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Mechanisms of Immunity in Hydatid Disease: Implications for Vaccine Development¹

Wenbao Zhang,* Allen G. Ross,[†] and Donald P. McManus^{2*}

The *Echinococcus* organisms, the cause of echinococcosis (hydatid disease), are parasitic helminths with life cycles involving a carnivorous definitive host (usually dog or fox) and an intermediate host (human, ungulate, or rodent). They are complex multicellular pathogens that, despite being under constant barrage by the immune system, are able to modulate antiparasite immune responses and persist and flourish in their mammalian hosts. Understanding how the immune system deals with these parasites is a major challenge. Recent application of modern molecular and immunological approaches has revealed insights on the nature of immune responses generated during the course of hydatid infection, although many aspects of the *Echinococcus*-host interplay remain unexplored. This review summarizes current understanding of the immunology of echinococcosis, indicates areas where information is lacking, and shows how knowledge of host protective immunity has been translated into the design and development of anti-*Echinococcus* vaccines for application in intermediate hosts. *The Journal of Immunology*, 2008, 181: 6679–6685.

H ydatid disease is a chronic, cyst-forming, parasitic helminthic disease of human beings as well as domestic and wild animals. It is caused by infection with the larval (metacestode) stages of dog/fox tapeworms (cestodes) belonging to the genus *Echinococcus* (family Taeniidae) and is also referred to as echinococcosis. The two major species of medical and public health importance are *Echinococcus granulosus* and *Echinococcus multilocularis*, which cause cystic echinococcosis (CE)³ and alveolar echinococcosis (AE), respectively. Human cystic echinococcosis is the most common presentation and probably accounts for >95% of the estimated 3 million global cases, with human alveolar echinococcosis causing ~0.3–0.5 million cases (all in the Northern Hemisphere).

The life cycle of the two tapeworms is shown in Fig. 1. Hydatid cysts of *E. granulosus* develop in internal organs (mainly liver and lungs) of humans and intermediate hosts (herbivores such as sheep, horses, cattle, pigs, goats, and camels) as unilocular fluid-filled bladders. These consist of two parasite-derived layers, an inner nucleated germinal layer and an outer acellular laminated layer surrounded by a host-produced fibrous capsule. Brood capsules and protoscoleces bud off from the germinal membrane. Definitive hosts are carnivores such as dogs, wolves, and foxes. Sexual maturity of adult *E. granulosus* occurs in the host small intestine within 4–5 wk of ingesting offal containing viable protoscoleces. Gravid proglottids or released eggs are shed in the feces and, following their ingestion by a human or ungulate host, an oncosphere larva is released that penetrates the intestinal epithelium into the lamina propria. This is then transported passively through blood or lymph to the target organs where it develops into a hydatid cyst.

The alveolar cyst or metacestode of *E. multilocularis* develops differently than that of *E. granulosus*, being a complex, tumor-like, multivesicular infiltrating structure consisting of numerous small vesicles embedded in stroma of connective tissue. The larval mass usually contains a semisolid matrix rather than fluid and granulomatous infiltration of mononuclear cells around the parasitic vesicles, a hallmark of AE, culminating in irreversible fibrosis. Adult worm infections of *E. multilocularis* occur mainly in red and arctic foxes, although dogs and cats can also act as definitive hosts. Small mammals (usually microtine and arvicolid rodents) act as intermediate hosts.

The proliferative larval stages of *E. granulosus* and *E. multilocularis* can “leak” out of a ruptured cyst (*E. granulosus*) or metastasize (*E. multilocularis*) to another organ or tissue, naturally producing a condition known as secondary CE or AE, respectively. This also allows the parasites to be passaged serially from one intermediate host to another by i.p. implantation of the larvae, simplifying the technical difficulties that would have been involved in cyclic passage through both definitive and intermediate hosts.

