Triggering and modulation of the host-parasite interplay by *Echinococcus multilocularis*: a review

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SUMMARY

As more facts emerge regarding the ways in which *E. multilocularis*-derived molecules trigger the host immune response and modulate the host-parasite interplay, it becomes possible to envisage how the parasite can survive and proliferate in its intermediate host, while in other hosts it dies out. Through effects on cells of both the innate and adaptive arms of the immune response, *E. multilocularis* can orchestrate a range of outcomes that are beneficial not only to the parasite, in terms of facilitating its intrahepatic proliferation and maturation, and thus life cycle over all, but also to its intermediate host, in limiting pathology. The present review deals with the role of metacestode surface molecules as well as excretory/secretory (E/S) metabolic products of the parasite in the modulation of the host responses such as to optimize its own survival.

Key words: *Echinococcus multilocularis*, alveolar echinococcosis, immunology, metabolites, immune modulation.

INTRODUCTION

Alveolar echinococcosis (AE) is one of the most severe helminthic diseases affecting humans. Infection is acquired upon ingestion of eggs of the fox tapeworm *Echinococcus multilocularis*. As a result, the metacestode (larval) stage of the parasite grows as a tumour-like tissue in the liver of its host. At a later stage, metastasis formation in adjacent and peripheral sites may cause detrimental obstruction of the respectively affected organs. Late diagnosis and non-treatment may result in case fatality. The natural intermediate hosts involved in the life cycle of the parasite, however, include predominantly small rodents. Similarly, humans contract AE accidentally, but with no subsequent involvement of the definitive hosts. Thus, the laboratory mouse is an excellent model to study the host-parasite interplay. Experimental intraperitoneal inoculation of metacestodes is referred to as secondary infection. In the peritoneal cavity of AE-infected mice, inter-visceral tumour-like growth of the metacestode overcomes the immune system and subsequently establishes a chronic phase of infection, which persists approximately between 2–6 months p.i. Through effects on cells of both the innate and adaptive arms of the immune response, the parasite can orchestrate a range of outcomes that are beneficial not only for metacestode establishment, but also in terms of facilitating its proliferation and maturation. In addition, the complex host-parasite interaction leads to only limited pathology. Thus, a higher survival potential for both host and parasite is achieved.

In the host-parasite interplay, metacestode surface molecules as well as excretory/secretory (E/S) metabolic products are considered to function as important key players (reviewed in Gottstein and Hemphill, 2008). The intraperitoneal murine infection model of AE offers the opportunity to study the direct effect of metabolic metacestode molecules on periparasitic peritoneal cells, including especially dendritic cells (DCs), but also other immunologically relevant populations such as macrophages (MØ), lymphocytes and other (inflammatory) cells that will play a significant role in the putative control of (or respective failure to control) the metacestode proliferation, and thus triggering of disease development.

In AE, the involvement of cellular immunity in controlling the infection is strongly suggested by studies in immunocompromised hosts. Immunodeficient athymic nude (Playford and Kamiya, 1992) and SCID mice (Playford *et al*. 1992) as well as HIV-co-infected patients (Sailer *et al*. 1997; Zingg *et al*. 2004) exhibited high susceptibility to infection and disease, thus suggesting that the cell mediated immune response plays an important role in suppressing larval growth.

E. MULTILOCULARIS METACESTODE METABOLITES

The *E. multilocularis* metacestode actively secretes or expresses molecules that putatively have potent effects on the immune system of the murine host.

