MINIREVIEW

Recent advances in the immunology and diagnosis of echinococcosis

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Abstract

Echinococcosis is a cosmopolitan zoonosis caused by adult or larval stages of cestodes belonging to the genus Echinococcus (family Taeniidae). The two major species of medical and public health importance are Echinococcus granulosus and Echinococcus multilocularis, which cause cystic echinococcosis and alveolar echinococcosis, respectively. Both cystic echinococcosis and alveolar echinococcosis are serious diseases, the latter especially so, with a high fatality rate and poor prognosis if managed inappropriately. This review highlights recent advances in immunity to infection and vaccination against both parasites in their intermediate and definitive hosts and procedures for diagnosis of cystic echinococcosis and alveolar echinococcosis, including the value of immunodiagnostic and DNA approaches. There is discussion also of progress in genomics and related technologies that is providing valuable insights on the functional biology of the Echinococcus organisms. These studies will underpin future research that will reveal a better understanding of the Echinococcus-host interplay, and suggest new avenues for the identification of additional targets for diagnosis, vaccination and chemotherapy.

Introduction

Echinococcosis is a cosmopolitan parasitic 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 Echinococcus multilocularis, which cause cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively. E. granulosus has a cosmopolitan distribution (McManus et al., 2003) and E. multilocularis, which is distributed in the northern hemisphere, is recognized as an emerging zoonosis in some regions of Europe and Eurasia (Craig, 2003).

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 a parasite-derived 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. Sexual maturity of adult E. granulosus occurs in the host small intestine within 4–5 weeks of ingesting offal containing viable PSC. Gravid proglottids or released eggs are shed in the faeces 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. Since the life cycle relies on carnivores eating infected herbivores, humans are usually a ‘dead-end’ for the parasite. 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 metacestode of E. multilocularis 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 (Eckert & Plazes, 2004). CE and AE are
both serious diseases, the latter especially so, with a high fatality rate and poor prognosis if careful clinical management is not carried out.

In contrast to E. multilocularis, which appears to exhibit very limited genetic variation, an important feature of the biology of E. granulosus is that it comprises a number of intraspecific variants or strains that exhibit considerable variation at the genetic level. 10 distinct genetic types (genotypes G1–10) have been identified and this categorization follows very closely the pattern of strain variation emerging based on biological characteristics. The extensive variation in nominal E. granulosus may influence life cycle patterns, host specificity, development rate, antigenicity, transmission dynamics, sensitivity to chemotherapeutic agents and pathology with important implications for the design and development of vaccines, diagnostic reagents and drugs. A detailed account of genetic variation in Echinococcus and its implications can be found in (McManus, 2006), so this important topic will not be considered further here.

In our earlier review on the immunology and diagnosis of echinococcosis (Zhang et al., 2003a), we considered immunity to infection in the intermediate and definitive hosts, innate resistance, evasion of the immune system, development of vaccines for use in intermediate and definitive hosts and, in particular, we emphasized procedures for diagnosis of CE and AE, including the value of immunodiagnostics and molecular approaches. Here we provide an update of recent progress in research on E. granulosus and E. multilocularis, especially highlighting advances in immunology, vaccine development, diagnosis and functional expression of key molecules.

**Immunity to infection in the intermediate host**

Although the host–parasite interplay, in most cases of echinococcosis appears to be harmonious and clinically asymptomatic for a long period after infection, the host does produce a significant immune response against the early stages of infection, while the parasite adapts highly effective evasive strategies to aid in survival.

**Cystic echinococcosis (CE)**

Clinical symptoms of CE reflect the presence of one or more unilocular fluid-filled cysts. About 70% of cysts are formed in the liver, followed by the lungs (20%), with the remainder involving other organs such as kidney, spleen, brain, heart and bone. Clinical manifestations are mild in the early stage, while as the cyst gradually grows, the parasite may physically damage tissues and organs, which can become dysfunctional at the later stages of echinococcosis. As discussed previously (Zhang et al., 2003a), immune responses against early E. granulosus infection have mainly been investigated using experimental infections of mice and sheep. After the oncosphere locates a target organ, the small hydatid cyst that commences development is immediately confronted by the host immune responses, which are mainly cell-mediated, especially involving infiltration of macrophages and eosinophil cells, and low-level polarized Th1 responses. Antibody responses are weak and are, normally, undetectable in the early two to three weeks following infection.

There are extensive data on immune responses against the established cyst both from studies on patients with echinococcosis and from experimentally infected animals (Zhang et al., 2003a). The established parasite produces significant quantities of antigens that modulate the immune responses and these include polarized Th2 responses, balanced with Th1 responses. The coexistence of elevated Th1 cytokines, especially interferon (IFN)-γ, and Th2 cytokines including IL-4, IL-5, IL-6 and IL-10, has been recorded in most hydatid patients where cytokine levels have been measured. In addition, IgG, especially IgG1 and IgG4, IgE and IgM are elevated as the cyst grows and becomes established. When a cyst dies naturally, is killed by chemotherapy treatment or is removed by surgery, Th2 responses drop rapidly, and Th1 responses become dominant. IgG levels can be maintained in humans for several years after the cyst has been removed. Once a patient suffers relapse, the Th2 responses regenerate very quickly.

In the early stages of echinococcal development, cellular responses may play a crucial role in protection against infection. A repeat challenge experiment showed that mice given a second oncospheral challenge 21 days after the primary infection with E. granulosus produced very high levels of protection (Zhang et al., 2001) but with a very low antibody response (Zhang et al., 2003d) at the time of the secondary challenge. Early experiments in vitro showed that neutrophils, in association with antibody, can bring about the killing of E. granulosus oncospheres (Rogan et al., 1992), suggesting a possible role for antibody-dependent cell-mediated cytotoxicity (ADCC) reactions although antibody levels against this stage are low and/or cell-mediated immunity may induce killing. This is an important area that needs to be further explored as it may provide an understanding of the mechanisms of protection against the oncosphere with benefits for future vaccine design.

A remarkable feature of CE infection is the coexistence of both Th1 and Th2 responses. It is likely due to the presence on echinococcal antigens of distinct epitopes for each T-cell subset as it has been shown that a single recombinant protein can stimulate both types of response. The C-terminal region of a heat shock protein, Eg2HSP70, induced significantly greater amounts of tumor necrosis factor (TNF)-α, IFN-γ, and IL-10 in Eg2HSP70-stimulated peripheral blood mononuclear cells (PBMC) from CE patients compared with unstimulated cultures in all patients (Ortona...
et al., 2003). Furthermore, the antigens EgA31 and EgTrp stimulated a significant amount of IL-12, IFN-γ, IL-10 and IL-6 cytokines in cytokine-producing splenocytes of BALB/c vaccinated with the two molecules compared with controls (Fraize et al., 2005).

