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Review

Molecules released by helminth parasites involved in host colonization

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Parasites are designed by evolution to invade the host and survive in its organism until they are ready to reproduce. Parasites release a variety of molecules that help them to penetrate the defensive barriers and avoid the immune attack of the host. In this respect, particularly interesting are enzymes and their inhibitors secreted by the parasites. Serine-, aspartic-, cysteine-, and metalloproteinases are involved in tissue invasion and extracellular protein digestion. Helminths secrete inhibitors of these enzymes (serpins, aspins, and cystatins) to inhibit proteinases, both of the host and their own. Proteinases and their inhibitors, as well as helminth homologues of cytokines and molecules containing phosphorylcholine, influence the immune response of the host biasing it towards the anti-inflammatory Th2 type. Nucleotide-metabolizing enzymes and cholinesterase are secreted by worms to reduce inflammation and expel the parasites from the gastrointestinal tract. An intracellular metazoan parasite, *Trichinella spiralis*, secretes, among others, protein kinases and phosphatases, endonucleases, and DNA-binding proteins, which are all thought to interfere with the host cellular signals for muscle cell differentiation. Secretion of antioxidant enzymes is believed to protect the parasite from reactive oxygen species which arise from the infection-stimulated host phagocytes. Aside from superoxide dismutase, catalase (rarely found in helminths), and glutathione peroxidase (selenium-independent, thus having a poor activity with H_2O_2), peroxiredoxins are probably the major H_2O_2-detoxifying enzymes in helminths. Secretion of antioxidant enzymes is stage-specific and there are examples of regulation of their expression by the concentration of reactive oxygen species surrounding the parasite. The majority of parasite-secreted molecules are commonly found in free-living organisms, thus parasites have only adapted them to use in their way of life.

Keywords: helminths, proteinases, proteinase inhibitors, Th2 immune response, kinases, phosphatases, phosphorylcholine, acetylcholinesterase, cytokines, ROS, superoxide dismutase, peroxiredoxins

The success of host colonization by a parasite depends on its abilities to subvert the host immune defense and to survive in the host for extended periods. The most striking features of parasitic helminths are long-term persistence within the host, the ability to elicit protective immunity only after many years of exposure, and complex developmental cycles often involving stage-specific antigens.

Among the numerous parasite species, helminths (nematodes, cestodes and trematodes) (Table 1) appear to follow extremely varied and complicated routes of infection of the host tissues. There are, however, some patterns of similarities in the migratory routes of various parasites, largely dictated by the anatomical make up of the mammalian host. Infections mainly originate in the ingestion

Abbreviations: *Ac*-API, *Ancylostoma caninum* aspartyl proteinase inhibitor; AchE, acetylcholinesterase; BMP-1, bone morphogenetic protein-1; CEI, chymotrypsin/elastase inhibitor; ES, excretory/secretory; E-64, cysteine proteinase-specific inhibitor; GH, growth hormone; IEC, intestinal epithelial cell; IGF, insulin-like growth factor; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MIF, migration-inhibitory factor; mMCP, mouse mast cell proteinase; PGF, plerocercoid-produced growth hormone-like factor; PBMC, peripheral blood mononuclear cells; PC, phosphorylcholine; PRX, peroxiredoxin; SAP, sphingolipid activator protein; *Tco*-API; *Trichostrongylus colubriformis* aspartyl proteinase inhibitor; *Ts*-TCI, *Trichuris suis* trypsin/chymotrypsin inhibitor; VIP, vasoactive intesinal polypeptide.

Table 1. Characterization of parasite helminths discussed in the text

Taxon		Parasite	Description	Invasive form	Location of adult parasite	Disease and symptoms
Nematoda	Trichocephhalida	*Trichinella*	muscle parasite, wide host range	L1	small intestine	Trichinellosis
		Trichuris	human, mouse, pig whipworms	L1	caecum	Trichuriasis
	Ascaridida	*Anisakis*		L3		
		Ascaris	human, swine gut parasite	L3	small intestine	Ascariasis
		Toxocara	canine and cat gut parasite	L2	small intestine	in humans visceral larva migrans
	Spirurida	*Onchocerca volvulus*	human filarial parasite	L3	skin nodules	River blindness
		Wuchereria bancrofti	human filarial parasite	L3	lymph nodes	Elephantiasis
		Brugia	human, monkey cat, lymphatic parasite	L3	lymph nodes	Elephantiasis
		Dirofilaria immitis	canine heart worm	L3	heart, eye, peritoneal cavity in humans: heart, lungs	
		Acanthocheilonema viteae	rodent filarial worm	L3		
	Rhabditida	*Strongyloides*	human, rodent threadworms	L3	small intestine	vomiting, skin lesions, diarrhea
		Caenorhabditis elegans	free-living			
	Strongylida	*Ancylostoma caninum, Necator americanus*	dog and human hookworms, respectively	L3	small intestine	*A. caninum* in man: cutaneous larva migrans
		Ostertagia ostertagi, Haemonchus contortus	sheep, cattle gut parasites	L3	stomach	Ostertagiosis; physical weakness, death
		Trichostrongylus colubriformis	ruminant parasite	L3	stomach, small intestine	
		Nippostrongylus brasiliensis	rodent gut parasite	L3	intestine	pulmonary granulomatous lesions
Trematoda		*Schistosoma*	blood, bladder flukes, human	cercariae	blood	Schistosomiasis
		Fasciola	liver flukes, herbivorous mammals	metacercariae	bile ducts	Fascioliasis
Cestoda		*Spirometra*	dog, cat	plerocercoids	small intestine	Sparganosis in man, diarrhea, anemia,
		Taenia	man, pig, cat, dog	oncospheres	small intestine	Taeniasis

of eggs/larvae (oral route) or in active penetration of the skin by parasite larvae or their vectors (percutaneous route). After oral infection and a brief occupation of the gastrointestinal tissues by the larval stag-

es, some parasitic nematodes, roundworms (*Haemonchus*, *Ostertagia*, and *Trichostrongylus*), remain in the gastrointestinal tract for the rest of their life, being localized in the lumen as adults. The infective larvae of some helminth parasites, however, penetrate the intestinal tissue and are transported by the venous blood flow to the liver. Most nematode larvae that follow this route undergo larval moult in the liver. Then, they are transported by venous blood flow to the heart and, *via* the pulmonary artery, to the lungs. In the lungs, most nematode larvae are temporarily arrested in the capillaries and alveoli. Subsequently, they ascend the respiratory tree to enter the pharynx where they are coughed up and re-swallowed. Their life cycle ends in the intestine where they mature to the adult stage. This migratory route is typical for *Ascaris*, *Ancylostoma* hookworm and *Toxocara canis* in young dogs. Several nematode parasites like *Ancylostoma* and *Toxocara* can follow both the oral and percutaneous infection routes to arrive in the lungs of the host.

Filarial nematodes are vectored by arthropods; they mature and mate in specific host tissues. Adult filariae dwell in various human tissues where they can live for several years. The lymphatic filariae *Wuchereria bancrofti* and *Brugia malayi* reside in lymphatic vessels and lymph nodes. Developing males and females of *Onchocerca volvulus* accumulate in subcutaneous tissue where they usually induce formation of nodules. Adult female worms produce microfilariae which circulate in the blood, except for those of *Onchocerca volvulus* and *Mansonella streptocerca*, occurring in the skin. The microfilariae infect biting arthropods (mosquitoes for the agents of lymphatic filariasis, blackflies for *O. volvulus*). Inside the arthropod, the microfilariae develop in one to two weeks into infective filariform (third stage) larvae. During subsequent blood consumption by the insect, the larvae infect the vertebrate host. The larvae migrate to the appropriate site of the host body, where they develop into adults. It is a slow process which can last as long as one year.

The migration profiles of the major trematode parasites (flukes) differ significantly from those of gastrointestinal nematodes. The blood flukes (schistosomes) also follow the percutaneous route to the lungs, but are then carried to and settle in the portal vein *(S. mansoni)* or in mesenteric veins *(S. haematobium* and *S. japonicum)*. Pairing of male and female worms takes place before they migrate to the small mesenteric veins and egg laying commences. Eggs lodge in the intestinal capillaries (or in the capillaries of the urinary bladder in the case of schistosomosis japonicum) and pass through the wall of the intestine (or of the urinary bladder) into the lumen of these organs, aided by an inflammatory process surrounding the eggs. A proportion of the eggs laid in the mesenteric veins are swept through the hepatoportal bloodstream to the liver where a similar inflammatory process causes the major pathology associated with the infection. The liver flukes (*Fasciola hepatica* and *F. gigantica*) do not take advantage of the host's circulatory system for their transport. Newly excysted juvenile flukes actively migrate through the intestinal wall into the peritoneal cavity. Either by chance or through an as yet unknown chemotactic process, the juvenile flukes find the liver and penetrate the liver capsule. After several weeks of active burrowing through the liver tissue, they localize in the bile ducts where they mature and lay eggs that drift with the bile into the intestine. The flukes of medical importance reproduce sexually in definitive vertebrate hosts and asexually in snail intermediate hosts. Flukes have a variety of different life cycle stages. Hatched, free-swimming miracidia infect snails, in which they give rise to sporocysts and rediae. The snails emit cercariae, which infect vertebrate hosts either directly or *via* an encysted form known as a metacercaria.

The life cycle of cestodes (tapeworms) involves definitive and one or more intermediate hosts. If the intermediate host is a mammal – and this may include man as an accidental host – the hooked larva penetrates the gut wall and is distributed throughout the body *via* the blood and the lymphatic system. In the sites of predilection of the intermediate host it develops into an infective cyst. The cyst, which already contains a rudimentary scolex (head), may then for example be ingested with the raw flesh of the intermediate host by the final host (dog, cat). In the intestinal tract of the final host the scolex becomes exposed and attaches itself to the intestinal mucosa, where the tapeworm develops into the adult form. Each type of life cycle has specialized larval forms (cysticercus, cysticercoid, coenurus, hydatid cyst, coracidium, procercoid, plerocercoid).

Although the term "helminth parasites" covers polyphyletic groups such as nematodes on one hand, and trematodes and cestodes on the other, all parasitic organisms have a common history of life with the rest of free-living creatures, thus they must use universal molecules in a wide variety of adaptive functions. This review will focus on the enzymes and other molecules secreted by helminths, which are thought to assist in host tissue colonization. Many of these molecules are immunogenic but this issue is beyond the scope of the present review.

