action of complement on protoscoleces *in vitro* is effected through the alternative pathway, and not via the classical pathway. Binding of C3 to protoscoleces was demonstrated by indirect fluorescent antibody assay.

Complement component C3 was detected by immunofluorescent techniques on the outer surface of the germinal layer, but not on the laminated layer of cysts from 12 week-old *E. multilocularis* cysts in C57 BL/6J mice[5].

5. Granulocytes

Injection of *E. multilocularis* protoscoleces into the peritoneal cavity is followed by a rapid infiltration of granulocytic cells. Between 1 and 3 weeks P.I. the total number of peritoneal cells (PEC) ranged from 15.5×10^6 to 50.1×10^6 in C57BL/6J mice[18]. Eosinophils began to increase steadily from day 3 P.I., and reached their peak numbers (38×10^6) at three weeks P.I. In another experiment using C57BL/6J mice, it was reported that "eosinophils constituted a minor fraction of PEC and intra-LCM leucocytes throughout the course of infection. Eosinophil counts in the PEC of hydatid-mice remained significantly higher than those in the LCMs throughout the course of infection"[78].

Neutrophil responses in these experiments showed a markedly different pattern. It was shown that neutrophil numbers in the peritoneal cavity remained comparatively low until the progressive growth phase of the LCM[18]. Numbers peaked at 12.8×10^6 cells at 10 weeks P.I., declining to 4×10^6 by 14 weeks P.I. Treves and Ali-Khan[78] showed a slightly different pattern, with neutrophil numbers peaking at 12×10^6 cells or 65% of total WBCs at about 7 weeks P.I. Kroeze & Tanner[43] demonstrated similar lymphocytic and granulocytic responses to larval *E. multilocularis* infection in Mongolian gerbils.

The actual effect of eosinophils and neutrophils on the LCM has not been demonstrated, but Alkarmi and Behbehani[7] suggest that it is the inability of neutrophils and macrophages to respond to chemotaxis in the progressive phase of LCM growth that allows larval cyst development to proceed unimpeded. Ali-Khan & Siboo[3] observed a high percentage of degenerating neutrophil-bound cysts in C57L/J mice infected subcutaneously with *E. multilocularis* cysts. In an experiment which successfully maintained partially matured *E. multilocularis* protoscoleces in the lungs of

prednisolone-treated golden hamsters, Sato and Kamiya[73] state that 'lack of neutrophil influx against the cestode..., may be important for parasite survival and atypical development in the pulmonary alveolar environment'. Sakamoto[70] reported detecting eosinophil granule components in hydatid fluid isolated from cases of bovine *E. granulosus* infection. He conjectured that eosinophils infiltrating the adventitial tissue play an important role in the formation of regressive hydatid cysts in cattle.

6. Macrophages

Macrophages have been shown to participate in large numbers in the intial cellular immune reaction to protoscoleces intraperitoneally. Devouge and Ali-Khan[18] report that in C57BL/6J mice, peritoneal monocyte/macrophage numbers reached 38×10^6 cells at 3 weeks post-infection. Peritoneal macrophages from infected mice, when assessed by morphologic criteria, appeared activated, having larger size, more ruffled surfaces and containing more cytoplasmic vacuoles than those from control mice[78].

Peritoneal macrophages obtained from *E. multilocularis*-infected mice or from mice injected with BCG were capable of killing up to 85% of fresh protoscoleces when co-cultured with them *in vitro*[12]. Protoscolicidal activity was enhanced when protoscoleces were pre-incubated in immune serum. The authors of this study commented that the possible role of the antibody is to identify the protoscolex target to the effector cell.

Alveolar hydatid cyst antigens were surmised to have potent neutrophil and macrophage chemotactic factors that are overwhelmed in the later stage of the infection by various processes as yet unidentified[7]. Interestingly, Bresson-Hadni et al.[13] observed less macrophages in the LCM of 'resistant' mice than 'sensitive' mice. They said "this could be due to qualitative differences in the functional activity of macrophages in sensitive and resistant strains, or to a deleterious effect of cytotoxic macrophages on normal liver cells followed by an enhanced capacity of parasitic cells to progress in the liver parenchyma". Liew[47] describes a similar phenomenon in experimental *Leishmania* infection in mice.

Rakha et al.[63] demonstrated that macrophages can also play a suppressor role in *Echinococcus* infection. Lymphocyte transformation induced by *E. multilocularis*-derived mitogens, or by Con A *in vitro* was suppressed when macrophages from *E. multilocularis*-infected mice were added to the culture.

7. Natural Killer Cells

The role of Natural Killer (NK) cells in secondary *E. multilocularis* infection was assessed by Oku et al.[59], by injecting protoscoleces into NK-deficient beige mice. The development of the parasite was similar to that in heterozygote mice, and it was concluded that NK cells may not play an important role in resisting infection by *E. multilocularis*. Nawa et al.[56] found *Strongyloides ratti* infection in beige mice to proceed more quickly than normal mice in the early stages, but beige mice were still able to expel adult worms from the intestine. Vuitton, et al.[81] noted a lower NK cell

activity in the peripheral blood of human patients suffering from alveolar echinococcosis compared with healthy age-and sex-matched blood donors.

