

Parasite survival strategies and new treatment options for Echinococcosis

Prof. Dr. A. Hemphill

Faculty of Philosophy and Natural Sciences,
Institute for Parasitological, University of Bern,
Hochschulstrasse, Bern, Switzerland

Abstract

Echinococcosis is a chronic disorder in that signs and symptoms best occur after numerous years of infection. The parasite evolved strategies which includes modulation, suppression and evasion of the hosts' immune reaction to continue to exist and reproduce in spite of the state-of-the-art vertebrate immune machine. The host immune response in Echinococcosis is characterised via a strongly polarized CD4+ T-helper (Th2) cell participation, which correlates with a modern direction of the disease. But, the induction of a Th1- dominated immune response is crucial for safety in opposition to the sickness. The outer floor of the parasite, the so-referred to as laminated layer (LL) is in steady contact with host immune and non-immune cells and therefore crucially worried in parasite host- interplay. It represents the primary source of delivering molecules which might be the primary tools for the parasite to apply its immunomodulatory method. We have isolated this sort of thing, named Em492, of the LL, characterized it, and investigated its consequences on the useful level. Em492 is a heterogeneous, carbohydrate-wealthy fraction secreted with the aid of the LL. It suggests an in vitro immunosuppressive impact on splenocytes. Experiments, designed to elucidate the mechanism of the observed immunosuppressive effect, indicated that Em492 induces activated macrophages to provide increased levels of nitric oxide (NO). For this reason, extended levels of NO can be liable for the cellular immune suppression. We also located that Em492 profoundly enhance the charge of apoptosis in a described T-cellular line. Our consequences indicate that Em492 antigen can be modulating the periparasitic mobile surroundings at some point of E.Multilocularis contamination via or greater as but not completely recognized mechanisms. Consequently Em492 may want to make contributions to immunosuppressive activities that occur on the host-parasite interface.

On a part of the host there is some proof that most important histocompatibility complex (MHC) polymorphism is associated with the outcome of the sickness. A few HLA-sorts may confer resistance or

sensitivity against an contamination. But, the severity and fatality of the ailment if left untreated, makes it urgently vital to locate powerful and a success treatment options. Modern-day treatment requires surgical intervention, if viable radical, combined with chemotherapy. Benzimidazole carbamate derivatives (albendazole and mebendazole) are currently used in chemotherapy. There are most important weaknesses of the existing remedy. First, the compounds have a parasitostatic rather than a parasitocidal effect. Second, in certain cases chemotherapy has been confirmed to be useless and the reoccurrence price is as an alternative excessive, as soon as remedy is stopped. Consequently the improvement of novel treatment alternatives of echinococcosis is predicted. In latest studies of our group it has been shown that nitazoxanide NTZ (2- acetolyloxy-N-(5-nitro 2-thiazolyl) benzamide) has an in vitro parasitocidal effect on *E. Multilocularis* metacestodes and in vivo experiments in mice have shown that it is as effective as albendazole. The blended remedy of NTZ and albendazole (ABZ) turned into proven to be the simplest remedy. We have now investigated the in vitro effects of NTZ towards *E. Granulosus*, and as our consequences were very promising, an in vivo observe in mice is currently finished. For *E. Multilocularis* a first clinical look at with NTZ, involving patients from Germany, Switzerland and Austria, is underneath way.

Intruduction

Echinococcus spp. Are endoparasitic flatworms belonging to the class Cestoda. Four species in the genus *Echinococcus* are diagnosed, i.E. *Echinococcus multilocularis*, *E. Granulosus*, *E. Vogeli* and *E. Oligarthrus* [1]. Individuals of this genus are small enteric tapeworms simplest 1-7mm in period which have hardly ever extra than five proglottids [1]). A top level view of the type and the taxonomically identified species is given in desk

1. The grownup worms stay inside the intestinal mucosa in their respective very last host (mainly puppies for *E. Granulosus*, foxes, puppies and cats for *E. Multilocularis*) where sexual reproduction and subsequent egg production takes region. The eggs are shed into the surroundings thru the faeces, wherein they may be by chance taken up by means of appropriate intermediate hosts. *Echinococcus* is capable of expand in a wide range of intermediate hosts together with rodents for *E. Multilocularis*, and cattle, horses, pigs, camels and sheep for *E. Granulosus*. The eggs incorporate the first larval degree, the oncosphere. At some stage in the stomach passage the oncosphere receives activated and leaves the protective egg. It actively penetrates the intestinal lining, and migrates thru blood and lymphatic vessels to

the visceral organs. Those are normally the liver for *E. Multilocularis*, and the liver, lung and other goal organs inside the case of *E. Granulosus*. There, those oncospheres become metacestodes, which constitute the second larval degree. Inside those metacestodes, protoscoleces improvement takes place, and upon oral uptake by means of the respective final host, protoscoleces attach to the intestinal epithelium and grow to be grownup worms, accordingly concluding the lifestyles cycle (Fig.1) [2].