This review is addressed to biochemists. Its main aim is to show that experimental biochemistry does not need to be based only on mammalian models. Parasitic invertebrates are also worth the trouble. Many aspects of their physiology are more universal and easier to study than one might expect.

PROTEINASES

Proteinases hydrolyze peptide bonds. On the basis of important chemical groups in their active site, proteinases are separated into major classes: serine, aspartic, metallo- and cysteine proteinases. Proteinases catalyze a broad spectrum of important biological reactions leading to activation of enzymes, hormones and peptide trophic molecules. These enzymes are involved in blood coagulation and fibrinolysis, protein metabolism, immune reactions, and tissue remodeling (for review: McKerrow, 1989; Tort *et al.*, 1999).

Secretion of enzymes is a common feature of both free-living and parasitic organisms. Proteinases are required for the emergence both of free-living and parasitic protozoa, helminths and arthropods from protective cysts, eggs or cuticles. In the case of nematodes, which are moulting animals, this process is controlled hormonally (like in insects) and proteolytic enzymes are involved in the digestion of proteins associated with the cuticle and, in some instances, in resorption of the old cuticle proteins. These are leucine aminopeptidases, zinc metalloproteinase and cysteine proteinases (Page in Kennedy & Harnett, 2001). The ability to secrete proteinases hydrolyzing cuticle collagens is shared by the free-living nematode *Caenorhabditis elegans* (Wada *et al.*, 1998) and parasitic nematodes. Proteinase secretion occurs also during cell migration accompanying growth of gonads in *C. elegans* (Moerman, 1999). Maternal cysteine proteinase is essential for *C. elegans* embryogenesis, as loss of the enzyme activity leads to aberrant processing and/or conformational changes in yolk proteins, resulting in abnormal platelet fusion (Britton & Murray, 2004). Thus, secretion of proteinases for these purposes is universal among free-living and parasitic organisms.

However, the use of proteinases to degrade the extracellular matrix appears to be unique to parasitic organisms, since larvae and adults of the free-living *C. elegans* do not secrete proteinases and do not degrade the extracellular matrix (Lackey *et al.*, 1989). Specific release of digestive enzymes after infection of a host serves an integral function in the transition of a free-living larva to parasitism (Hawdon *et al.*, 1995; Gamble & Mansfield, 1996). In parasites, proteinases facilitate invasion of host tissues and digest host proteins. Additionally, they help parasites to evade the host immune response, prevent blood coagulation (McKerrow, 1989) and have potentiating effects on growth (Phares, 1996) (Table 2).

Extracellular protein digestion

Metallo- and serine proteinases are known to act in tissue/cell invasion processes in parasitic organisms, but recently cysteine proteinases have been implicated in invasion by many helminths (Sajid & McKerrow, 2002). Some of these enzymes are secreted by helminths that invade and/or feed on tissues, such as *Haemonchus contortus, Nippostrongylus brasiliensis, Strongyloides ratti* and *Ancylostoma caninum.* Cysteine proteinases of parasitic helminths function in a broader chemical environment than the homologous host enzymes. Mammalian lysosomal cysteine proteinases are active at low pH, but relatively unstable at neutral pH when compared with the parasite orthologues (reviewed in Sajid & McKerrow, 2002), the exception being mammalian cathepsin S. In marked contrast to the vertebrate proteinases the parasite enzymes are more active and remain stable at neutral pH. This broad pH profile of the parasite cysteine proteinases is consistent with the numerous extra lysosomal functions that have been characterized. Molecular phylogeny suggests that cysteine proteinases arose early in evolution to degrade proteins both intra- and extracellularly (Sajid & McKerrow, 2002). There is a wealth of examples of degradation of exogenous proteins in parasitic protozoans, helminths, and arthropods (McKerrow *et al.*, 1993; Tort *et al.*, 1999).

Digestion of hemoglobin

Two of the best-characterized parasite proteinase systems that catalyze the degradation of host proteins are the hemoglobin degrading activity of a falcipain 2 of the malarial parasite *Plasmodium falciparum* in its digestive vacuoles (Shenai *et al.*, 2000) and of the fluke *Schistosoma mansoni* cathepsin-B1 in the worm's gut (Dalton *et al.*, 1995; Fig. 1). It is likely that an analogous hemoglobinolytic proteinase system exists also in another fluke *Fasciola hepatica*, where activation of a number of cysteine proteinases including cathepsin B-like, cathepsin L-like, dipeptidylpeptidase I and asparaginyl endopeptidase have been identified. A similar pathway for hemoglobin and also fibrinogen degradation may occur in excretory/secretory (ES) products of blood-feeding nematodes.

The adult *Necator americanus* parasitizes the small intestine of man. The worms hold onto the intestinal wall and feed on blood and tissue exudates. Adult *N. americanus* ES products contain a heterogeneous mixture of proteolytic activities *in vitro*; at least two cysteine proteinases, a cathepsin B-like proteinase and a cathepsin L-like one, aspartic- and serine proteinases (Brown *et al.*, 1995), additionally anti-coagulant properties have previously been ascribed to a metalloproteinase in ES from adult hookworms (Hotez & Cerami, 1983). It is believed that proteolytic enzymes are necessary for adult hookworms for two reasons. They digest host tissue and

Table 2. Proteinases of helminths known as important for host-parasite relationships

Proteinase families			Parasites and description
Cysteine proteinases	Family C1 Papain-like	Cathepsin B-like	*Schistosoma, Fasciola, Ancylostoma, Necator, Ascaris, Strogyloides, H. contortus, Ostertagia, Trichuris, O. volvulus, C. elegans*
		Cathepsin L-like	*Paragonimus, Spirometra, Schistosoma, Fasciola, Strongyloides, H. contortus, Brugia, Toxocara, Ancylostoma, Ascaris, C. elegans*
		Cathepsin S	*Spirometra mansoni* (plerocercoids)
		Cathepsin C =Dipeptidyl peptidase 1	*Schistosoma japonicum, Haemonchus contortus*
	Family C2 Calpain-like		*S. mansoni, S. japonicum, C. elegans*
	Family C13 Legumain-like	Asparaginyl endopeptidase	*S. mansoni, S. japonicum, F. hepatica, C. elegans*
Serine proteinases	Family S1 Chymotrypsin	Elastase	*Schistosoma, Onchocerca lienalis*
		Chymotrypsin-like	*Schistosoma* (cercariae), *Schistocephalus solidus* (procercoids; collagenolytic activity), *Spirometra mansoni* (plerocercoids; cleaves IFN-γ)
		Trypsin-like	*Anisakis simplex, Spirometra mansoni* (plerocercoids; collagenolytic activity), *Schistosoma* (cercariae; elastase activity)
		Kallikrein-like	*Trichuris muris, Onchocerca volvulus, Hymenolepis diminuta, Schistosoma mansoni*
	Family S8 Subtilisin	Blisterase	*Onchocerca volvulus*
	Family S10 Carboxypeptidase C	Cathepsin A	*Nippostrongylus brasiliensis*
Aspartic proteinases	Family A1 Pepsin	Cathepsin D	*Schistosoma japonicum*
		Cathepsin E	*Onchocerca volvulus*
		Aspartic endopeptidase	*Schistosoma mansoni*
Metalloproteinases	Family M12 Astacin	Astacin	*Strongyloides stercoralis, Trichinella spiralis, Ancylostoma caninum*
	Other helminth metalloproteinases	Collagenase	*Brugia malayi, Schistosoma mansoni, Gymnorhynchus gigas*
		Dipeptidyl peptidase III	*Schistosoma mansoni*
		Metalloaminopeptidase	*Schistosoma mansoni*
		Metalloproteinases with elastase activity	*Onchocerca volvulus, Strongyloides stercoralis*

also impede the potentially damaging host-derived coagulation events (Brown *et al.,* 1995). *Haemonchus contortus* is a blood-sucking nematode occurring in the fourth stomach of sheep and other ruminants. The L4 larvae and adults of *H. contortus* cause considerable damage to the mucosal lining of the abomasum of infected sheep, resulting in extensive hemorrhages and severe chronic anemia. *H. contortus* would appear to adopt a similar feeding strategy to hookworms. ES proteinases from adult *H. contortus* also degrade fibrinogen and plasminogen and this degradation is, in part, due to cysteine- and aspartic proteinases (Karanu *et al.,* 1993; Knox, 1994). It is interesting that the uptake of radiolabeled hemoglobin by adult parasites *in vitro* is not inhibited by a cysteine proteinase inhibitor, although hemoglobin breakdown in the culture medium is reduced by 50% (Fetterer & Rhoads, 1997b). Thus it is possible that cysteine proteinases are functional in the extra corporeal digestion of the blood meal but are not

required for the uptake of the products. The latter function may be mediated by metallo- and serine proteinases.

Nematode proteinases in host tissue invasion

Connective tissue, as well as blood vessel walls, contain abundant quantities of elastin and collagen, thus secreted collagenolytic and elastinolytic enzymes could potentially facilitate the parasite's access to host tissues.

Adult *H. contortus* and L4 larvae degrade the extracellular matrix produced by smooth-muscle cells *in vitro* (Rhoads & Fetterer, 1996). Degradation of matrix involves the specific action of cysteine proteinase and is not simply the result of mechanical disruption by motile worms. Significantly, both live adult parasites in culture and adult ES products degrade the glycoprotein elastin and collagen components of the matrix produced *in vitro* by smooth muscle cells. The secreted cysteine proteinases are thus able to hydrolyze glycoproteins, which function as anchoring proteins essential in maintaining the structural integrity of the matrix. In addition, the ability of cysteine proteinases to degrade both elastin and collagen components of the matrix is similar to that of mammalian cathepsin L. However, the *H. contortus* enzyme is active over a broad range of acidic and alkaline pH (Karanu *et al.*, 1993; Rhoads & Fetterer, 1995). Thus, this property might ensure the parasite flexibility to modify or modulate host proteins or affect processes involving tissue destruction, anticoagulation, nutrition, and immune evasion. Cysteine proteinase activity could be important for the penetration of the abomasal mucus layer. It is worth mentioning that proteinases released by *Ostertagia ostertagi*, a bovine abomasal nematode, have been found to degrade bovine mucin (Geldhof *et al.*, 2000).

Trichinella spiralis is a nematode with an amazing lack of host specificity. The infective-stage larvae after being released from their capsules in the host's stomach penetrate into the epithelial cells of the small intestine. Therefore, degradation of the extracellular matrix is an essential requirement for tissue invasion and feeding (Appleton in Kennedy & Harnett, 2001). The L1 larva of *T. spiralis* has no oral appendages or a stylet. The larvae invade epithelial cells "head first". It has been shown that larvae wound the plasma membranes of rat intestinal epithelial cell line (IEC-6); that is, they create transient breaches in the membrane. Wounding is considered to be a common occurrence in intestinal epithelia. The ES products of the infective stage larvae of *T. spiralis* contain serine, aspartic and cysteine proteinases (Criado *et al.*, 1992; Moczoń & Wranicz, 1999; Lun *et al.*, 2003) and zinc-dependent metalloproteinases, with a substantial contribution of metalloendoproteinases, e.g., collagenase (Lun *et al.*, 2003). The deduced amino-acid sequence of this 58 kDa metalloproteinase of *T. spiralis* shows significant homology to both the precursor and active forms of the zinc metalloproteinases TOH-2 of *C. elegans* and various protein members of the astacin family, e.g., tolloid and BMP-1. Astacin metalloproteinases synthesized as zymogens are post-translationally activated (Stöcker *et al.*, 1995). They have diverse functions, e.g., food digestion (Stöcker & Zwilling, 1995), eggshell hatching (Yasumasu *et al.*, 1992) and hydrolysis of extracellular matrix components such as type I collagen (Yan *et al.*, 2000). During the intestinal phase of worm development, the astacin metalloproteinase should be a key effector molecule for such functions. It is less obvious, however, why adult *Trichinella,* which reside within the cytoplasm of columnar epithelial cells in the small intestine (Wright, 1979) should require degradation of fibrinogen and plasminogen (Todorova *et al.*, 1995). The authors suggest that secreted proteinases may play an anti-coagulant role, contributing to the pathology of trichinellosis. The elastase activity found in secretions of adult worms may play a role in the degradation of intestinal tissues and, hence, facilitate either the penetration of the parasites into host enterocytes or the release of nutrients (Todorova & Stoyanow, 2000).

In another species of Trichocephalida, *Trichuris muris*, secretion of two major peptidases of 85 and 105 kDa, respectively, which are serine peptidases (possessing some degree of specificity for collagen-like molecules) was observed to be time-dependent. No peptidase activity was detected in worm extracts, suggesting that these enzymes are activated during or following secretion from worms (Drake *et al.*, 1994). The *in vivo* functional role of these enzymes remains unclear. They may function in a nutritive capacity. Also, the ability of live worms to degrade basement membrane proteins (Drake *et al.*, 1994) is suggestive of an involvement in the invasive process. These enzymes may aid in the production and subsequent maintenance of the parasite syncytial habitat as the anterior portion of the adult worm becomes embedded within a syncytial tunnel derived from host caecal epithelium (Panesar, 1981). It is possible that action of secreted peptidases disrupts the integrity of epithelial cell membranes leading to cell leakage and thus contributing to the disease pathology (Cooper *et al.*, 1992).

The filaria *Onchocerca volvulus* is an important nematode parasite of humans affecting the skin and eyes. Two important events in the infection by *Onchocerca* involve cutaneous tissue migration by larval stages. L3 larvae migrate from the blackfly bite site to subcutaneous locations for adult filariae develop-

ment, and microfilariae migrate from subcutaneous nodules to distant regions of the skin and sometimes the eye (Lackey *et al.*, 1989). Serine and metalloproteinase activities in ES products of microfilariae and adult males degrade components of the dermal extracellular matrix, collagen type IV, fibronectin and laminin (Haffner *et al.*, 1998). According to the authors, the proteolytic activity in ES products of microfilariae and males is responsible for the degradation of elastic fibers of host tissue as observed in chronic onchocerciasis. Proteinase activity is absent in ES products of females. This could result from different behavior of worms; the infective larvae microfilariae and males must migrate through host tissue while adult females reside in nodules. Stage-specific secretion of *Onchocerca* 43-kDa serine elastase has been shown in *O. lienalis*, with the enzyme being secreted by L3 larvae but not adult worms. Thus, the serine proteinase of L3 larvae probably plays an important function, facilitating L3 migration from the blackfly bite site to distant regions of the body where adult filariae will develop (Lackey *et al.*, 1989). It is worth mentioning that apart from proteinases, also chitinases could play a role in filarial parasitism.

Filarial chitinases

Chitinases are enzymes that hydrolyze chitin, a homopolymer of poly-β (1-4) linked *N*-acetylglucosamine monomers. Chitin is a part of the exoskeleton of arthropods and is also found in various fungi and bacteria. It has been described as a constituent of the nematode eggshell and eggshell-derived structures (Wharton, 1983). Chitinase activity is associated with nematode eggs, uterine stages and female worms (Adam *et al.*, in Kennedy & Harnett, 2001). The coincidence between the appearance of chitinase on the sheath of microfilaria (the first larval stage of filarial nematodes) and the ability to infect the arthropod vector is indicative of a role for this protein in liberation of the microfilariae from their sheaths (Fuhrman, 1995). However, infective larvae (L3) of filariae produce and store chitinase inside the intermediate host (Adam *et al.*, 1996), while secretion is triggered by environmental conditions of the vertebrate host and occurs during the early phase of infection and during moulting (Wu *et al.*, 1996). The role of L3 chitinase is not fully understood and different scenarios are possible. Firstly, the protein could contribute to the egress of L3 larvae from the chitinous mouthparts of the vector during the blood meal. Secondly, since the release of chitinase occurs during the first days of culture under vertebrate conditions, the enzyme may act on host molecules during an early stage of infection. It is possible that chitinase interacts with, for example, elements of the extracellular matrix, facilitating the migration through host tissues (Adam *et al.*, in Kennedy & Harnett, 2001).

Platyhelminth proteinases in invasion

Unlike the nemathelminthan worms, the platyhelminths (cestodes and trematodes) do not moult. Their body is covered with a syncytial tegument (McLaren & Hockley, 1977). The tegumental surface is constantly being regenerated and sloughed off into the host bloodstream (Wilson & Barnes, 1979). The tegument is not only a protective shield for the parasite but performs other important functions at the interface between the parasite and its host (Skelly & Shoemaker, 1996)

Parasitic trematodes migrate through tissues in one or more stages of their life cycle; however, there are few examples of cysteine proteinases that are involved in tissue migration. The cathepsin L-like proteinases secreted into the gut and regurgitated from *F. hepatica* degraded laminin, collagen and other matrix proteins (Halton, 1997), and a cysteine proteinase located in the cercarial penetration glands of *Diplostomum pseudospathaceum* is thought to be involved in skin penetration of aquatic birds (Moczoń, 1994). A very interesting study of Fishelson *et al.* (1992) elucidated how the parasitic blood fluke *Schistosoma mansoni* synthesizes, stores, and releases a serine proteinase during differentiation of its invasive larvae. *In situ* hybridization with a cDNA probe allowed to localize the proteinase mRNA in acetabular cells, the first morphologically distinguishable parasite cells that differentiate from embryonic cell masses present in the intermediate host snail. Antiproteinase antibody binding showed that the proteinase progressively accumulated in these cells and was packaged in vesicles of three morphologic types. Extension of cytoplasmic processes containing proteinase vesicles formed "ducts" which reached the anterior end of fully differentiated larvae. During invasion of human skin, groups of intact vesicles were released through acetabular cytoplasmic processes and ruptured within the host tissue. Ruptured proteinase vesicles were noted adjacent to degraded epidermal cells and dermal-epidermal basement membrane, as well as along the surface of the penetrating larvae themselves. These observations are consistent with the proposed dual role for the enzyme in facilitating invasion of host skin by larvae and helping to release the larval surface glycocalyx during metamorphosis to the next stage of the parasite.

Cestodes reside in the gut of their host but the larval stages are involved in tissue invasion. Metalloproteinases are most likely the enzymes facilitating tissue invasion, and has been shown that metalloproteinase present in the migrating larval stage of the cestode *Proteocephalus ambloplitis* parasit-

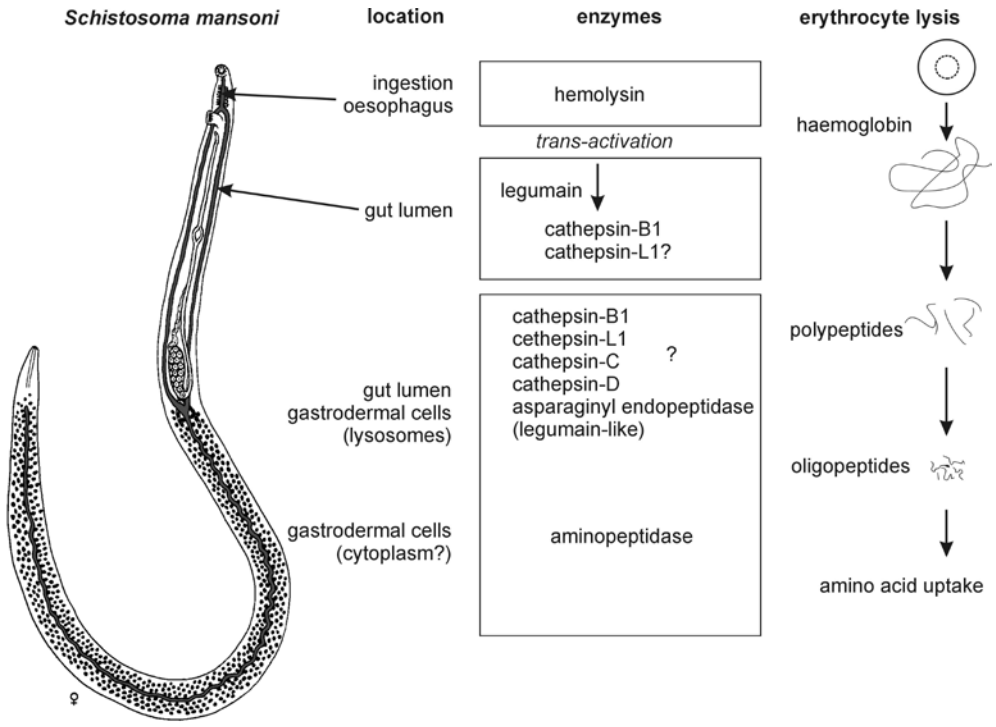

Figure 1. Schematic presentation of hemoglobin digestion by the liver fluke *Schistosoma mansoni* (based on the Sajid & McKerrow, 2002).

ic in fishes, has collagenolytic, hemoglobinolytic and slight elastinolytic activity. It is suggested that this proteinase (the only enzyme class present in this larval stage) is secreted from the apical organ during the few-hour migration into host visceral organs (Polzer *et al.*, 1994). In the cestode *Schistocephalus solidus* parasitizing birds, a chymotrypsin-like proteinase with collagenolytic activity was present in procercoids (but not plerocercoids or adults), possibly being necessary for the penetration of host intestinal wall (Polzer & Conradt, 1994). Studies *in vitro* have demonstrated that oncospheres of *Hymenolepis diminuta* that invade meal beetles by penetrating the wall of their midgut, just after emerging from their embryophores release a serine proteinase from penetration glands (Moczoń, 1996). Plerocercoids (spargana) of another tapeworm *Spirometra mansonoides* are known to cause sparganosis, a disease caused by granulomatous lesions made around the worm and the tortuous migration track of the sparganum. Sparganosis involves mainly subcutaneous tissues, but may invade visceral organs and the central nervous system. It has been demonstrated that excretory granules from gland cells and myelin-like bodies, synthesized in subtegumental cells of plerocercoids, are released during migration (Osaki, 1990). Within the host tissue, the excreted/secreted proteinases of spargana degrade the extracellular matrix, collagen, fibronectin and myosin, resulting in tissue damage and facilitating migration of the larvae (Song & Chappell, 1993). It has been documented that excretory/secretory products of *Spirometra mansonoides* plerocercoids contain tissue-lysing enzymes: cysteine proteinases of 21 kDa and 28 kDa, trypticases of 104 and 198 kDa, as well as chymase activity corresponding to 36 kDa (Cho *et al.*, 1992; Song & Chappell, 1993).

Although loss of weight by the host is commonly associated with parasitism, especially among mammals, Mueller (1963) has found that when spargana of *S. mansonoides* are injected subcutaneously into young female mice, the infected mice show accelerated gain in weight when compared to uninfected mice. This intriguing observation, which cannot be accounted for by the weight of the parasites, has initiated 33-year lasting studies of the unusual host-spirometrid tapeworm relationship. They culminated in the discovery by Phares (1996) that plerocercoids of *S. mansonoides* secreted plerocercoid-produced growth hormone-like factor (PGF).

Growth hormone-like factor of *Spirometra*

The growth hormone-like factor of *Spirometra* plerocercoids (PGF) stimulates growth by increasing the level of IGF-1 (somatomedin/insulin-like growth factor-1). PGF binds to hormone receptor(s) to stimulate growth and lipogenesis. Surprisingly, the biological actions of PGF appear to be more similar to those of human growth hormone than of other vertebrate growth hormones. PGF evokes a dramatic lactogenic response, whereas non-primate growth hormones do not have an ability to bind prolactin receptors and stimulate the lactogenic activity. Ple-

rocercoids of another species, *Spirometra erinacei*, also produce and release a growth hormone-like factor (PGF-e). There are distinct differences in its biological characteristics when compared to PGF from *S. mansonoides*. PGF and PGF-e both express growth hormone-like activities by binding growth hormone receptors, increasing IGF-1 activity and stimulating growth. However, PGF expresses only the insulin-like activities of GH, whereas PGF-e shows only anti-insulin/diabetogenic activities, presumably due to differential activation of receptors. Purification of PGF to homogeneity (Phares & Kubik, 1996) showed the product to be a single protein of 27.5 kDa with human growth factor-like activity. Comparison of the sequence of the plerocercoid protein revealed no homology to human growth factor or to any other hormone. However, the predicted sequence of the 215 amino acids contained in the 27.5 kDa protein does share 40–50% homology with cysteine proteinases. A fascinating conclusion that the 27.5 kDa plerocercoid protein is both a cysteine proteinase and a growth hormone-like factor is supported by data of Wang *et al.* (1995) who showed that *S. erinacei* also expresses PGF-e, a 27 kDa protein with both growth hormone-like and proteinase characteristics.

Addition of a cysteine proteinase-specific inhibitor (E-64) in a concentration sufficient to inhibit all hydrolytic activity, blocks receptor binding of PGF by up to 80%. Also the ability of PGF-e to stimulate proliferation of hepatocytes was inhibited by proteinase inhibitors (Tsuboi *et al.,* 1992). Whereas the 27.5 kDa protein is clearly the most abundant protein in plerocercoids (Phares & Kubik, 1996), its distribution is restricted to the plerocercoid stage, as no growth-promoting activity of this protein is detected in adult tapeworms or procercoids. The mechanism by which PGF activate the growth hormone receptor is not known.

Wang *et al.* (1995) demonstrated that PGF-e is present extensively on the external surface of the tegument and in subtegumental cells of plerocercoids of *S. erinacei*. This finding is in line with various reports of continuous release of PGF into the environment of plerocercoids (cf. Phares, 1996). Of the general protein substrates tested, collagen was the most efficiently hydrolysed one by PGF/proteinase. Therefore, based on its distribution on the surface of the worm and preference for collagen as a substrate, it is reasonable to conclude that an important function of PGF/proteinase is to facilitate tissue penetration by plerocercoids. The proteinase also appears to be involved in sloughing of the plerocercoid body, which occurs after the worms are ingested, but before penetration of the scolex out of the gut into the body cavity of mice (Wang *et al.,* 1995). The availability of an effective collagenase would seem especially important to plerocercoids of *Spirometra* as they have no hooks or other structural features to enhance tissue penetration. Furthermore, PGF/ proteinase may play an important role for plerocercoids in evasion of the host immune defense system by cleaving IgG, which attaches to the parasite surface (both PGF and PGF-e cleave IgG). A potential role of the GH-like function of PGF/proteinase in completing *Spirometra mansonoides* complex life cycle is proposed by Phares (1996): PGF stimulates growth, suppresses endogenous growth hormone levels, but does not duplicate the stimulatory effect of growth hormone on immune function (Sharp *et al.,* 1982). Even after obtaining considerable evidence that the 27.5 kDa plerocercoid protein functioned both as a growth hormone-like factor and a cysteine proteinase, no molecules with structural similarity to human growth hormone have been found. PGF is a neutral cysteine proteinase that also binds and activates growth hormone receptors from a variety of species. The serendipitous observation by Mueller in 1963 that plerocercoid-infected mice grow significantly larger than their uninfected littermates has led to the discovery of an equally unique biochemical phenomenon of a cysteine proteinase which is a partial growth hormone agonist.

Helminth plasminogen receptors: **enolase**

Plasminogen is a 92 kDa plasma proenzyme of the serine proteinase plasmin, which plays important roles in processes like fibrinolysis and degradation of extracellular matrices (Plow *et al.,* 1995). One of the best-characterized cellular plasminogen receptors in mammals is the glycolytic enzyme enolase (Redlitz *et al.,* 1995). Eukaryotic enolases have been shown to be multifunctional proteins presenting a variety of activities besides the glycolytic one (Pancholi, 2001). Enolase has also been localized on the surface of several pathogens like bacteria, fungi or protozoa (Pancholi, 2001) and very recently in helminths like *Onchocerca volvulus* (Jolodar *et al.,* 2003) and *Fasciola hepatica* (Bernal *et al.,* 2004). Enolase from *F. hepatica* excretory-secretory products binds human plasminogen *in vitro* and this suggests that enolase may function as a plasminogen receptor. These studies suggest an important role of this association for the invasion of host tissues by the pathogenic agent (Pancholi, 2001) as recent reports have pointed to an enhanced activation of plasmin(ogen) upon interaction with the pathogen-derived enolase (Lähteenmäki *et al.,* 2001; Jong *et al.,* 2003). The plasminogen-binding property of *O. volvulus* and *F. hepatica* enolase may support plasmin-mediated proteolysis, including degradation of the hosts' extracellular matrix, thereby promoting the migration of larval stages through tissues. The recognition of enolase by antibodies in sera of *O. volvulus*-infected

persons indicates an involvement of this protein in the interaction between the parasite and the human host.

IMMUNOEVASION

Investigations of different parasitic infections have provided general statement that resistance to intracellular parasite infections is associated with production of pro-inflammatory cytokines (interferon γ, IL-2, tumor necrosis factor β) by the lymphocyte subset of $CD4^+$ T helper cells known as Th1 cells evoking Th1-type of immunological response (Fig. 2). Susceptibility to infection has been associated with the Th2 type response originating from a subset of Th2 cells (Street & Mosmann, 1991). Most helminth infections of humans and animals induce immune responses which are characterized by the production of Th2-associated cytokines IL-4, IL-5, IL-9, IL-10, IL-13, and of antibodies (IgG1 in mouse, IgG4 in man, IgE in both species) by B lymphocytes. This type-2-biased immune phenotype generally persists for the duration of the infection. Among the numerous reported activities helminth-induced type-2-associated immune responses have been linked to the expulsion of gastrointestinal nematodes and the formation of circumoval granulomas in the course of schistosomosis (Hoffmann *et al.*, 2002). The regulation of Th1 and Th2 responses has been well studied (Artis & Grencis in Kennedy & Harnett, 2001) but a detailed account of Th1/Th2 responses in parasitic infections is beyond the scope of this review. It should be mentioned, though, that these responses are influenced by the type of antigen-presenting cells involved, the presence of co-stimulatory molecules and the cytokine environment present during T-cell receptor engagement. Whereas a number of studies have suggested a role for excretory/secretory products in modulation of host immune responses, both of lymphocytes and macrophages, only very few parasite molecule(s) involved have been identified.

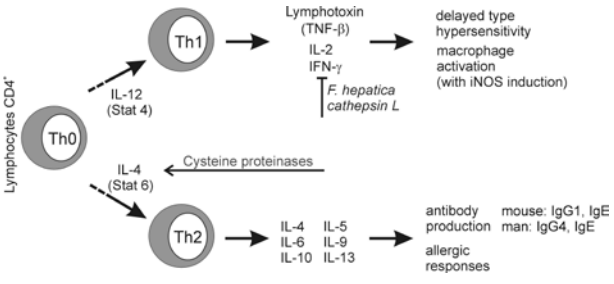

Figure 2. Polarization of Th1/Th2 immune responses under influence of parasitic infection.
Induction of IL-4 and inhibition of IFN-γ by helminth cysteine proteinase is marked with gray.

Induction of Th2 response

Several studies have proposed a role for cysteine proteinases in inhibiting Th1 immunity through the induction of IL-4. Interleukin-4 that is secreted by $CD4^+$ T cells (Fig. 2), as well as by some minor subpopulations of T lymphocytes and cells of the mast cell lines, is the main cytokine responsible for Th2 differentiation. Machado *et al.* (1996) who suggested that cysteine proteinases secreted by various pathogens down-regulate Th1 responses by a mechanism involving IL-4, demonstrated that these proteinases induce mast cells and basophils to degranulate and secrete IL-4. In accord, it has been shown that in mice, cathepsin L (a cysteine proteinase) of *F. hepatica* suppresses production of IFN-γ, a cytokine of Th1-type response (induced by *Bordetella pertusis*) (Fig. 2). Moreover, molecules secreted by *F. hepatica* induce production of IL-4 and IL-10, cytokines of Th2-type response (O'Neill *et al.*, 2001). Other studies with the nematode *Brugia pahangi* (Osborne & Devaney, 1999) and the trematode *Schistosoma haematobium* in urinary schistosomiasis (King *et al.*, 1996) revealed that parasite-reactive Th1 cells were suppressed by a mechanism that involved an up-regulation of IL-10 production which, in turn, coincided with cellular hyporeactivity.

Modulation of $CD4^+$ expression

A surface molecule, $CD4^+$ of T helper lymphocytes plays an essential role in interactions of lymphocytes with antigen-presenting cells in the generation of T helper responses. By decreasing $CD4^+$ expression, a parasite may interfere with the generation of the host immune response and hence increase their survival. *Fasciola hepatica* cathepsin L suppresses sheep T cell proliferation and reduces surface $CD4^+$ expression on both human and ovine T cells (Prowse *et al.*, 2002). A cysteine proteinase found in ES products of *Taenia solium* metacestodes can decrease $CD4^+$ expression on human lymphocytes. These data suggest that metacestodes may modulate $CD4^+$ expression as a way of controlling the host inflammatory response (Molinari *et al.*, 2000). Of note, two metalloproteinases from the *Leishmania* protozoan have been shown to exhibit similar effects (Hey *et al.*, 1994). Considering the earlier known *T. solium* metacestode factor, shown to inhibit IL-2, IFN-γ, IL-4 and TNF-α synthesis as well as lymphocyte proliferation (Arechavaleta *et al.*, 1998), the cysteine proteinase secreted into the host tissue may modulate both the humoral and the cellular host responses. In accord, in histological sections of both human and porcine cysticercosis viable metacestodes are surrounded by a lim-

ited inflammatory reaction (reviewed in Molinari *et al.,* 2000).

Innate immunity and helminths

Macrophages are effector cells of innate immunity and their activation brings about among others, production of IL-1β, TNF-α and free radicals: superoxide anion, produced by NADPH oxidase and nitric oxide produced by inducible nitric oxide synthase (iNOS). These free radicals have bactericidal and antiparasitic activity, well documented in the case of intracellular parasites. *Spirometra erinaceieuropaei* infects domestic and wild cats and dogs (Mueller, 1974). In human, an accidental host, the plerocercoids cause sparganosis. Studies of the influence of plerocercoid ES products on macrophages revealed suppression of macrophage pro-inflammatory response induced by a combination of lipopolysaccharide (antigen from the cell wall of Gram⁻ bacteria) and IFN-γ. Plerocercoid ES products suppressed proinflammatory response of macrophages reducing iNOS mRNA expression and production of nitric oxide (Fukumoto *et al.,* 1997). ES products suppressed IL-1β mRNA expression, as well as TNF-α gene expression and TNF production (Dirgahayu *et al.,* 2002; 2004), and IL-6 mRNA level (Tanihata, 1996). Further studies demonstrated that these ES products (possibly a protein of molecular mass > 97 kDa) interfered with signaling pathways inhibiting ERK1/2 and p38 MAPK phosphorylation induced by lipopolysaccharide (Dirgahayu *et al.,* 2002). The suppression of pro-inflammatory cytokine expression such as IL-1β and TNF-α may be among the mechanisms by which plerocercoids can successfully survive within the host (Fukumoto *et al.,* 1997).

In mice showing Th1 or Th2 response, two different types of macrophage activation have been described, M-1 (classical) involving induction of iNOS and nitric oxide production and M-2 (alternative) involving arginase induction (Mosser, 2003). We have shown that *T. spiralis* infection of guinea pigs does not induce nitric oxide production in lung macrophages (Dzik *et al.,* 2002a; 2002b) exposed to larval antigens when newborn larvae pass to the muscles. Instead, arginase activity is enhanced in these cells (Dzik *et al.,* 2004), concomitantly with respiratory burst induction (Dzik *et al.,* 2006), both being elements of the non-classical type of response. Recently, it has been shown that thioredoxin peroxidase secreted by *F. hepatica* induces the alternative activation of macrophages characterized by the production of high levels of IL-10, a Th2-type cytokine (Donnelly *et al.,* 2005). In view of the foregoing, helminth-induced anti-inflammatory response concerns macrophages as well.

Do helminths produce cytokines?

The intestinal nematode *Trichuris muris* has been shown to secrete a protein with homology to IFN-γ, which binds IFN-γ receptors on host lymphocytes and mediates cellular changes similar to those induced by IFN-γ itself (Grencis & Entwistle, 1997). Similarly, one of the ES products (p66) of the tapeworm *Taenia crassiceps* larvae possesses activities that mimic some characteristics of murine IFN-γ, such as the ability to induce spleen T-cell proliferative responses, and upregulation both of IFN-γ and IL-10 production in these cells. Moreover, p66 was able to upregulate nitric oxide production in macrophage cell line (Spolski *et al.,* 2002). The significance both of p66 and the *Trichuris muris* IFN-γ homologue in immunoregulation remains to be elucidated.

Also, two TGF-β homologues have been identified in *Brugia malayi* and one of them, secreted by adult parasites *in vitro,* is able to bind to host TGF-β receptors. In the context of a helminth infection, the most intriguing role for a parasite TGF-β would be down-regulation of host inflammatory response, such as ablation of nitric oxide generation (Maizels *et al.,* 2001). It is interesting to mention that the cestode *Mesocestoides corti* releases proteins with homology to stress proteins, which have an ability to alter the isotype profile of the host antibody response (Estes & Teale, 1991).

A mammalian cytokine, macrophage migration-inhibitory factor (MIF), is involved in the initiation of adaptive immune response and is an essential regulator of T-cell activation (Bacher *et al.,* 1996). The MIF monomer has a secondary structure similar to that of the dimer of IL-8 (Kato *et al.,* 1996). MIF is genetically (Esumi *et al.,* 1998) and structurally (Sugimoto *et al.,* 1999) related to D-dopachrome tautomerase and shares with it the spurious enzyme activity. The physiological substrates of MIF are phenylpyruvate and *p*-hydroxyphenylpyruvate.

A helminth homologue of mammalian MIF was first isolated from *Trichinella spiralis* (Pennock *et al.,* 1998). MIF homologues have also been found in several filarial nematodes *Brugia pahangi, B. malayi, Wuchereria bancrofti* and *Onchocerca volvulus,* and the intestinal nematodes *Trichuris muris* (Pennock *et al.,* 1998; Pastrana *et al.,* 1998) and *T. trichiura* (Tan *et al.,* 2001). Immunocross-reactive material was also detected in *Ascaris lumbricoides* (Pastrana *et al.,* 1998). Mammalian and filarial MIFs have conserved cysteinyl residues, which have been implicated in redox reactions (Kleemann *et al.,* 1998). A comparison of the primary structures of MIFs from *T. spiralis* and *T. trichiura* with those of mammals and other nematodes shows that *T. spiralis* and *T. trichiura* MIFs share between 25% and 46% amino-acid sequence

identity with other MIFs, including all of the uniformly conserved residues, but are notably lacking in cysteine residues (Tan *et al.*, 2001). Both *T. spiralis* MIF and human MIF show extremely potent inhibition of monocyte migration and chemotactic activity in serum-free medium (Tan *et al.*, 2001). The amount of MIF secreted by *T. spiralis* larvae and related to total secreted protein in ES products is much higher (18–55 ng/mg of protein) than the MIF level in normal human plasma (0.04–0.4 ng/mg of plasma protein) (reviewed in Tan *et al.*, 2001) and might therefore be sufficient to affect monocyte migration locally

Table 3. Naturally occuring proteinase inhibitors of helminths

Inhibitor			Enzyme inhibited
Cysteine proteinase inhibitors (cystatins)		*Brugia malayi Bm*CPI-1	
		*Brugia malayi Bm*CPI-2	Cathepsin S, B, L, asparaginyl endopeptidase
		Acanthocheilonema viteae Av 17	
		Onchocerca volvulus onchocystatin	Cathepsin L, S, cysteine proteinase in moulting
		Haemonchus contortus cystatin	
Aspartic proteinase inhibitors (aspins)		*Onchocerca volvulus Ov* 33	Pepsin, cathepsin E
		Ascaris suum PI-3	Pepsin, cathepsin E
		Ancylostoma canium Ac-API	
		Trichostrongylus colubriformis aspin	
Serine proteinase inhibitors	Serpins	*Ascaris suum As*-SPN	
		Brugia malayi Bm-SPN-1, *Bm*-SPN-3	
		Brugia malayi Bm-SPN-2	Cathepsin G, neutrophil elastase
		Onchocerca volvulus Ov-SPN-1, *Ov*-SPN-2	
		Onchocerca ochengi Oo-SPN	
		Trichostrongylus vitrinus Tv-SPN	Mast cell proteinase among others host cell proteinases
		Trichinella spiralis serpin	Trypsin
		Caenorhabditis elegans SRP-2	Granzyme B, lysosomal cysteine proteinases (cathepsin K, L, S, V)
		C. elegans Ce-SPN-1–SPN-10,	
		*Schistosoma mansoni Smpi*56	Elastase
		Schistosoma haematobium SHSP1	
	Smapins	*Anisakis simplex* ASPI-1, ASPI-2	Elastase
		Anisakis simplex ASPI-3	
		Ascaris suum ICE-1, ICE-2-5	Chymotrypsin, elastase
		Ascaris suum ITR-1, ITR-2	Trypsin
		Ancylostoma caninum AcAP5	Factor Xa, XIa
		Ancylostoma caninum AcAP6	Factor Xa
		Ancylostoma caninum AcAPc2	FactorVIIa/TF
		Ancylostoma caninum KI	Trypsin chymotrypsin, pancreatic elastase
		Onchocerca volvulus Ov-SPI-1, *Ov*-SPI-2	
		Trichuris suis Ts-TCI	Trypsin, chymotrypsin
		Trichuris suis Ts-CEI	Chymotrypsin, elastase, chymase, cathepsin G

in infected tissue. Taken together, the elegant studies on the structural, catalytic and cell-migration-inhibitory properties of *T. spiralis* MIF indicate that it is partially orthologous to mammalian MIF. The occurrence of MIF orthologues in parasitic helminths might contribute to subversion of host defences.

Digestion of immunoglobulins

One of the important enzymatic activities of helminth proteinases is digestion of immunoglobulins. Cleavage of immunoglobulins by helminth parasite proteinases is important, not only because of the potential for immune evasion of antibody-dependent cell cytotoxicity (Carmona *et al.,* 1993) but also due to the fact that degradation of immunoglobulin G (IgG) produces biologically active material that binds to receptors on immune effector cells and induces cytokine release (Kinet, 1989). Thus, *in vivo* immunoglobulin cleavage may affect the outcome of some helminth infections. For example, a trypsin-like proteinase or aminopeptidase of *Schistosoma mansoni* schistosomula can cleave off the Fab fragment when the Fc receptor of the worm binds IgG (Auriault *et al.,* 1981). Immature *Fasciola hepatica* also release cathepsin B- or L-like proteinases, which cleave mammalian IgG *in vitro* (Chapman & Mitchell, 1982; Carmona *et al.,* 1993), and *F. hepatica* cathepsin L has been shown to prevent antibody-mediated attachment of eosinophils to juvenile flukes *in vitro* (Carmona *et al.,* 1993). The cathepsin S-like proteinase secreted by *Spirometra mansoni* plerocercoid cleaves IgG (Kong *et al.,* 1994). Metallo-, aspartic-, and cysteine proteinase activities were found in extracts from *Taenia solium* metacestodes and IgG digestion detected *in vitro* by these extracts was reported (White *et al.,* 1992). Also, a serine proteinase of *Dirofilaria immitis* microfilariae was reported to cleave IgG (Tamashiro *et al.,* 1987).

PROTEINASE INHIBITORS OF HELMINTHS

Proteinases exist in all living organisms and they are involved in various physiological and pathological processes, therefore their activity, if uncontrolled, can be destructive to the cell or organism and must be precisely regulated by endogenous inhibitors. This paragraph is devoted to the inhibitors of cysteine-, serine-, and aspartic proteinases of helminthic origin (Table 3).

Cysteine proteinase inhibitors: cystatins

Cystatins are reversible, tight-binding inhibitors of cysteine proteinases (Nicklin & Barrett, 1984) that share some fundamental features, the most prominent being thermostability (Abrahamson, 1994). Cystatins are divided in three major families: the stefins with no disulfide bridges and displaying a mean molecular mass of 11 kDa, the cystatins with two disulfide bridges and molecular mass of approx. 14 kDa, and kininogens, which are glycoproteins with a relatively high molecular mass ranging from 60 to 120 kDa (Abrahamson, 1994).

The overall consensus regarding the functional aspects of the cystatins is that they act similarly to zymogens, regulating proteinases mainly as inhibitors within the cytoplasm, prior to the release of the active form of the enzymes (Morales *et al.,* 2004). Cystatins are found in mammals but cystatin-like molecules are also present in mammals and parasites (Vray *et al.,* 2002).

Modulation by cystatins of antigen processing and presentation

The first described cystatin of parasite origin was the "onchocystatin" of the human filarial nematode *Onchocerca volvulus* (Lustigman *et al.,* 1992). This protein was initially thought to regulate parasite proteinases during the moulting of the nematode. However, additional functions outside the moulting process are underlined by the fact that cystatin of the rodent filaria *Acanthocheilonema vitae* is secreted by male worms and blood-stage microfilariae that do not moult (Hartmann *et al.,* 1997). Investigation of the features of cystatins from both filarial (Schonemeyer *et al.,* 2001) and gastrointestinal nematodes (Dainichi *et al.,* 2001; Newlands *et al.,* 2001) showed them to inhibit the cysteine proteinases cathepsin L and S that are involved in the proteolytic processing of polypeptides. Moreover, filarial cystatins possess an additional motif that is required to inhibit a distinct class of cysteine proteinases, the legumains (asparaginyl endopeptidases) and it was shown that *B. malayi* cystatin has the capacity to inhibit legumain-like proteinases (Manoury *et al.,* 2001). These inhibition profiles imply that the studied nematode cystatins might have a dual function, inhibiting nematode cysteine proteinases as well as host proteinases.

The legumain-like proteinases are involved in the degradation of proteins within the endosomal-lysosomal compartment of antigen-presenting cells, as well as in the cleavage of the MHC class II-associated invariant chain by aspartic and cysteine proteinases, such as cathepsin S, cathepsin L (Nakagawa & Rudensky, 1999) and cathepsin F (Shi *et al.,* 2000). In this respect, *N. brasiliensis* cystatin inhibits *in vitro* processing of ovalbumin by cathepsin B and cathepsin L, suggesting that the same effect occurs in the lysosome during antigen degradation (Dainichi *et al.,* 2001).

Modulation of cytokine production and T cell proliferation

Apart of their capacity to inhibit proteinases, nematode cystatins seem to have a profound effect on cytokine production (Fig. 3). Filarial cystatins have been shown to induce the production of several cytokines causing anti-inflammatory responses (Hartmann *et al.*, 1997; Schonemeyer 2001). In this regard, prominent differences were found by an analysis of the cytokine profile of the peripheral blood mononuclear cells (PBMC) exposed to filarial (*O. volvulus*) or *C. elegans* cystatins. *O. volvulus* cystatin induced the release of TNF-α (an event that usually leads to proinflammatory responses), followed by a downregulation of IL-12 production and massive increase of IL-10 production by these cells, a hallmark of Th2 (anti-inflammatory) response (Schonemeyer *et al.*, 2001). *C. elegans* cystatins induced only the production of the Th1 cytokines (TNF-α, IL-12) of human PBMC (Schierack *et al.*, 2003). In this regard, *C. elegans* cystatins had an effect similar to that of chicken cystatin (Das *et al.*, 2001). The molecular basis for these differing effects are currently unclear.

The most obvious difference between filarial cystatins and cystatins of *C. elegans* was seen in experiments on the proliferative capacity of T cells. While the filarial cystatins of *O. volvulus* or *Acanthoheilonema viteae* interfered with the proliferation of human or murine T cells, *C. elegans* cystatins had no inhibiting effect (Schierack *et al.*, 2003). Inhibition of antigen presentation, as well as T cell proliferation, by filarial cystatins (Hartmann *et al.*, 1997; Schonemeyer *et al.*, 2001) contributes to parasite survival in the host.

It appears that cystatins of both parasitic and free-living nematodes differ substantially with regard to their immunomodulatory properties. This suggests that cystatins have evolved during co-evolution of the parasites and their hosts and acquired anti-inflammatory properties (Hartmann & Lucius, 2003).

Effects on inducible nitric oxide production

Interestingly, cystatins of nematodes, both parasitic and free-living, share with other members of the cystatin superfamily the potential to upregulate the nitric oxide production of IFN-γ-activated macrophages (Verdot *et al.*, 1996; Schierack *et al.*, 2003). The upregulation of NO by application of chicken cystatin was potent enough to cure mice from potentially fatal visceral leishmaniasis (Das *et al.*, 2001). Nitric oxide was also associated with suppression of antigen-specific T cell proliferation in a murine model of filariasis (O'Connor *et al.*, 2000). Because filarial cystatins are strong inducers of NO, it is not excluded that they are indirectly linked to killing of microfilariae and inhibition of the T cell responses.

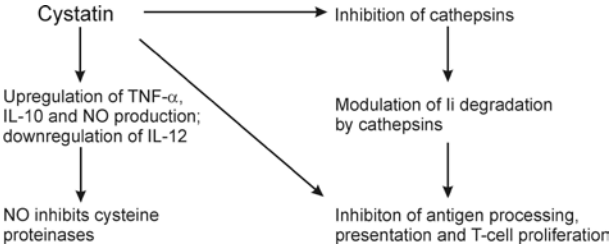

Figure 3. Simplified presentation of immunomodulatory action of nematode cystatins (based on Vray *et al.*, 2002). NO, nitric oxide; Ii, the MHC class II-associated invariant chain.

Serine proteinase inhibitors

Serine proteinase inhibitors are categorized according to primary sequence, structural motifs and mechanism of binding (Potempa *et al.*, 1994). Serpins are large proteins, more than 100 of which have been identified in vertebrates, insects, plants and viruses (Marshall, 1993). In mammals, they are key regulatory proteins involved in essential extracellular functions such as complement activation, fibrinolysis, coagulation and inflammation (Potempa *et al.*, 1994). In helminth genome, sets of serine proteinase inhibitor genes (available from accessible databases) are found in *C. elegans*, many parasitic nematodes as well as the trematode *Schistosoma* (Zang & Maizels, 2001; Table 3). Among them a novel, distinct family of smapins, small serine proteinase inhibitors (less than 100 amino-acid residues) was recognized (Table 3). The most striking characteristic of the smapin family is the universal presence of ten cysteine residues that form five disulfide bonds.

Serpins

Although nematode serpins have low overall homology to the serpins from mammalian species, their sequences are identical or conserved at most of the key amino-acid positions (Zang & Maizels, 2001). In view of the key role that mammalian serpins play in the regulation of biological processes, one can imagine that pathogens might themselves encode serpins and use them to block host-defense functions. The only nematode serpin for which experimental evidence indicates an evasive function is *Brugia malayi* serpin-2 (*Bm*-SPN-2). It specifically inhibits two human neutrophil-derived serine proteinases, cathepsin G and elastase (Zang *et al.*, 1999). It is interesting that *Bm*-SPN-2 is expressed and secreted at a single point in the parasite life cycle by the blood-born microfilariae. The consequences of *Bm*-SPN-2 inhibition *in vivo* have yet to be studied, but neutrophil-derived cathepsin G is known to be an important chemokinetic stimulator of T lymphocytes and a chemoattractant for monocytes (Chertov *et al.*, 1997). *Schistosoma* serpins are found either on or

within the worm surface tegument and are present in higher-molecular-mass forms, indicating complex formation, perhaps with cognate proteinase (Ghendler *et al.,* 1994). *Schistosoma* serpin, Smpi56, inhibits both schistosome and neutrophil elastase (Ghendler *et al.,* 1994). Consequently, its role could include both the physiological control of elastase within the schistosomes, and protection of the parasite from activated neutrophils during inflammation. Physiological roles of *C. elegans* serpins have yet to be established. The assumption will be that their primary role is the regulation of endogenous serine proteinases (Zang & Maizels, 2001).

Smapins

The role of smapin molecules has been clarified in two distinct parasite systems. In *Ascaris*, a parasitic nematode that resides within the human and pig intestinal tract, smapins protect the worm from proteolytic degradation by the host's digestive enzymes (Martzen *et al.,* 1985). These inhibitors are located at the surface of the developing eggs, larvae and the epithelial surface of the worm's own gut, combining with host proteinases to form inactive enzyme-inhibitor complexes. It is possible that this mechanism not only protects *Ascaris* within the degradative environment but could also mask the surface of developing larvae, permitting them to evade the host's immune system as they migrate from the intestine to the liver and lungs. In hookworms, the smapins are responsible for the long-known anticoagulant properties of these blood-feeding parasites. In this respect three smapins have been identified in the dog hookworm *Ancylostoma caninum* (Capello *et al.,* 1995). Each inhibitor specifically inhibits a different range of blood coagulation serine proteinases (Stassens *et al.,* 1996). Thus the strategy of *A. caninum* to interfere with mammalian blood coagulation pathway is distinct from those used by other hematophageous parasites or the mammalian host (reviewed in Zang & Maizels, 2001). Two smapins: trypsin/chymotrypsin inhibitor, *Ts*-TCI, and chymotrypsin/elastase inhibitor, *Ts*-CEI, were purified from the adult stage of *Trichuris suis,* an intestinal parasite of swine. *Ts*-CEI inhibits chymotrypsin, pancreatic and neutrophil elastases, chymase (mouse mast cell proteinase-1, mMCP-1) and cathepsin G (Rhoads *et al.,* 2000). The serine proteinase inhibitors of *T. suis* may function as components of the parasite defense mechanism by modulating intestinal mucosal mast cell-associated, proteinase-mediated, host immune response (Rhoads *et al.,* 2000). In addition, the serine proteinase inhibitor "taeniastatin", isolated from the larval stage of the cestode *Taenia taeniaeformis*, has long been known to inhibit endogenous IL-1 and IL-2 production of murine lymphocytes, presumably in order to escape host immune defense (Leid *et al.,* 1986).

These serine proteinase inhibitors are phylogenetically ancient, as inhibitors from *T. suis* share some similarity of sequence with proteinase inhibitors from other nematodes: *Anisakis simplex, Ascaris suum* (Rhoads *et al.,* 2000; Table 3) as well as insects and amphibians (Rhoads *et al.,* 2000).

Helminth aspartic proteinase inhibitors: **aspins**

These inhibitors have been identified in several parasitic nematodes and *C. elegans*. The structural features common to nematode aspins include the presence of a signal peptide sequence and conservation of all four cysteine residues in the mature protein (Shaw *et al.,* 2003). The function of aspins is unclear. *Ov*33 from *O. volvulus* (Tume *et al.,* 1997) and PI-3 from *Ascaris suum* (Martzen *et al.,* 1990; Kageyama, 1998) inhibit the *in vitro* activity of aspartic proteinases such as pepsin and cathepsin E. PI-3 from *Ascaris* has been suggested to protect against digestion by host proteinases in the stomach and thus could enhance the survival of infective larvae. However, an *Ancylostoma* aspartic proteinase inhibitor-1, *Ac*-API (Delaney *et al.,* 2005) as well as the proteinase inhibitor *Tco*-API-1 of another parasite of alimentary tract *Trichostrongylus colubriformis* did not inhibit the activity of porcine pepsin (Shaw *et al.,* 2003). More likely, *Ac*-API functions as an inhibitor of an endogenous aspartic proteinase. Several nematodes possess non-lysosomal aspartic proteinases that are detected in excretory/secretory products (Jolodar & Miller, 1998; Geldhof *et al.,* 2000) and may be secreted as inactive complexes with their appropriate aspartic proteinase inhibitors. Dissociation of the inhibitor would presumably activate the proteinase. As *Ac*-API is transcribed at all stages of development, and is released in excretory/secretory products of the adult, it would interact with the host immune system. Nematode APIs have been suggested to evoke Th2 immune responses in hosts, as *O. volvulus* aspin, *Ov*33 induced IgE and IgG4 antibodies production (Garraud *et al.,* 1995). Considering the finding of a pronounced inhibitory activity against cathepsin E (Kageyama, 1998) this might be an important target *in vivo.* Cathepsin E has long been implicated as playing an important role in the processing of exogenous antigens for presentation to cells of the immune system on class II MHC proteins (expressed on the surface of an antigen presenting cells) (Bennett *et al.,* 1992; Riese & Chapman, 2000).

ACETYLCHOLINESTERASES

Acetylcholine is an important neurotransmitter in both free-living and parasitic nematodes and

is associated with the neuromuscular system. The classical role of acetylcholinesterase (AchE) is to terminate transmission of neuronal impulses by rapid hydrolysis of acetylcholine. Cholinesterases secreted by many parasitic nematodes of (predominantly) the alimentary tract or other mucosal tissues are true acetylcholinesterases when analyzed by substrate specificity, inhibitor sensitivities and primary structure (reviewed in Lee, 1996). In the first two respects, they resemble vertebrate acetylcholinesterases, whereas the somatic (and therefore presumably neuronal) enzymes of nematodes analyzed to date display enzymatic properties similar to those of other invertebrate acetylcholinesterases (reviewed in Lee, 1996).

The amount of secreted enzymes varies from species to species, and between the sexes, it can also vary from larvae to adults. The isoenzyme pattern of acetylcholinesterase could change during the course of the infection as it has been shown for *Nippostrongylus brasiliensis* (reviewed in Lee, 1996). The reasons for those changes are not known. Possibly, by changing the form of the secreted AChEs the nematode is able to evade the action of antibodies directed against the earlier forms of the secreted enzyme (reviewed in Lee, 1996).

Influence on intestinal peristalsis

Certain species of nematodes that inhabit the alimentary system of animals can affect contractions of the wall of the alimentary tract and alter the movement of gut contents along the tract (Lee & Foster, 1995). Thus, it was reasonable to conclude that the parasite's AChE could act as a "biochemical holdfast" enabling the nematodes to stay in their preferred site and that earlier-mentioned protective antibodies would inhibit this holdfast mechanism. However, Foster *et al.* (1994) have shown that AChE from the electric eel does not affect the amplitude of contractions of uninfected rat intestine segments maintained *in vitro,* as does supernatant of homogenates and ES from adult *Nippostrongylus*. It would appear that ES products of *Nippostrongylus* contain a vasoactive intestinal polypeptide-like protein (VIP-like protein) (Lee & Foster, 1995; Foster & Lee, 1996). Its mammalian homologue directly inhibits intestinal smooth muscle (Bitar & Makhlouf, 1982) and VIP-ergic neurons are important inhibitory neurons in the gastrointestinal tract of mammals (Costa & Furness, 1983).

Influence on intestinal transport processes

It is well known that the enteric nervous system does not simply regulate smooth muscle contraction, but is intimately involved in the control of transport processes in enterocytes. Thus, according to a model for cholinergic signaling in the intestinal mucosa, acetylcholine released from enteric cholinergic motor neurons stimulates chloride secretion (Cooke, 1984), mucus secretion (Specian & Neutra, 1980), and Paneth cell exocytosis through muscarinic receptors (Satoh *et al.,* 1992). It is likely that these secretory events contribute to expulsion of pathogens. Fluid and mucus secretion are stimulated during infection with nematode parasites. It is therefore an attractive proposition that AChEs secreted by nematode parasites of the gastrointestinal tract act to inhibit secretory responses by hydrolyzing acetylcholine released from the enteric nervous system (Selkirk *et al.,* in Kennedy & Harnett, 2001). It was shown that the expression of muscarinic acetylcholine receptors increased progressively on cells in the *lamina propria* after entry of *N. brasiliensis* parasites into rat jejunum (Selkirk *et al.,* in Kennedy & Harnett, 2001). These alterations in receptor expression may provide a lead to understanding the reasons for acetylcholinesterase secretion by parasitic nematodes.

Immunomodulation

It has been suggested that one of the functions of acetylcholinesterases secreted by the nematodes is to modulate the immune system of the host (Rhoads, 1984; Pritchard *et al.,* 1993). Acetylcholinesterase produced by nematodes, such as *Haemonchus* and *Ostertagia* that inhabit the stomach or abomasum, might reduce inflammation and local ulceration by hydrolyzing acetylcholine which stimulates gastric acid secretion. Acetylcholine has been recorded to have numerous effects on leukocytes, including stimulation of chemotaxis and lysosomal enzyme secretion by neutrophils, inflammatory mediators, histamine and leukotriene release by mast cells, and augmentation of lymphocyte-mediated cytotoxicity (reviewed in Lee, 1996). Plasma cells can respond to acetylcholine by increasing secretion of immunoglobulins (Brink *et al.,* 1994). Thus, acetylcholinesterase activity would help to prevent stimulation of cellular and humoral response to parasite infection.

In conclusion, the function of the secreted enzymes remains undefined, but may be related to the regulation of physiological responses that promote expulsion of parasites by cholinergic elements of the enteric nervous system or to the modulation of the host's inflammatory and/or immune response.

MUCINS

Invasion of *Toxocara canis* occurs in all mammalian species, but only in canid hosts do larvae

progress along a typical ascarid nematode developmental pathway. In other hosts the larvae remain in the tissue-migratory phase (for even 9 years after infection) without ever developing. The ability of arrested-stage larval parasites to survive in the tissues for many years must depend on potent immune-evasive and anti-inflammatory mechanisms operated by the parasite. *Toxocara* has an exceptional ability to withstand attack by the immune system, most probably due to specific glycoproteins that are found in secretory glands and on the surface of the parasite. The external surface of the *T. canis* larva is covered by a carbohydrate-rich surface coat, which is a common feature of nematodes, both parasitic and free-living (Maizels & Loukas in Kennedy & Harnett, 2001). The surface coat appears to play a primary role in immune evasion, as it is shed when the parasite is bound by granulocytes or antibodies. A principal class of surface coat molecules are secreted mucins, MUC-1, MUC-2 and MUC-3 (Maizels & Loukas in Kennedy & Harnett, 2001). Mucins are large glycoproteins characterized by high charge density from sialic acid and surface residues, as well as by proteinase resistance and hydration of molecules (Moncada *et al.,* 2003). Adult worms of another nematode, *Strongyloides venezuelenzis*, secrete mucin-like substances which are a key component enabling the parasites to invade and establish in the host epithelial layer (Maruyama & Nawa, 1997).

LECTINS

In helminths, several surface and/or secreted C-type lectins and S-type lectins (galectins) have been identified; they are speculated to play a role in immunomodulation, but their probable interaction with host immune cells remains hypothetical (Loukas & Maizels, 2000), although the activation of immune cells by components from protozoan parasites has been well documented (Moncada *et al.,* 2003).

C-type or Ca^{+}-dependent lectins are a family of carbohydrate-binding proteins that bind carbohydrates which range from simple monosaccharides to complex glycoconjugates, in Ca^{+}-dependent fashion (Weis *et al.,* 1998). Lectins are involved in activation of innate immunity in both vertebrates and invertebrates. Helminth C-type lectins, sharing sequence and structural similarity with mammalian immune cell lectins, have recently been identified from *T. canis* larvae.

Arrested stage larvae secrete lectins: TES-32 and TES-70. TES-32 has been localized to the epicuticule of larval *T. canis* (Page *et al.,* 1992). The C-terminal domain of TES-32 shows similarity to host immune lectins like macrophage mannose receptor, E-selectin, macrophage binding protein A (Loukas *et al.,* 1999). TES-70 lectin binds to the surface of mammalian epithelial cells which suggests that host glycans, possibly those involved in immunity, are ligands for these TES lectins. It is hypothesized that secreted nematode C-type lectins might bind to selectin ligands that are up-regulated during tissue damage, and thus compete with L-selectin, inhibiting its binding to leukocytes. Lectins might inhibit infiltration of leukocytes to sites of inflammation by binding to ligands expressing sialyl-Lewis^{x} antigen (Loukas *et al.,* 2000).

Hematophagous nematodes such as hookworms are often in intimate contact with the mucosa and lectins secreted at the site of attachment might dampen the local immune response during feeding. Anticoagulation of host blood is another potential role for C-type lectins secreted by blood-feeding nematodes as it is known that snake venom contains C-type lectins that inhibit clotting of blood (Takeya *et al.,* 1992). In accord, two cDNAs encoding C-type lectins have been identified from expressed sequence tags of the human hookworm *Necator americanus* (Daub *et al.,* 2000) and *Nippostrongylus brasiliensis* (Harcus *et al.,* 2004). Expressed sequence tags encoding C-type lectin-like proteins have been identified from *Ancylostoma ceylanicum, Ascaris suum* and *Haemonchus contortus* (reviewed in Loukas & Maizels, 2000). All these genes share sequence similarity with mammalian immune cell lectins.

There is the good biochemical evidence for the presence of surface/secreted lectins at most stages of schistosome life cycle. These surface lectins might adsorb host antibodies and complement components (Tarleton & Kemp, 1981), as well as MHC I class antigens, on their surface to avoid immune recognition (Simpson *et al.,* 1983). Thus host lectins are required to bind to carbohydrate "danger" signals in order to initiate inflammatory influx around the parasite, and the release of a parasite lectin may block this process. Recently, Sm60, a mannose-binding protein from *S. mansoni*, was recovered from adult worm tegument and cercariae. Sm60 induced *in vitro* migration of human neutrophils and degranulation of mast cells (Coelho-Castelo *et al.,* 2002).

PHOSPHORYLCHOLINE

Phosphorylcholine (PC) is a structural component of a variety of prokaryotic and eukaryotic pathogens that also has immunomodulatory properties. In bacteria, it was found to be associated with a polysaccharide component of the cell wall and cell membrane (reviewed in Harnett *et al.,* 2003). Both in prokaryotes and eukaryotes, PC is attached directly to sugar residues (reviewed in Harnett & Harnett, 1999). Some species of nematodes as filariae

(reviewed in Harnett & Harnett 1999) or *T. spiralis* (Takahasi *et al.*, 1993) contain PC on their cuticle. PC is also a component of certain glycoproteins actively secreted by filarial nematodes. One of these molecules, ES-62, is a major secreted glycoprotein of the rodent filarial nematode *Acanthocheilonema viteae* (Harnett *et al.*, 1989), and another homologue of ES-62 is produced by the feline filarial nematode *Brugia pahangi* (Nor *et al.*, 1997) and *Onchocerca* spp. (Haslam *et al.*, 1999) and has been found in expressed sequence tags in the human filarial nematode *Brugia malayi* (Stepek *et al.*, 2004). The amino-acid sequence of ES-62 shows 37–39% identity with a family of six other proteins, some of which have been predicted to be amino- or carboxypeptidases. ES-62 has been shown to possess a weak aminopeptidase activity *in vitro* against synthetic substrates (Harnett *et al.*, 1999).

Interaction of immune system cells with phosphorylcholine-containing molecules

The glycoprotein ES-62 is able to interact with a number of immune system cells, specifically with B- and T-lymphocytes, macrophages and dendritic cells. Cells expressing PAF receptor (known to interact with the phosphorylcholine moiety of PAF) are able to interact with phosphorylcholine-containing bacteria. The interaction can be blocked by PAF-receptor antagonists and such antagonists can prevent bacteria-induced pneumonia in mice (Cundell *et al.*, 1995). Our unpublished results show that phosphorylcholine antigens of *T. spiralis* exert their effect through the PAF receptor. We observed that specific blocking of alveolar macrophage PAF receptors with an antagonist (CV 6209) prevented oxidative burst evoked by homogenates derived from *T. spiralis* newborn as well as muscle larvae.

Peritoneal macrophages pre-exposed to ES-62 have impaired production of IL-12, IL-6 and TNF-α following subsequent stimulation with IFN-γ and LPS (reviewed by Harnnett *et al.*, 2003). The PC component of filarial nematode extracts was shown to induce IL-10 production in peritoneal B-1 cells (Palanivel *et al.*, 1996). On the other hand, PC-containing glycosphingolipids from *Ascaris suum* were found to inhibit IL-12 production by peritoneal macrophages of mice (Deehan *et al.*, 2002). The reduced production of Th1-inducing cytokine IL-12, as well as pro-inflammatory TNF-α and IL-6, biases immune responses towards the Th2/anti-inflammatory phenotype. Likewise, dendritic cells, which mature in the presence of ES-62, acquire a phenotype allowing them to induce anti-inflammatory, Th2-type responses (reviewed by Harnnett *et al.*, 2003).

Lymphocytes exposed to ES-62 both *in vitro* and *in vivo* are less able to proliferate in response to ligation *via* the antigen receptor. Studies of the interaction of ES-62 in lymphocytes show that it is associated with activation of certain signal transduction molecules, including a number of protein tyrosine kinases and mitogen activated protein kinases (MAP kinases). Whereas such activation is insufficient to induce proliferation, it serves to almost completely desensitize the cells to activation of phosphoinositide 3-kinase (PI-3-kinase) and Ras/MAP kinase pathways by antigen-receptor ligation, events critical for lymphocyte proliferation. Such desensitization reflects ES-62-primed recruitment of a number of negative regulators of these pathways, such as the phosphatases SHP-1 and Pac-1 (reviewed by Harnnett *et al.*, 2003).

NEMATODE NUCLEOTIDE-METABOLIZING ENZYMES

Tissue damage results in a variety of molecular signals that activate elements of the immune system. Key regulators of these events are extracellular nucleotides that signal through purinergic receptors. Nucleotides may be secreted in a regulated manner or released upon mechanical stimulation, whereas tissue damage results in their massive release into extracellular fluids. In hematopoietic cells, signaling through nucleotide receptors stimulates a multitude of responses of the innate immune system (Linden, 2001; Hasko & Cronstein, 2004). Hematophagous insects and ticks secrete enzymes in their saliva that degrade nucleotides, thus inhibiting hemostasis and minimizing the ensuing pain and inflammatory reactions provoked by these mediators (reviewed in Gounaris & Selkirk, 2005). The discovery of an enzymatic cascade of nucleotide-metabolizing enzymes in secreted products of *T. spiralis* suggests that endoparasites use similar mechanisms to modulate host purinergic receptor signaling (Fig. 4).

Recently, *T. spiralis* has been shown to secrete nucleoside diphosphate kinase, 5′-nucleotidase and adenosine deaminase (Gounaris, 2002; Gounaris *et al.*, 2001). The 5′-nucleotidase of *T. spiralis* exhibits unique properties in catalyzing the hydrolysis of nucleoside 5′-diphosphates in addition to nucleoside 5′-monophosphates, but shows no activity against nucleoside 5′-triphosphates (Gounaris *et al.*, 2004; Fig. 4). Close homologues of this enzyme are present in the *Trichuris muris* expressed sequence tag dataset (Gounaris & Selkirk, 2005). Apyrase (nucleoside triphosphate dephosphorylase), 5′-nucleotidase and nucleoside diphosphate kinase activities were identified in secreted products of *Nippostrongylus brasiliensis* (Gounaris & