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Echinococcus spp. and echinococcosis

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Summary

Echinococcus spp. are cestode parasites commonly known as small tapeworms of carnivorous animals. Their medical importance lies in the infection of humans by the larval stage of the parasites, predominantly including *Echinococcus granulosus*, which is the causative agent of cystic echinococcosis (CE) and *Echinococcus multilocularis*, which causes alveolar echinococcosis (AE).

A few other species or genotypes are only very rarely or not at all found in humans. Due to the emerging situation in many parts of Europe, the present article will predominantly focus on *E. multilocularis* .

The natural life cycle of *E. multilocularis* involves predominantly red and arctic foxes as definitive hosts, but domestic dogs can also become infected and represent an important infection source for humans in highly endemic areas. In the definitive host, egg production starts as early as 28 days after infection. After egg ingestion by a rodent or a human, larval maturation will occur practically exclusively within the liver tissue. The geographic distribution of *E. multilocularis* is restricted to the northern hemisphere. In Europe, relatively frequent reports of AE in humans occur in central and eastern France, Switzerland, Austria and Germany. Within the past ten years, the endemic area of Europe now includes many more countries such as Belgium, The Netherlands, Italy, and most former Eastern countries as far as up to Estonia. The Asian areas where *E. multilocularis* occurs include the whole zone from the White Sea eastward to the Bering Strait, covering large parts of Siberia, western and central parts of China and northern Japan. Worldwide there are scant data on the overall prevalence of human AE. Some well-documented studies demonstrate a generally low prevalence among affected human populations. The annual mean incidence of new cases in different areas including Switzerland, France, Germany and Japan has therefore been reported to vary between 0.1 and 1.2/100,000 inhabitants. The incidence of human cases correlates with the prevalence in foxes and the fox population density. Recently, a study documented that a fourfold increase of the fox population in Switzerland resulted in a statistically significant increase of the annual incidence of AE cases [1] (Schweiger et al., 2007). This dramatic increase in red fox populations has also been reported throughout Europe, especially in urban areas. The so-called city-fox phenomenon and, thereafter, the increased proximity of foxes with humans and an urban domestic dog – rodent cycle may, therefore, have significant public health implications [1-3].

In infected humans the *E. multilocularis* metacestode (larva) develops primarily in the liver. Occasionally, secondary lesions form metastases in the lungs, brain and other organs. The typical lesion appears macroscopically as a dispersed mass of fibrous tissue with a conglomerate of scattered vesiculated cavities with diameters ranging from a few millimeters to centimeters in size. In advanced chronic cases, a central necrotic cavity containing a viscous fluid may form, and rarely there is a bacterial superinfection. The lesion often contains focal zones of calcification, typically within the metacestode tissue. Histologically, the hepatic lesion is characterized by a conglomerate of small vesicles and cysts demarcated by a thin PAS-positive laminated layer with or without an inner germinative layer [4]. Parasite proliferation is usually accompanied by a granulomatous host reaction, including vigorous synthesis of fibrous and germinative tissue in the periphery of the metacestode, but also necrotic changes centrally. In contrast to lesions in susceptible rodent hosts, lesions from infected human patients rarely show protoscolex formation within

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vesicles and cysts. Genetic and immunologic host factors are responsible for the resistance shown by some patients in whom there is an early 'dying out' or 'abortion' of the metacestode [5,6]. Therefore, not every individual infected with *E. multilocularis* is susceptible to unlimited metacestode proliferation and develops symptoms in the average within 5–15 years after infection. The host mechanisms modulating the course of infection are most likely of an immunologic nature, including primarily suppressor T cell interactions. Thus, the periparasitic granuloma, mainly composed of macrophages, myofibroblasts and T cells, contains a large number of CD4+ T cells in patients with abortive or died-out lesions, whereas in patients with active metacestodes the number of CD8+ T cells is increased. An immunosuppressive process is assumed to downregulate the lymphoid macrophage system. Conversely, the status of cured AE is generally reflected by a high in-vitro lymphoproliferative response. The cytokine mRNA levels following *E. multilocularis* antigen stimulation of lymphocytes show an enhanced production of Th2-cell cytokine transcripts IL-3, IL-4 and IL-10 in patients, including a significant IL-5 mRNA expression in patients and not in healthy control donors. A lack or deficiency of Th cell activity such as in advanced AIDS is associated with a rapid and unlimited growth and dissemination of the parasite in AE, recovery of the T cell status in AIDS is prognostically favorable.

More detailed information about the host-parasite interplay that decides about the outcome of infection has been achieved with the murine model of AE. The involvement of cellular immunity in controlling the infection is strongly suggested by the intense granulomatous infiltration observed in the periparasitic area of lesions. Immunodeficient athymic nude and SCID mice exhibited high susceptibility to infection and disease, thus suggesting that the host cell mediated immune response plays an important role in suppressing the larval growth. *E. multilocularis* appears to induce skewed Th2-responses. Based on *in vitro* and *in vivo* studies, Th2 dominated immunity was more associated with increased susceptibility to disease, while Th1 cell activation through IL-12, IFN .γ, TNF.α and IFN.α was suggested to correlate with a more protective immunity in AE. Nevertheless, effective suppression of larval growth by means of an immunological attack is hampered by the fact, that the parasite synthesizes a carbohydrate-rich laminated layer in order to be protected from host effector mechanisms, as outlined above.

Basically, the larval infection with *Echinococcus multilocularis* begins with the intrahepatic postoncospheral development of a metacestode that – at its mature stage - consists of an inner germinal and the outer laminated layer as discussed above [4]. Several lines of evidence obtained *in vivo* and *in vitro* indicate the important bio-protective role of the laminated layer, e.g. as to protect the germinal layer from nitric oxide produced by periparasitic macrophages and dendritic cells, and also to prevent immune recognition by surrounding T cells. On the other hand, the high periparasitic NO production by peritoneal exudate cells contributes to periparasitic immunosuppression [7], explaining why iNOS deficient mice exhibit a significantly lower susceptibility towards experimental infection [8]. The intense periparasitic granulomatous infiltration indicates an intense host-parasite interaction, and the involvement of cellular immunity in control of the metacestode growth kinetics is strongly suggested by experiments carried out in T cell deficient mouse strains [9]. Carbohydrate components of the laminated layer, as the Em2 (G11) and Em492 components discussed above, yield immunomodulatory effects that allow the parasite to survive in the host. I.e., the IgG response to the Em2 (G11)-antigen takes place independently of alpha-beta +CD4+ T cells, and in the absence of interactions between CD40 and CD40 ligand [10]. Such parasite molecules also interfere with antigen presentation and cell activation, leading to a mixed Th1/Th2-type response at the later stage of infection. Furthermore, Em492 [11] and other (not yet published) purified parasite metabolites suppress ConA and antigen-stimulated splenocyte proliferation. Infected mouse macrophages (AE-MØ) as APCs exhibited a reduced ability to present a conventional antigen (chicken ovalbumin, C-Ova) to specific responder lymph node T cells when compared to normal MØ [12].

Echinococcus granulosus parasitizes as a small tapeworm the small intestine of dogs and occasionally other carnivores. The shedding of gravid proglottids or eggs in the feces occurs within 4–6 weeks after infection of the definitive host. Ingestion of eggs by intermediate host animals or humans results in the development of a fully mature metacestode (i.e. hydatid cyst) over a period of several months to years. Infections with *E. granulosus* occur worldwide, however predominantly in countries of South and Central America, the European and African part of the Mediterranean area, the Middle East and some sub-Saharan countries, Russia and China. Most cases observed in Central Europe and the USA are associated with immigrants from highly endemic areas. Various strains of *E. granulosus* have been described, and differ especially in their infectivity for intermediate hosts such as humans. The most important strains for human infection include sheep (G1) and cattle (G5) as intermediate hosts.