8. T cells

Evidence exists that T cells play an important role in secondary echinococcosis in mice. Infection in congenitally athymic nude mice proceeded very rapidly with little host reaction[34,35]. Host cellular immune response was limited to "a few macrophages, eosinophils and fibroblasts" appearing in the adventitious tissue 4 weeks P.I. Thymectomy alone dose not reduce the extensive lag phase of the infection in mice, and weights of cysts were not affected in experiments using thymectomised mice[11], though the mean number of cysts was greater. Antithymocyte serum (ATS) on the other hand, had a permissive effect on both mean cyst weight and mean number of metastases. Combining thymectomy with ATS produced a marked increase in the mean cyst weight compared to the controls.

Lymphocyte response to secondary hydatid infection in mice is rapid, being detectably greater than controls within 48 hours, peaking at about 3 weeks P.I. and sustaining high levels for the duration of the infection,[78]. In human patients with alveolar echinococcosis of the liver, i.e. chronic infection, $CD8^+$ lymphocytes were predominant in the periparasitic granuloma[81]. However, healing parasitic stages were associated with high numbers of $CD4^+$ lymphocytes. In mice, the distribution and numbers of T cell subsets identified by monoclonal antibodies in the periparasitic granuloma was investigated in 'sensitive' (C57BL/6J and AKR) and 'resistant' C57BL.10 mice[13]. The L3T4 (CD4 or helper T) subpopulation was predominant at the beginning of larval proliferation in all three strains, and remained so up to 6 months P.I. in the resistant strain. It was progressively replaced by the $Ly2^+$ (CD8) T cell subpopulation between 1 and 4 months P.I. in sensitive strains. In this study, resistance appeared to be associated with a relatively lower number of macrophages, and lower development of fibrosis. Riley & Dixon[69] observed a significant increase in T suppressor (CD8) cells in the lymph nodes of *E. granulosus*-infected BALB/c ('sensitive') mice from 8 through to 84 days P.I., with the T helper cell population decreasing rapidly from 14 days P.I.

Kizaki et al.[42] observed a depression in proliferative responses and IL-2 production induced by Con-A *in vitro* by spleen cells of BALB/c mice infected with *E. multilocularis* protoscoleces. This depression was linked to the presence of large numbers of $CD8^{dull}$ subpopulation of T cells in the spleen of infected mice 2 weeks after inoculation (detected by flow cytometry). Addition of $CD8^{dull}$ cells to normal spleen cells in culture resulted in marked suppression of the Con A responses.

9. Delayed-type hypersensitivity response

Delayed-type hypersensitivity (DTH) is mediated by lymphokines from antigen-sensitized T lymphocytes[29]. As detected by the response to *E. multilocularis* antigen injected into the footpad, DTH was significantly lower in infected mice 12 weeks

P.I. compared to 6 weeks P.I.[2]. This result is presented as proof that hydatid infection depresses *in vivo* cell-mediated immunity (CMI) responses, and the author states that the slow intial growth of the LCM (i.e. prior to 6 weeks) reflects the host's "intact and functional" CMI. Liance et al.[44] compared the footpad response in strains of mice that were ascertained to be 'resistant' (C57BL.10), or 'sensitive' (AKR and C57BL.6) to *E. multilocularis* secondary infection. They found that the 'resistant' strains had a significantly greater DTH response than the two 'sensitive' strains, and that this continued for the duration of the infection. In a more recent experiment, it was also found that Cyclosporin A treatment of mice before *E. multilocularis* infection enhanced cyst growth while depressing DTH responses. Treatment after infection, however, did not increase larval cyst growth[45].

Discussion

A summary of host factors effective in combating *Echinococcus multilocularis* infection in mice is presented in Table 1. Growth of the LCM of *E. multilocularis* in rodents varies between species and strain of hosts. Experimentally-infected mice have been variously classified as 'resistant' or 'sensitive' (Liance[44]) on the basis of 'receptivity' to the parasite, or as type 1 (suitable host) or type 2 (non-suitable) on the basis of morphology of the parasite and host reaction (Ohbayashi[57]). Liance et al.'s definition of 'receptivity' rests on the percentage of mice that contract *E. multilocularis* infection after intrahepatic inoculation, ('resistant' strains=30%, 'sensitive' strains=80–90%), while Ohbayashi's definition includes the speed of parasite development and extent of host tissue reaction. Other researchers use the terms 'susceptibility'[1,52], 'resistance'[31], and 'suitable'[41], to describe variation between mouse strains in their responses to an identical inoculating dose. It must be remembered though, that the mouse is not a natural intermediate host for *Echinococcus*, and any classification of parasite behaviour in this host is somewhat artificial.

Several immune components, including antibody[41], complement[33] and macrophages[12] have been demonstrated to have direct protoscolicidal activity *in vitro*. Contact of these components with larval tissue *in vivo* would be possible at the time of inoculation, or with oncospheres in a natural infection at the time of penetration of the intestinal wall, but later is severely limited by the formation of the larva's laminated layer. Kassis & Tanner[40] have shown that the calcareous corpuscles of the larvae are anti-complementary, and thus may be able to protect the young parasite from attack. However measures of protoscolicidal activity would only give an indication of the immune factor's effect on cyst growth *in vivo*, as larval cyst growth is mediated not by protoscoleces but by avesicular cysts and buds of germinal epithelium[23,76].

Granulocytes have not been proved to be capable of suppressing the growth of larval *E. multilocularis* cysts, but indirect evidence suggests they do contribute to combating infection[3,70,73]. Neutrophils and eosinophils have been shown to be capable of direct killing of other helminth parasites such as *Onchocerca*[28] and *Schistosoma*[15].

Other cellular immune components, particularly T lymphocytes, have been shown to be vital in suppressing parasite growth *in vivo*. Thymectomy and treatment with anti-thymocyte serum allowed the parasite to increase in weight and disseminate[11]. The number of peritoneal exudate cells was inversely proportional to the weight of the larval cyst mass during the course of infection[18]. T and B lymphocyte-deficient scid mice had much greater cyst growth than immunocompetent mice after intra-peritoneal inoculation[62]. Not just presence, but also function of immune cells seems to be important to the outcome of infection. Thus, decline in lysosomal enzyme content of macrophages and decrease in the percentage of macrophages and neutrophils bearing Fc and C3d receptors was linked to the rapid growth of the parasite[79]. Similarly, macrophages from infected animals were significantly more effective at killing *E. multilocularis* protoscoleces *in vitro* than those from uninfected animals, although BCG-activated (i.e. non-specifically activated) macrophages were also capable of protoscolicidal activity[12]. BCG treatment of cotton rats one week prior to intraperitoneal inoculation with *E. multilocularis* protoscoleces drastically restricted the growth and metastasis of the LCM (av. 0.5g versus 14.0g in untreated controls[64]).

The normal process for activation of macrophages *in vivo* is by T helper lymphocytes. Mice and humans with higher numbers of T helper cells were shown to be more resistant to *Echinococcus* infection than those with lower numbers[13,14]. Moreover, mouse strains with higher DTH responses were shown to be more resistant to infection[44]. This is because the subset of T helper cells responsible for DTH responses (T_H^1) also produce the potent macrophage activating factor Interferon-gamma (IFNγ)[55]. High T_H^1 activity then, would be expected to confer on mouse strains a greater resistance to *Echinococcus* infection than those with low activity, as is the case in mouse leishmaniasis[25,50]. Gottstein et al.[22a] observed that human patients who had undergone 'self-cure' of alveolar echinococcosis had low levels of antibody against *E. multilocularis*, but showed high *in vitro* lymphoproliferative responses to *E. multilocularis* antigen. Similar results were reported by Vuitton[80]. This would indicate a dominance of IL-2, IFNγ producing T_H^1 cells in these patients over the T_H^2 subset, usually associated with high antibody and eosinophil levels[55].

Suppression of LCM growth in mice has been shown to be associated with granuloma formation[62]. This observation is consistent with the findings that T cells and macrophages are important in controlling cyst growth, as both these cells are essential for the production of granulomas[29]. Recently, granuloma formation in *Schistoma*-infected mice was linked to the action of Tumor Necrosis Factor alpha (TNFα)[10], a cytokine released mainly from cells of the monocyte line in response to stimuli from pathogens and cytokines[30].

Immunosuppression during the course of infection has been noted by many researchers. The earliest manifestation of this is probably the rise in immunoregulating $CD8^{dull}$ T cells in the spleen from about 2 weeks post-infection[42]. The inability to

migrate to specific (from 8 weeks P.I.) and non-specific (from 12 weeks P.I.) chemotactic factors is displayed by neutrophils and macrophages in infected mice[7], a feature that may be associated with the abovementioned functional changes in these cells. Moreover, macrophages isolated from mice 2–3 months P.I. inhibited the mitogen-induced transformation of lymphocytes *in vitro*[63]. Suppressor lymphocyte and macrophage action would decrease the number of functional T cells available, inhibiting T helper activation of macrophages, and thus hampering CMI. Plasmacyte infiltration followed by regression of the cortical follicles and loss of lymphocytes in the paracortex of lymph nodes draining *E. multilocularis* lesions was observed by Ali-Khan & Siboo[4]. One or a combination of these immunosuppressive mechanisms could be responsible for the biphasic growth of the LCM seen in some strains of mice. The evidence for this association is circumstantial, but is suggested by the close time link between the immunosuppressive effects reported and the rapid growth of the LCM from about 6 weeks post-infection. However, biphasic growth of the LCM has also been noted in lymphocyte-deficient scid mice[62], indicating that it may be unrelated to either immune complex deposition or suppressor cell activity.

Factors secreted by the larval parasite must also be considered when investigating the host response. Parasite factors have been shown to be chemotactic for macrophages and neutrophils[7], cytotoxic for macrophages[27], mitogenic for lymphocytes, induce suppressor activity in macrophages[63], and contribute to the immune complexes which block receptors and may diminish the ability of leucocytes to bind and cytolyse the opsonized cysts[79].

Acknowledgements

The authors would like to thank Prof. M. Ohbayashi and Drs. Y. Oku and H. K. Ooi of the Dept. of Parasitology, Prof. M. Onuma of the Dept. of Epizootiology, Faculty of Veterinary Medicine, and Dr. K. Ishiwata, Dept. of Bacteriology, Faculty of Medicine, Hokkaido University for their suggestions and review of this manuscript.

This work was supported by the Japanese Government Ministry of Science, Education & Culture grant nos. 02404083 & 03044016.

Table 1. Host factors involved in combating larval *Echinococcus multilocularis* infection in mice.

Macrophages	
-protoscolicidal *in vitro*, activation potentiates protoscolicidal effects	Baron & Tanner, 1977
-BCG treatment before infection restricted cyst growth in cotton rat	Rau & Tanner, 1975
-numbers increase during infection	Devouge & Ali-Khan, 1983
T cells	
-absence increases susceptibility	Kamiya *et al.* 1982
-high numbers CD4+ cells linked to healing in humans	Bresson-Hadni *et al.* 1990
-CD8+ dominance over CD4+ cells in infected mice related to susceptibility	Riley & Dixon, 1987
-high CD4+ cell-dependent DTH response associated with resistance	Liance *et al.* 1990
Antibody	
-titers not related to susceptibility	Ali-Khan, 1974
-protoscolicidal *in vivo* or when injected into cysts of infected animals	Kassis & Tanner, 1976b
-may promote adherence of effector cells *in vitro*	Rau & Tanner, 1976
Complement	
-protoscolicidal *in vitro*	Kassis & Tanner, 1976a
-acts via alternative pathway	Kamiya *et al.* 1980
Granulocytes	
-rapid response post-infection	Devouge & Ali-Khan, 1983
-effect on parasite survival suspected but not confirmed	Sato & Kamiya, 1990; Sakamoto 1992
Natural Killer cells	
-absence did not affect susceptibility of mice	Oku *et al.* 1984
-(may be effective in activating macrophages in early infection but functions masked by T cells in immunocompetent animals)	

References

1) Ali-Khan, Z. (1974): Host-Parasite relationship in *Echinococcus* I. Parasite biomass and antibody response in three strains of inbred mice against graded doses of *Echinococcus multilocularis* cysts. *J. Parasitol.*, **60**; 231–235
2) Ali-Khan, Z. (1978): *Echinococcus multilocularis*: Cell-mediated Immune response in early and chronic alveolar murine hydatidosis. *Exp. Parasitol.*, **46**; 157–165
3) Ali-Khan, Z. & R. Siboo (1980a): Pathogenesis and host response in subcutaneous alveolar hydatidosis I. Histogenesis of alveolar cyst and a qualitative analysis of the inflammatory infiltrate. *Z. Parasit.*, **62**; 241–254
4) Ali-Khan, Z. & R. Siboo (1980b): Pathogenesis and host response in subcutaneous alveolar hydatidosis II. Intense plasmacellular infiltration in the paracortex of draining lymph nodes. *Z. Parasit.*, **62**; 255–265
5) Ali-Khan, Z. & R. Siboo (1981): *Echinococcus multilocularis*: Distribution and Persistence of specific host Immunoglobulins on cyst membranes. *Exp. Parasitol.*, **51**; 159–168
6) Ali-khan, Z. & R. Siboo (1983): Immune complexes in experimental alveolar hydatidosis. *Tropenmed. Parasitol.*, **34**; 187–192
7) Alkarmi, T. & K. Behbehani (1989): *Echinococcus multilocularis*: Inhibition of murine neutrophil and macrophage chemotaxis. *Exp. Parasitol.*, **69**; 16–22
8) Alkarmi, T., Z. Alsharkarchi & K. Behbehani (1988): *Echinococcus multilocularis* : the non-specific binding of different species of immunoglobulins to alveolar hydatid cysts grown *in vivo* and *in vitro*. *Parasit. Immunol.*, **10**; 443–457
9) Allan, D., P. Jenkins, R. J. Connor & J. B. Dixon (1981): A study of immunoregulation of BALB/c mice by *Echinococcus granulosus equinus* during prolonged infection. *Parasit. Immunol.*, **3**; 137–142
10) Amiri, P., R. M. Locksley, T. G. Parslow, M. Sadick, E. Rector, D. Ritter & J. H. Mckerrow (1992): Tumor Necrosis Factor α restores granulomas and induces parasite egg-laying in schistosome-infected scid mice. *Nature*, **356**; 604–607
11) Baron, R. W. & C. E. Tanner (1976): The effect of immunosuppression on secondary *Echinococcus multilocularis* infection in mice. *Int. J. Parasitol.*, **6**; 37–42
12) Baron, R. W. & C. E. Tanner (1977): *Echinococcus multilocularis* in the mouse: The *in vitro* protoscolidal activity of peritoneal macrophages. *Int. J. Parasitol.*, **7**; 489–495
13) Bresson-Hadni, S., M. Liance, J. P. Meyer, R. Houin, J. L. Bresson & D. A. Vuitton (1990): Cellular immunity in experimental *Echinococcus multilocularis* infection. II. Sequential and comparative phenotypic study of the periparasitic mononuclear cells in resistant and sensitive mice. *Clin. exp. Immunol.* **82**; 378–383
14) Bresson-Handi, S., D. A. Vuitton, D. Lenys, M. Liance, E. Racadot & J. P. Miguet (1989): Cellular immune responses in *Echinococcus multilocularis* infection in humans. II. Lymphocyte reactivity to *Echinococcus* antigens in patients with echinococcosis. *Clin. exp. Immunol.*, **78**; 61–66
15) Capron, A., J. P. Dessaint, M. Capron, J. H. Ouma & A. E. Butterworth (1987):

Immunity to Schistosomes: Progress towards a vaccine. *Science*, **238**; 1065–1072

16) DELABRE, I., C. GABRION, F. CONTAT, A.-F. PONTAVY & S. DEBLOCK (1987): The susceptibility of the Mongolian gerbil (*Meriones unguiculatus*) and the OF1 mouse strain to *Echinococcus multilocularis*-ultrastructural aspects of the cysts. *Int. J. Parasitol.*, **17**; 773–780

17) DEMPSTER, R. P., G. B. L. HARRISON, M. V. BERRIDGE & D. D. HEATH (1992): *Echinococcus granulosus*: Use of an intermediate host mouse model to evaluate sources of protective antigens and a role for antibody in the immune response. *Int. J. Parasitol.*, **22**; 435–441

18) DEVOUGE, M. & Z. ALI-KHAN (1983): Intraperitoneal murine Alveolar Hydatidosis: Relationship between the size of the Larval Cyst Mass, Immigrant inflammatory cells, splenomegaly and Thymus involution. *Tropenmed. Parasit.*, **34**; 15–20

19) DOTTORINI, S., C. TASSI, V. D. NARBO & G. ROSSI (1978): A study on the antibody response in the experimental hydatid disease. *Rivist. Parasit.*, **39**; 71–76

20) ECKERT, J., R. C. A. THOMPSON & H. MELHORN (1983): Proliferation and metastases formation of larval *Echinococcus multilocularis* I. Animal model, macroscopical and histological findings. *Z. Parasit.*, **69**; 737–748

21) GOTTSTEIN, B. (1992): *Echinococcus multilocularis* infection: Immunology and immunodiagnosis. *Adv. in Parasitol.*, **31**; 321–380

22) GOTTSTEIN, B., N. MÜLLER, S. J. CRYZ Jr., M. VOGEL, I. TANNER & T. SEEBECK (1990): Humoral and cellular immune response in mice and dogs induced by a recombinant *Echinococcus multilocularis* antigen produced by a *Salmonella typhimurium* vaccine strain. *Parasit. Imm.*, **12**; 163–174

22a) GOTTSTEIN, B., B. MESARINA, I. TANNER, R. W. AMMAN, J. F. WILSON, J. ECKERT & A. LANIER (1991). Specific cellular and humoral immune responses in patients with different long-term courses of alveolar echinococcosis (infection with *Echinococcus multilocularis*). *Am. J. Trop. Med. Hyg.* **45** (6): 734–742.

23) GOTTSTEIN, P., P. DEPLAZES & M. AUBERT (1992): *Echinococcus multilocularis*: Immunological study on the "Em2-positive" laminated layer during *in vitro* and *in vivo* post-oncospheral and larval development. *Parasitol. Res.*, **78**; 291–297

24) HEATH, D. D. (1986). Immunobiology of *Echinococcus* infection. *The Biology of Echinococcus and Hydatid disease.* Ed. R. C. A. THOMPSON George, Allen & Unwin, London. 164–188

25) HOLADAY, B. J., M. D. Sadick, Z. E. Wang, S. L. Reiner, F. P. Heinzel, T. G. Parslow & R. M. Locksley (1991): Reconstitution of *Leishmania* immunity in Severe Combined Immunodeficicent mice using Th–1 and Th-2 like cell lines. *J. Immunol.*, **147**; 1653–1658

26) IIDA, H., K. ICHIKAWA & I. NAKAGAWA (1961): II. Studies on the sero-diagnosis of *Echinococcus* disease. *Rep. Hokk. Inst. Pub. Hlth.*, 109–112 (In Japanese)

27) JANNSEN, D., A. OSUNA, J. LAZUEN & P. H. DE RYCKE (1992): Comparative cytotoxicity of secondary hydatid cysts, protoscoleces, and *in vitro* developed microcysts of *Echinococcus granulosus. J. Helminthol.*, **66**; 124–131

28) JOHNSON, E. H., S. LUSTIGMAN, B. BROTMAN, J. BROWNE & A. M. PRINCE (1991):

Onchocerca volvulus: *In vitro* killing of microfilaria by neutrophils and eosinophils from experimentally infected chimpanzees. *Tropenmed. Parasitol.*, **42**; 351–355

29) JONES, T. C. & R. D. HUNT (1983). Immunopathology, Chapter 7, *Veterinary Pathology*. Lea and Febiger, Philadelphia.

30) JÄÄTTELÄ, M. (1991): BIOLOGY of DISEASE: Biologic activities & mechanisms of action of Tumor Necrosis Factor α/cachectin. *Lab. Invest.*, **64**; 724–742

31) KAMIYA, H. (1972): Studies on *Echinococcus* XXIV. Age difference in resistance to infection with *Echinococcus multilocularis* in AKR strain of mice. *Jap. J. Vet. Res.*, **20**; 69–76

32) KAMIYA, H., S. FUKUMOTO & Y. OKU (1982): Studies on the host resistance to infection with *Echinococcus multilocularis* IV. Observation of lesions due to the lytic effect of complement on the protoscoleces and pre-adults in vitro by scanning electron microscope. *Jap. J. Parasitol.*, **31**; 479–486

33) KAMIYA, H., M. KAMIYA & M. OHBAYASHI (1980): Studies on the host resistance to *Echinococcus multilocularis* 2. Lytic effect of complement and its mechanism. *Jap. J. Parasitol.*, **29**; 169–179 (In Japanese)

34) KAMIYA, H., M. KAMIYA, M. OHBAYASHI & T. NOMURA (1980): Studies on host resistance to infection with *Echinococcus multilocularis* I. Difference of susceptibility of various rodents, especially of congenitally athymic nude mice. *Jap. J. Parasitol.*, **29**; 87–100 (In Japanese)

35) KAMIYA, H. & M. OHBAYASHI, (1981): Studies on host resistance to infection with *Echinococcus multilocularis* III. Effect of castration on male mice. *Jap. J. Parasitol.*, **30**; 73–79 (In Japanese).

36) KAMIYA, M., Y. OKU, H. KAMIYA & T. NOMURA (1982): Characteristic responses of Nude Mice in Angiostrongylosis and Echinococcosis. *Proceedings of the Third International Workshop on Nude Mice*. Gustav Fisher, New York. 133–145

37) KAMIYA, M., H. K. OOI, Y. OKU, M. OKAMOTO, M. OHBAYASHI & N. SEKI (1987): Isolation of *Echinococcus multilocularis* from the liver of swine in Hokkaido, Japan. *Jap. J. Vet. Res.*, **35**; 99–107

38) KAMIYA, M. & H. SATO (1990): Complete life-cycle of the canid tapeworm, *Echinococcus multilocularis*, in laboratory rodents. *FASEB J.*, **4**; 3334–3339

39) KASSIS, A. I., S. L. GOH & C. E. TANNER(1976): Lesions induced by complement *in vivo* on the protoscoleces of *Echinococcus multilocularis*: a study by electron microscope. *Int. J. Parasitol.*, **6**; 199–211

40) KASSIS, A. I. & C. E. TANNER (1976a): The role of complement in Hydatid disease: *in vitro* studies. *Int. J. Parasitol.*, **6**; 25–35

41) KASSIS, A. I. & C. E. TANNER (1976b): Novel approach to the treatment of Hydatid disease. *Nature*, **262**; 588

42) KIZAKI, T., S. KOBAYASHI, K. OGASAWARA, N. K. DAY, R. A. GOOD & K. ONOE (1991): Immune suppression induced by protoscoleces of *Echinococcus multilocularis* in mice. Evidence for the presence of CD8 dull suppressor cells in spleens of mice infected intraperitoneally with *E. multilocularis*. *J. Immunol.*, **147**; 1695–1666

43) KROEZE, W. K. & C. E. TANNER (1986): *Echinococcus multilocularis*: Responses to

infection in Mongolian gerbils. *Exp. Parasitol.*, **61**; 1–9

44) LIANCE, M., S. BRESSON-HADNI, J. P. MEYER, R, HOUIN & D. A. VUITTON (1990): Cellular immunity in experimental *Echinococcus multilocularis* infection I. Sequential and comparative study of specific *in vivo* delayed type hypersensitvity against *E. multilocularis* antigens in resistant and sensitive mice. *Clin. exp. Immunol.*, **82**; 373–377

45) LIANCE, M., S. BRESSON-HADNI, D. A. VUITTON, D. LENYS, J. P. CARBILLET & R. HOUIN (1992): Effect of Cyclosporin A on the course of murine alveolar echinococcosis and on the specific cellular and humoral immune responses against *Echinococcus multilocularis*. *Int. J. Parasitol.*, **22**; 23–28

46) LIANCE, M., D. A. VUITTON, S. GUERRET-STOCKER, J. P. CARBILLET, J. P. GRIMAUD & R. HOUIN (1984): Experimental alveolar echinococcosis. Suitability of a murine model of intrahepatic infection by *Echinococcus multilocularis* for immunological studies. *Experientia*, **40**; 1436–1439

47) LIEW, F. W. (1986): Cell-mediated Immunity in experimental Cutaneous Leishmaniasis. *Parasitol. Today*, **2**; 264–270

48) LIGHTOWLERS, M. W. (1990): Immunology and Molecular Biology of *Echinococcus* infections. *Int. J. Parasitol.*, **20**; 471–478

49) LIU, D., M. W. LIGHTOWLERS & M. D. RICKARD (1992): Examination of murine antibody response to secondary hydatidosis using ELISA and immunoelectrophoresis. *Parasit. Immunol.*, **14**; 239–248

50) LOCKSLEY, R. M. & P. SCOTT (1991): Helper T-cell subsets in mouse leishmaniasis: induction, expansion and effector function. *Imm. Today*, **12**; A58–A61

51) LUBINSKY, G. (1964): Growth of the vegetatively propagated strain of larval *Echinococcus multilocularis* in some strains of Jackson mice and their hybrids. *Can. J. Zool.*, **42**; 1099–1103

52) LUBINSKY, G. & S. DESSER (1963): Growth of the vegetatively propagated strain of larval *Echinococcus multilocularis* in C57L/J, B6AF and A/J mice. *Can. J. Zool.*, **41**; 1213–1216

53) MELHORN, H., J. ECKERT & R. C. A. THOMPSON (1983): Proliferation and metastases of larval *Echinococcus multilocularis* II. Ultrastructural investigations. *Z. Parasitenkund*, **69**; 749–763

54) MIYAUCHI, T., M. SAKUI, M. ISHIGE, S. FUKUMOTO, A. UEDA, M. ITO & M. OHBAYASHI (1984): A case of multilocular echinococcosis in a horse. *Jap. J. Vet. Res.*, **32**; 171–173

55) MOSMANN, R. & K. W. MOORE (1991): The role of IL-10 in crossregulation of Th1 and Th2 responses. *Imm. Today*, **12**; 49–53

56) NAWA, Y., T. ABE, J. IMAI & H. MARUYAMA (1988): Impaired natural defence of beige (Chediak-Higashi syndrome) mice against tissue-migrating larvae of *Strongyloides ratti* and its reconstitution by bone marrow cells. *Parasit. Immunol.*, **10**; 117–126

57) OHBAYASHI, M. (1960): Studies on Echinococcosis X. Histological observations on experimental cases of multilocular echiococcosis. *Jap. J. Vet. Res.*, **8**; 134–160

58) OHBAYASHI, M., R. L. RAUSCH & F. FAY (1971): On the ecology and distribution of

Echinococcus spp. (Cestoda : Taeniidae) and characteristics of their development in the intermediate host II. Comparative studies on the development of larval *E. multilocularis* Leuckart 1863 in the intermediate host *Jap. J. Vet. Res.*, **19**; 1–53

59) OKU, Y., H. K. OOI, M. KAMIYA & M. OHBAYASHI (1984) : Larval development of *Echinococcus multilocularis* in beige mice with the Chediak-Higashi syndrome. *Jap. J. Vet. Res.*, **32**; 83–86

60) ORIHARA, M. (1969) : Studies on Echinococcosis XXII. Changes of serum titres in mice infected experimentally with *E. multilocularis*. *Jap. J. Vet. Res.*, **17**; 121–127

61) PAULUZZI, S. (1969) : Serologic response of mice and rats to secondary experimental Hydatid disease. *Am. J. trop. Med. & Hyg.*, **28**; 7–12

62) PLAYFORD, M. C., H. K. OOI, Y. OKU & M. KAMIYA (1992) : Secondary *Echinococcus multilocularis* infection in the scid mouse : Biphasic growth of the larval cyst mass. *Int. J. Parasitol.*, (in press)

63) RAKHA, N. K., J. B. DIXON, S. D. CARTER, P. S. CRAIG, P. JENKINS & S. FOLKARD (1991) : *Echinococcus multilocularis* antigens modify accessory cell function of macrophages. *Immunology*, **74**; 652–659

64) RAU, M. E. & C. E. TANNER (1975) : BCG suppresses growth and metastasis of Hydatid infection. *Nature*, (Lond.) **256**; 318–319

65) RAU, M. E. & C. E. TANNER (1976) : *Echinococcus multilocularis* in the cotton rat : The *in vitro* protoscolicidal activity of peritoneal cells. *Int. J. Parasitol.*, **6**; 195–198

66) RAUSCH, R. L. (1986) : Life-cycle patterns and geographic distribution of *Echinococcus* species. *The Biology of Echinococcus and Hydatid disease*. Ed. R. C. A. THOMPSON George, Allen and Unwin, London. 44–80

67) RAUSCH, R. L. & J. WILSON (1973) : Rearing of the adult *Echinococcus multilocularis* Leuckart, 1863, from sterile larvae from man. *Am. J. trop. Med. Hyg.*, **22**; 357–360

68) RICKARD, M. D. (1974) : Hypothesis for the long term survival of *Taenia pisiformis* in rabbits. *Z. Parasitenk.*, **44**; 203–209

69) RILEY, E. M. & J. B. DIXON (1987) : Experimental *Echinococcus granulosus* in mice : immunocytochemical analysis of lymphocyte populations in local lymphoid infections during early infection. *Parasitol.*, **94**; 523–532