In vitro lifestyle of Echinococcus metacestodes

As it is presently now not viable to attract definite conclusions about factors involved in modulation of parasite differentiation from in vivo research most effective, it is vital to have the correct in vitro version gadget (reviewed in [3, 4]). Confounding host elements e.G. In studying gene expression, gene regulation and molecules of the parasite itself can be constrained to a minimal. Another critical argument that makes the improvement and use of in vitro cultivation so exciting is the truth, that a large range of experimental animals can be saved and in vivo experiments may be changed to a degree. Particularly in initial drug trying out the usage of the suitable in vitro culture device is perfect. Hence, in *Echinococcus* many distinctive in vitro tradition fashions for one-of-a-kind existence levels were developed so far [5-9]. The in vitro proliferation of *E. Multilocularis* metacestodes had already been described by using Rausch and Jentoft in 1957 [10]. However, those in vitro generated vesicles had been devoid of the laminated layer. Emery and co-employees had been capable of cultivate metacestodes in vitro only up to 10 days [11]. Inside the final couple of years distinctive techniques for the in vitro cultivation of the metacestode level of *Echinococcus* have been developed [7, 12, 13] with the goal to resolve primary parameters of parasite proliferation and differentiation, to research the position of heterologous cells, to localize parasite antigens, to analyze the effect of host derived increase elements and to dissect the ultra structure and composition of the acellular laminated layer (LL) of the parasite [14- 18]. The work supplied in this thesis depended basically on material of in vitro cultured parasite fabric. First, in vitro drug checking out of novel compounds become accomplished [19, 20] and 2d, described additives of the LL have been isolated and functionally analyzed [21].

Novel remedy alternatives for CE and AE

To be able to optimize remedy of CE and AE, novel possibly active compounds should be tested out. In vitro testing serves as a primary evaluation of parasite susceptibility to certain compounds. Moreover you'll have a look at drug uptake and respective effects on the parasite (36). The maximum

promising compounds determined to be superb in in vitro assessments, can then be utilized in in vivo experiments. Promising compounds in in vitro evaluation of drugs against *E. Granulosus* were cetrimide, [55], praziquantel [56-58], and the ionophore monensin [59], all with a protoscolicidal motion however with nearly no effect on metacestodes. Two classical anti-nematode pills, levamisole and ivermectin exhibited in vitro sports corresponding to benzimidazoles [60]. In *E. Multilocularis*, the antiviral drug isoprinosine showed to be effective towards in vitro cultivated metacestodes [61], but still now not as powerful because the benzimidazoles MBZ [62] and ABZ, ABZSN [63]. But, all of these capsules failed within the bioassay. As already noted above, Amphotericin B was recently proven to be powerful in vitro and to a point in vivo. One of the very promising new pills in opposition to AE and CE is nitazoxanide (2-acetyloxy- N-(5nitro 2-thiazolyl) benzamide, NTZ), a extensive spectrum anthelmintic also used in remedy of enteric micro organism, *Giardia* and *Cryptosporidium*. In vitro NTZ-dealt with *E. Multilocularis* metacestodes showed big alterations of the germinal layer and most importantly these handled metacestodes have been non-possible while delivered into susceptible mice [19]. Moreover, NTZ additionally triggered intense alterations and harm of *E. Granulosus* protoscoleces and germinal layer of metacestodes [20]. Examined in vivo in the mouse model, NTZ by myself or in aggregate with ABZ exhibited profound anti-parasitic outcomes. The aggregate of the two drugs supplied the most promising effects [64]. Pharmacokinetic evaluation of corresponding serum tiers in mice show the utility of a blended NTZ/ABZ treatment considerably increases the ABZSO-tiers and prolongs the lifespan of ABZSO [64]. Presently in vivo experiments are carried out to check the efficacy of NTZ against *E. Granulosus* in infected mice. In a clinical have a look at the potential of NTZ as a drug in treatment of human AE may be investigated.

