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Concepts in Immunology and Diagnosis of Hydatid Disease

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INTRODUCTION

Echinococcosis is a cosmopolitan zoonosis caused by adult or larval stages of cestodes belonging to the genus *Echinococcus* (family Taeniidae). Larval infection (hydatid disease, hydatidosis) is characterized by long-term growth of metacestode (hydatid) cysts in the intermediate host. The two major species of medical and public health importance are *Echinococcus granulosus* and *E. multilocularis,* which cause cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively. This review emphasizes recent advances in the immunology and diagnosis of CE, but comparative reference is also made to AE infection and to earlier important findings concerning both diseases. The biology, life cycle characteristics, and etiology of *Echinococcus* have been described comprehensively in a recent review (245), and so only a brief overview is presented here.

Hydatid cysts of *E. granulosus* develop in internal organs (mainly the liver and lungs) of humans and intermediate hosts (herbivores such as sheep, horses, cattle, pigs, goats and camels) as unilocular fluid-filled bladders (Fig. 1 to 3). 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 (PSC) bud off from the germinal membrane. Definitive hosts are carnivores such as dogs, wolves, and foxes (Fig. 1). Sexual maturity of adult *E. granulosus* occurs in the host small intestine within 4 to 5 weeks after the host ingests offal containing viable PSC. 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 and enters the lamina propria. The larva is then transported passively through the blood or lymph to the target organs, where it develops into a hydatid cyst. Since the life cycle relies on carnivores eating infected herbivores, humans are usually a dead end for the parasite. Adult worm infections by *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 metacestode of *E. multilocularis* (Fig. 2) is a 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 (245). CE and AE are both serious diseases, the latter especially so, with a high fatality

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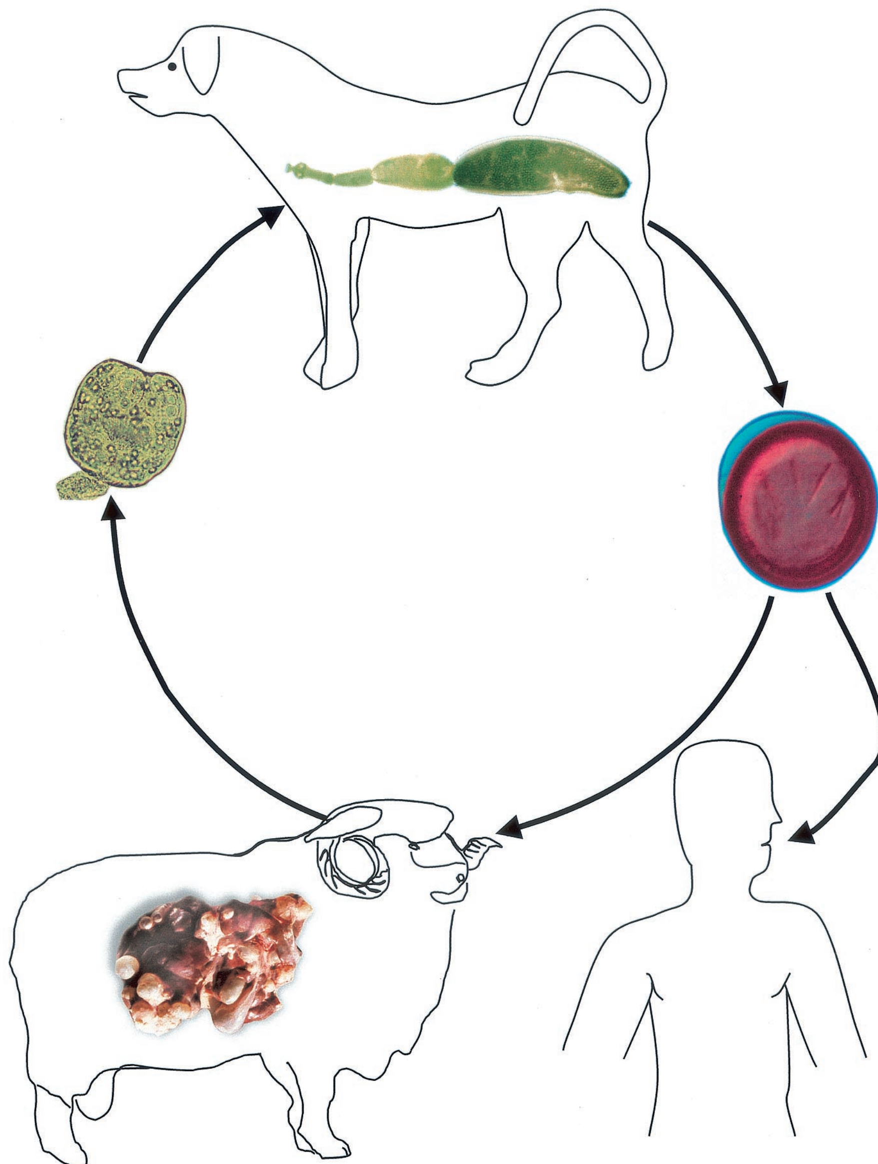

FIG. 1. Life cycle of *E. granulosus. Echinococcus* spp. require two mammalian hosts for completion of their life cycle. Segments containing eggs (gravid proglotitids) or free eggs are passed in the feces of the definitive host, a carnivore. The eggs are ingested by an intermediate host, in which the metacestode stage and protoscoleces develop. The cycle is completed if the metacestode and protoscoleces are eaten by a suitable carnivore.

rate and poor prognosis in the absence of careful clinical management.

IMMUNITY IN THE INTERMEDIATE HOST

The immunology of hydatid disease has been divided conceptually into preencystment and postencystment phases (210), which are differentiated by the formation of the laminated layer around the hydatid cyst. This occurs between 2 and 4 weeks postinfection in the animal intermediate or human host following ingestion of the egg and release of the oncosphere.

Innate Resistance and Early Immunity

Primary infection. Very little is known about the factors affecting innate susceptibility to infection with *E. granulosus* following ingestion of the infective egg stage and establishment of the primary cyst. Host age, sex, and physiological state may influence the innate susceptibility or resistance to infection (210). Furthermore, experimental infections of mice with eggs or oncospheres of *E. granulosus* showed that susceptibility varies with different strains of mice (56). It is noteworthy that although cattle are naturally susceptible to infection with *E. granulosus,* the resultant cysts are invariably infertile and do

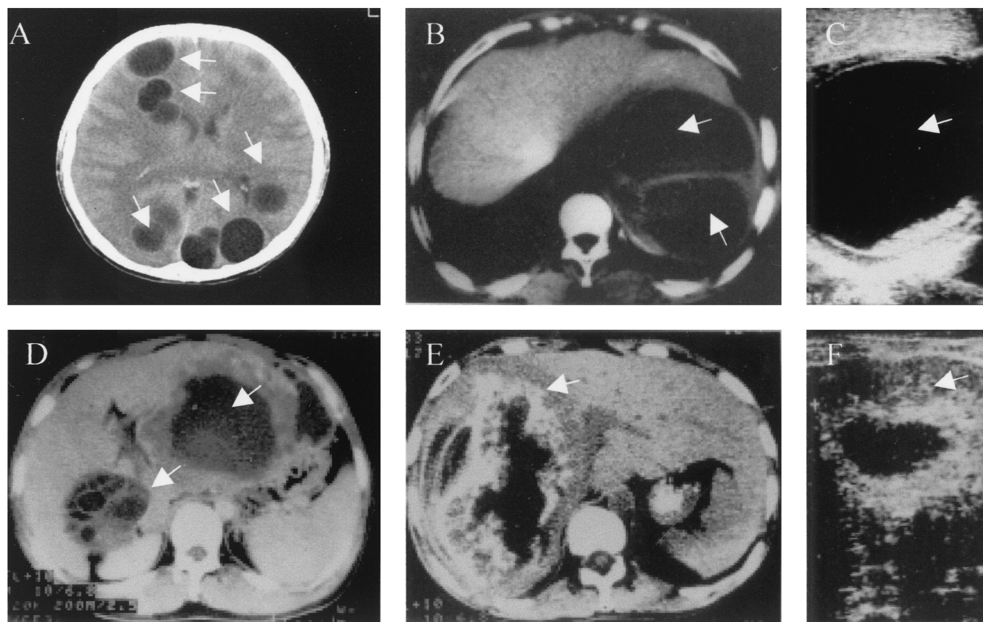

FIG. 2. Clinical images of CE and AE. (A) CT scan of a brain with CE. (B) CT scan of a liver with CE. (C) Sonogram of a liver with CE. (D) CT scan of a liver with both AE and CE (note the presence of daughter cysts). (E) CT scan of a liver with AE. (F) Sonogram image of a liver with AE. Cysts (CE) or cyst masses (AE) are arrowed.

not produce brood capsules or PSC (246). In contrast, sheep cysts are generally fully fertile, with brood capsules asexually budding from the germinal layer and PSC developing from the inner wall of the brood capsules. It has been suggested that this difference may be due to parasite strain variation, but the same situation applies in cattle and sheep from the same area of endemic infection (41, 146, 273). This implies that cattle may have some natural immunity that inhibits the development and growth of PSC. In contrast, sheep appear to be highly susceptible to infection. Experimental infection of sheep with eggs showed that a high percentage (32 to 48%) of oncospheres survived and developed, suggesting that naive sheep may have only a limited resistance to primary infection (266).