The polarized T-cell responses are modulated by echinococcal antigens. Dematteis et al. (2003) analysed whether the cytokine responses in early and late experimental infection with E. granulosus depend on the dose of parasites to which the host is exposed. To this purpose BALB/c mice were inoculated intraperitoneally (i.p.) with either 500 or 2000 protoscoleces. Splenocytes of mice were obtained at days 3, 7, 14 and 21 and also on week 37 post-infection and were cultured in vitro with protoscolex antigens. Type-1 and type-2 cytokines were analysed in supernatants by enzyme-linked immunosorbent assay (ELISA). The lower number of protoscoleces induced an early type-0 cytokine response, whereas the inoculation of 2000 protoscoleces induced an early Th2 response. Parasite growth was lower in the group inoculated with the low infective dose, which stimulated type-0 cytokine responses that may be protective, while more protoscoleces may inhibit the protective Th0 or Th1 responses, invoking Th2 responses, which may be beneficial for parasite growth.

To further investigate the role of T lymphocytes in the immune response to E. granulosus, Rigano et al. (2004) generated T-cell lines from patients with active, transitional and inactive hydatid cysts and stimulated these using sheep hydatid fluid (SHF) and antigen B (AgB). The cell lines from a patient with an inactive cyst had a Th1 profile, while the T-cell lines derived from seven patients with active and transitional hydatid cysts had mixed Th1/Th2 and Th0 clones. The results showed that Th1 lymphocytes contribute significantly to the inactive stage of hydatid disease, with Th2 lymphocytes being more important in the active and transitional stages. This group (Rigano et al., 1995) had previously shown that, in human subjects undergoing pharmacological treatment with albendazole/mebendazole, a Th1 cytokine profile, rather than a Th2 profile, typically dominates, indicating that Th1 responses have a role in the process of cyst degeneration. An increased Th1-type cytokine IFN-γ response has been suggested as a marker for monitoring AE patient treatment (Dvoroznakova et al., 2004), whereas measurement of serum IL-4 may be a useful marker for the follow up of patients with CE (Rigano et al., 1999).

Ultrasound can be used to classify cysts into different clinical types according to the progression of cyst status (Wang et al., 2003). There are no data showing cytokine profiles associated with these cyst categories and this is clearly an area for future study. One aspect that is likely to be important is the influence of CD4+ T-helper lymphocytes on the control of such immunological mechanisms as they may impact on treatment (Vuitton, 2004). Self-cure is common for CE infection in sheep (Zhang & Zhao, 1992; Cabrera et al., 2003), and it most likely also happens in human populations in hyperendemic areas as patients with calcified cysts are often reported (Macpherson et al., 2004; Moro et al., 2005). Cytokines are likely to play a key role in the process of self-cure.

Hypersensitive immune responses/allergic reactions (urticaria, itching and anaphylactic shock) often complicate the course of CE. Some CE patients have elevated IgE levels to certain antigens, such as EgEF-1β/δ, AE21 and EgTeg, that appear to be associated with these allergic reactions (Colebrook et al., 2002; Ortona et al., 2003; Vuitton, 2004).

**Alveolar echinococcosis (AE)**

Human AE is a chronic and often fatal disease characterized by slowly developing cysts, mainly in the liver. Pathologically, 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. Like CE infection, Th1 responses predominate in the early stages of AE infection, with the immune response switching to a Th2 polarized profile in later progression (Shi et al., 2003; Wei et al., 2004).

Another pathological characteristic of AE infection is the strong cellular immune response elicited by E. multilocularis. This results in a large granulomatous infiltrate surrounding the parasitic lesions (Vuitton et al., 1989; Ricard-Blum et al., 1996; Grenard et al., 2001), which is a process similar to granuloma formation and the resulting fibrosis associated with the pathology of schistosomiasis (Wynn et al., 2004). The cells involved in the formation of the periparasitic granuloma are mainly macrophages, myofibroblasts and T lymphocytes. In patients with abortive or dead lesions, a large number of CD4(+) T lymphocytes are present, whereas patients with active metacestodes display a significant increase in activation of predominantly CD8(+) T cells (Manfras et al., 2002), indicating that CD4(+) T cells play a role in the killing mechanism. This is supported by mouse experiments undertaken by Dai et al. (2004), who infected mice with E. multilocularis of different genetic backgrounds including micro MT, nude, T-cell receptor (TCR)-β−/−, major histocompatibility complex (MHC)-I−/− and MHC-II−/− mice and found that at 2 months post-infection, the parasite mass was more than 10 times higher in nude, TCR-β−/− and MHC-II−/− mice than in infected C57BL/6 wild-type (WT) mice; furthermore these T-cell-deficient mice started to die of the high parasite load at this time-point. In contrast, MHC-I−/− and micro MT mice exhibited parasite growth rates similar to those found in WT controls. These findings clearly point to the major role that CD4(+) T cells play in the process of host parasitism.
T cells play in limiting E. multilocularis proliferation, while CD8(+) T and B cells appeared to play a minor role in the control of parasite growth. In the absence of T cells, especially CD4(+) or γδβ(+) T cells, the cellular immune response to infection was decreased which resulted in the lack of hepatic granuloma formation around the parasite. In addition, in T-cell-deficient mice, the expression of IFN-γ and other inflammatory cytokines (except for interleukin-6) were increased in association with a high parasite load. Thus, the relative protection mediated by CD4(+) γδβ(+) T cells against E. multilocularis infection seems not to be IFN-γ dependent, but rather relies on the effector functions of CD4(+) γδβ(+) T cells (Dai et al., 2004).

The precise role that each of the cytokines play in fibrosis and development of AE lesions remains to be determined. Nevertheless, pretreatment of mice with interleukin (IL)-12 was extremely efficient in preventing the development of lesions and led to abortive parasitic vesicles surrounded by fully efficient periparasitic immune cell infiltration and fibrosis (Emery et al., 1998). Also, 75% of mice treated with IFN-γ-2a had no hepatic lesions and half were fully protected. IFN-γ-2a treatment markedly decreased the abnormally elevated production of IL-10 in both spleen cell cultures and peritoneal macrophage cultures from infected mice and restored phagocytosis and oxidative metabolism of macrophages. The treatment also inhibited IL-6 and IL-13 antigen-induced secretions in spleen cell cultures (Godot et al., 2003) and may be useful for treatment of AE patients; how these two Th1 cytokines may impact on the progression of AE is unknown but they likely act via inhibition of Th2 responses. IL-13 has clearly been shown to be a major factor of AE is unknown but they likely act via inhibition of Th2 responses. IL-13 has clearly been shown to be a major factor in granuloma formation and the resulting fibrosis in schistosomal infection in the mouse model (Pearce & MacDonald, 2002), but there are no similar studies aimed at determining the role this cytokine plays in the hepatic fibrosis caused by AE.