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The production of these molecules and their chemical compositions might depend on the stage of the parasite (oncosphere, early vesicle or fully mature metacestode). However, little is known about biological effector molecules arising metabolically or somatically from the intrahepatic stages of the metacestode, although various *E. multilocularis* antigens, their epitopes and respective genes have been characterized. Among the main antigens described, a major carbohydrate named Em2 (G11) localizes on the surface of the laminated layer of the metacestode (Gottstein *et al.* 1992). Another polysaccharide-containing antigen C has been isolated and characterized from crude metacestode extract (Sato and Furuya, 1994). Similar investigations have yielded the finding of EmP2 (Ingold *et al.* 1998), a high molecular mass glycan as a part of the major structural elements of the laminated layer (Ingold *et al.* 2000). Biological activity of a high molecular mass carbohydrate molecule, called Em492, was demonstrated by Walker *et al.* (2004), from which they concluded that the Em492-antigen could be one of the factors contributing to immunosuppressive events that occur at the host-parasite interface. Another neutral glycosphingolipid has been identified as suppressor of human PBMCs proliferation following stimulation by phytohemagglutinin (Persat *et al.* 1992, 1996). Structure determination of this glycosphingolipid fraction had revealed that it belongs to the neogala series (Galβ1→6Gal). Huelsmeier and co-workers (2002) had then isolated novel mucin-type glycoforms from the metacestode of *E. multilocularis*, and these glycoforms contained mucin-type core-I type and core-II type structures that were further diversified by addition of GlcNAc or Gal residues. Recently, Koizumi *et al.* (2009) reported on the synthesis of the glycan portions of a glycoprotein antigen of *E. multilocularis* in order to elucidate the interactions between oligosaccharides and sera of AE by enzyme-linked immunosorbent assay (ELISA). Stereocontrolled synthesis of branched tri-, tetra-, and pentasaccharides displaying a Galβ1→3GalNAc core in the glycan portion of the glycoprotein antigen was achieved, which may become an interesting tool for further studies on their putative biological function.

With regard to metabolized proteins, an *E. multilocularis* protoscolex-associated antigen of 62 kDa (Auer *et al.* 1988), two 70 and 90 kDa proteins (Korkmaz *et al.* 2004), and several recombinant *E. multilocularis*-proteins (such as antigen II/3 (Vogel *et al.* 1988) and its subfragments II/3-10 (Müller *et al.* 1989) and Em18 (Ito *et al.* 1995), EM10 (Frosch *et al.* 1991)), have all been published and discussed in view of a potential biological role. However, these antigens were mainly used to investigate respective immune responses with emphasis on immunodiagnosis of AE, and their biological functions have not been appropriately studied. Siles and coworkers (1998) identified and cloned a 14-3-3-gene of *E. multilocularis*, which appeared to play a key role in basic cellular events related to cellular proliferation, including signal transduction, cell-cycle control, cell differentiation and cell survival (Siles *et al.* 1998; Siles and Gottstein, 2003). *E. multilocularis* rec14-3-3 protein, used as a vaccine, was highly protective (97%) against primary challenge infection with *E. multilocularis* eggs (Siles *et al.* 2003). Gauci *et al.* (2002) identified an *E. multilocularis* cDNA encoding an antigen (designated EM95), which demonstrated that a respective EM95 recombinant protein could be used to induce significant levels of protection against challenge infection with *E. multilocularis* eggs in mice. In a similar context, Kouguchi *et al.* (2007) identified a cDNA clone, designated as EMY162 that encoded a putatively secreted protein. EMY162 shared structural features with the EM95 antigen, e.g. 31% amino acid sequence identity to EM95. RT-PCR analysis revealed that EMY162 gene expression was significantly higher than EM95 at each life cycle stage. Recombinant EMY162 antigen induced a significant level of host protection (74·3%) upon experimental challenge infection with *E. multilocularis* eggs in mice.

HOST RESPONSE TO METABOLITES AND SOMATIC PARASITE MOLECULES OF *E. MULTILOCULARIS*

In murine infections with *E. multilocularis*, the involvement of cellular immunity in controlling the infection is strongly suggested by the intense granulomatous infiltration observed in the periparasitic area of lesions (Bresson-Hadni *et al.* 1990; Emery *et al.* 1996). *E. multilocularis* appears to induce skewed Th2-responses (Emery *et al.* 1996). Based on *in vitro* and *in vivo* studies, Th2 dominated immunity was associated with increased susceptibility to disease, while Th1 cell activation through IL-12 (Emery *et al.* 1996), IFNγ (Jenne *et al.* 1998; Liance *et al.* 1998), TNFα (Amiot *et al.* 1999) and IFNα (Godot *et al.* 2003) was suggested to induce protective immunity in AE (Emery *et al.* 1998; Vuitton, 2003). 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 (Dai *et al.* 2004). At the time of initial encounter with its murine host, the metacestode might modulate the immune response; the changes that it induces are dynamic and depend on the stage of development, e.g. ranging from oncosphere, to early stage vesicles up to a fully mature and fertile metacestode. Dendritic cells (DCs) and macrophages (MØs) are among the first cells encountered by the parasite, which, by secreting and expressing certain molecules, has evolved mechanisms to suppress the

major inflammatory and thus immunopathological pathway. Interaction of parasite metabolites with Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) that are expressed largely, but not exclusively, on DCs and MØs is assumed to result in phenotypic changes and modification of the cytokine profiles produced by these cell types, but this has not yet been shown experimentally at the early post-oncospheral stage of infection of murine AE.

DENDRITIC CELLS AND MACROPHAGES (MØ)

DCs, the most important antigen-presenting cells (APCs) in the initiation of a type 1 or type 2 immune response, in dependence of the nature of the antigen(s) (Foti *et al*. 2006), range among the first players in the elaboration of a specific immune response. In the frame of a Th1 immune orientation, it is largely accepted that DCs are activated mostly by bacterial or viral pathogens via Toll-like receptor (TLR) ligation to produce IL-12 and TNF-α, both pro-inflammatory cytokines inducing a Th1 oriented response (Boonstra *et al*. 2003; Takeda *et al*. 2003). Th1-associated DC activation by microbial products evokes rapid phenotypic changes, including up-regulation of MHC class II, CD80, CD86 and CD40 (Reis e Sousa *et al*. 1999; Romagnoli *et al*. 2004). Thereafter, DCs have the ability to fully activate effector T cells. There is no mirror-image signature of cytokine and surface ligands that DCs express to stimulate Th2 differentiation. However, exposure of DCs to some helminthic antigens, including the products of filarial *Acanthocheilonema viteae* (ES-62), *Schistosoma mansoni* soluble egg antigen (SEA), and the schistosome-associated glycan lacto-N-ficopentaose III (LNFPIII), was found to pulse DCs to prime CD4+ T cells into Th2 type cells, and this occurred in the absence of increased MHC class II expression and co-stimulation molecule up-regulation (Whelan *et al*. 2000; MacDonald *et al*. 2001; Thomas *et al*. 2003). Ingold *et al*. (2000) had revealed the presence of high molecular mass glycans that form the major structural elements on the laminated layer of the metacestode of *E. multilocularis*. Whether exposure of DCs to these AE-glycans would pulse them to prime naïve CD4+ T into Th2 differentiated cells needs to be addressed.

Those helminth antigens mentioned above (ES-62, SEA, glycan LNFPIII) do not appear to induce DCs to produce IL-12 (MacDonald *et al*. 2001). Thus, the Th2 response could result as a default-pathway that occurs in the absence of IL-12 production (Sher *et al*. 2003). DCs did not display any new phenotype following stimulation with the respective parasite antigens. Thus DC-dependent Th2 immunity appeared to result from antigen presentation in the absence of DC activation and/or maturation (Maizels *et al*. 2004). Indeed, it has been previously shown that immature DCs did not mature upon exposure to unfractionated crude metacestode antigen of *E. multilocularis* (Maizels *et al*. 2004). DCs that can induce tolerance may need to be resistant to maturation-inducing factors (Morelli and Thomson, 2007). A primary early source of IL-4 is needed to drive the priming of naïve CD4+ T cells into differentiated Th2 type cells (Abbas *et al*. 1996; O'Garra, 1998). It has been shown earlier in several models of ecto- or endo-parasitic infections that IL-4 might be produced early by different cell types, including DCs and other cells such as keratinocytes, T$\gamma\delta$, mast cells and basophils (Mbow *et al*. 1994; Falcone *et al*. 1996). Aumüller *et al*. (2004) used extracts from metacestodes of *E. multilocularis* to induce basophil degranulation, as well as the secretion of histamine, IL-4 and IL-13, in a dose-dependent manner. They concluded that *E. multilocularis* induces a Th2 response upon IL-4 release from basophils.