After infection, the earliest detectable immunoglobin G (IgG) response to hydatid cyst fluid (HCF) antigens occurs after 2 to 11 weeks in mice and sheep, respectively (247, 266), and after 4 weeks in vervet monkeys (227). Early infections may be associated with a significant cellular inflammatory response (172, 210) that may cause pathologic changes (12, 80) since there is an increased leukocytosis, mainly of eosinophils, lymphocytes, and macrophages (195). With oncospheres, necrosis of surrounding cells is followed by infiltration of neutrophils and macrophages 3 to 5 days after infection in sheep (195). Experiments in vitro have shown also that neutrophils, in association with antibody, can bring about the killing of *E. granulosus* oncospheres (226), suggesting a possible role for antibody-dependent cell-mediated cytotoxicity reactions. At the early stages of disease, there is a marked activation of cell-mediated immunity to the parasite (81).

Secondary infection. In experimentally induced secondary infections in mice, intraperitoneally injected PSC are surrounded by a considerable cellular infiltration within 3 days, initially involving activated macrophages and subsequently including neutrophils, eosinophils, and lymphocytes (208, 221, 222). Interleukin-10 (IL-10), IL-4, and IL-5 secreted in vitro by splenocytes can be detected as early as week 1 postinfection (55). High levels of tumor necrosis factor alpha (TNF), gamma interferon (IFN), IL-6, and specific IgG1 were detectable in serum, and IgG3 was measurable in the peritoneal cavity using protoscolex somatic antigens (55, 111). These data suggest that polarized Th2 reactions are evoked at the very beginning of the immune response to secondary infection. *E. granulosus* PSC contain immunogenic T-independent antigens (29). Primary antibody responses to protoscolex somatic antigens and the production of IgM and IgG3 in early infection appear to be stimulated mainly by a T-independent mechanism (29).

Similar to *E. granulosus,* differences in susceptibility to *E. multilocularis* have been shown in both primary and secondary infection of different mouse strains (26, 53, 74, 75, 106, 184). Susceptibility and resistance are based on the activation of different $CD4^+$ T-cell immune responses (26, 53, 198). Experiments with mice infected with eggs showed that IFN-gamma-, IL-2-, and IL-4-expressing cells in the parasitic lesions were not detectable at the early phase of the infection but were present at the end (26). Similarly, low levels of cytokines in the sera were measurable at the beginning of the infection and high levels were detectable subsequently (26). IL-10 was the most prominent cytokine measurable throughout the course of the infection. Correspondingly, only small amounts of IgM, IgG1, IgG2a, and IgG3 could be detected early on, and higher levels were detectable later (190). A strong, specific intestinal immune response was found in the early stage (190). Both subsets of $CD4^+$ T cells (Th1 and Th2) are involved in primary murine alveolar echinococcosis (26, 74).

In secondary AE in mice, very low levels of Th2 cytokines and IgG1, IgG2a, IgG3, and IgM are produced at the early infection stage, but these levels subsequently increase signifi-

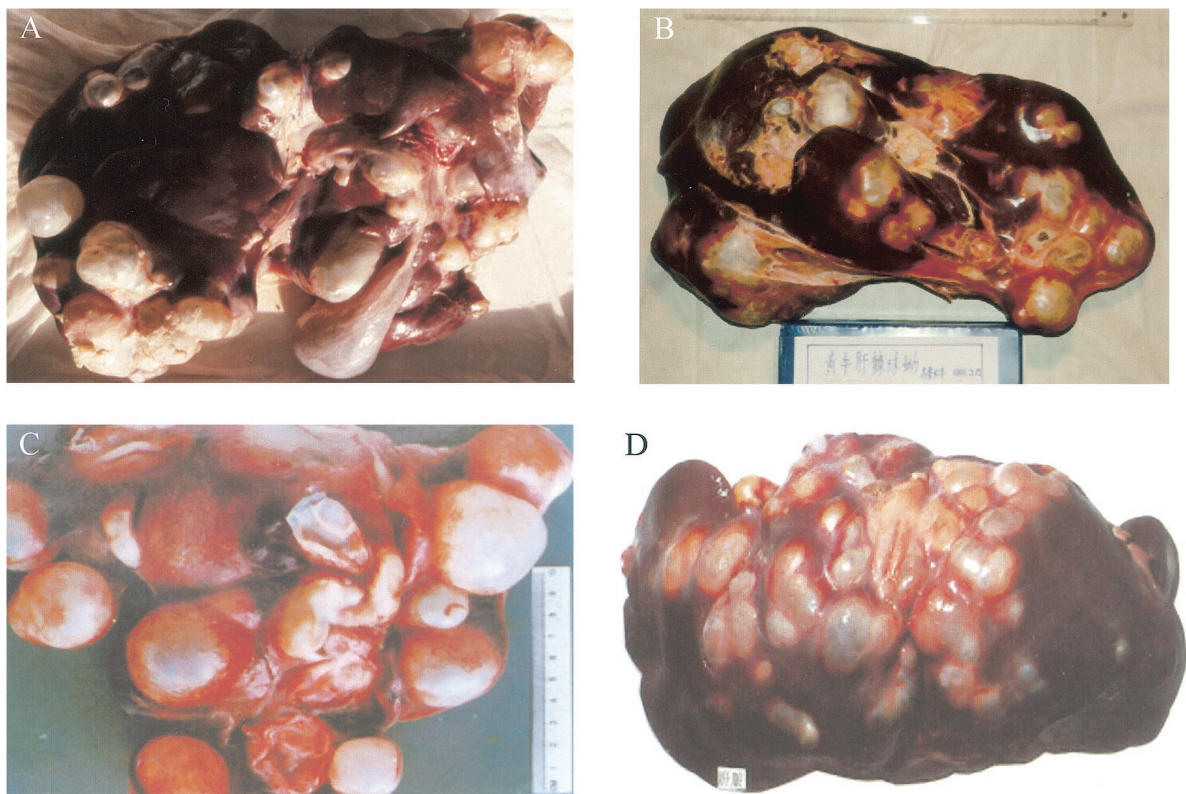

FIG. 3. Cystic hydatid disease in the livers of various intermediate hosts. (A) sheep; (B) cow; (C) camel; (D) yak.

cantly (73). The degree of antibody response by the murine host does not correlate with susceptibility to *E. multilocularis* (198).

Established Cysts

Compared with events occurring during early infection, the immune response to established cysts has received much more attention. In humans there is frequent occurrence of elevated antibody levels, particularly of the IgG, IgM, and IgE isotypes (46, 52, 64, 113, 114, 197, 206, 243). In seropositive individuals, there tends to be a predominance of IgG1 and IgG4 antibody recognition of antigen 5 (Ag5) and antigen B (AgB) (see below), respectively (2, 52, 129, 233, 243, 258). This is of some value with respect to immunodiagnosis and is discussed below.