Echinococcus multilocularis vesicle antigens have been shown to induce pro-inflammatory, regulatory and chemokine release by PBMC from patients (Eger et al., 2003). The pro-inflammatory cytokines IL-1β and IL-18 were reduced in echinococcosis patients; regulatory IL-10 was similar, but parasite vesicle-induced IL-8 was dominant and clearly elevated in patients. Such selective and opposite dynamics of inflammatory cytokines and chemokine release may prevent overwhelming and pathogenic inflammation, and constitute an appropriate response for attraction of effector cells into the periparasitic tissues with the capacity to limit E. multilocularis metacestode proliferation and dissemination.

The production of nitric oxide (NO) by intraperitoneal macrophages of mice during secondary infection with E. multilocularis mediates immunosuppression at the early and late stages of infection (Dai et al., 2003). NO production parallels the production of TNF-α (Shi et al., 2003) indicating that NO levels are enhanced by this cytokine. Antigens in the laminated-layer of the cyst decrease NO production in vitro, indicating that E. multilocularis produces molecules that can modulate the host immune responses (Andrade et al., 2004).

Antibody levels were shown to be low early on in murine (BALB/c) E. multilocularis infection but the levels of IgG1 and IgG3 increased significantly 8 weeks after challenge, and remained elevated throughout the 25 week period of observation (Shi et al., 2003). The production of IL-2R and TNF-α by spleen cells from infected mice stimulated with EmAg also increased significantly 8 weeks after infection, while IL-2R sharply decreased after 12 weeks of infection. During the period of 2–12 weeks after infection there was an increase in IL-1 secretion. The levels of IL-1 and TNF-α rapidly increased during the 16 weeks postinfection. A high level of IFN-γ was detected during the period of observation, and showed a peak at 12 weeks. These data indicate, as for E. granulosus, that Th1 is the major response in the early stage of infection, which is replaced by a Th2 response in the later phase of infection.

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. IgE stripping and resensitization of basophils indicating that the mechanism of IL-4 induction requires the presence of IgE on the cells (Aumuller et al., 2004). Echinococcus multilocularis may thus induce a Th2 response in their hosts by the induction of IL-4 release from basophils.

Vaccines and vaccination

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

Vaccination of intermediate hosts

Substantial progress has been made towards developing a practical, recombinant vaccine (EG95) for use against E. granulosus in sheep (Gauci et al., 2005). The EG95 vaccine, cloned from the oncosphere, produces high levels of protection in terms of the reduction in the numbers of hydatid cysts in sheep, goats and cattle following experimental challenge in a number of countries, and natural challenge of sheep in China (Lightowlers & Heath, 2004). First described nearly a decade ago, the EG95 vaccine has,
however, yet to be introduced as part of any government-supported control program in areas endemic for hydatid disease. Economic and political factors will likely impede the large-scale use of the vaccine (Gauci et al., 2005). Further developments in recombinant protein production and in delivery of the EG95 vaccine may make it more amenable to widespread use. In this context, a commercial process was recently described for producing the recombinant vaccine in E. coli (Manderson et al., 2005). Furthermore, Marsland et al. (2003) described the construction of a recombinant orf (Parapoxvirus) virus expressing EG95 levels comparable to that achieved by a similar vaccinia virus recombinant. This recombinant virus will be a valuable tool with which to assess the potential of recombinant orf viruses to deliver vaccine antigens to sheep. If the virus can pass vertically from one generation to another, this would be a major step forward in the use of EG95 for the control of hydatid disease globally (Marsland et al., 2003).

Recent research indicates that the EG95 encoding gene belongs to a gene family of at least seven related genes, that at least some of the proteins encoded by the eg95 gene family are expressed in other stages (immature and mature adult worms, protoscoleces) as well as in the oncosphere (Zhang et al., 2003b; Chow et al., 2004; Gauci et al., 2005). As described earlier, E. granulosus exhibits extensive strain variation, and variability of the eg95 gene in different isolates of E. granulosus may directly impact the effectiveness of the EG95-based vaccine. Analysis of the eg95 gene family from E. granulosus collected in Xinjiang, in northwest China, where hydatid disease is hyperendemic, showed the eg95 gene family was shown to comprise two basic cDNA sequence types but very limited sequence variation was evident in the EG95 protein from oncospheres (Zhang et al., 2003b). This high degree of sequence conservation predicts that the vaccine will continue to be effective in China and elsewhere, although this needs to be fully substantiated, particularly should failures of the EG95 vaccine occur in the future.

A protein containing characteristic fibronectin III domains, related to EM95 (a homologue of EG95), that can induce significant levels of protection against challenge infection with E. multilocularis eggs in mice, has been identified in E. multilocularis (Merkelbach et al., 2003). Another recombinant E. multilocularis molecule, the 14–3–3 protein, elicited a high degree of protection (97%) against a primary egg challenge infection but no protection against secondary infection in vaccinated mice (Siles-Lucas et al., 2003); the results suggest the protective mechanisms were effective only against the oncosphere.

**Vaccination of definitive hosts**

In comparison with intermediate hosts of Echinococcus, immunization of canines has received very little attention in the past two decades. As the major definitive hosts for E. granulosus, dogs play a pivotal role in the transmission of hydatid disease. Interruption of the parasite life cycle in the dog host can provides a very acceptable and cost-effective complementary method for control by vaccination as there are far fewer dogs than sheep on farms so that fewer animals need vaccination and less vaccine doses would be necessary. The fact that old animals have a lower abundance and/or prevalence rates of E. granulosus compared with young dogs (Lahmar et al., 2001; Torgerson et al., 2003; Budke et al., 2005; Buishi et al., 2005; Moro et al., 2005) provides epidemiological evidence that canines may become resistant to reinfection in nature. Similar results have been described with E. multilocularis in naturally infected foxes (Hofer et al., 2000; Yimam et al., 2002; Losson et al., 2003). A mathematical model has indicated that there is significant herd immunity in dogs under a relatively high infection pressure (Torgerson, 2006).

It was found that when dogs were infected with E. granulosus, local and systemic specific antibodies and cellular responses were raised (Carmena et al., 2004, 2005; Moreno et al., 2004). While there was no relationship between serum IgA responses and parasite burden at the end of the infection, an inverse association of antiparasite IgE and parasite load appeared to exist. No differences were observed in the numbers of intestinal mast cells and goblet cells among all infected dogs (Moreno et al., 2004). Dogs infected with E. multilocularis also produced specific antibodies and T-cell responses to parasite antigens, although some modification of lymphocyte responses was apparent (Kato et al., 2005a), a situation also prevailing in the Mongolian gerbil, a prednisolone-untreated rodent definitive host model (Kato et al., 2005b).