Cystic echinococcosis (CE) is clinically related to the presence of one or more well-delineated spherical primary cysts, most frequently formed in the liver, but other organs such as the lungs, kidney, spleen, brain, heart and bone may be affected too. Tissue damage and

organ dysfunction result mainly from this gradual process of space-occupying displacement of vital host tissue, vessels or parts of organs. Consequently, clinical manifestations are primarily determined by the site, size and number of the cysts, and are therefore highly variable. Accidental rupture of the cysts can be followed by a massive release of cyst fluid and hematogenous or other dissemination of protoscolices. This can result in anaphylactic reactions and multiple secondary cystic echinococcosis (as protoscolices can develop into secondary cysts within the same intermediate host). The parasite evokes an immune response, which is involved in the formation of a host-derived adventitious capsule. This often calcifies uniquely in the periphery of the cyst, one of the typical features found in imaging procedures. In the liver there may be cholestasis. Commonly, there is pressure atrophy of the surrounding parenchyma. Immunologically, the coexistence of elevated quantities of interferon IFN-γ, IL-4, IL-5, IL-6 and IL-10 observed in most of hydatid patients supports Th1, Th17 and Th2 cell activation in CE. In particular, Th1 cell activation seemed to be more related to protective immunity, whereas Th2 cell activation was related to susceptibility to disease.

Prevention of both CE and AE focuses primarily on veterinary interventions to control the extent and intensity of infection in definitive host populations, which may indirectly be approached by controlling the prevalence in animal intermediate hosts also. The first includes regular pharmacologic treatment and taking sanitary precautions for handling domestic dogs and to prevent infection and egg excretion, respectively. Regular praziquantel treatment of wild-life definitive host may contribute to lower the prevalence in affected areas.

For diagnosis, imaging procedures together with serology will yield appropriate results [13,14]. Sonography is the primary diagnostic procedure of choice for hepatic cases [15], although false positives occur in up to 10% of cases due to the presence of nonechinococcal serous cysts, abscesses or tumors. Computerized tomography is the best investigation for detecting extrahepatic disease and volumetric follow-up assessment; magnetic resonance imaging (MRI) assists in the diagnosis by identifying changes in the intra- and extrahepatic venous systems. Ultrasonography is also helpful in following up treated patients as successfully treated cysts become hyperechogenic. Calcification of variable degree occurs in about 10% of the cysts. Aspiration cytology appears to be particularly helpful in the detection of pulmonary, renal and other nonhepatic lesions for which imaging techniques and serology do not provide appropriate diagnostic support. The viability of aspirated protoscolices can be determined by microscopic demonstration of flame cell activity and trypan blue dye exclusion. Immunodiagnostic tests to detect serum antibodies are used to support the clinical diagnosis of both AE and CE.

Assessing the parasite viability in vitro following therapeutic interventions may be of tremendous advantage when compared with the invasive analysis of resected or biopsied samples. Such alternatives may be offered by magnetic resonance spectrometry or positron emission tomography (PET). The latter technique has recently been used for assessing the efficacy of chemotherapy in AE. PET positivity actually demonstrates periparasitic inflammatory processes due to a remaing activity of the metacestode tissue. Serologic tests are more reliable in the diagnosis of AE than CE. The use of purified *E. multilocularis* antigens such as the Em2 antigen and recombinant antigens from the family of EMR-proteins (EmII/3-10, EM10, EM4 and Em18, all four of them harbouring an identical immunodominant oligopeptide sequence) exhibits diagnostic sensitivities ranging between 91% and 100%, with overall specificities of 98–100%. These antigens allow discrimination between the alveolar and the cystic forms of disease with a reliability of 95%. Seroepidemiologic studies reveal asymptomatic preclinical cases of human AE as well as cases in which the metacestode has died at an apparently early stage of infection (see above). Serologic tests are of value for assessing the efficacy of treatment and chemotherapy only when linked to appropriate imaging investigations. Prognostically, disappearance of anti-II/3–10 or anti-Em18 antibody levels coupled to PET negativity indicates innactivation of AE. The management of CE and AE follows the strategy recommended in the manual on echinococcosis published in 2001 by the Office International des Epizooties and the World Health Organisation.

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