Helminths and host manipulation

Manipulation of the immune reaction: possible techniques

The reaction of mammalian hosts against helminth infection is strikingly comparable, in spite of the great diversity of helminths. There may be still a debate about why and the way this consistent response in opposition to bug infection advanced (evaluation in [41, 65-68]). Helminths can persist of their host for an extended period of time and are thereby manifestly capable of one way or the other deal with the immune protection of the host. These parasites can also regularly be making use of a strategy that leads to a stability between self-protection, exploitation and inconspicuousness [66]. Possibly accordingly,

some of extraordinary and complex techniques appear to have developed (evaluation in [66]). To rapidly indicate some of them:

(i) Interference with antigen processing. Protease inhibitors in, as an instance, *Brugia malayi*, *Nippostrongylus brasiliensis*, or *Onchocerca volvulus* hinder antigen processing through intracellular proteases in dendritic cells. For this reason, antigen presentation by means of dendritic cells may be compromised [69-71].

(ii) Cytokine interference and mimicry. A TGF- β -like ligand has been noted within the cestode *E. Granulosus*, and TGF- β -like proteins are also acknowledged in, as an instance, *B. Malayi* [72, 73]. An crucial function in TGF- β signaling is played by means of Smad elements. Novel Smad proteins have been recently identified and characterized in *E. Multilocularis* [74]. Some other set of helminth cytokines are the macrophage migration inhibition factors (MIFs). Exclusive MIFs had been characterized, and their outcomes on host immune cells have been studied [75-78]. It's miles, for instance, hypothesized that a *B. Malayi* encoded MIF may set off an anti inflammatory pathway (as its mammalian homologue does) and thereby interferes with the host response [79]. An IFN- γ -like protein changed into said to be secreted by using *Trichuris muris* [80] and a neutrophil chemoattractant with chemokine-like binding residences has been discovered in *Ascaris suum* [77].

(iii) blockading effector mechanisms. The chemokine eotaxin is, for example, degraded with the aid of proteases from hookworms. Thereby, the parasite inhibits the recruitment of immune cells from the blood [81]. Analogously, serpins from numerous helminth parasites can inhibit the hobby of certain host proteases (e.G. Of cathepsin B, [82]). In the cestode *E. Granulosus*, the activation of supplement is inhibited by using uptake of the host inhibitory aspect H onto hydatid cyst wall [83].

(iv) Modulation of antigen presenting cells. Parasite-derived prostaglandins can block the manufacturing of IL-12 via dendritic cells. They thereby act as a Th2 cell selling issue [84], or they could inhibit the migration of epidermal Langerhans cells [85]. Predominant glycans of schistosome eggs (Lacto-N-fucopentaose III and lacto-N-neotetraose) set off macrophages to produce IL-10. Such as a substitute activated macrophages adopt to a suppressive phenotype which could block T-cell proliferation [86, 87]. Some other look at investigated the impact of the schistosome glycan Lacto-N-fucopentaose (LNFPIII) on naïve dendritic cells. LNFPIII promoted the differentiation into the DC2-type, a phenotype of dendritic cells that has been linked to a Th2 reaction [88].

(v) Activation of T regulatory cells. About 10% of the CD4+ T cells belong neither to the Th1 nor to Th2 type. This set of CD4+ T cells is called regulatory T cells (Treg). They specific CD25 alpha chain for the IL-2 receptor, and additionally they specific T cell receptor (TCR) and co-stimulatory molecules. A strong point is the excessive stage of IL- 10 and reworking boom element B (TGF β) secretion, both with immunosuppressive homes. It's far hypothesized that immunological tolerance is not simplest because of thymic deletion or peripheral T-mobile anergy however additionally by way of Treg cells. Yazdanbakhsh and associates [89] hypothesized that there might be a hyperlink between Treg hobby and the manipulate of irritation and pathology in each allergic reactions and infectious sickness. They concluded that Treg cells should have an impact on allergic reactivity either with the aid of inducing an anti-inflammatory milieu or as a response to epitope pass-reactive between allergens and helminth derived molecules. A primary function of Treg cells is the suppressive impact they've on traditional CD4+ cells [90-92]. Additionally, they're also able to suppress Th1 responses in an IL-10 based manner [93, 94]. Relying on the form of antigen dendritic cells are stimulated with, they activate the improvement of naïve T cells into distinctive phenotypes. The schistosomal lipid phosphatidylserine was proven to be a stimulator of innate immunity and polarized dendritic mobile populations [95]. The phosphatidylserine induced dendritic cells to set off Th2 cells and IL-10 producing Tregcells. In vivo experiments showed that contamination with schistosomes leads to the improvement of subpopulations of CD4+ cells, Th2 and Treg , and both CD4+ populations were able to inhibit Th1 responses [96].