Macrophages from AE-infected mice (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 from non-infected mice (Mejri and Gottstein, 2006). This obstructed activity in antigen presentation of AE-MØ appeared to trigger an unresponsiveness of T cells, which in turn led to the suppression of their clonal expansion during the chronic phase of AE infection. In a similar context it was shown that high periparasitic NO production by peritoneal exudate cells, mainly AE-MØs, also contributed to periparasitic immunosuppression (Dai and Gottstein, 1999; Andrade *et al*. 2004). Parasite-derived molecules also interfered with antigen presentation and cell activation, leading to a mixed Th1/Th2-type response at the later stage of infection. This correlated with the marked depression of the cell mediated immune response that had been observed in chronic AE (Devouge and Ali-Khan, 1983; Kizaki *et al*. 1991, 1993).

T AND OTHER CELLS

Cells of the innate immune system are not the only targets of these immunomodulatory parasite-derived molecules. Endothelial cells (in the skin, lungs, intestine and liver) can also be induced to express and secrete anti-inflammatory mediators, such as IL-10 and prostaglandins (Zaccone *et al*. 2008). In this way, the parasite not only reduces its likelihood of elimination but can also minimize local host-tissue damage, with coincidental and paradoxical benefits for the host. By inducing functional changes in DCs and MØs, the metacestode can achieve important shifts in T cell subsets. An initial acute inflammatory Th1 response is subverted gradually to a Th2 response during the chronic phase of AE. Cytokines, such as IL-4, IL-5, IL-9 and IL-13, secreted largely

by immune cell types in response to parasite antigens, not only down-modulate the Th1 response but can also promote parasite expulsion and tissue renewal and repair (Pennock and Grencis, 2006). The metacestode most likely achieves the Th2 expansion through the induction of regulatory cytokines, such as IL-10 and TGF-β (Zhang *et al*. 2008). As mentioned above, in murine AE, the host cell mediated immune response plays an important role in controlling the metacestode proliferation.

In the past decade, the Th1–Th2 paradigm has been revisited continually and alternative T cell lineages have been proposed. In particular, the recent discovery of the IL-17 cytokine family has added a new dimension to the balance of inflammation and tolerance during parasite infections. The presence of IL-17-secreting CD4+ T (Th17) lymphocytes correlates with high hepatic pathology in murine schistosomiasis (Rutitzky *et al*. 2005), which prompts a more detailed similar investigation in murine AE. Another member of the IL-17 cytokine family, IL-25 (or IL-17E), also has an important role in parasitic infections. Fallon *et al*. (2006) demonstrated that IL-25 was important for mounting an appropriate Th2 response to *Nippostrongylus brasilensis*, and also for efficient parasite clearance. The sources of IL-25 include activated Th2 cells, mast cells and a non-B non-T cells (Fort *et al*. 2001). IL-25 as a new player will have to be the focus of respective investigations in murine AE, too.

EOSINOPHILS

One of the striking features observed in experimental murine AE (and also in naturally acquired AE of humans) is the absence of any eosinophilia. The mobilization of eosinophils is known to be a crucial immunological event that plays an important role in the host defence against helminths. Eotaxin, a CC-proinflammatory chemokine, is one of several described chemo-attractants for eosinophils. In addition, also IL-5 may mobilize these cells (Yamaguchi *et al*. 1988) but its role remains controversial. In many examples of nematode infections, eosinophilia is a marked characteristic, and eosinophils directly cause profound damage to the worm tegument, such as in *Strongyloides ratti* and in *T. spiralis*, in which a marked reduction of fertility and longevity was observed (Machado *et al*. 2005). On the other hand, IL-5 and eosinophils had no detectable effects on the infection with *Mesocestoides corti*, *Hymenolepis diminuta* and *Fasciola hepatica* (Ovington and Behm, 1997). An extravasation of eosinophils causing eosinophilia in the peritoneal cavity has been demonstrated to be beneficial for the host by causing damage to the immigrant immature *Fasciola hepatica*, resulting in the erosion of the tegumental syncytium (Burden *et al*. 1983). Eosinophils possess granules containing a variety of toxic molecules (major basic protein (MBP), peroxidase, neurotoxin, histaminase and others) which are active against many multicellular parasites, in particular helminths (Saraswathi *et al*. 2003). To elucidate the situation in experimental murine AE, we demonstrated that metacestode antigens (VF and E/S) exhibit proteolytic activity on eotaxin *in vitro* (Mejri and Gottstein, 2009). Inhibition of eotaxin activity may suppress the mobilization of eosinophils into the peritoneal cavity of intraperitoneally AE-infected mice. Eotaxin is considered as one of the main activator and chemo-attractant of resident eosinophils secreted principally by epithelial cells of the intestine (Mishra *et al*. 1999). A putative inhibition of granulocytic eosinophil infiltration may be relevant for parasite survival, as this type of cells is particularly important in the defence against helminths (Ovington and Behm, 1997). In experimental murine AE, the detected eotaxin inactivation by VF and E/S products may contribute to explain the absence of eosinophils within the peritoneal cavity of AE-secondary infected mice. Absent eosinophils thus may be a part of a series of events that maintain a low level of inflammation displayed within the peritoneal cavity of experimentally infected mice.