Also involved in the establishment phase is cellular infiltration, which includes eosinophils, neutrophils, macrophages, and fibrocytes (8, 9, 17, 18, 208, 219, 222, 240). However, this generally does not result in a severe inflammatory response and aged cysts tend to become surrounded by a fibrous layer that separates the laminated layer from host tissue. Eosinophilia and the production of high levels of IgE are the common consequences of infection by helminths (30, 37). It has been suggested that the eosinophil has evolved especially as a defense against the tissue stages of parasites that are too large to be phagocytosed (112) and that the IgE-dependent mast cell reaction has evolved primarily to localize eosinophils near the parasite and then enhance their antiparasitic functions (30). Eosinophils are less phagocytic than neutrophils, but, like neutrophils, they can kill larval stages of parasites (204) such as *Echinococcus* (179) by both dependent and independent mechanisms; their activities are also enhanced by cytokines (36).

Like other helminth infections (12, 80, 159, 180, 193), echinococcosis induces two very distinct Th1 and Th2 cytokine secretion patterns. Th1 cells produce IL-2, IFN-γ, and lymphotoxin, whereas Th2 cells express IL-4, IL-5, IL-6, IL-10, and an induced gene (*p600*) of unknown function. In tissue culture, the Th1- and Th2-cell patterns are well defined and stable. They are generally cross-inhibitory. IFN-γ inhibits Th2-cell proliferation, whereas IL-10 inhibits the synthesis of Th1 cytokines. In hydatid infections, both cell population profiles remain highly expressing, at least in cysts that survive the immune response. Elevated levels of IL-4, IL-5, IL-6, and IFN-γ are produced in vitro by peripheral blood mononuclear cells (PBMC) isolated from infected human subjects and stimulated by HCF antigens (213–219). Elevated cytokine levels were also measurable in the sera from hydatid disease patients with lung and liver involvement (249). The coexpression of IL-10 and IFN-γ at high levels in human hydatidosis suggests that the immune response to *E. granulosus* infection is possibly regulated by both Th1 (or Th0) and Th2 profiles. It is not understood why hydatid infection can induce high levels of both Th1 and Th2 cytokines since they usually down-regulate each other (193). It may be due to the very complex mixture of antigens in HCF (177), which probably contain distinct epitopes for each T-cell subset. However, the situation with human subjects is difficult to explain since the involvement of Th0 cells in a late chronic infection is rare (1).

In human subjects undergoing chemotherapy treatment, a

Th1 cytokine profile, rather than a Th2 profile, typically dominates (215). It has been suggested that this could be one of the killing mechanisms that set in during the later stages of infection (225). Significantly, increased production of IL-4 and IL-10 in hydatid disease patients corresponds to high levels of IgE and IgG4 (215). Therefore, both IL-4 and IFN-γ regulate the IgE and IgG4 responses (153, 154, 180). AE patients experiencing a relapse of the disease have a tendency to increased production of IL-5 but lower IFN-γ production accompanied by significantly higher levels of IgE and IgG4 compared to patients with a primary infection (94).

In addition to IL-4 and IL-10 production, two other Th2 cytokines, IL-5 and IL-6, are produced in large quantities by hydatid disease patients. IL-5 was shown to be specifically induced by parasite antigens in 90% of patients while control subjects were negative (219). Other studies have shown that IL-5 is associated with the regulation of specific IgE and IgG4 expression (219). In general, IL-5 also regulates the eosinophilic response (45, 125). However, some patients infected with *E. granulosus* (11%) (218) and most patients infected with *E. multilocularis* have limited eosinophilia (219, 244).

Sera from patients with active cysts have a range (2 to 500 U/ml) of concentrations of IL-6. The major role of this cytokine is to induce differentiation of B cells into plasma cells, thus contributing to the development of antigen-specific humoral responses (254).

CE patients with relapsing disease have high levels of IgE and IgG4, increased levels of IL-5, IL4, and IL-10, and low levels of IFN-γ produced in vitro by PBMC compared to patients with a primary infection (215, 219). Patients with a primary infection have higher levels of IL-2, IFN-γ, and IL-5. The high level of IL-5 is in agreement with the high levels of IgG4 and IgE observed (219). IFN-γ and IL-6 activities were undetectable in sera from two liver hydatidosis patients who relapsed (250). There is a significant correlation between IgE and IgG4 production in sera from patients with hydatid disease and a trend toward increased IL-4 and IL-10 levels in patients who are high producers of IgE and IgG4 (214).

When specifically stimulated with HCF antigen, PBMC from hydatid disease patients produced higher levels of IL-2 did than those from uninfected donors (125). The apparent bias toward a Th2 response appeared to be related to clinical status and was suggestive of a putative role of Th2-like responses in susceptibility to reinfection by *E. granulosus* (125). Clearly, these results merit further study.

Primary and secondary infections elicit similar responses, which include elevated levels of TNF-α, IL-1α, IFN-γ, IL-6, and IL-10 (111) and detectable levels of specific IgG1 and IgG3 isotypes (55). The levels of IgM and IgG2a are slightly increased following infection and remain elevated throughout the first 18 weeks of infection. During the 129- to 209-day period following the onset of infection, there is an increase in the level of secreted IL-10 and a slow decrease in the levels of IL-6 and IFN-γ. IgM, IgG, IgG1, and IgG2a levels plateau during this period, whereas IgG3 and TNF-α levels peak on day 190 postinoculation. These data suggest that induction of Th2 antibody-mediated immunity with a parallel expansion of Th1-mediated inflammatory responses is an important mechanism of host defense against the metacestode (111). Local inflammatory reactions to PSC at the site of injection are intense, involving neutrophils, eosinophils, macrophages, and mast cells (221).

Significantly higher levels of IL-10 (257) and IL-5 (222, 244) have been found in AE patients than in controls. In contrast, IL-4 was measurable in only a minority of patients and controls. IL-12 levels were comparable between AE patients and controls and showed a similar distribution pattern to IL-10 with regard to disease progression. These studies suggest that a Th2-dominated immune response occurs in AE in vivo. AE infection results in a strong Mac-1^+ cell infiltration of the peritoneal cavity and spleen (53). Peritoneal cells from mice infected with AE at the 1-month stage were rich in macrophages and expressed significantly higher levels of transcripts for the inflammatory cytokine IL-1β and for TNF-α and inducible nitric oxide synthase (257).

Inhibition of Cyst Growth

It is generally accepted that *Echinoccocus* is unaffected by the immune response during the developing stage. However, natural infections in sheep indicate that some cysts can be killed during the latter stages of development (275), with the relatively frequent occurrence of dead, calcified metacestodes or necrotic cysts. These are due to the primary cyst having degenerated, leaving the cavity full of host leukocytes and protoscolex-derived daughter cysts (224). There is no direct evidence that the death of such cysts is due to an immunological phenomenon, but it is a likely possibility. If a progression in cyst degeneration does take place, then the immune response may play a role in the death of the parasite. This may signify increased immunological stimulation with cyst progression. Unfortunately, there are no detailed studies of immunological events associated with the degeneration of different types of cyst, and it is therefore unknown which mechanisms may be involved. This is clearly an area for future study. One aspect that is likely to be important is the influence of $CD4^+$ Th lymphocytes on the control of such immunological mechanisms. In addition, IFN-γ and nitric oxide production may play a role (249).

Complement through C5-mediated effectors contributes to host defenses by both restricting the establishment of infection and controlling the growth of established cysts. This contribution may be associated with the ability of C5a to promote eosinophil infiltration (65, 266). Lysis in both immune and normal serum is antibody dependent and complement mediated (116). Protoscoleces of *E. multilocularis* and *E. granulosus* are lysed by fresh serum of many different species of mammals (121, 152). The presence of *Echinococcus* cysts appears to deplete host complement (121, 150, 151). The rapid development of *E. multilocularis* infection is associated with depletion of serum complement; the use of cobra venom factor to deplete complement results in faster growth of *E. multilocularis* cyst masses.

In hydatid infections, IL-6 seems to be produced nonspecifically (254) whereas IL-5 production appears antigen specific. The effect of IL-5 on human B cells is controversial (45), but a significant correlation between IL-5 production and IgE and IgG4 expression has been found in hydatid disease patients (219). When CE cysts grow, IgG1 and IgG4 levels are elevated, whereas the concentrations of specific IgG1 and IgG4 decline

TABLE 1. Proposed mechanisms of avoidance from host-protective responses by the metacestode stage of *Echinococcus*[a]

Mechanism	Reference(s)
Sequestration and antigenic disguise	33, 54, 67, 68, 170, 174
Molecular mimicry	68, 83
Antigen or DNA polymorphism	35, 39, 43, 98, 230, 235, 268–270
Production of proteases	66, 79, 175, 248, 260
Protease inhibitor	235
Interference with complement activity	44, 79
Alteration of macrophage and leukocyte funtion	16, 198, 205, 223
Alteration of lymphoid organ architecture	3, 4, 6, 7, 9, 220–222
Depletion of T lymphocytes	34, 155–157, 198, 255
Induction of suppressor cells	67, 155–157
Alteration of antibody responses	5, 28
Alteration of lymphocyte proliferative responses	26, 28, 74, 157, 198
Inhibition of effector cell chemotaxis	198, 212

[a] Modified from reference 256.

in cases characterized by cyst infiltration or calcification. This indicates that the IgG4 antibody response is also associated with cystic development and growth and with disease progression whereas the IgG1, IgG2 and IgG3 responses occur predominantly when cysts became infiltrated or are destroyed by the host (52).

In experimental infection, fewer than 10% of PSC survive to form cysts (194, 272). The majority of parasite killing occurs within the first 2 weeks postinfection. Activated macrophages are involved in the killing of *Echinococcus* PSC (8, 9, 24, 142). Studies in vitro indicate that macrophage-dependent killing of PSC can be increased by IFN-γ (148) and decreased by some cytokines such as IL-10 or IL-4 (142). Therefore, it seems likely that during a secondary infection, an initial Th0 or Th1 response effective in killing parasites becomes polarized to a Th2-type response and that this response seems less effective. This is supported by studies with patients undergoing albendazole chemotherapy who responded better to treatment when they possessed a more dominant Th1-type cytokine profile than when they had a more dominant Th2-type profile (125).

Evading the Immune System

The life span of hydatid cysts of *E. granulosus* can be as long as 53 years in humans (241) and 16 years in horses (228). The ability of the parasite to survive for such a long time in hosts with the potential to resist infection implies that the parasite possesses strategies for subverting or avoiding protective immune responses. Theoretically, there are two types of mechanisms to subvert the host immune response: passive escape, in which the parasite avoids the damaging effects of an immune response, and immunomodulation, which is an active interaction with the immune system to reduce the impact of a response to the parasite (211). Some of the proposed mechanisms of immune evasion used by the *Echinococcus* organisms (256) are summarized in Table 1.

A notable feature of the metacestode of *E. granulosus* is the formation of two capsules. One is the cyst-derived acellular laminated capsule. The other is the host fibrous capsule, which typically surrounds fully developed viable cysts of *E. granulosus* and is formed probably by filtration of eosinophils (240) and of fibroblasts and mesothelial cells (208). These structures protect the parasite both physically and from immune attack.

The initial response of the host abates with time and is minimal 6 months after infection (81). IFN-γ-, IL-2-, and IL-4-expressing cells could not be detected in lesions of the early phase of infection (26), possibly indicating host immunosuppression. Furthermore, about 30% of CE patients have undetectable antibody levels in their sera (40, 48, 216), a feature which also appears to occur in ovine infections (145, 169, 265). The mechanisms behind this are unclear. Circulating parasite antigen could be mopping up specific antibody, since both circulating antigen and immune complexes are detectable in some seronegative individuals (46). The possibility of antigen-induced specific immunological tolerance has also been raised, suggesting that antibody production during the course of the infection may be regulated, perhaps through periodic release of antigen from cysts and/or general down-regulation of B cells through Th-cell activity.

When using susceptible C57BL/6 mice, spleen cells supplemented with peritioneal cells from *E. multilocularis*-infected mice induced a complete suppression of splenic proliferation at the early and late stages of infection, and this suppression was reversed to a large extent by the addition of N^G-monomethyl-L-arginine and partially by anti-IFN-γ antobodies (53). Spleen cells from late-stage-infected mice express only background levels of IL-10 but greatly increased levels of inducible nitric oxide synthase. The immunosuppression observed in chronic AE is not primarily dependent on IL-10 but rather on nitric oxide production by macrophages from infected animals (53, 74).

Proliferative responses and IL-2 production induced by concanavalin A (ConA) in spleen cells from BALB/c mice are significantly depressed at an early stage after infection (157). With *E. multilocularis* PSC, addition of plastic-adherent cells from normal syngeneic mice to the nonadherent spleen cells from infected mice did not restore the depressed ConA responsiveness. On the other hand, exogenous IL-2 completely reconstituted the proliferative responses to ConA. Flow cytometry analysis revealed that the number of $CD4^-$ $CD8^+$ cells with a low density of CD8 antigen ($CD8^{dull}$ cells) increased in spleens from infected mice 2 weeks after inoculation. Addition of the spleen cell subpopulation containing the $CD8^{dull}$ cells, but not that depleted of the $CD8^{dull}$ cells, to normal spleen cells resulted in marked suppression of the ConA responses. These findings suggest that the $CD8^{dull}$ cells detected in the

spleens of mice inoculated with *E. multilocularis* PSC may play a key role in the suppressive regulation of immune responses (157).

Little is known about antigenic drift or shift in *Echinococcus* infections compared with some other parasites (50, 104, 105, 108–110). The production of proteases has been reported for a range of helminths and is considered important for the conversion of host tissues into nutrients (128), for host invasion (126), and for migration through host tissues (127). It is unknown whether proteases from *Echinococcus* function in the cleavage of IgG, as has been recorded in other helminths (19, 20, 158, 202, 203). Nevertheless, secretions from the penetration glands of hatched and activated oncospheres of *E. granulosus* cause lysis of host tissues; these secretions may protect the parasite against the host immune response while the laminated layer develops (126).

IMMUNITY IN THE DEFINITIVE HOST

Immune reactions of canid definitive hosts to *Echinococcus* infections have been comprehensively reviewed (113, 114). There is now quite an extensive literature, but until the 1980s, little research had been performed on immune responses to *Echinococcus* and other taeniid cestodes in their definitive hosts. This may be because the adult worms, being parasites of the gut lumen, were thought unlikely to evoke systemic immune responses and also because of the lack of knowledge of host-protective immune responses to reinfection with taeniid cestodes at the time (163). Mucosal immunity in animals is now recognized as an important phenomenon. In sheep, clearance of the parasitic nematodes *Trichostrongylus colubriforms* and *Haemonchus contortus* is associated with the sensitization of mucosal mast cells (MMC), measured by the release of sheep mast cell protease and the number of globular leukocytes, which are possibly degranulated by MMC (242). These cell types are more numerous in the regions of the gastrointestinal tract where these parasites reside (31).

An increase in the level of immune mediators, sheep mast cell protease larval migration inhibition components, and peptidyl leukotriene is correlated with the clearing of *T. colubriforms* infection (144) and a reduction in fecal egg counts (69). The secretion of leukotrienes and larval migration inhibition from MMC is thought to be a major mechanism of parasite removal (70). IgA and IgE are important in mucosal immunity since they bind directly to antigens and also attract effector cells that bind the constant region of the antibody. Eosinophils and MMC bind immunoglobulin constant regions via Fc receptors, becoming activated to degranulate when bound to the opsonized parasite. This method of antibody-dependent cell-mediated cytotoxicity is well established as an important mechanism by which the host can damage a multicellular parasite (211).

As reviewed by Heath (113, 114), the scolex of adult *Echinococcus* worms is normally in close contact with the canine intestinal submucosa, but mucosal immune responses, leading to the production of neutralizing IgA antibodies to deal with the secretions of the strobila, have no effect on the scolex. The scolex is in intimate contact with the systemic circulation, even in the Peyer's patches, and it appears to maintain its privileged integrity by suppression of cytotoxic and effector cell activity in the region of the scolex.

Experiments with immunosuppressed golden hamsters subsequently infected with *E. multilocularis* showed that worms developed faster than in normal animals (147). In addition, dogs that were immunosuppressed and then challenged with PSC of *E. granulosus* were shown to harbor more worms than did nontreated dogs, suggesting that the definitive host may have some innate resistance to infection by adult worms (D. Heath, personal communication).