Manipulation of the immune reaction: the molecules

Parasite-derived proteins, lipoconjugates and glycans all appear to play their function within the parasite-imposed Th2 bias within the host immune response (reviewed in [66-68]). Some of the studies, if now not to say a majority, were carried out within the schistosome version machine. So this segment will recognition particularly on schistosome research. In schistosomes, soluble egg antigens (SEA), specially glycans and glycolipids, play a critical function in such occasions. In advance research in schistosomes already started to elucidate the relation of SEAs and the induction of the commonly found Th2 reaction (e.G. [97-99]). Similarly research on this area showed the importance of glycans in immunomodulatory occasions. Induction of the Th2 response with in general excessive degrees of IgE turned into found to be largely due to the oligosaccharide lacto-N-fucopentaose (LNFPIII) [100]. In some other test, the equal glycoconjugate caused the expansion of a suppressor Gr1+ macrophage population that suppresses proliferation of naïve CD4+ T-cells [86]. Fucose residues became out to be important for the discovered

effects of LNFPIII. Multiple fucosylation on a carbohydrate seems to set off very green Th2 responses [68]. In a study by van der Kleij et al. [101], in which the authors synthesized neoglycoconjugates, difucosylated glycoconjugates (LDN-DF) became out to be most green in inducing cytokine production by way of monocytes.

Furthermore, measurement of cytokine manufacturing in peripheral blood mononuclear cells (PBMC) in response to the LDN-DF neoglycoconjugate unveiled LDN-DF as a strong inducer of Th2-related cytokines. Candidate glycan motifs like □ three-fucosylation of a chitobiose (GlcNac₂) core and □ 2-xylosylated core sugar have been identified in the helminths *S. Mansoni*, *Haemonchus contortus*, and *Caenorhabditis elegans*. Curiously in hypersensitive reactions evoked by honeybees and some vegetation, N-glycans containing the middle □ 1-three-fucose are suspected to be crucially worried inside the hypersensitivity [102]. Van Die et al. [103] finish that this conserved glycan may constitute a commonplace IgE epitope. Faweeuw et al. Could show that the presentation of schistosomal glycoconjugates by means of dendritic cells (DCs) to T-cells leads to the induction of Th2 response and to egg-precipitated granulomatous response in the liver tissue [104]. Experiments in other helminth parasites like, as an example, *Taenia crassiceps* affirm the immunological adjuvant properties of N- and O-connected glycans. Excessive tiers of IgG1, IL-6, and IL-10 factors which might be associated with the Th2 kind are favoured in reaction to the parasite glycans [105].

Manipulation of the immune reaction: the receptors

Ligands that comprise so-known as pathogen-specific molecular styles (PAMPs) are typically of bacterial origin [106]. They're recognized via sample reputation receptors (PRRs) as, for instance, the recently found toll like receptors (TLRs) [106]. TLRs are gift on antigen imparting cells along with macrophages and dendritic cells and consequently are crucially involved in signaling and initiation of innate immune response. To this point ten mammalian TLRs are defined and that they understand an expansion of microbial merchandise (assessment in [107]). There may be a bulk of facts to be had on ligands of bacterial origin worried in APC activation thru PAMP recognition (e.G. [108, 109] and the following induction of pro-inflammatory responses. However, tremendously little is thought on PAMP-activation of APCs main to a Th2 response. Van der Kleij et al. Established that a schistosomal precise lipid turns on TLR2 and affects immune polarization closer to Th2 response [95]. A later take a look at in schistosomes confirmed that the glycan LNFPIII induces the maturation of dendritic mobile 2 phenotype (DC2) the use of a TLR4 structured mechanism [88]. And in a recently published experiment it was

proven that *Taenia crassiceps* carbohydrates stimulate naïve macrophages to specific IL-6 mainly through TLR4 and to a lesser quantity via TLR2 [110]. It became not clear till lately whether or not any other elegance of receptors, the C-type lectins expressed by way of dendritic cells, can be involved in helminth carbohydrate recognition. Structural analysis of carbohydrate recognition domains on C-type lectin dendritic mobile- unique ICAM-three-grabbing nonintegrins (DC-signs) set the idea of selective recognition of oligosaccharides [111]. Appelmelk et al. Should show that DC-signs and symptoms bind to synthetic mannose- and fucose-containing glycoconjugates [112]. What makes this result exciting within the context of helminth glycan popularity by APCs is the fact that those carbohydrate systems are abundantly expressed in pathogens along with e.G. *Leishmania mexicana* and *S. Mansoni*. In an inhibition test, the identical research organization proven the major role of DC-sign receptor in recognition of the glycan antigen Lewisx of *S. Mansoni* [113]. Helminth parasites like, for instance, the roundworm *Toxocara canis* additionally explicit and secrete C-kind lectins (C-TL) ([114]). C-TLs possess a purposeful carbohydrate recognition domain (CDR) [115]. Most proteins that include C-TL domains are mobile floor receptors that are crucial inside the cell signaling and specifically in the activation of immune responses. On the series level, the CRD of the parasites' C-TL became out to be very just like mammalian C-TLs such as macrophage receptors. Above, the take a look at with the aid of Loukes et al. [116] suggests that a bunch glycan is the local ligand for the parasite lectin. It's far hypothesized that parasite homologues can also interfere in host cell signaling pathways in competitively inhibiting host immune mobile lectins from recognizing their ligands.

There are some of in addition surface receptors of macrophages and DCs inclusive of macrophage mannose receptor, DEC-205, and galactose-GalNAc that bind pathogen glycans [117].

Conclusion

The LL is of incredible excessive physical and chemical stability [16]. With the aid of appearing as a bodily barrier, the LL become consequently proposed to defend the parasite from nitric oxide (NO) produced by using peri-parasitic macrophages and dendritic cells [16, 118]. The LL has also been postulated to prevent immune recognition by surrounding T cells [27]. The LL, and extra particularly the immunodominant mucin-kind Em2-antigen in the LL, also seems to be involved in antibody maturation and in the modulation of antigen recognition and T cell activation, and may hence strongly impact cytokine production at the host-parasite interface [17].

Dematteis et al. Have isolated a carbohydrate-rich fraction named E4+ from *E. Granulosus* protoscoleces [46]. This fraction can also have a role inside the induction and maintenance of the Th2-type response at some stage in experimental *E. Granulosus* contamination in a murine model. E4+ is described via immunoreactivity with the monoclonal antibody mAbE492/G1 [46]. Current studies on the immune response in humans have recommended a conjectural role for E4+ throughout the route of infection with *E. Granulosus* [119].

In our paintings, the mAbE492/G1-binding fraction of *E. Multilocularis* metacestodes, sooner or later particular as Em492-antigen, was isolated from in vitro and in vivo generated parasites and become in addition characterised [21]. Our look at indicates that Em492- antigen is secreted, then transiently localized at the metacestode surface before it is released, and for that reason will be one of the elements contributing to the modulation of mobile immunity for the duration of murine alveolar echinococcosis (AE). Em492-antigen is specially of carbohydrate nature and reacts with a sequence of lectins. The epitope identified by means of mAbE492/G1 is sensitive to sodium-periodate, however is not laid low with protease treatment. Anti-Em492-IgG1 and –IgG2, and decrease levels of IgG3, have been found in sera of mice stricken by experimentally brought about secondary, however not primary, AE. But, with reference to cellular immunity, a suppressive effect on concavalin A or crude parasite extract-precipitated splenocyte proliferation in these mice turned into discovered upon addition of Em492-antigen, however Trypan-blue exclusion exams and TEM failed to reveal any cytotoxic effect in Em492-antigen-handled spleen cells [21]. Preliminary studies on the effect of Em492 on macrophages showed that nitric oxide (NO) manufacturing by means of peritoneal macrophages (macs) was considerably more suitable in macs co cultured with Em492 in comparison to sham treated (Walker, Spicher, Brunner and Hemphill, unpublished consequences). This could be a primary indication that NO plays a function in suppressing spleen cellular proliferation. In advance experiments in our group completed with wild type and iNOS-KO mice (inducible NO synthase knock out mice) established that excessive levels of NO production discovered throughout persistent infection contributes to the immunosuppressive phenomenon [118, 120].

We in addition studied the mechanism that might be involved within the NO-mediated immunosuppression. Induction of apoptosis by way of activated T-lymphocytes is one opportunity. It's been shown that NO and different intermediators are cytotoxic and might set off apoptosis in lots of mobile sorts inclusive of macs or T-lymphocytes [121],[86]. To test whether Em492 immediately brought about apoptosis in T-cells, we finished in vitro experiments with the T cellular hybridoma A1.1 (A1.1.

Cells). In this cell line, apoptotic signaling is mediated via stimulation of dying inducing molecules at the cellular surface inclusive of Fas/FasL [122-124]. Upon activation by means of \square CD-three, A1.1. Cells go through apoptosis [122]. First effects confirmed that incubation of A.1.1 cells with \square CD-three collectively with Em492 complements the rate of apoptotic cells via greater than 20% in comparison to A1.1. Cells handled only with \square CD-3 (Walker, Spicher, Brunner and Hemphill, unpublished results). These observations suggest that Em492-antigen modulates the peri-parasitic mobile environment all through contamination thru as but unidentified mechanisms. Consequently, Em492 can be one of the factors contributing to immunosuppressive events that arise at the host-parasite interface.

References

1. Eckert, J. (1997). Alternatives to animal experimentation in parasitology. *Veterinary Parasitology* 71, 99-120.
2. Howell, M.J., and Smyth, J.D. (1995). Maintenance and cultivation of *Echinococcus* species *in vivo* and *in vitro*. In *Echinococcus* and Hydatid Disease, R.C.A. Thompson and A.J. Lymbery, eds. (Wallingford, UK: CAB INTERNATIONAL), pp. 201-227.
3. Thompson, R.C.A., and Eckert, J. (1982). The production of eggs by *Echinococcus multilocularis* in the laboratory following *in vivo* and *in vitro* development. *Zeitschrift für Parasitenkunde* 68, 227-234.
4. Jura, H., Bader, A., Hartmann, M., Maschek, H., and Frosch, M. (1996). Hepatic tissue culture model for study of host-parasite interactions in alveolar echinococcosis. *Infection and Immunity* 64, 3484-3490.
5. Ingold, K., Gottstein, B., and Hemphill, A. (2000). High molecular mass glycans are major structural elements associated with the laminated layer of *in vitro* cultivated *Echinococcus multilocularis* metacestodes. *International Journal for Parasitology* 30, 207-214.
6. Stettler, M., Fink, R., Walker, M., Gottstein, B., Geary, T.G., Rossignol, J.F., and Hemphill, A. (2003). *In vitro* parasitocidal effect of nitazoxanide against *Echinococcus multilocularis* metacestodes. *Antimicrobial Agents and Chemotherapy* 47, 467-474.
7. McManus, D.P., Zhang, W.B., Li, J., and Bartley, P.B. (2003). Echinococcosis. *Lancet* 362, 1295-1304.
8. Kern, P. (2003). *Echinococcus granulosus* infection: clinical presentation, medical treatment and outcome. *Langenbecks Arch Surg* 388, 413.

9. Vuitton, D.A. (2003). The ambiguous role of immunity in echinococcosis: protection of the host or of the parasite? *Acta Tropica* 85, 119-132.
10. Maizels, R., Balic, A., Gomez-Escobar, N., Nair, M., Taylor, M.D., and Allen, J.E. (2004). Helminth parasites-masters of regulation. *Immunological Reviews* 201, 89.
11. Sato, C., Kawase, S., and Yano, S. (1999). Monoclonal antibodies specific to carbohydrates of *Echinococcus multilocularis*. *Japanese Journal of Infectious Diseases* 52, 156-159.
12. d Riddler, C.R. (1986). Praziquantel adversely affects protoscolices of *Echinococcus granulosus in vivo*. *Journal of Helminthology*, 279.
13. Rogan, M.T., and Richards, K.S. (1986). *Echinococcus granulosus: in vivo* effect of monensin on the tegument of the protoscolex. *Parasitology* 93, 347.
14. Martinez, J., Perez-Serrano, J., Bernadina, W.E., and Rodriguez-Caabeiro, F. (1999). *Echinococcus granulosus: in vivo* effects of ivermectin and praziquantel on hsp60 and hsp70 levels. *Experimental Parasitology* 93, 171-180.
15. Lawton, P., Walchshofer, N., and Sarciron, M.E. (2001). *In vivo* effects of isoprinosine and a dipeptide methyl ester on *Echinococcus multilocularis* protoscoleces. *Journal of Helminthology* 75, 251.
16. Casado, N., Perez-Serrano, J., Denegri, G., and Rodriguez-Caabeiro, F. (1996). Development of chemotherapeutic model for the *in vivo* screening of drugs against *Echinococcus granulosus* cysts: the effects of an albendazole-albendazole sulphoxide combination. *International Journal for Parasitology* 26, 59.
17. Pearce, E.J., and MacDonald, A.S. (2002). The immunobiology of schistosomiasis. *Nature Reviews Immunology* 2, 499-511.
18. Dainichi, T., Maekawa, Y., Ishii, K., Zhang, T.Q., Nashed, B.F., Sakai, T., Takashima, M., and Himeno, K. (2001). Nippocystatin, a cysteine protease inhibitor from *Nippostrongylus brasiliensis*, inhibits antigen processing and modulates antigen-specific immune response. *Infection and Immunity* 69, 7380- 7386.
19. Gomez-Escobar, N., Lewis, E., and Maizels, R.M. (1998). A novel member of the transforming growth factor-beta (TGF- beta) superfamily from the filarial nematodes *Brugia malayi* and *B. pahangi*. *Experimental Parasitology* 88, 200-209.
20. Gomez-Escobar, N., Gregory, W.F., and Maizels, R.M. (2000). Identification of tgh-2, a filarial nematode homolog of *Caenorhabditis elegans* daf-7 and human transforming growth

- factor beta, expressed in microfilarial and adult stages of *Brugia malayi*. *Infection and Immunity* 68, 6402-6410.
21. Zavala-Gongora, R., Kroner, A., Wittek, B., Knaus, P., and Brehm, K. (2003). Identification and characterisation of two distinct Smad proteins from the fox- tapeworm *Echinococcus multilocularis*. *International Journal for Parasitology* 33, 1665-1677.
 22. Pennock, J.L., Behnke, J.M., Bickle, Q.D., Devaney, E., Grecis, R.K., Isaac, R.E.,
 23. Falcone, F.H., Rossi, A.G., Sharkey, R., Brown, A.P., Pritchard, D.I., and Maizels, R.M. (2001). *Ascaris suum*-derived products induce human neutrophil activation via a G protein-coupled receptor that interacts with the interleukin-8 receptor pathway. *Infection and Immunity* 69, 4007-4018.
 25. Zang, X.X., Taylor, P., Wang, J.M., Meyer, D.J., Scott, A.L., Walkinshaw, M.D., and Maizels, R.M. (2002). Homologues of human macrophage migration inhibitory factor from a parasitic nematode - Gene cloning, protein activity, and crystal structure. *Journal of Biological Chemistry* 277, 44261-44267.
 26. Kleemann, R., Hausser, A., Geiger, G., Mischke, R., Burger-Kentischer, A., Flieger, O., Johannes, F.J., Roger, T., Calandra, T., Kapurniotu, A., Grell, M., Finkelmeier, D., Brunner, H., and Bernhagen, J. (2000). Intracellular action of the cytokine MIF to modulate AP-1 activity and the cell cycle through Jab1. *Nature* 408, 211-216.
 27. Grecis, R.K., and Entwistle, G.M. (1997). Production of an interferon-gamma homologue by an intestinal nematode: functionally significant or interesting artefact. *Parasitology* 115, S101-S106.
 28. Culley, F.J., Brown, A., Conroy, D.M., Sabroe, I., Pritchard, D.I., and Williams, T.J. (2000). Eotaxin is specifically cleaved by hookworm metalloproteases preventing its action *in vitro* and *in vivo*. *Journal of Immunology* 165, 6447-6453.
 29. Zang, X.X., and Maizels, R.M. (2001). Serine proteinase inhibitors from nematodes and the arms race between host and pathogen. *Trends in Biochemical Sciences* 26, 191-197.
 30. Diaz, A., Ferreira, A., and Sim, R.B. (1997). Complement evasion by *Echinococcus granulosus* - Sequestration of host factor H in the hydatid cyst wall. *Journal of Immunology* 158, 3779-
 31. Yazdanbakhsh, M., van den Biggelaar, A., and Maizels, R.M. (2001). Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. *Trends in Immunology* 22, 372-377.

32. Green, D.R., Flood, P.M., and Gershon, R.K. (1983). Immunoregulatory T-cell pathways. *Annual Review of Immunology* 1, 439-463.