In order to identify genes expressed only in mature adult worms that may encode targets for inhibiting egg production, thus forming the basis for developing a transmission blocking vaccine applicable to dogs, differences in mRNA expression between immature adult worms and mature adult worms of E. granulosus were determined using polymerase chain reaction-based differential display (DDRT-PCR) (Zhang et al., 2003c). As a result, examination of the deduced amino acid sequence of three of the corresponding complimentary DNAs (cDNAs) (egM4, egM9 and egM123) indicated they were cysteine-rich and contained a 24 amino acid repeat sequence, repeated four to six times. The repeat regions were predominantly α helical in nature with interspersed turns, forming alternating zones of positive and negative charge. The functional significance of each of the cDNAs identified was unclear as none had significant sequence similarity to genes of known function. Nevertheless, polypeptides encoded by egM4 and egM123 were recognized by antibodies in a serum pool from dogs experimentally infected with E. granulosus, suggesting they
could prove of value in serodiagnosis of definitive hosts. In addition, pilot vaccine/challenge experiments in dogs with proteins encoded by egM4, egM9 and egM123 showed encouraging results in terms of reduced egg production (Zhang, 2003), suggesting they may prove of value as components of a vaccine effective in the definitive host.

**Diagnosis**

Early diagnosis of CE and AE can provide significant improvements in the quality of the management and treatment of both diseases. In most cases, the early stages of infection are asymptomatic, so methods that are relatively easy to use and that are cheap are required for large-scale epidemiological surveillance of populations at high risk. Immunodiagnosis provides such an approach and can, additionally, confirm clinical findings. The definitive diagnosis for most human cases of CE is by physical imaging methods, such as radiology, ultrasonography, computed axial tomography (CT scanning) and magnetic resonance imaging although such procedures are often not readily available in isolated communities. Immunodiagnosis can also play an important complementary role. It is useful not only in primary diagnosis but also for follow-up of patients after surgical or pharmacological treatment. Additional advantages of immunodiagnosis include screening of large populations in communities from endemic areas, rapid testing of individuals in remote areas where imaging equipment may not be readily available, for follow-up monitoring of subjects in endemic areas, and for confirmation of CE or AE cases when physical imaging does not provide a definitive diagnosis (see Qaqish et al., 2003; Hernandez et al., 2004). Immunodiagnosis can also play a major role in the detection of AE infection, which is very important for early commencement or treatment, because of the high associated mortality.

**CE serodiagnosis**

The detection of circulating *Echinococcus granulosus* antigens in sera is less sensitive than antibody detection, which remains the method of choice. CE serology 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 between the various tests, both in specificity and sensitivity. As the sensitivity of a test increases, so generally does the demand for improved antigens in order that sufficient specificity can be achieved to take advantage of the greater sensitivity. An optimum test should be specific with high sensitivity. Insensitive tests have been replaced by the ELISA and immunoblotting (IB) in routine laboratory applications (see, e.g. Nasrith & Abdel-Hafez, 2004) although the choice of diagnostic antigens exhibiting the requisite specificity and sensitivity is ever critical.

The lipoproteins antigen B (AgB) and antigen 5 (Ag5), the major components of hydatid cyst fluid (HCF), have been the two molecules that have received wide attention in regards 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 sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). 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. Cross-reactivity with antigens from other parasites, notably other taeniid cestodes, is a major problem.

**Antigen B**

Antigen B is a polymeric lipoprotein with a molecular weight of 120 kDa. It can be measured in patient blood as circulating antigen and it has been suggested that AgB has an important role in the biology of the parasite and its relationship with the host. AgB is a highly immunogenic molecule, a characteristic that underpins its value in serodiagnosis. It appears ladder-like under reduced condition on SDS-PAGE with three bands (subunits AgB1, AgB2 and AgB3) with molecular sizes of approximately 8 or 12, 16 and 24 kDa, suggestive that it comprises polymers of 8 kDa subunits. The smallest subunit has proved the most useful target in diagnostic studies. A possible new AgB subunit (AgB4) was recently identified (Arend et al., 2004) and recent work shows that AgB is encoded by a multigene family (Haag et al., 2006). Furthermore, AgB presents homology to, and shares apparent structural similarities with, helix-rich hydrophobic ligand binding proteins (HLBPs) from other cestodes, and has fatty acid binding properties (Chemale et al., 2005).

Two residues in the AgB sequence are critical for diagnosis (Gonzalez-Sapienza & Cachau, 2003). The N-terminal extension of the major subunit of AgB concentrates the immunoreactive B-cell epitopes of the native molecule. The nature of this immunodominance was analyzed using four monoclonal antibodies (mAbs) defining overlapping epitopes in this region of the AgB molecule. The minimal epitope requirements of these mAbs were determined using phage display peptide libraries. The consensus sequences isolated with the mAbs, and alanine replacement analysis with synthetic peptides mapped the relevant molecular contacts within a short stretch corresponding to residues 17–24 of the AgB major subunit. Substitution of two critical residues within this stretch produced a dramatic loss of antigenicity, as determined using patient sera.
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. Peptide antigens have been considered as a way to enhance specificity and efforts have been made to define discrete epitopes of AgB and other molecules that could be mimicked by synthetic peptides. Two of the recombinant subunit components (rAgB8/1 and rAgB8/2) of AgB have been used widely in diagnosis. Virginio et al. (2003) showed that, of six purified recombinant proteins tested in ELISA for specific IgG with a panel of sera from patients with surgically confirmed or immunologically diagnosed CE, AgB8/2 provided the highest diagnostic sensitivity (93.1%) and specificity (99.5%). Furthermore, different IgG isotypes showed dominance in the response for each of the recombinant antigens. There was a clear predominance of IgG4 response for all antigens tested, indicating that this would be the subclass of choice to be assessed for these, and possibly other, recombinant proteins. The detection by immunoblotting of antibodies specific for the 8 kDa subunit of antigen B and in particular the IgG4 subclass expression, has also been advocated by Siracusano et al. (2004) as a promising serodiagnostic tool.

To compare variability between laboratories, six South American groups double-blind tested six antigens against the same serum collection (Lorenzo et al., 2005). High intercenter reproducibility was attained and the results showed that HCF, native AgB and its recombinant AgB8/1 subunit had almost the same efficiency at 81.4, 81.3 and 81.9%, respectively, with the diagnostic efficiencies for an AgB-derived synthetic peptide peptide, the recombinant AgB8/2 subunit, and recombinant cytosolic malate dehydrogenase from E. granulosus (EgMDH) being less efficient at 76.8, 69.1 and 66.8%, respectively. Different regional batch preparations of HCF yielded different diagnostic performances but the group recommended that recombinant AgB should be used as the standard antigen in laboratory analysis.

Using a novel approach, Chen et al. (2003) generated a genetically engineered antibody against recombinant AgB that may have potential implications in immunological treatment and drug targeting delivery.

**Antigen 5**

There have been few studies on Ag5 in recent years. Ag5 is a very high molecular weight (c. 400 kDa) lipoprotein complex composed of 57 and 67 kDa components that under reducing conditions dissociate into 38 and 22–24 kDa subunits. Historically, one of the most used immunodiagnostic procedures for CE was the demonstration of serum antibodies precipitating antigen 5 (arc 5) by immunoelectrophoresis or similar techniques. Although they did not carry out any serodiagnostic studies, Lorenzo et al. (2003a) cloned the Ag5 gene by reverse transcription-PCR on the basis of the amino acid sequences of tryptic fragments. The nucleotide sequence indicated that Ag5 is synthesized as a single polypeptide chain that is afterwards processed into single disulphide-bridged 22 and 38 kDa subunits. Whereas the 22-kDa component contains a highly conserved glycosaminoglycan-binding motif that may help to confine Ag5 in the host tissue surrounding the parasite, the 38 kDa subunit is closely related to serine proteases of the trypsin family (Lorenzo et al., 2003a). However, neither proteolytic activity nor binding to protease inhibitors could be detected using native purified Ag5. Thus it may be possible that Ag5 possesses a highly specific physiological substrate or, more likely, that trypsin-like folding has been recruited to fulfill novel functions. Subsequently, this group (Lorenzo et al., 2005) prepared two recombinant forms of the antigen, namely, rAg5 (corresponding to the unprocessed polypeptide chain of the antigen) and rAg5–38s (corresponding to its 38 kDa subunit). Their antigenicities were compared to that of the native antigen using a human serum collection. There was a major drop in the reactivity of the sera, particularly against rAg5–38s, which was confirmed by analysis of the cross-reactivity of two panels of monoclonal antibodies specific for rAg5–38s and the native antigen. Using the chemically deglycosylated native antigen, these authors demonstrated that the reduced antigenicity of the recombinants was due to the loss of the sugar determinants, and not to their misfolding. Inhibition experiments using phosphorylcholine confirmed that this moiety also contributes to the reactivity of the antigen, but to a much lesser extent. The presence of immunodominant highly cross-reactive glycan moieties in the Ag5 molecule may involve a parasite evasion mechanism, as was earlier shown for AgB (see Zhang et al., 2003a).

Studies by Mahmoud & Abou Gamra (2004) have shown that a purified alkaline phosphatase (EgAP) extracted from E. granulosus hydatid cyst membranes possesses exceptional diagnostic characteristics with 100% specificity without any decrease in sensitivity (100%) with significant potential for use in routine diagnosis and follow-up of CE patients. This mirrors the diagnostic value previously shown for purified alkaline phosphatase (pAP) from E. multilocularis metacestodes (see Zhang et al., 2003a).

**Other antigens and approaches for immunodiagnosis**

In a significant advance, Li et al. (2003) used a pool of serum samples from mice infected with oncospheres (eggs) of E. granulosus to screen a cDNA library constructed with RNA extracted from protoscolex larvae from sheep hydatid cysts. One immunoreactive clone, designated EpC1, was shown to encode a protein of 76 residues. The cDNA fragment was
subcloned into an expression vector, pET-41b(+), and the resulting recombinant EpC1 glutathione S-transferase (GST) fusion protein (rEpC1–GST) was expressed in *Escherichia coli* and was affinity purified against the GST tag. Immunoglobulin G was the dominant antibody isotype generated against rEpC1–GST. A total of 896 human serum samples were used to evaluate the diagnostic sensitivity and specificity of the fusion protein by immunoglobulin G immunoblotting; 324 serum samples from patients with CE, 172 from patients with neurocysticercosis, 89 from patients with alveolar echinococcosis, and 241 from patients with other infections or clinical presentations, as well as 70 from confirmed-negative control subjects, yielded an overall sensitivity of 92.2% and an overall specificity of 95.6%. The combined levels of sensitivity and specificity achieved with the rEpC1–GST fusion protein for diagnosis of CE were unprecedented, taking into account the large panel of serum samples that were tested (Table 1). In addition to testing recombinant antigen B subunits, Virginio *et al.* (2003) assessed the diagnostic potential in ELISA of a cytosolic isoform of malate dehydrogenase (EgMDH), an EF-hand calcium-binding protein (EgCaBP2), and a full-length (EgAFFPt) and a truncated form (EgAFFPt, aa 261370) of an actin filament fragmenting protein. These recombinant antigens yielded sensitivities between 58.6 and 89.7%, and three of them were considered of complementary value.

Purified recombinant thioredoxin peroxidase of *E. granulosus* (TPxEg) was used to screen sera from heavily infected mice and patients with confirmed hydatid infection. Only a portion of the sera reacted positively with the EgTPx-GST fusion protein in Western blots (69.3% specificity and 39% sensitivity with human sera), suggesting that EgTPx may form antibody-antigen complexes or that responses to the EgTPx antigen may be immunologically regulated (Li *et al.*, 2004). A dipstick assay has been developed that exhibited 100% sensitivity and 91.4% specificity with 26 CE sera and 35 other parasite infection sera using camel hydatid cyst fluid as antigen (Al-Sherbiny *et al.*, 2004). Since the dipstick assay is extremely easy to perform with a visually interpreted result within 15 min, in addition to being both sensitive and specific, the test could be an acceptable alternative for use in clinical laboratories lacking specialized equipment and the technological expertise needed for western blotting or ELISA.

### Application of serodiagnosis for evaluation of infection risk in populations

An important area for study is the evaluation of the diagnostic potential of serodiagnostic procedures in the field. Qaqish *et al.* (2003) used ELISA to determine the seroprevalence of CE in different communities in Jordan. The rural-agricultural subjects were significantly more likely to be seropositive (11.4%) than the semi-Bedouin (5.0%) or Bedouin (3.7%). In another study, Hernandez *et al.* (2005) screened villagers in an area with high prevalence of CE in rural Tacuareumbo, Uruguay. The correlation between serological data and the incidence of risk factors carried out on 480 individuals who were examined by means of abdominal sonography (local prevalence = 0.8%). Serum samples (305) were analysed by ELISA to determine specific IgG against crude antigens from *E. granulosus*. A total of 27 individuals exhibiting no detectable changes in abdominal sonographic examination were found to be seropositive (‘ultrasound normal group’). Of these individuals, nine were seroreactive against purified AgB. A significant degree of correlation was found between seroreactivity and the incidence of some risk factors (CE antecedent in the family, *P* < 0.005 and use of rural water, *P* < 0.0001) among this group. Follow-up of individuals of the ‘ultrasound normal group’ was carried out after 2 years to evaluate the implications of this serological reactivity. No predictive value for cyst development was assessed with complementary image study; in contrast

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**Table 1.** Performance comparison of recombinant EpC1-GST protein with native hydatid cyst fluid antigen B (HCF-AgB) for human cystic echinococcosis (CE) serodiagnosis by immunoblotting (data summarized from Li *et al.*, 2003, 2004)

<table>
<thead>
<tr>
<th>Disease/clinical presentation</th>
<th>Number of serum samples</th>
<th>Positive serum samples; number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EpC1-GST</td>
</tr>
<tr>
<td>CE, before surgery</td>
<td>116</td>
<td>107 (92.2)</td>
</tr>
<tr>
<td>AE</td>
<td>89</td>
<td>4 (4.5)</td>
</tr>
<tr>
<td><em>Taenia solium</em> NCC</td>
<td>172</td>
<td>16 (9.3)</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>78</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Other parasitic infection</td>
<td>116</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>Non-infectious diseases</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>70</td>
<td>3 (4.3)</td>
</tr>
</tbody>
</table>

*Partially tested.
CE, cystic echinococcosis; AE, alveolar echinococcosis; NCC, neurocysticercosis.
transient antibodies were observed with both crude and purified antigen as approximately 60% of individuals became negative when re-sampled. This study showed that serodiagnostic data can prove useful for evaluating the efficacy of CE control efforts.

Deutz et al. (2003) investigated the seroprevalence of a range of zoonotic pathogens, including E. granulosus and E. multilocularis, in hunters originating from the south-eastern Austrian federal states of Styria and Burgenland, and compared the results with other predisposed occupational groups. The high seroprevalences for CE and AE, in addition to several other pathogens, demonstrated that hunters are particularly exposed to zoonotic pathogens, which is perhaps not surprising giving their occupation and lifestyle (Deutz et al., 2003).

Challenges for immunodiagnosis

Despite the development of sensitive and specific techniques, such as immunoblotting and ELISA, the immunodiagnosis of CE in clinical practice and population studies remains a complex task and three major problems still remain. The first problem is that most available screening tests give a high percentage of false negative results which can be as much as 50% with sera taken from communities in population surveys (Moro et al., 2005). To explore possible factors associated with false negative antibody response in immunodiagnosis of CE patients, Xu et al. (2002) determined IgG subclasses (IgG1, IgG2, IgG3, IgG4) and IgA, IgM and IgE in the sera of individuals with a negative total IgG response; they concluded that the seronegative response of total IgG in CE patients might be due to low levels of specific IgG, variant IgG antibody expression and/or formation of circulating immune complexes, and that the combined detection of IgG1+IgA+IgM could enhance the sensitivity of serological tests in CE patients (Xu et al., 2002).

The second issue relates to putative false positive reactions, the causes for which may be very complex. Cross-reactivity with antibodies from other infections may be one reason for this false-positivity, but it may also be due to the fact that CE antibodies can remain in serum for long periods following surgical removal or effective drug treatment of cysts, or even if the infection self-cures as discussed earlier.

The third problem is how to distinguish active or progressive cases of echinococcosis from cured individuals. It has been shown in a number of studies that IgG, especially IgG1 and IgG4, can remain circulating in the human blood system for more than 5 years (Bulut et al., 2001; Li et al., 2003; Nasrieh & Abdel-Hafez, 2004), with IgA and IgM also detectable in serum 3 years after surgical cyst removal (Doiz et al., 2002). In addition, significantly high levels of specific IgE serum antibodies determined by ELISA were still detectable one year after surgery (Bulut et al., 2001).

In order to address this issue, Lawn et al. (2004) investigated whether concentrations of CE-specific IgG subclasses 1–4 in ELISA using crude horse hydatid cyst fluid as an antigen correlated better with disease activity than total IgG. They studied a cohort of patients with symptomatic CE treated with anthelminthic drugs and surgery and who were followed up clinically and radiologically for several years (Lawn et al., 2004). Changes in concentrations of antibodies were correlated with clinical and radiologic outcome. At diagnosis, concentrations of CE-specific total IgG, IgG1 and IgG2 antibodies were significantly elevated in a greater proportion of patients compared with IgG3 and IgG4 antibodies. The fact that using cyst fluid antigen rather than purified antigen (AgB or Ag5) yielded better responses could be that crude antigens enhance detection of subclass antibodies with different antigen specificities with IgG2 antibodies being most sensitive. Importantly, during post-treatment follow up, the IgG2 antibody response provided the best correlate of disease activity, with serum concentrations of CE-specific antibodies showing that IgG2 correlated most strongly with clinical outcome (Lawn et al., 2004).

Immunodiagnosis of CE in animal intermediate hosts

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, 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. 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. In sheep, the principal intermediate host of E. granulosus in most endemic regions of the world, antibodies to various antigens including antigen 5 are detectable in the sera of some, but not all infected sheep (‘nonresponders’). As with human CE, the detection of circulating antigen does not appear to be useful for diagnostic purposes.

Ibrahim et al. (2002) used AgB, partially purified from hydatid cyst fluid from camels or sheep, and a recombinant form of AgB (r-AgB) in an ELISA, to screen panels of serum samples from slaughtered camels and sheep naturally infected with CE (Ibrahim et al., 2002). Seroreactivity, however, was variable. Native AgB gave the highest sensitivity (97%) in ELISA for camel CE. In contrast, r-AgB gave lower sensitivity for camel (84%) and sheep (28%) CE. The r-AgB-ELISA was, however, highly specific, yielding 90 and 95%
specificity, respectively, for natural camel and sheep CE infections (Ibrahim et al., 2002).

Kittelberger et al. (2002) carried out a very extensive study aimed at developing an immunological method for the identification of sheep infected with *E. granulosus* which would allow the monitoring of animals imported into countries free from hydatidosis, and as an aid to countries where control schemes for the disease are in operation. Three ELISAs were developed and validated, using as antigen purified 8 kDa AgB hydatid cyst fluid protein (8kDaELISA), recombinant EG95 oncosphere protein (OnELISA) or a crude protoscolex preparation (ProtELISA). Sera used for the assay validations were obtained from 249 sheep that were infected either naturally or experimentally with *E. granulosus* and from 1012 non-infected sheep. The highest diagnostic specificity was obtained using the ProtELISA at 62.7 and 51.4%, depending on the cut-off. Assay sensitivities were lower for the 8kDaELISA and the OnELISA. Diagnostic sensitivities were high, ranging from 95.8 to 99.5%, depending on the ELISA type and cut-off level chosen. A few nastic specificities were high, ranging between 77 and 92%. In addition, serology for antibodies against the Em2 antigen have been shown to be a useful method for identifying animals including cynomolgus mokeys (*Macaca fascicularis*) (Bacciarini et al., 2004; Rehmann et al., 2005) and lowland gorillas (*Gorilla g. gorilla*) (Rehmann et al., 2003) that might be infected with *E. multilocularis* and are therefore at risk of developing fatal AE.

The Em2plus ELISA, a combination of Em2 with a recombinant protein designated II/3–10 (also termed EM10), increased the sensitivity to 97% (Gottstein et al., 1993). The Em2plus assay exhibits cross-reaction with CE in (25.8% of cases) which is higher than the individual Em2 (5.6%) and 11/3–10 (6.5%), but limited cross-reactivity with other diseases. The Em2plus-ELISA has been commercialized for clinical diagnosis of AE and for population screening (Gottstein et al., 1993).

EM10 (Em II/3–10) shares almost complete identity to the *E. granulosus* protein sequences EG10 and EGII.3 (Sako et al., 2002). Although the two species have similar sequences, recombinant EM10 protein showed very high specificity to distinguish AE infection from CE infection in human patients. It is not clear why these homologous molecules exhibited such a different pattern of sero-recognition. Sako et al. (2002) suggested that EG10 may be expressed at a very low level in larval *E. granulosus*, but it may equally be that the transcription of EG10 is low or silenced. An 18 kDa antigen (Em18) from protoscolecates 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 (Ito et al., 1995; Akira, 1997). Subsequently, EM18 was shown to be a fragment of the C-terminal of EM10 and the recombinant protein was recognized by 87.1 and 90.3% of 31 serum samples from AE patients in ELISA and immunoblotting, respectively (Sako et al., 2002). Recombinant Em18-ELISA and Em18-immunoblot assays have proved invaluable for differentiating AE from CE infection (Ito et al., 2002), the former also being useful for evaluating the efficacy of treatment in patients with AE (Fujimoto et al., 2005). Epitope mapping indicated that the part of the ReEm18 antigen sequence necessary for AE diagnosis occurs in the N-terminal half to two-thirds of the entire sequence (Jiang et al., 2004).

**AE serodiagnosis**

The diagnosis of AE is based on similar findings and criteria as in CE. These include case history, clinical findings, morphological lesions identified by imaging techniques, PCR or immunofluorescence/immunohistochemistry, and immunodiagnosis. Like CE, serodiagnosis of alveolar echinococcosis 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. As we have emphasized earlier, AE is a very serious disease with a high fatality rate, so early detection is paramount in order that successful management and treatment can commence.
Full-length cDNA and genomic DNA encoding an 8-kDa subunit of antigen B from *E. multilocularis* (designated EmAgB8/1) were isolated from an *E. multilocularis* metacercoid cDNA library and a protoscolex genomic DNA library, respectively (Mamuti et al., 2004). Reverse transcription-PCR analysis revealed that the clone encoding EmAgB8/1 is predominantly transcribed in larval *E. multilocularis*. The mature form was expressed in *Escherichia coli*, and its antigenic reactivity was compared with that of its counterpart 8-kDa subunit of *E. granulosus* AgB (EgAgB8/1), by Western blotting and ELISA with serum samples from patients confirmed to have CE and AE. The sensitivity of EmAgB8/1 was comparable to that of EgAgB8/1 for the serodiagnosis of echinococcosis. It is noteworthy that there was no cross-reaction with sera from patients with cysticercosis, which often cross-react when native antigens are used for serodiagnosis.

Direct detection or amplification of *E. multilocularis* nucleic acids in clinical samples is also considered useful for the primary diagnostic identification of parasite materials in biological specimens resected or biopsied from patients, and also for the assessment of the viability of parasite samples after chemotherapy or other treatment. Infections of humans with *E. multilocularis*, have been described with increasing frequency in Poland since 1994. In the attempt to verify these reports, specimens were obtained from a group of Polish patients (Myjak et al., 2003). Liver lesions in patients with AE, diagnosed on the basis of results of histological and serological tests, were shown, by the presence of specific microsatellite sequences and mitochondrial 12S rDNA, to contain *E. multilocularis* DNA. These data provided unequivocal proof that human infections with *E. multilocularis* occur in Poland. PCR-amplification of specific *E. multilocularis* DNA has proved useful also for helping to diagnose unambiguously AE infection in monkeys (Bacciarinini et al., 2004).

**Diagnosis of echinococcosis in definitive hosts**

The detection of *Echinococcus* infections in canines is important for epidemiological surveillance and evaluation of echinococcosis control programs. Diagnosing *Echinococcus* infections in dogs and other definitive hosts is problematical as the eggs of taeniid cestodes are extremely similar, and thus identification by microscopic examination of the faeces is risky and non-specific. Two major diagnostic methods have been extensively used in dogs, purgation with aracoline compounds and necropsy of the small intestine. Necropsy is the method of choice for foxes and other final hosts. Two 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 faeces. Overall, the available ELISA-based methods for detection of circulating antibodies in canines have poor sensitivity, specificity is unclear and there is no correlation with worm burden so their usefulness, other than in population-based studies of canine hosts, is questionable (Zhang et al., 2003a).

Much more success has been achieved using the other major approach to immunological diagnosis of *Echinococcus* infection in the definitive host by detection of adult worm products in faeces using sandwich ELISA methodology and this technique has been widely used with successful detection of *E. granulosus* and *E. multilocularis* (Lopera et al., 2003; Sanchez Thevenet et al., 2003; Benito & Carmena, 2005; Cavagnion et al., 2005; Moro et al., 2005; Reiterova et al., 2005). Although these tests for diagnosis of canine echinococcosis provide high specificity and sensitivity, little is known of the characteristics of antigenic molecules present in faeces from infected animals. While initial attempts to determine the molecular weights of *E. granulosus* coproantigens by SDS-PAGE and Western blotting with coproantigen reactive capture antibodies were equivocal, they suggested the presence of a significant carbohydrate component. Elayoubi et al. used SDS-PAGE and western blot to show that the coproantigens are highly glycosylated and contain β-galactose and N-acetyl-β-glucosamine (Elayoubi et al., 2003). Subsequently, supernatants prepared from *E. granulosus*-infected dog faecal samples and fractionated by size-exclusion fast protein liquid chromatography (FPLC) showed that the antigens are large molecular weight molecules that may be derived from the carbohydrate-rich surface glycolyx of adult worms, and are shed, released or secreted during the life-span of the tapeworm (Elayoubi & Craig, 2004).

Copro-antigen detection allows *in vitro* diagnosis of *Echinococcus* infections but, as well, several PCR-based protocols have been developed that allow identification of *Echinococcus* DNA from eggs or from adult parasites. These provide a highly complementary approach for positive and highly specific diagnosis of canines infected with *E. granulosus* and *E. multilocularis* (Cabrera et al., 2002; Abbasi et al., 2003; Deplazes et al., 2003; Casulli et al., 2004, 2005; Stefanic et al., 2004; Varvasi et al., 2004; Reiterova et al., 2005) and environmental detection of *Echinococcus* eggs in soil samples (Shaikenov et al., 2004).