70) SAKAMOTO, T. (1992) : Epidemiological survey on the unilocular hydatidosis occurring in Australia. *Epidemiological survey on the new unilocular echinococcosis occurring in tropical Queensland of Australia*. Yamaguti-Hokushyuu Insatu, Morioka, Japan

71) SAKUI, M., M. ISHIGE, S. FUKUMOTO, A. UEDA & M. OHBAYASHI (1984) : Spontaneous *Echinococcus multilocularis* infection in swine in North-Eastern Hokkaido, Japan. *Jap. J. Parasitol.*, **33**; 291–296

72) SARCIRON, M. E., S. AL-NAHHAS, S. WAIDAUM, G. RAYNAUD & A. F. PETAVY (1991) : Treatment of experimental alveolar echinococcosis : comparative study of mebendazole, Isoprinosine & a mebendazole Isoprinosine association. *Trop. Med. Parasitol.*, **42**; 417–419

73) SATO, H. & M. KAMIYA (1990) : Extraintestinal strobilar development of immature *Echinococcus multilocularis* in laboratory rodents following intratracheal inoculation of the protoscoleces. *Int. J. Parasitol.*, **20**; 689–692

74) SCHWABE, C. W., L. A. SCHINAZI & A. KILEJIAN (1959): Host Parasite relationships in Echinococcosis II. Age resistance to secondary Echinococcosis in the white mouse. *Am. J. trop. Med. Hyg.*, **8**; 29–36

75) SWEATMAN, G. K., R. G. ROBINSON & B. W. MANKTELOW (1963): Comparative observations on the scolex and germinal membranes of *Echinococcus granulosus* as a source of secondary hydatid cysts. *Amer. J. trop. Med. Hyg.*, **12**; 199–203

76) THOMPSON, R. C. A. (1986): Biology and Systematics of *Echinococcus*. *The Biology of Echinococcus and Hydatid disease*. Ed. R. C. A. THOMPSON George, Allen and Unwin, London. 5–43

77) THOMPSON, R. C. A., P. DEPLAZES & J. ECKERT (1990): Uniform strobilar development of *Echinococcus multilocularis* in vitro from protoscolex to immature stages. *J. Parasitol.*, **76**; 240–247

78) TREVES, S. & Z. ALI-KHAN (1984a): Characterization of the inflammatory cells in progressing Tumor-like Alveolar Hydatid Cysts I. Kinetics and composition of Inflammatory Infiltrates. *Tropenmed. Parasit.*, **34**; 183–188

79) TREVES, S. & Z. ALI-KHAN (1984b): Characterization of the inflammatory cells in progressing tumor-like Alveolar Hydatid Cyst II. Cell surface receptors, endocytosed Immune complexes and lysosomal enzyme content. *Tropenmed. Parasit.*, **35**; 231–236

80) VUITTON, D. A., A. LASSÉGUE, J. P. MIGUET, P. HERVÉ, T. BARALE, E. SEILLÉS & A. CAPRON (1984): Humoral and cellular immunity in patients with hepatic alveolar echinococcosis. A 2 year follow-up with and without flubendazole treatment. *Parasit. Immunol.*, **6**; 329–340

81) VUITTON, D. A., S. BRESSON-HADNI, L. LAROCHE, D. KAISERLIAN, S. GUERRET-STOCKER, J. L. BRESSON & M. GILLET (1989): Cellular immune response in *Echinococcus multilocularis* infection in humans II. Natural killer cell activity and cell subpopulations in the blood and in the periparasitic granuloma of patients with alveolar echinococcosis. *Clin. exp. Immunol.*, **78**; 67–74

82) YAMASHITA, J. (1968a): Development of *Echinococcus* in laboratory animals. *Bull. W. H. O.*, **39**; 127–130

83) YAMASHITA, J. (1968b): Natural resistance to Echinococcosis and biological factors responsible. *Bull. W. H. O.*, **39**; 121–122

84) YAMASHITA, J., M. OHBAYASHI & R. DOI (1963): Studies on Echinococcosis XV. Secondary multilocular echinococcosis by intrahepatic inoculation *Jap. J. Vet. Res.*, **11**; 55–60

85) YAMASHITA, J., M. OHBAYASHI & S. KONNO (1956): Studies on Echinococcosis IV. Experimental infection of the white mouse. *Jap. J. Vet. Res.*, **4**; 125–128

86) YAMASHITA, J., M. OHBAYASHI & S. KONNO (1957): Studies on Echinococcosis VI. Secondary *Echinococcosis multilocularis* in mice. *Jap. J. Vet. Res.*, **5**; 197–202

87) YAMASHITA, J., M. OHBAYASHI & T. SAKAMOTO (1960): Studies on Echinococcosis XI. Observations on secondary echinococcosis *Jap. J. Vet. Res.*, **8**; 315–322

88) YAMASHITA, J., M. OHBAYASHI & T. SAKAMOTO, M. ORIHARA, K. SUZUKI & M. OKUGI(1963): Studies on Echinococcosis XIV. Further observations on the difference of susceptibility to *Echinococcus multilocularis* among uniform strains of the mouse. *Jap. J. Vet. Res.*, **11**; 50–54