Cells from Peyer's patches of dogs infected with *E. granulosus* produce specific immunoglobulin in vitro (61). Infection depresses the ability of unstimulated cells to proliferate in response to HCF protein but enhances the response to ConA (10). Dogs with enhanced reactivity to ConA and another mitogen, phytohemagglutinin, have significantly fewer worms and a lower number of mature worms than do dogs with less reactivity. After infection, the concentrations of IgG and IgA increased in serum and IgA levels increased in feces. Dogs with high-titer anti-HCF antigen serum antibodies were better protected than were dogs with low titers in serum (10).

VACCINES AND VACCINOLOGY

As indicated above, the life cycles of *E. granulosus* and *E. multilocularis* include two hosts: an intermediate host and a definitive host. Effective CE control programs show that prevention of transmission to either host can reduce or even eliminate the infection in human and livestock populations (see below). Therefore, if either or both hosts can be vaccinated, the effect will be to improve and more rapidly expedite control (116). The sylvatic nature of the life cycle of *E. multilocularis* makes a vaccination approach to control unlikely.

Vaccination of the Intermediate Host

Vaccination of the intermediate host is a burgeoning area that has moved forward considerably in recent years following the development of a recombinant vaccine against *Taenia ovis* infection in sheep (164). A similar approach has been applied successfully to develop a recombinant vaccine against *E. granulosus* (164–167). Earlier, a range of different antigens including cyst fluid (51, 62, 63, 118, 191), cyst membranes (191), and PSC (63, 124, 253) had been used as prototype vaccines against *E. granulosus.* However, oncospheres or oncospheral antigens induce much higher levels of protection in sheep (91, 117, 119, 189) and mice (56, 272) against challenge. One fraction (25 kDa) separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) from a crude preparation of oncospheral antigens was also shown to stimulate a similar level of resistance (117). Lightowlers et al. (167) used antibody prepared against this fraction to screen a cDNA library prepared from oncospheres; selected cDNA clones subsequently expressed in *Escherichia coli* as a fusion protein with glutathione *S*-transferase (GST) were tested for protective efficacy by vaccinating sheep and then challenging them with *E. granulosus* eggs (167). One 16.5-kDa recombinant protein (termed EG95)—50 μg of the GST fusion protein formulated in oil adjuvant or made up with Saponin, Quil A or ISA70 adju-

vant—elicited significant protection (mean, 96 to 98%) against the development of hydatid cysts (164, 167).

The immunity (mediated by complement-fixing antibodies) generated by two injections of the vaccine given 1 month apart persists for at least 12 months (115). Thereafter, annual vaccination of domestic livestock is recommended (115, 167). The shelf life of the formulated vaccine is at least 12 months (115). One liter of *Escherichia coli* culture can yield more than 10,000 vaccine doses, which means that the vaccine could be manufactured cheaply if produced on a large scale. Other noteworthy features of the EG95 vaccine are that immunity can be transferred passively to neonates with antibody from vaccinated dams (115) and that the protection induced is associated with conformational epitopes (261–264). Furthermore, the vaccine conferred a high degree of protection against challenge with different geographical isolates of *E. granulosus* (166), indicating that it could have wide applicability as a new tool for use in hydatid disease control campaigns. The vaccine therefore provides a valuable new tool to aid in the control of transmission of this important human pathogen and also has the potential to prevent hydatid disease directly through vaccination of humans. Recent research indicates that the EG95-encoding gene belongs to a gene family of six or more genes (43). A closely related protein (designated EM95) that can induce significant levels of protection against challenge infection with *E. multilocularis* eggs in mice has also been identified in *E. multilocularis* (90).

Vaccination of the Definitive Host

Compared with the major advances in vaccinating sheep against *E. granulosus,* attempts to vaccinate canid definitive hosts have yet to achieve a similar level of success. Nevertheless, a series of experiments to induce immunity in dogs through vaccination have been carried out, with some encouraging results. Irradiated *E. granulosus* PSC used in vaccine trials showed that the preparations could induce inhibition of the growth of worms in the canine intestine (14, 181, 182). Protoscoleces or antigens from PSC can stimulate dogs to reduce the numbers or suppress the growth of worms (92, 251, 252, 274). Protection was also obtained with other antigen sources, such as cyst fluid (14), cyst membranes (93, 251), adult worm extracts (92, 251), and worm secretions (122, 123). There is some evidence for the development of acquired immunity to *E. multilocularis* in foxes, although detailed knowledge is unavailable (71).

DIAGNOSIS

Early diagnosis of CE and AE can result in significant improvements in the quality of the management and treatment of both diseases. In most cases, the early stages of infection are asymptomatic, so that methods that are cheap and relatively easy to use are required for large-scale screening of populations at high risk. Immunodiagnosis provides such an approach and can also, confirm clinical findings.

The immunodiagnosis of echinococcosis has been comprehensively reviewed in a series of early articles by Schantz and Gottstein (232), Rickard and Lightowlers (209), Lightowlers and Gottstein (165) and Gottstein (100). Here, more recent progress in the development and application of specific diagnosis of *Echinococcus* infection in humans, animal intermediate hosts and definitive hosts is assessed. Readers should also refer to excellent recent comprehensive reviews of the field (192). Over the past decade, diagnosis of CE and AE has improved due to the use of new or more optimal methods for purification of *Echinococcus* antigens from somatic materials, by the application of molecular tools for parasite identification and the synthesis of recombinant diagnostic antigens and immunogenic peptides. These approaches have not only improved the sensitivity and specificity of tests for diagnosis of AE and CE but also allowed more reliable characterization of the biological status of parasite materials (reviewed in references 237 and 245).

Immunodiagnosis of Cystic Echinococcosis in Humans

The definitive diagnosis for most human cases of CE is by physical imaging methods, such as radiology, ultrasonography, computed tomography (CT scanning) and magnetic resonance imaging (165), although such procedures are often not readily available in isolated communities. Immunodiagnosis can also play an important complementary role. It is useful not only for primary diagnosis but also for follow-up of patients after surgical or pharmacological treatment. Detection of circulating *E. granulosus* antigens in sera is less sensitive than antibody detection, which remains the method of choice.

Hydatid serological testing has a very long history, and almost all serological tests that have been developed have been used in the diagnosis of human cases. There are considerable differences among the various tests in both specificity and sensitivity. As the sensitivity of a test increases, so generally does the demand for improved antigens so that sufficient specificity can be achieved to take advantage of the greater sensitivity. An optimum test should be specific with high sensitivity. Insensitive and nonspecific tests, including the Cassoni intradermal test, the complement fixation test, the indirect haemagglutination test, and the latex agglutination test, have been replaced by the enzyme-linked immunosorbent assay (ELISA), the indirect immunofluorescence antibody test, immunoelectrophoresis (IEP), and immunoblotting (IB) in routine laboratory application (165).

Chordi and Kagan (42) were the first to analyze antibody responses in human hydatid infection by IEP with sera from patients and HCF of sheep origin as antigen. Further extensive studies have also focused on HCF antigens that are still considered an invaluable source of antigenic material for immunodiagnosis (Table 2). This research evaluated the immunoreactivity of these antigens with sera from patients with hydatid infection and resulted in the development of new techniques for the preparation of purified antigens (160, 183, 186, 196, 200, 201, 259). Antigen prepared from human HCF was found to be unsuitable for diagnosis because it contains host proteins such as IgG (27). Sheep HCF obtained from fertile cysts has been used routinely to prepare and standardize antigen. Bovine HCF can be used as an alternative antigen source; indeed, it can improve diagnostic sensitivity (130, 206). HCF of camel origin has also been used as antigen in an ELISA format to measure total *E. granulosus*-specific IgG antibodies and IgG subclasses (206). The diagnostic value of measuring IgG1

TABLE 2. Features of assays for immunodiagnosis of CE based on HCF antigens[a]

No. of subjects tested			Antigen source	Assay method	Sensitivity (%)	Specificity (%)	Ig isotype	Reference
CE patients	Healthy subjects	Patients with other diseases						
204	90	53	HFF	IHA	54	100	Ig	188
119	37	54	HBLF	LA	86	87.9	Ig	23
204	90	53	HFF	IEP	31	100	Ig	188
70	30	73	ppHCF	ELISA	89	40.8	IgG	143
204	90	53	HFF	ELISA	72	97	IgG	188
90	28	88	FBHCF	ELISA	84	60	IgG	22
111 (Li)	0	0	sWHF	ELISA	89	ND	IgG	21
122 (Lu)	0	0	sWHF	ELISA	78	ND	IgG	21
35	200	339	HCF	ELISA	91	82.3	IgG	199
52p	200	339	HCF	ELISA	96	82.3	IgG	199
119	37	54	FBHCF	ELISA	83	86.8	IgG	23
204	90	53	HFF	IB	80	96	IgE	188

[a] Abbreviations: HFF, hydatid fluid fraction, rich in Ag5 and AgB; HBLF, heparin-binding lipoprotein fraction; ppHCF, partially purified HCF; FBHCF, fertile bovine hydatid cyst fluid; Li, liver; Lu, lung; sWHF, sheep whole-hydatid-cyst fluid; IHA, indirect hemagglutination assay; LA, latex agglutination assay; ND, not done.

(97.7%), as assessed by a rating index (J) for combined sensitivity and specificity, was superior to the use of total IgG (65.1%) and IgG2 to IgG4 (77.8, 57.9, and 39.6%, respectively) (206). These findings have set the stage for field evaluation of the IgG1 assay in areas where human CE is endemic.

The lipoproteins antigen B (AgB) and antigen 5 (Ag5) (185), the major components of HCF, have received the most attention with regard to diagnosis. Along with HCF, they are the most widely used antigens in current assays for immunodiagnosis of CE. Both antigens have been well characterized by immunoblotting and/or by immunoprecipitation of radiolabeled antigen and SDS-PAGE (13, 168, 234, 236).

Antigen B. AgB is a polymeric lipoprotein with a molecular mass of 120 kDa (185). It can be measured in patient blood as circulating antigen (149, 171), and it has been suggested that it plays an important role in the biology of the parasite and its relationship with the host (212, 235). AgB is a highly immunogenic molecule (42, 186), a characteristic that underpins its value in serodiagnosis (Table 3). It appears ladder-like under reduced condition on SDS-PAGE, with three bands with molecular sizes of approximately 8 or 12, 16, and 24 kDa (42, 162, 168, 186, 236), suggesting that it comprises polymers of 8-kDa subunits (95, 168). The smallest subunit has proved the most useful target in diagnostic studies (188, 229).

TABLE 3. Features of assays for immunodiagnosis of CE using native AgB[a]

No. of subjects tested			Antigen	Assay method	Sensitivity (%)	Specificity (%)	Ig isotype	Reference
CE patients	Healthy subjects	Patients with other diseases						
204	90	53	Gel-EF	ELISA	74	100	IgG	188
90	28	86	pp	ELISA	77	85	IgG	97
191	50	133	pp	ELISA	79	98	IgG	233
81		98	pp	ELISA	89	86	IgG	258
31	29	87	AEC	ELISA	77	82	IgG	229
90	28	88	MAb-AP	ELISA	77	86	IgG	22
81		98	pp	ELISA	58	92	IgG1	258
191	50	133	pp	ELISA	57	100	IgG1	233
81		98	pp	ELISA	53	94	IgG2	258
81		98	pp	ELISA	46	95	IgG3	258
210	47	79	pp	ELISA	63	81	IgG4	178
191	50	133	pp	ELISA	38	99	IgG4	233
81		98	pp	ELISA	73	91	IgG4	258
210	47	79	pp	ELISA	63	81	IgG4	178
69	82	63	18 kDa	IB	10	77	IgG	143
35	200	339	8 kDa	IB	71	97	IgG	199
52p	200	339	8 kDa	IB	60	97	IgG	199
173	29	66 (AE)	8/29/34 kDa	IB	85	65	IgG	132
35	200	339	8/29/34 kDa	IB	91	94	IgG	199
52p	200	339	8/29/34 kDa	IB	81	94	IgG	199
204	90	53	Gel-EF	IB	66	100	IgE	188
158	29	152	pp	IB	92	69	IgG	132
173	29	115	pp	IB	92	100	IgG	132
210	47	79	pp	dELISA	93	65	IgG	178

[a] Abbreviations: Gel-EF, eluted fractions from SDS-PAGE; pp, partial purification; AEC, anion-exchange chromatography; MAb-AP, affinity purification by monoclonal antibody; p, posttreatment; dELISA, dot ELISA.

TABLE 4. Features of assays for immunodiagnosis of CE based on using recombinant AgB and AgB peptides[a]

No. of subjects tested			Antigen	Assay method	Sensitivity (%)	Specificity (%)	Ig isotype	Reference
CE patients	Healthy subjects	Patients with other diseases						
210	47	79	rAgB.MBP	ELISA	65	91	IgG4	178
64	39	105	EG55-GST	ELISA	89	72	IgG	120
31	29	87	rAgB8/1	ELISA	55	80	IgG	229
31	29	87	rAgB8/2	ELISA	84	98	IgG	229
119	44	123	rEgPS-3-GST	ELISA	74	87	IgG	161
90	28	86	P176	ELISA	80	93	IgG	97
90	28	86	P175	ELISA	49	94	IgG	97
90	28	86	P177	ELISA	38	92	IgG	97
90	28	86	P65	ELISA	44	96	IgG	97
90	28	86	Gu4	ELISA	18	98	IgG	97
31	29	87	GU4	ELISA	26		IgG	229
90	28	88	p65#	ELISA	34–48	80–97	IgG	22
90	28	88	pGU4#	ELISA	12–18	96–100	IgG	22
210	47	79	rAgB.MBP	dELISA	74	88	IgG	178
25	9	8	p65	dELISA	64	100	IgG	161
204	90	53	rAgB-GST	IB	72	100	IgG	188

[a] Abbreviations: dELISA, dot ELISA; rEgPS, recombinant PSC protein; rAgB, recombinant AgB; MBP, maltose binding protein; #, coated with different buffer.

To date, several AgB cDNAs have been cloned, expressed as recombinant proteins, and used for diagnosis; in addition, a number of AgB peptides have been synthesized and used in ELISA for diagnostic purposes (Table 4). Peptide antigens have been considered for use to enhance specificity, and efforts have been made to define discrete epitopes of AgB and other molecules that could be mimicked by synthetic peptides. Shepherd et al. (235) cloned a C-terminal fragment from a PSC cDNA library and expressed this as a 12-kDa protein (235); the complete sequence, termed EgAgB8/1, was cloned subsequently (82). The sequence is highly conserved (82), a fact that underscores its utility for application in immunodiagnosis. Subsequently, another fragment named EgAgB8/2 was also proposed to be an 8-kDa subunit, with 38% similarity to the EgAgB8/1 clone (77).

Another recombinant clone, EG55, also corresponding to the smallest subunit of AgB, was expressed as a GST fusion molecule and tested in a sandwich ELISA for its ability to detect specific serum antibodies in CE patients (Table 4). The antigen cross-reacted mainly with sera from AE parients (39.2% subjects reacted) (120). A 12-kDa EgPS-3 recombinant antigen, also corresponding to the smallest subunit of AgB, was similarly tested in ELISA as a GST fusion protein for diagnosis of human CE; 74% of patients recognized the protein, but there was cross-reactivity with sera from AE- and *Schistosoma japonicum*-infected patients (161). The EgPS-3 recombinant antigen was explored further for diagnostic value with three synthetic peptides prepared based on its predicted amino acid sequence; p65, a 27-mer peptide corresponding to residues 12 to 39 of AgB8/1, showed increased specificity but slightly reduced sensitivity in ELISA (161) (Table 4).

In further efforts to standardize CE diagnosis, Barbieri et al. (22) compared the diagnostic value of p65 and GU4, a 34-mer synthetic peptide corresponding to the C-terminal end of the AgB8/2 subunit (Tables 3 and 4) with that of p89-122, a synthetic peptide derived from Ag5 (Table 5). The p65 peptide provided three- to fourfold higher sensitivity but 30% lower specificity than the other two peptides. A further study showed that a highly antigenic region of AgB resides in the N-terminal extension of the AgB8/1 subunit; an ELISA based on the use of a single peptide designated p176, a 38-mer peptide from the N -terminus of the AgB8/1 subunit, exhibited a diagnostic

TABLE 5. Features of assays for immunodiagnosis of CE based on using native, recombinant and synthetic peptides of Ag5[a]

No. of subjects tested			Antigen	Assay method	Sensitivity (%)	Specificity (%)	Ig isotype	Reference
CE patients	Healthy subjects	Patients with other disease						
35	200	289	Arc5	IEP	63	97.2	Ig	199
52p	200	289	Arc5	IEP	58	97.2	Ig	199
90	28	88	MAb-AP	ELISA	50	92	IgG	22
39	29	51	MAb-AP	ELISA	54	89	IgG	96
90	28	88	P89-122#	ELISA	14–21	77–100	IgG	22
39	29	51	rP-29	ELISA	61	80	IgG	96
39	29	51	P89-122#	ELISA	44	100	IgG	96
111 (Li)	ND	ND	pAg 5	ELISA	89	ND	IgG	21
122 (Lu)	ND	ND	pAg 5	ELISA	78	ND	IgG	21

[a] Abbreviations: Li, liver; Lu, lung; MAb-AP, affinity purified using monoclonal antibody; #, coated with different buffer; pAg5, purified Ag5; ND, not determined; p, posttreatment.

performance that was superior to that obtained by the use of native AgB (97). The results of this and other studies investigating the sensitivity and specificity of recombinant AgB and AgB peptides in CE diagnosis are summarized in Table 4.

Antigen 5. Ag5 is a very-high-molecular-mass (approximately 400-kDa) lipoprotein complex composed of 57- and 67-kDa components that under reducing conditions dissociate into 38- and 22- to 24-kDa subunits (168). Historically, one of the most widely used immunodiagnostic procedures for CE was the demonstration of serum antibodies precipitating antigen 5 (arc 5) by immunoelectrophoresis or similar techniques. Early work suggested absolute diagnostic specificity for detecting *E. granulosus* infection, but subsequent studies showed that antigen 5 is cross-reactive with human antibodies to other taeniid cestodes, most notably *E. multilocularis* and *Taemia solium,* and, indeed, other helminths (236).

Using pooled hydatid disease-specific sera highly reactive with Ag5, a partial cDNA sequence termed Eg6 was isolated (76). The recombinant protein fragment encoded by the sequence was recognized by a monoclonal antibody specific for Ag5 (38). In addition, antibodies eluted from this recombinant protein recognized the 38-kDa subunit of Ag5. Another clone, designated Eg14, was selected and shown to code for an amino acid sequence partially homologous to Eg6 identified with the same monoclonal antibody. Using Eg6 sequence primers, a novel sequence coding for a 29-kDa antigen (termed P-29) was amplified from PSC of *E. granulosus.* The sequence has 100% identity to the amino acid sequence encoded by Eg6. Additional work has shown that P-29 and Ag5 are immunologically related but are nevertheless different proteins, raising questions about the current state of knowledge of Ag5 (96). Results of studies investigating the sensitivity and specificity of native and recombinant Ag5 and Ag5 peptides in CE diagnosis are summarized in Table 5.

Limitations of Current Tests

Although AgB and Ag5 have proved to be diagnostically valuable, there are difficulties related to their lack of sensitivity and specificity and problems with the standardization of their use (21). Cross-reactivity with antigens from other parasites, notably other taeniid cestodes (165, 171, 188, 199, 229), is a major problem. In addition, results from one- and two-dimensional electrophoresis and microsequencing have suggested that AgB and Ag5 are composed of a family of proteins in cyst fluid, which may complicate their use in diagnosis (271). Furthermore, the older traditional methods still used to purify AgB and Ag5 may limit the purity of the antigens. *Echinococcus* antigenic components have been isolated and purified through the application of a variety of methods, such as anion-exchange chromatography (13, 95), affinity purification with protein A or monoclonal antibodies (95, 101, 271), isoelectric focusing (132), and affinity chromatography (23, 271).

One recent study highlights the need to standardize techniques and antigenic preparations and to improve the performance of immunodiagnosis by characterizing new antigens and detecting distinct immunoglobulin classes. The diagnostic sensitivity and specificity of IEP, ELISA, and IB in detecting IgG antibodies to native and recombinant AgB and a hydatid fluid fraction in patient sera were compared (188). Sera tested were from patients who had CE grouped according to their type of cysts, from patients with other parasitic diseases, lung or liver carcinomas, or serous cysts, and from healthy controls. Hydatid fluid fraction-IB gave the highest sensitivity (80%) followed by ELISA (72%) and IEP (31%). The diagnostic sensitivity decreased significantly as cysts matured (from type I–II to type VII, classified by ultrasonography). Recombinant and native AgB-IB yielded similar sensitivities (74%), but a large number of clinically or surgically confirmed CE patients (20%) were negative. In these patient sera, the use of IB to assess the usefulness of another recombinant *E. granulosus* molecule (elongation factor 1β/δ [EF-1β/δ]) in detecting IgE antibodies yielded 33% positivity (188).

The results of this and other studies suggest that hydatid serological testing may be improved by combining several defined antigens (including synthetic peptides) and by designing new *E. granulosus*-specific peptides that react with otherwise false-negative sera.

Diagnosis for Monitoring Treatment of Cystic Echinococcosis in Humans

Patients with CE need to be carefully monitored after surgery or drug treatment to ensure that they remain free from infection and disease. Antibody detection is a valuable method of monitoring a patient after treatment. In patients from whom cysts have been removed successfully, the IgG4 subclass becomes negative soon after surgery (107). In contrast, patients with relapsing disease maintain high IgG4 titers in ELISA (107, 215, 219), which suggests that the IgG4 subclass is a good marker for hydatidosis follow-up. Specific IgE and IgM ELISAs are also useful in this respect (215, 219, 267).

PBMC isolated from CE patients can be driven in vitro by HCF antigens (125, 216) to produce large amounts of cytokines. IL-4 detection may be useful in the follow up of patients with CE. Furthermore, this can be combined with reverse transcriptase PCR to determine the mRNA expression of cytokines in PBMC to complement the biological assays in the follow-up (213). Detection of circulating antigens is also relevant as a method of post-surgical follow-up of patients and for monitoring the growth dynamics and/or the activity of cysts (78, 165, 207).

EF-1β/δ, a parasite protein present both in PSC and hydatid fluid, is a sensitive marker of infection (176). The higher percentage of humoral immune responses to EF-1β/δ observed in CE patients with calcified cysts than in patients with active cysts suggests that the protein is released into the hydatid fluid after the degeneration of PSC and indicates its possible use in immunosurveillance of CE. Furthermore, EF-1β/δ may play a key role in the allergic disorders (urticaria, itching, and anaphylactic shock) that often complicate the course of CE (176, 187).

Brief Comments on the Diagnosis of Alveolar Echinococcosis in Humans

The diagnosis of AE is based on similar findings and criteria to those for the diagnosis of CE. These include case history, clinical findings, morphological lesions identified by imaging techniques, PCR, or immunofluorescence/immunohistochem-

TABLE 6. Features of assays for immunodiagnosis of AE using different antigens[a]

No. of subjects tested			Antigen	Assay method	Sensitivity (%)	Specificity (%)	Ig isotype	Reference
AE patients	Healthy subjects	Patients with other disease						
37	37	95	pAP	ELISA	100	100	IgG	207
44	30	99	Em18	ELISA	91	89.1	IgG	143
140	500	348	Em2	ELISA	89.3	98.0	IgG	103
74	39	95	rEm10	ELISs	93.2	96.5	IgG	120
140	500	348	II/3–10	ELISA	86.4	96.8	IgG	103
140	500	348	Em2^{plus}	ELISA	97.1	90.2	IgG	103
28		72	rEm13	ELISA	82.1	100	Ig	84
66	29	259	Em18	IB	97	96.9	IgG	132
33	82	99	Em18	IB	91	92.3	IgG	143

[a] See the text for details.

istry, and immunodiagnosis. The area has been comprehensively reviewed (192). Like CE, serodiagnosis of AE provides a complementary role to other procedures in early detection of the infection. The methods are similar to those used for CE, but serological tests for antibody detection are generally more reliable. AE is a very serious disease with a high fatality rate, so that early detection is vital for successful management and treatment (15).

Em2, a species-specific native antigen isolated from the metacestode of *E. multilocularis* (101), has been used for immunodiagnosis of human AE with encouraging results (99). The sensitivities of Em2 in ELISA varied depending on the geographical origin of the patient; they ranged between 77 and 92% (103). The Em2^{plus} ELISA, a combination of Em2 with a recombinant protein designated II/3-10, increased the sensitivity to 97%. The Em2^{plus} assay exhibits cross-reaction with CE (in 25.8% of cases), which is higher than for the individual Em2 (5.6%) and 11/3-10 (6.5%), but limited cross-reactivity with other diseases. The Em2^{plus} ELISA has been commercialized for clinical diagnosis of AE (103) and for population screening (32). More recently, an 18-kDa antigen (Em18) from PSC of AE was reported as being a highly species-specific (96.8%) and sensitive (97%) antigen with potential not only for differentiation of AE from either CE or other helminth infections but also for differentiation of active from inactive AE (131–136, 143). Both Em2^{plus} ELISA and Em18 in an IB format have been used for long-term follow-up monitoring of AE patients following pharmacological treatment (173). Furthermore, Em13 and Em10, recombinant proteins expressed from cloned cDNAs from PSC of *E. multilocularis,* are also valuable in serodiagnosis of AE (Table 6). A purified alkaline phosphatase from *E. multilocularis* metacestodes has been shown to have exceptional diagnostic characteristics, with 100% specificity without any decrease in sensitivity (100%), and has significant potential for use in routine diagnosis and follow-up of AE patients (231).

Immunodiagnosis of Cystic Echinococcosis in Animals

In comparison with investigations in humans, relatively little research has been directed toward the development of immunodiagnostic techniques for *E. granulosus* infection in domesticated animals such as sheep and cattle. Currently, the diagnosis of CE in intermediate hosts is based mainly on necropsy procedures. Accurate serological diagnosis of CE infection in livestock is difficult due to serological cross-reactions with several other species of taeniid cestodes including *Taenia hydatigena* and *Taenia ovis* (165, 266). Furthermore, natural intermediate host animals produce very poor antibody responses to infection compared with the relatively high levels of specific antibody seen in human infection (165). In sheep, the principal intermediate host of *E. granulosus* in most regions of endemic infection worldwide, antibodies to various antigens including Ag5 are detectable in the sera of some but not all infected sheep ("nonresponders") (139). As with human CE, detection of circulating antigen does not appear to be useful for diagnostic purposes (71).

ELISA techniques using a variety of antigens have been applied to the immunodiagnosis of animal CE (138, 141, 266). In experimentally infected sheep, antibodies to hydatid antigens can be detected as early as 4 to 6 weeks postinfection (266) and persist for at least 4 years (138). However, as referred to above, serological cross-reactions between *E. granulosus* and other cestodes limit the specific diagnosis of hydatid infection by ELISA with crude parasite antigens (138, 266). Affinity purification of crude antigens with antibodies from animals immunized with homologous antigen (141) or affinity depletion of cross-reactive antigens with monoclonal antibody (138) only partly reduces the cross-reactivity. Components of ovine HCF can bind to sheep immunoglobulin nonspecifically and contribute to false-positive reactions, even with sera from cestode-free animals (138). After affinity depletion of crude antigen with both monoclonal antibody and sheep immunoglobulin from animals not infected with hydatid disease, background reactions were greatly reduced. Using this affinity-depleted antigen, it was possible to differentiate serologically between a flock of sheep with hydatid infection and uninfected sheep from the same locality; however, specific diagnosis of infection in individual sheep from another locality was low, and variation in antibody responses to different parasite strains was suggested as a possible cause of these differences (138).

Polysaccharide antigens from either the secretions produced during in vitro cultivation of *E. granulosus* PSC or from mouse hydatid cyst membranes by phenol extraction have been used to test sera from sheep (140). Although the antibody responses were significantly higher than those of sheep infected with *T. hydatigena* or *T. ovis,* very high cross-reacting antibody responses in the sera from *T. hydatigena*-infected animals were detected with the antigenic secretions from PSC. Neither an-

TABLE 7. Dynamics of the coproantigen ELISA for diagnosis of CE and AE in canine hosts

Host/no. tested	Species	No. of parasites detectable	Sensitivity (%)	Specificity (%)	Reference
Dogs/94	*E. granulosus*	3–10,000	61.5	91	72
	E. granulosus	>20	87.5		72
	E. granulosus	>100	100		72
Dogs/242	*E. granulosus*	Variable	63	97	60
	E. granulosus	<100	29		60
	E. granulosus	>100	92		60
Dogs/15	*E. granulosus*	Variable	56	98[a]	59
	E. granulosus	>200	87		59
	E. granulosus	<200	10		59
Dogs/660	*E. multilocularis*			99.5	57
Dogs/661	*E. multilocularis*	Variable	80	99.5	58
Foxes/55	*E. multilocularis*	4–60,000	83.6		57
	E. multilocularis	>20	93.3		57
Foxes/20	*E. multilocularis*			95	58
Foxes/35	*E. multilocularis*	Variable	80		58
	E. multilocularis	>55	93		58
Foxes/59	*E. multilocularis*	Variable	79.7	98[a]	59
	E. multilocularis	>1,000	100		59
	E. multilocularis	<1,000	73.9		59
Cats/263	*E. multilocularis*			98	57

[a] From 155 carnivores including dogs, foxes, and cats.

tigen was sufficiently sensitive or specific for routine serodiagnostic use (140).

DNA techniques are now available that allow the identification of *Echinococcus* species and of *E. granulosus* strains by using metacestode material from intermediate hosts (245).

Diagnosis of Echinococcosis in Definitive Hosts

Two major diagnostic methods have been extensively used in dogs: purgation with arecoline compounds and necropsy of the small intestine. Necropsy is the method of choice for foxes and other final hosts. Two main immunodiagnostic approaches have been developed for diagnosis of *E. granulosus* and *E. multilocularis* infection in definitive hosts: assays for specific serum antibody and detection of parasite products (coproantigens) in feces.

Specific serum antibodies were shown to be detectable in the blood of dogs after experimental infection with taeniid cestodes, including *E. granulosus,* using metacestode antigen preparations in ELISA (138–141). Subsequently, others confirmed the appearance of specific antibodies, following experimental infection with *E. granulosus* in dogs, detected using antigens derived from the oncosphere (25, 238, 239). These latter results suggested that some of the eggs released into the small intestine, following apolysis of proglottids, may hatch and penetrate the intestinal wall, resulting in immunological stimulation of the host. Detection of circulating anti-Em2 antibodies by ELISA may be useful for primary screening of fox populations, but antibody prevalence does not correlate with the actual prevalence of the *E. multilocularis* intestinal infection (58, 102). Overall, the available ELISA-based methods have poor sensitivity, the specificity is unclear, and there is no correlation with worm burden (85–89); therefore, their usefulness, other than in population-based studies of canine hosts, is questionable.

The other major approach to diagnosis of *Echinococcus* infection in the definitive host is through detection of adult worm products in feces by using the sandwich ELISA. Craig et al. (47, 49) used a monoclonal antibody specific for an antigen on the surface of *E. granulosus* oncospheres to distinguish *E. granulosus* eggs in perianal swabs or samples from environmental sites. This indirect immunofluorescence test is relatively cumbersome, and its sensitivity can be affected by the periodic absence of eggs in fecal samples (47). Nevertheless, the approach has been developed further and used successfully by a number of groups to detect coproantigens of *Echinococcus* spp. in canine host feces by antibody capture ELSA (11, 59); at least two commercial ELISA kits are now available. Although the various tests that have been developed show some cross-reactivity with other cestode infections (72), they exhibit a high probability of correlation with current infection (57, 59, 137). Further, these tests are capable of detecting patent and prepatent infections with a high degree of sensitivity and specificity, making them reliable tools for epidemiologic investigations (11, 57–60, 72, 184). Some details of investigations showing the sensitivity and specificity of the coproantigen ELISA are shown in Table 7.

There is also interest in detecting parasite DNA (copro-DNA) in fecal samples. No test is available for *E. granulosus,* but a PCR-based assay has been developed for detecting DNA of *E. multilocularis* in fecal samples of foxes after isolation of the parasite eggs by a sieving procedure (58); in a total of 55 infected foxes, the specificity was 100% and the sensitivity was 94%. For field application, the coproantigen ELISA has the potential for replacing necropsy examinations, and the copro-PCR is a valuable method for confirmation of positive coproantigen results and for diagnosis in individual animals (58).

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