The efficacy of two PCR-based methods to detect patent and prepatent infection in dogs experimentally infected with *E. granulosus* was recently compared (Naichich et al., 2006). The detection was based on amplification of a fragment of the mitochondrial cox1 gene (Mit-PCR) and a repetitive element (Rep-PCR) from the genome of *E. granulosus*. The ability of both methods to detect several genotypes of the parasite were assessed. Both PCR methods could detect *E. granulosus* during both prepatent and patent periods, even...
when microscopical observation of eggs in faecal samples was negative. The Mit-PCR produced the same amplification pattern for all the parasite genotypes tested while the amplification patterns with the Rep-PCR differed among groups of strains. In addition, faecal samples collected from dogs from an endemic area in Argentina were diagnosed with more sensitivity using the PCR methods than by arecoline hydrobromide purgation. These copro-PCR tests can thus be applied in the confirmation of coproantigen-positive faecal samples and to verify the success of control programs.

**Outlook: genomics and the functional expression of genes from Echinococcus**

A series of recent studies have investigated the functional expression of genes from *E. granulosus* and *E. multilocularis*. All parasitic helminths, including *Echinococcus* process a large subset of their mRNA molecules via spliced leader trans-sping (Fernandez et al., 2002). In an important contribution, Brehm et al. (2003) described a rapid and efficient method, ‘spliced leader differential display’, to analyse gene expression patterns of *in vitro* cultivated protoscoleces and metacestode vesicles from *E. multilocularis*. The approach was further used to identify genes which are induced in the *E. multilocularis* metacestode stage under growth-promoting conditions in the presence of host hepatocytes. One mRNA, encoding a putative member of the epidermal growth factor family of mitogens, was shown to be 10-fold induced upon co-incubation of metacestode vesicles with host cells which indicated a possible involvement of parasite-determined, epidermal growth factor-like signal transduction systems in metacestode proliferation and development. Shortly thereafter, an *E. multilocularis* candidate receptor for Egfd was identified by Spiliotis et al. (2003). The identified molecule, EmER, displayed clear homologies to EGF receptors from different phylogenetic origins and was expressed in metacestode and protoscoleces. It has not yet been experimentally shown that Egfd and EmER form a corresponding ligand–receptor pair. However, the fact that *emer* is the only *E. multilocularis* gene that encodes an EGF receptor strongly suggests that this is the case.

In another approach, Rosenzvit et al. (2006) used the signal sequence trap technique to identify genes coding for secreted and membrane-bound proteins from *E. granulosus*. A number of isolated clones showed significant similarity with amino acid transporters, Krebs cycle intermediates transporters, presenilins and vacuolar protein sorters proteins. Other cDNAs encoded secreted proteins without homologues. All the mRNAs were expressed in protoscoleces and adult worms, but some of them were not found in oncospheres. These identified putative *E. granulosus* secreted and membrane-bound proteins are likely to play important roles in the metabolism, development and survival of the parasite in the host.

A series of more conventional studies have been undertaken on the cloning, characterization and functional expression of a number of molecules from both *E. granulosus*

### Table 2. Examples of molecules isolated from *Echinococcus* and their function

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Main function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ras and Raf</td>
<td>Regulatory factors</td>
<td>Spiliotis et al. (2005)</td>
</tr>
<tr>
<td>Em5kip</td>
<td>SNW/SKIP transcriptional regulator</td>
<td>Gelmedin et al. (2005)</td>
</tr>
<tr>
<td>SNW/SKIP</td>
<td>Transcriptional co-regulators</td>
<td>Gelmedin et al. (2005)</td>
</tr>
<tr>
<td>Thioredoxin peroxidase</td>
<td>An element in the thioredoxin system</td>
<td>Li et al. (2004)</td>
</tr>
<tr>
<td>PDZ-1</td>
<td>NERMAD-specific interaction partner of the ERM protein</td>
<td>Hubert et al. (2004)</td>
</tr>
<tr>
<td>Mitochondrial malate dehydrogenase (mMDH)</td>
<td>Krebs cycle enzyme</td>
<td>Freire et al. (2004)</td>
</tr>
<tr>
<td>N-acetylgalactosaminyltransferase</td>
<td>SNW/SKIP transcriptional coregulator</td>
<td>Gelmedin et al. (2005)</td>
</tr>
<tr>
<td>EmSmad</td>
<td>Smad family, important role in transforming growth factor signaling cascade</td>
<td>Zavalá-Gongora et al. (2003)</td>
</tr>
<tr>
<td>Thioredoxin glutathione reductases</td>
<td>Reducing thioredoxin and glutathione in the antioxidation system</td>
<td>Agorio et al. (2003)</td>
</tr>
<tr>
<td>Tyrosine kinase</td>
<td>Phosphorylation</td>
<td>Konrad et al. (2003)</td>
</tr>
<tr>
<td>EmIR</td>
<td>Insulin receptor, signaling</td>
<td>Konrad et al. (2003)</td>
</tr>
<tr>
<td>Ag5</td>
<td>Proteases of the trypsin family</td>
<td>Lorenzo et al. (2003b)</td>
</tr>
<tr>
<td>EgAFFP</td>
<td>Actin filament fragmenting and nucleating activities</td>
<td>Cortez-Herrera et al. (2001)</td>
</tr>
<tr>
<td>H-FABPs</td>
<td>Heart group of fatty acid binding protein</td>
<td>Alvite et al. (2001)</td>
</tr>
<tr>
<td>Egact</td>
<td>Actin, promoting transcription</td>
<td>Gimba et al. (2000)</td>
</tr>
<tr>
<td>EM10</td>
<td>Ezrin–radixin–moesin (ERM)</td>
<td>Hubert et al. (1999)</td>
</tr>
</tbody>
</table>
and *E. multilocularis* which include regulatory factors, signalling factors, receptors and enzymes (Table 2).

Currently, genomic and expressed sequence tag (EST) information on *Echinococcus* spp. is very limited compared with studies on the filaria and schistosomes although plans are in place to produce 60 000 ESTs to characterize genes from *E. granulosus* and *E. multilocularis* metaccestodes (http://www.sanger.ac.uk/Projects/Echinococcus/). This new information will add significantly to the available *Echinococcus* genomic and cDNA sequences in GenBank and the current collection of >3000 EST clusters for trans-spliced and non-trans-spliced cDNAs of *E. granulosus* and *E. multilocularis* available on the LophoDB search base (http://zeldia.cap.ed.ac.uk/Lopho/LophDB.php), established by John Parkinson (Toronto, Canada).

The new developments in genomics, proteomics (Cemelal *et al.*, 2003) and microarray analysis that are underway or planned for the *Echinococcus* organisms will underpin future advances on the functional biology of these important, emerging (Eckert & Deplazes, 2004) pathogens, provide a better understanding of the parasite–host interplay in echinococcal infections, and new avenues for the identification of additional targets for diagnosis, vaccination and chemotherapy.

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