FROM MURINE TO HUMAN AE

The conventional course of AE as a disease in humans resembles strongly that of the naturally infected mouse, in that untreated AE will, in many but not all cases, finally lead to fatality. The first detection of naturally 'aborted' calcified liver lesions in *E. multilocularis*-infected persons (Godot *et al*. 2000; Gottstein *et al*. 2001) have indicated that not all infected individuals permit *E. multilocularis* metacestode development (Vuitton, 2003). That cell mediated immunological parameters play a crucial role also in human AE became clinically obvious when immunosuppressive status such as after liver transplantation (Bresson-Hadni *et al*. 2003) or during AIDS (Sailer *et al*. 1997; Zingg *et al*. 2004), resulted in a dramatically increased disease severity. Most studies so far have stressed a role for CD8+ T cells and for Interleukin-10 in the development disease susceptibility (Vuitton *et al*. 2003). A spontaneous secretion of IL-10 by PBMCs seemed to be the immunological hallmark of patients with progressive forms of AE. IL-10-induced inhibition of effector macrophages but also of antigen-presenting dendritic cells may be operating to protect the parasitic growth and survival (Vuitton *et al*. 2003). Susceptibility to infection in humans associates with predominantly TH2-related immunity (Wellinghausen *et al*. 1999), including IL-10 (Godot *et al*. 1997; 2000), IL-4 (Kilwinski *et al*. 1999), IL-5 (Sturm *et al*. 1995) production, especially during chronic stage of infection. Thus, in terms of Th polarization and associated cytokine expression, man

and mouse appear to respond to infection quite similarly. Kocherscheidt *et al*. (2008) studied chemokine responses in AE patients at different states of infection (progressive, stable and cured AE). The production of CC and CXC chemokines which are associated with inflammation (MIP-1 alpha/CCL3, MIP-1 beta/CCL4, RANTES/CCL5 and GRO-alpha/CXCL1) was constitutively larger in all groups of AE patients than in controls (Kocherscheidt *et al*. 2008). A disparate cellular responsiveness was observed in all groups of AE patients to viable *E. multilocularis* vesicles; cluster 1 (GRO-alpha/CXCL1, MCP-3/CCL7, MCP-4/CCL13, TARC/CCL17, LARC/CCL20) and cluster 2 chemokines (PARC/CCL18, MDC/CCL22, MIG/CXCL9) were down-regulated, while cluster 3 chemokines (MIP-1 alpha/CCL3, MIP-1 beta/CCL4, RANTES/CCL5) appeared up-regulated (Kocherscheidt *et al*. 2008). The fact that *E. multilocularis* metacestodes selectively suppressed cellular chemokine production in AE patients may constitute an immune escape mechanism which reduces inflammatory host responses, prevents tissue destruction and organ damage, but may also facilitate parasite persistence.

FROM AE TO CE

In tandem with AE, the host response to infection with *E. granulosus* (cystic echinococcosis, CE) exhibits some similarities, but also striking dissimilarities. *E. granulosus* 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, and a marked difference to AE. On differentiation into the hydatid cyst, mechanisms inhibiting complement activation on the cyst wall have been elucidated, contributing to the understanding of how the inflammatory response is controlled during CE. Similarly to AE, immunoregulatory events have been linked to the generation of T suppressor populations and to impairing the accessory action of macrophages in lymphoproliferative responses (Riley and Dixon, 1987). *E. granulosus* was also shown to be a polyclonal activator of B cells inducing both transformation and differentiation, and the effect was thymus independent (Cox *et al*. 1989). The mechanism by which the hydatid cyst regulates potentially larvicidal effector mechanisms appeared to be based on the production of lymphokines suppressive for metacestode killing (Jenkins *et al*. 1990). Data obtained from experimental infections of *E. granulosus* supported the hypothesis that early IL-10, secreted by B cells in response to non-protein antigens, may favour parasite-survival and the establishment of a polarized type-2 cytokine response (Baz *et al*. 2006). The coexistence of elevated quantities of interferon gamma (IFN-g), interleukin (IL)-4, IL-5, IL-6 and IL-10 observed in most of CE patients supports Th1, Th17 and Th2 cell activation in CE. In particular, Th1 cell activation seemed to be more related to protective immunity, whereas patients with active and transitional cyst presented a rather mixed Th1/Th2 and Th0 orientation (Rigano *et al*. 2004). The latter may be actively triggered by the parasite itself, as demonstrated by the fact that the hydatid cyst secretes and exposes numerous immunomodulatory molecules to the host's immune system, similar to AE (Siracusano *et al*. 2008).

MODULATION OF HOST RESPONSE BY *E. MULTILOCULARIS* METABOLITES

The larval infection with *E. multilocularis* begins with the intrahepatic post-oncospheral development of a metacestode. In certain cases, an appropriate host immune response may inhibit parasite proliferation. Several lines of evidence obtained *in vivo* and *in vitro* indicate the important bio-protective role of the metacestode laminated layer (Gottstein *et al*. 2002). For instance, the laminated layer has been proposed 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 (Dai and Gottstein, 1999; Andrade *et al*. 2004), explaining why iNOS-deficient mice exhibit a significantly lower susceptibility towards experimental infection (Dai *et al*. 2003).

Carbohydrate components of the laminated layer, such as Em2(G11) and Em492, as well as other parasite metabolites 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 (Dai *et al*. 2001). 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 (Walker *et al*. 2004) as a purified parasite metabolite suppresses ConA and antigen-stimulated splenocyte proliferation.

Interesting insights into immunomodulation by the parasite were obtained with regard to human AE. Hübner *et al*. (2006) examined the production of cytokines, chemokines and the expression of CD molecules on peripheral blood mononuclear cells (PBMC) from AE patients and healthy controls in response to *E. multilocularis* metacestode culture supernatant, viable metacestode vesicles and vesicle fluid antigen *in vitro*. After 48 h of co-culture, the antigens depressed the release of the proinflammatory cytokine interleukin (IL)-12 by PBMC. This effect was dose-dependent and a suppression of

tumour necrosis factor (TNF)-α and IL-12 was observed even when PBMC were activated with lipopolysaccharide (LPS). Comparing proinflammatory cytokine release by AE patients and controls showed that the release of IL-12 and TNF-α was reduced in AE patients, which was accompanied by an increased number of CD4+CD25+ cells and a reduced release of the Th2 type chemokine CCL17 (thymus and activation regulated chemokine, TARC), suggesting an anti-inflammatory response to the metacestode in human AE patients (Hübner *et al*. 2006). Instead, the production of IFN-γ and the expression of CD28 on CD4+ T cells were increased in PBMC from AE patients when compared to controls. This was accompanied by a higher release of the Th2-type chemokine CCL22 (macrophage derived chemokine, MDC) supporting that *E. multilocularis* also generates proinflammatory immune responses. These results indicate that *E. multilocularis* antigens modulated both, regulatory and inflammatory, Th1 and Th2 cytokines and chemokines.

Sako *et al*. (2007) isolated two cDNA clones from *E. multilocularis* metacestodes encoding cysteine peptidases (EmCLP1 and EmCLP2). The authors showed that EmCLP1 and EmCLP2 are capable of degrading a variety of proteins, including components of the extracellular matrix, albumin and also IgG. The degradation of IgG appeared to interfere with the cytotoxic activity of infiltrating neutrophils and macrophages. These cells were attracted by IL-8 and MCP-1, respectively, following activation *in vitro* of PMN and PBMC from AE patients by parasite vesicles. Such production of chemokines in the presence of specific antibodies may not enhance cellular attacks on the parasite, but stimulate further emigration of effector cells into the inflammatory lesions surrounding proliferating *E. multilocularis* metacestodes (Dreweck *et al*. 1999). The degradation of humoral molecules might be closely related to the pathogenesis of intrahepatic AE. It has also been shown that the metacestode development in the murine liver is triggered by cell signaling originating from the intermediate host (Brehm *et al*. 2006). The phosphorylation of EmMPK1, a parasitic orthologue of the extracellular signal-regulated kinase (ERK) MAPK, is specifically induced in *in vitro* cultured *E. multilocularis* metacestode vesicles, in response to exogenous host serum, hepatic cells and/or human epidermal growth factor (EGF). The *E. multilocularis* metacestode is thus able to 'sense' host factors which results in an activation of the parasite MAPK cascade (Spiliotis *et al*. 2006). The fact the intrahepatic metacestode expresses signaling systems with significant homologies to those of the host raises the interesting question whether cross-communication between cytokines and corresponding receptors of host and parasite can occur during an infection, i.e. whether the parasite may also influence signaling mechanisms of host cells through the secretion of various molecules that might bind to host cell surface receptors. Such interactions could contribute to immunomodulatory activities of *E. multilocularis* or be involved in mechanisms of organotropism and/or in host tissue destruction or regeneration during parasitic development. Lin *et al*. (2009) have recently described three mitogen-activated protein kinases (MAPKs), namely p38, JNK and ERK1/2, that become activated in primary cultures of rat hepatocytes upon exposure to metacestode vesicle fluid. JNK activation by host-free supernatant of *E. multilocularis* cultures suggested that liver cell signaling pathways are actually activated by parasitic components. Hepatic proliferation in AE could thus be induced through a direct influence of the parasite and not only linked to the usual reaction of hepatic cells to the occupying process that takes place in the liver (Lin *et al*. 2009).

One prominent pathway in the cross-talk between *E. multilocularis* metacestodes in infected tissues and the immune effector cells involves the activating killer cell lectin-like receptor (NKG2D) and its ligands (major histocompatability complex class I chain–related molecules A and B [MICA/B] and UL16-binding proteins) (Bahram, 2000; Groh *et al*. 2001). The germinal layer of the parasite and especially its inner germinal layer, demonstrated strong staining with the anti-MICA/B Mab, implying its presence within these compartments. Hepatic cells of the liver parenchyma surrounding the metacestode expressed MICA/B, even in areas distant from the parasitic vesicles. Because parasite and host cells expressed high amounts of MICA/B proteins, they represent putative targets of liver-infiltrating CD8+ T cells and/or NK cells that constitutively express NKG2D on their surface. The strong expression of MICA/B in the liver of AE patients contrasted with the low number of NK cells and the lack of NKG2D expression on the numerous CD8+ T lymphocytes of the periparasitic infiltrate (Zhang *et al*. 2008). Some conventional cancer patients presented elevated levels of tumour-derived soluble MICA in their serum (Jinushi *et al*. 2005). This soluble MICA reduced surface expression of NKG2D and impaired NK and CD8+ T cell functions (Holdenrieder *et al*. 2006). Sustained expression of NKG2D ligands on tissue targets could also down-regulate surface expression of NKG2D and reduce general cytotoxicity (Doubrovina *et al*. 2003; Wu *et al*. 2005). Therefore, although soluble MICA was absent in the serum of AE patients, the strong sustained expression of MICA/B molecules by *E. multilocularis* metacestodes and host cells might lead to down-regulation of NKG2D, with subsequent inhibition of NKG2D-dependent CD8+ T cell–mediated cytotoxicity. This could contribute to the sustained growth of the parasite. Additionally, TGF-β, present in most T cells of the granuloma, down-regulate surface expression of

NKG2D and contribute to the reduction of general cytotoxicity. In previous studies, TGF-β has been shown to down-regulate expression of NKG2D in cancer patients, thereby impairing NKG2D-mediated immune surveillance and mediating immune escape of tumours (Castriconi *et al*. 2003). It would not be surprising if similar events occur in AE.

PROSPECTIVE STUDIES AND APPLICATION

The elucidation of the peritoneal cytokine profile characterizing the chronic infection mode of murine AE is crucial to explain some mechanisms behind the impairment of the immune response against the infective larval stage of *E. multilocularis*. The role of DCs, known to orchestrate the immune response, needs to be understood in more detail. Therefore, one needs to address the question whether peritoneal DCs are activated by monitoring their abundance and gene expression levels of cytokines such as TGF-β, IL-10 and IL-12 following AE-infection. It has been previously shown that immature DCs did not mature upon exposure to unfractionated crude metacestode antigen of *E. multilocularis* (Jenne *et al*. 2001), and lymphocytes in periparasitic infiltrates of AE-infected liver strongly expressed TGF-β, a pleitropic immunosuppressive cytokine (Zhang *et al*. 2008). TGF-β is able to attenuate CIITA gene expression and consequently inhibits HLA-DRA expression. Moreover, it inhibits the expression of co-stimulatory molecules CD80, CD86 and CD40 on APCs (Rojas *et al*. 1999). Therefore, one needs to study the integrity of the pathway used by MHC class II molecules within the AE-DCs and the formation of MHC class II-peptide complexes on the surface of AE-DCs, providing the first signal following recognition by CD4+ T helper cells (Germain, 1994). The gene expression levels of the different molecules implicated in the formation of MHC class II-peptides complexes, including CIITA, I-aβ chain, invariant chain (li), non classical class II molecule (H-2M) and cathepsin S enzyme (Cat-S) (Weenink *et al*. 1997) have to be assessed as well. Studies on the phenotype, in particular the surface expression of co-stimulatory molecules such as B7-1, B7-2 and CD40, will show whether AE-DCs provide the secondary signal required for T cell activation.

Several subpopulations of regulatory T cells have been described in other infection models, including suppressive CD8+ T cells (Honey, 2005), two types of regulatory T cells (Tr1) and T helper 3 (Th3) that might be induced at the mucosal surface to maintain tolerance. Both of them mediated regulation by secretion of soluble factors IL-10 (Tr1) and/or TGF-β (Th3). These regulatory cells were often found within the intestinal mucosa (Groux *et al*. 1997; Faria and Weiner, 2005). Another key regulatory T cell is the natural CD4+CD25+ T cell. These cells express Foxp3, a transcription factor protein that inhibits IL-2 production (Hori *et al*. 2003; Ghiringhelli *et al*. 2005). Herein it will interesting to investigate the generation and implication of regulatory CD4+ and CD8+ T cells in the suppression of the immune response of AE-infected mice. As it had already been reported that naïve CD4+CD25+ T cells do not proliferate unless supplemented with IL-2 or IL-4, the Th2 immune response, characterized by high expression levels of IL-4, provides, in the presence of TGF-β, favourable conditions to promote the propagation of regulatory AE-CD4+CD25+ pT cells. To clarify this issue, it will be interesting to determine the proportion of CD4+CD25+ reg T cells in the peritoneal cavity of AE-infected mice, and to elucidate whether these cells produce TGF-β.

We can by no means provide a complete picture of the range of molecules that may be produced by the *E. multilocularis* metacestode with the potential to modulate immune responses. It is assumed commonly that the production of such molecules serves to facilitate the growth and survival of the parasite. For example, the induction of TGF-β may serve two functions. Internal maturation of the metacestode tissue might depend on signaling through a TGF-β receptor, although this cytokine can also modulate the host immune response.

In terms of application of these findings to human AE, increasing our knowledge on how the parasite modulates the host immune response is crucially linked to the identification and mechanistic understanding of those parasite and host factors that interact with each other, serve in functions such as host-parasite communication, immunological cross-talk, and metacestode growth and proliferation. These are the metabolites that determine whether an infection leads to survival or death, and they represent a crucial prerequisite for the development of novel immunotherapeutic tools. Such tools could be applied for either prevention of infection and/or disease at an early stage of infection, or for the elimination of established infections, possibly in combination with appropriate chemotherapeutical measures.

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