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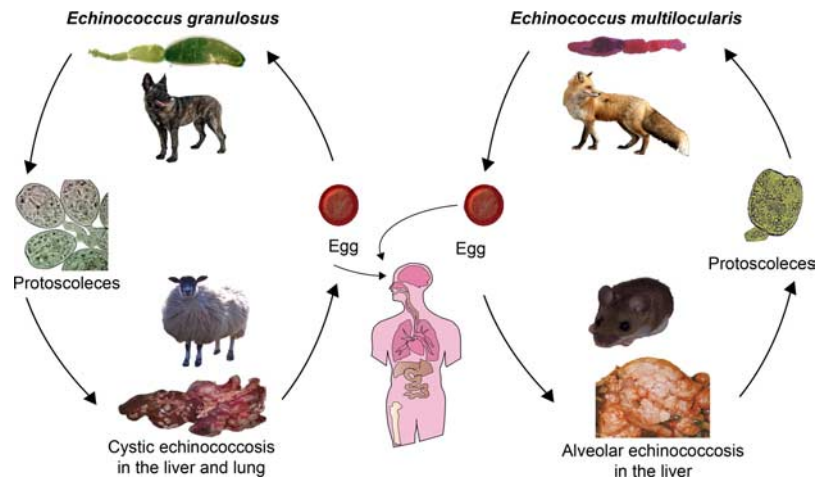
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³ Abbreviations used in this paper: CE, cystic echinococcosis; AE, alveolar echinococcosis; BCG, bacillus Calmette-Guérin; DC, dendritic cell; LL, laminated layer; s/r, susceptibility/resistance.

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FIGURE 1. Life-cycles of *E. granulosus* and *E. multilocularis*. This figure was published in *Trends Parasitol.*; vol. 24; Zhang W., McManus D.P.; Vaccination of dogs against *Echinococcus granulosus*: a means to control hydatid disease?; pp. 419–425. Copyright Elsevier. 2008.



CE and AE are both serious diseases, the latter especially so, with a poor prognosis if careful clinical management is not conducted. Late stage damage can be fatal, especially in AE, when the parasite destroys the liver parenchyma, bile ducts, and blood vessels, resulting in biliary obstruction and portal hypertension. In most late-stage cases a necrotic cavity, containing a viscous fluid, may form in the liver.

Human disease in CE reflects the development and growth of the fluid-filled hydatid cysts mainly in the liver and the lungs, although *E. granulosus* can affect the abdominal cavity, heart, bone, muscle, nervous system, or other locations. Growth of cystic larvae is slow, asymptomatic for a long period after infection, and well tolerated by the host, occasionally leading to large parasitic masses. By contrast, AE does not have well-defined external limits and infiltrates the surrounding parenchyma. A proportion of CE cases detected by field surveys have been reported to spontaneously regress. Seroepidemiological surveys showed that ~20% of villagers in the central highlands of Peru were seropositive to *E. granulosus* Ags but with only 3% having cysts (1). Similar findings were obtained in western China, where 26% of the population were shown to be *E. granulosus*-seropositive, whereas only 43/100,000 subjects had surgery to remove hydatid cysts (2). These epidemiological data suggest that most *E. granulosus* infections do not develop to disease and it is likely that immune responses play a pivotal role in limiting cystic larval development. Nevertheless, despite considerable research efforts, a number of key aspects of the host-parasite interplay that are important for understanding the immunology of infection remain unexplored.

Innate immunity

The factors involved in innate susceptibility/resistance (s/r) to *Echinococcus* infections are largely unknown. Different strains of mice infected with eggs, hatched eggs, or activated oncospheres of *E. granulosus* showed differences in s/r (3). Cotton rats (*Sigmodon hispidus*) treated with nonspecific Ags such as bacillus Calmette-Guérin (BCG), BCG cell walls, or phytohemagglutinin (4) were protected against inoculated proliferating *E. multilocularis* metacystodes, indicating that protection against echinococcosis can be induced nonspecifically, with protection correlating with increased numbers of monocytes and macrophages (5, 6). Congenic C5-sufficient mice were shown more resistant to *E. granulosus* infection than C5-deficient mice, suggesting that the alternative complement pathway

contributes to s/r (7). There is significant evidence showing that infiltration of neutrophils and macrophages occurs during the early phases of *E. granulosus* and *E. multilocularis* infection followed by leukocytosis, resulting in an increased number of myeloid cells such as eosinophils, lymphocytes, and macrophages (8, 9) (Fig. 2). Pronounced pathologic changes then follow (10–12). It is noteworthy that eosinophils have been implicated as potent effector cells in innate immunity against the infective larval stages, but not adults, of most helminth parasites, including *E. granulosus* (13).

In chronic stage CE, as well as neutrophils and macrophages, there is cellular infiltration of eosinophils and fibrocytes into the outer adventitial layer of human hydatid cysts, leading to

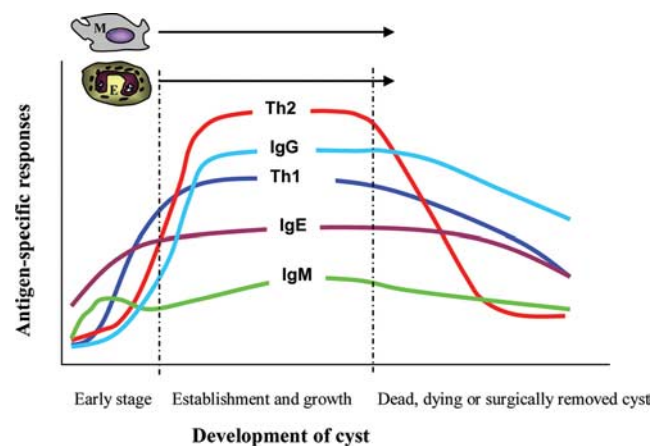


FIGURE 2. Immune responses during the development of a hydatid cyst of *E. granulosus* in the intermediate host. In the early stage of infection the oncosphere is transported to a host organ such as the liver or lung, where it develops into a hydatid cyst. The immature cyst has to overcome host, mainly cell-mediated, immune responses, especially the infiltration of macrophages and eosinophils and low level polarized Th1 responses. About 8- to 10-wk postinfection in mice, cyst growth is maintained and complex echinococcal Ags are released from the cyst. These Ags stimulate complex immune responses. These include polarized Th2 responses balanced with Th1 responses. At this time, the parasite produces significant quantities of Ags that help to modulate the immune response, which may benefit both host and parasite; IgG, especially IgG1, and IgG4, IgE, and IgM levels are elevated. When the cyst is dead, dying, or surgically removed, the Th2 responses drop rapidly whereas the Th1 responses drop slowly, then becoming polarized. IgG can be maintained in the human host for many years after the cyst is surgically removed. Once an infected patient has relapsed, the Th2 responses recover very quickly whereas other responses are elevated slowly. M, Macrophage; E, eosinophil.

fibrosis/necrosis and bile duct and vessel obstruction (14, 15). Eosinophils degranulate at the host-*Echinococcus* interface, and eosinophil cationic protein, a major component of eosinophil granules, reaches levels in hydatid cyst fluid harmful to the parasite (16).

There have been few studies that have investigated NK cells in *Echinococcus* infection, although these cells are known to be instrumental in innate immune responses against intracellular pathogens including viruses, bacteria, and protozoa (17). Patients with active CE cysts were shown to have proportionally more NK cells (CD56⁺CD8⁻) in their PBMC than controls (18), but no functional studies or in situ analysis of the cells at the periphery of the cysts were undertaken, so their role in the outcome of hydatid disease was not determined. In contrast, patients with AE infection had a lower level of PBMC NK activity than healthy controls and subjects with nonparasitic biliary disease; this could be due to a lower percentage of NK cells circulating in the blood of the AE patients or to the presence of serum inhibitory factors such as immune complexes or Abs (19). It has been hypothesized that in AE the MHC class I chain-related molecules A and B (MICA/B), induced by *E. multilocularis*, skew the NKG2D activation pathway on NK and CD8 T cells, inhibiting NKG2D-dependent cytotoxicity and thereby contributing to the longevity of the parasite (17).

A regulatory role for IL-12 in innate resistance in intermediate hosts against *Echinococcus* infection has been suggested. Mice injected with an expression vector encoding IL-12 (20) or treated with rIL-12 (21) were protected against secondary CE and AE, respectively, and this was accompanied by a Th1 response. TLR is believed to play a key role in the innate immune system, but there is limited information on its involvement in innate resistance against *Echinococcus* infection. As is discussed later, *E. granulosus* Ags can modulate the maturation of dendritic cells (DC) via TLR (22) thereby limiting antiparasite immune responses.

Adaptive immunity

Although the data are limited, there is, nevertheless, clear evidence from experiments with animals challenged with *E. granulosus*/*E. multilocularis* eggs or oncospheres that infected hosts produce significant immune responses, including Ab and T cell responses generated by lymphocytes. Understanding the mechanisms whereby these immune responses are produced, particularly the role of protective Abs against the oncosphere, has been of fundamental importance in developing highly effective recombinant vaccines against both *E. granulosus* and *E. multilocularis*.

Antibody responses. The earliest IgG response to hydatid cyst fluid and oncospherical Ags appears after 2 and 11 wk, respectively, in mice and sheep challenged with eggs or oncospheres of *E. granulosus* (23, 24). As will be described below, these anti-oncospherical Abs play a major role in parasite killing and are central to the protective immune response against *E. granulosus* and *E. multilocularis*. In the chronic phase of CE, elevated levels of Ab, particularly IgG, IgM, and IgE, occur in humans (25–29) (Fig. 2), with IgG1 and IgG4 being predominant (26, 30, 31). Ab responses to protoscolex Ags are relatively weak and delayed in the early stage of *E. multilocularis* infection in mice but are increased later (32, 33); IgG1 and IgG3 levels increase significantly at 8 wk postchallenge and remain elevated thereafter (34).

T cell responses. Early Th1-polarized cytokine production, which can kill the metacestode at the initial stages of development (35), shifts to a predominant Th2 cytokine response in the later chronic stage and is characteristic of *E. granulosus* and *E. multilocularis* infection (Fig. 2). Very little is known of cytokine production in the early phases of a primary (oral challenge with eggs) *E. granulosus* infection, although both Th1 and Th2 cytokine levels are low in the early stages of a primary *E. multilocularis* infection but are raised subsequently (32). As well, it is thought the Th2 cytokines are responsible for inhibition of parasite killing because of the anti-inflammatory action of IL-10 (32, 35).

A hallmark of chronic *Echinococcus* infection is the presence of high levels of IL-10 (17, 35), a cytokine typically associated with immunoregulation of effector responses (36). The principal function of IL-10 appears to be to limit and ultimately terminate inflammatory responses. IL-10 also regulates growth and/or differentiation of leukocytes. IL-10 plays a key role in differentiation and function of regulatory T cells, which may figure prominently in the control of immune responses and tolerance in vivo (36). By inducing the host to produce high levels of IL-10, *E. multilocularis* appears able to modulate the immune response so that the T cells infiltrating the periparasitic granuloma cannot participate in the effector phase of the cellular immune response (17, 35).

It is not known whether the significant cellular infiltration of macrophages and neutrophils occurring as the parasite develops results from the innate immune mechanisms of the host and the release of chemotactic substances by the parasite or whether it is dependent on Th0/Th1 cytokines. A large number of CD4⁺ T lymphocytes are present in AE patients with aborted or dead lesions, whereas patients with active parasites display a significant increase in activation of predominantly CD8⁺ T cells (37), indicating that CD4⁺ T cells may play a role in the killing mechanism. This is supported by experiments undertaken with genetically modified mice (38). Conversely, *E. multilocularis* is able to survive and persist in its host indefinitely for long periods of time. In fact, the murine immune response fails to clear infection even when presented with the lowest possible infection dose by injection with a single parasite vesicle (39).

Patients with chronic CE generate both Th1 and Th2 responses (40). As Th1 and Th2 cytokines usually down-regulate each other (41), this is likely due to echinococcal Ags containing distinct epitopes for each T cell subset (42, 43). Liver pathology in AE is characterized by the presence of a huge granulomatous infiltrate of mononuclear cells involving mainly macrophages, myofibroblasts, and T lymphocytes (19, 44). In the progressive forms of the disease, the T cell infiltrate within the periparasitic granuloma is mainly composed of CD8 T lymphocytes (45).

Relationship of cytokines with cellular responses and Ab production. Extracts from metacestodes of *E. multilocularis* cause basophil degranulation, as well as the secretion of histamine, IL-4, and IL-13 in a dose-dependent manner (46). IgE stripping and re-sensitization of basophils indicates that the mechanism of IL-4 induction requires the presence of IgE on the cells (46). *E. multilocularis* may thus induce a Th2 host response by the induction of IL-4 release from basophils.

There is a clear correlation between cytokine and Ab profiles in the chronic stage of human CE. There is increased production of IL-4 and IL-10 (47), concomitant with high levels of IgE and IgG4 (48, 49), and a significant correlation between IL-5

production and IgE/IgG4 expression in hydatid patients (50). IgG1 and IgG4 levels may be good markers for indicating the status of infection; when *E. granulosus* cysts grow, IgG1 and IgG4 levels are elevated, whereas the concentrations of specific IgG1 and IgG4 decline in CE cases characterized by cyst infiltration or calcification (26). Compared with patients with a primary infection, CE patients with relapsing disease have higher levels of IgE and IgG4 Abs and produce elevated quantities of IL-5, IL-4, and IL-10 with lower levels of IFN- γ (48, 50). IFN- γ levels were undetectable in the sera of relapsed patients (51).

The proinflammatory cytokines IL-1 β , IL-18, IL-12, and TNF- α are reduced, IL-8 is elevated, and regulatory IL-10 is unchanged in AE patients compared with controls (52). This is accompanied by an increased number of regulatory CD4⁺CD25⁺ cells and a reduced release of the Th2-type chemokine CCL17 (thymus and activation-regulated chemokine or TARC), suggesting an anti-inflammatory response. As well, the production of IFN- γ and the expression of CD28 on CD4⁺ T cells is increased as is the release of the Th2-type chemokine CCL22 (macrophage-derived chemokine or MDC), supporting the concept that *E. multilocularis* generates proinflammatory immune responses. These results indicate that *E. multilocularis* can modulate both regulatory and inflammatory Th1 and Th2 cytokines and chemokines. Such a mixed profile might be required for limiting parasite growth and for reducing periparasitic tissue and organ damage in the host (53).

NO synthase has been found in liver biopsies of CE patients; a correlation between nitrite and IFN- γ levels implied that NO production by the host was in response to an IFN- γ -activating signal (54). The production of NO may constitute a host defense against *E. granulosus* (55). As well, NO production by i.p. macrophages from mice during secondary infection with *E. multilocularis* mediates immunosuppression at the early and late stages of infection (56). NO production parallels the production of TNF- α in AE-infected mice (34), indicating that NO levels are enhanced by this cytokine. Ags in the laminated-layer of AE and CE cysts decrease NO production by activated rat macrophages in vitro, suggesting that *E. multilocularis* and *E. granulosus* produce molecules that can suppress its parasite inhibitory effect (57).

Ag-presenting cells. Macrophages, B cells, and DC present *Echinococcus* Ags to T cells. The importance of the role of DC in immunity to echinococcosis has only recently been appreciated. Crude *E. multilocularis* Ags were unable to induce DC maturation (58). However, CD11⁺CD123⁻ myeloid DC pulsed with *E. multilocularis* Ags were able to induce autologous CD8⁺ T cell proliferation (39), suggesting that the myeloid DC pathway is involved in CD8⁺ T cell proliferation. Macrophages from mice infected with *E. multilocularis* can act as APC to process and present conventional Ags such as chicken OVA (59). CD8⁺ T cells increase markedly during the course of *E. multilocularis* infection due to significant stimulation of a Th1-type cytokine IFN- γ response and effector macrophage function (60).

Immunomodulation. *E. granulosus* and *E. multilocularis* can survive for many years in their mammalian hosts (61, 62), suggesting that these parasites can modulate antiparasite immune responses. The physical barrier between *E. granulosus* and *E. multilocularis* cysts and their hosts is the parasite-derived lami-

nated layer (LL), characterized by its rich high molecular weight polysaccharide composition. In *E. multilocularis* the LL and especially its major carbohydrate Ag Em2(G11), appear as key to the parasite's survival, acting by modulating the host immune response by virtue of its T cell-independent nature (38). Em2(G11), another carbohydrate component of the LL (Em492), as well as other parasite metabolites, also interfere with Ag presentation and cell activation, leading to a mixed Th1/Th2-type response during late infection (63). In *E. granulosus*, the LL is bounded by a host-produced fibrous adventitious layer or capsule, which probably also helps to protect it physically from host immune attack. The capsule is the product of a three-layered host cellular inflammatory-type response initiated in the early stages of postoncospherical development by infiltration of eosinophils (64), fibroblasts, and mesothelial cells (65), although the precise immunological reactions involved require elucidation.

The *Echinococcus* organisms have evolved a range of additional strategies for immune evasion that include antigenic variation, shedding of surface protein, protease production, active modulation including immunosuppression, skewing of the Th1/Th2 cytokine profile, molecular masking and mimicry, T cell suppression and modulation, inhibition of effector cell chemotaxis, and the release of antigenic proteins, carbohydrates, and mitogenic components of the oncosphere/metacestode that interfere with Ag presentation (9, 17, 22, 49, 59, 63, 66–72).

CD8⁺ T suppressor cells have been detected in spleens of mice infected with protoscoleces of *E. multilocularis* (73, 74), suggesting that the parasite itself plays a key role in suppressing the immune response via inducing CD8⁺ regulatory (suppressive) T cells, although the active parasitic component(s) involved were not identified.

A notable example of an immune evasion molecule is Ag B, a 120-kDa polymeric lipoprotein consisting of various 8-kDa subunits, which is the major *E. granulosus* immunomodulatory Ag isolated from hydatid fluid. As well as inhibiting elastase activity and neutrophil chemotaxis, Ag B elicits a nonprotective Th2 cell response, thereby helping the parasite to evade the human response (22, 49). This escape of host immunosurveillance is achieved by Ag B and other *E. granulosus* Ags interfering with monocyte differentiation and by modulating DC maturation via TLR (22). As emphasized previously, Th1 responses are responsible for damaging the parasite whereas Th2-promoting cytokines, such as IL-4 and IL-13, are beneficial for parasite growth (40) and are responsible for the inhibition of parasite killing through the action of IL-10 and other mediators that can inhibit the effector phase of the cellular immune response (35). Consistently, a shift in the cytokine response toward a type 1 expression pattern in humans or mice reduces parasite growth (60, 63, 75). Hence, blocking those Ags responsible for inducing host Th2 cell responses and vaccination Ags inducing a Th1 cell response may be an important pointer for future vaccine design, should this be necessary.

Protective immune responses and identification of host-protective Ags

Considerable attention has been paid over a long period aimed at characterizing protective immune mechanisms in the intermediate hosts of *E. granulosus*, *E. multilocularis*, and other related taeniid cestodes (76). In the 1930s, some general concepts of protective immunity were formulated following pioneering experiments undertaken in rodents with *Taenia taeniaeformis*

Table I. Important, unresolved areas of hydatid immunology that may inform improved vaccine design

Required Tasks
Precise identification of the Ig isotypes involved in protective immunity
Define the role of T cells as effectors of protective immunity and their regulation
Unravel the relationship between T cell and B cell responses in protective immunity
Characterize memory B and T cell recognition of protective Ags, including the EG95 vaccine
Determine the role(s) of Ab-dependent cell-mediated immune effector mechanisms such as the involvement of neutrophils and the eosinophil/IgE/mast cell axis and neutrophils with Ab in the killing of the infective larval stage (the oncosphere) contained within the egg
Evaluate cellular inflammatory responses/cell-mediated immunity, the generation of associated cytokine/chemokines, and the role of regulatory T cells, neutrophils, macrophages, eosinophils, fibrocytes, and NK cells in the early stages of infection
Determine the involvement of Ab-dependent, complement-mediated lysis (by the classical or alternative pathway) and the role of neutrophils in the immune killing of oncospheres in vivo

and rabbits with *Taenia pisiformis* that have been confirmed subsequently to apply generally to natural infection with taeniid parasites, including *E. granulosus*. These extensive studies have shown that infection leads to a state of immunity to reinfection; naive hosts can be protected against an initial infection by immunization with nonliving parasite extracts; naive hosts can also be protected against an initial infection by passive transfer of serum or colostrum from either a previously infected host or a host that had been actively immunized; and a substantial degree of cross-reactivity exists between Ags from different taeniid cestode species, reflected in both active immunization and passive transfer of immunity using Ags from, or immune sera raised by, heterologous species (76).

The field did not advance much further until the 1970s and 1980s, when many of the earlier studies were revisited and the results confirmed but additional important mechanisms relating to the protective immune responses were also formulated. In particular, it was shown that concomitant immunity, whereby the presence of the developing metacestode causes destruction by Ab-dependent, complement-mediated lysis of any further oncospheres that attempt to invade, is a pivotal characteristic of infection with taeniid cestodes in their intermediate hosts. Indeed, Abs appear to be critical in immunity to taeniid metacestodes, with IgG1, IgG2a, IgG2b, and IgE Abs playing a major role in oncosphere killing, although the involvement of other mechanisms cannot be ruled out. In general, the results of these extensive investigations into the immunobiology of infection with taeniid cestodes have been shown to apply to *E. granulosus* (76–82). In particular, the antiparasitic effects of serum from either infected or actively immunized hosts of taeniid cestodes were reflected in the ability of the sera to kill activated oncospheres in vitro (49, 71, 72, 83, 84). This in vitro correlate has been applied particularly effectively in investigations of the nature of the protective immune response against *E. granulosus*,

which identified Ags from the parasites' infective larval stage contained within the oncosphere with the potential to induce high levels of protection in vaccinated hosts (84–86). The critical role for Abs in the immunity to taeniid metacestodes has provided a powerful method to screen cDNA libraries and to identify genes encoding specific, host-protective oncospherical Ags. This has resulted in the development of effective recombinantly derived vaccines not only against *E. granulosus* and *E. multilocularis*, but also against *Taenia ovis* in sheep, *Taenia saginata* in cattle, and *Taenia solium* in pigs (82).

It is not known whether cell-mediated responses play a role in host protection against *E. granulosus* and *E. multilocularis*, and this is clearly an area for future study. However, some indirect evidence has shown that these responses are likely to be important. Depletion of T cells enhanced metastasis of *E. multilocularis* in mice (87). In vitro experimentation has shown that neutrophils in association with Ab can bring about killing of *E. granulosus* oncospheres (88), indicating a possible role for Ab-dependent, cell-mediated cytotoxicity reactions. This remains to be shown in vivo. It has been suggested that parasite-specific T cell lines derived from patients at various clinical stages of CE may be used to identify Th1 protective epitopes on *Echinococcus* Ags (89).

Information on cellular responses generated by the *E. granulosus* and *E. multilocularis* recombinant vaccines is limited. The secondary *E. granulosus* hydatid cyst mass in mice immunized with the *E. granulosus* vaccine (EG95) coupled to BCG was reduced by nearly 93%, and this was associated with elevated levels of IL-2, IFN- γ , and TNF- α and decreased IL-4, suggesting that Th1 responses may play a major role against challenge infection in this vaccine model (90). Furthermore, mice immunized with *E. multilocularis* vaccines coupled to BCG (BCG-EmII/3 and BCG-Em14-3-3 vaccines) also induced significant Th1 responses in mice challenged with *E. multilocularis* protozoles (91).

Conclusions

The *Echinococcus* organisms are very complex multicellular pathogens. They are highly immunogenic, stimulating proinflammatory cellular responses, significant Ab production, and T cell- and other cell-mediated responses in their human and intermediate hosts. An understanding of these immune mechanisms is of fundamental importance for unraveling the underlying host protective responses. It is clear that host immunity plays a major role in the natural host-parasite relationship in echinococcosis, with this immunity being associated with protective Abs, a feature that has been used to develop the highly effective recombinant EG95 vaccine against *E. granulosus*. However, although some of the immune responses involved in infection have been addressed, including the involvement of Abs, the precise mechanisms underlining protection are largely unknown. Some important unresolved areas of hydatid immunobiology that may inform future vaccine design are presented in Table I. Understanding these mechanisms will benefit researchers working not only on cestode vaccines but also on vaccination against other helminth parasites as well.

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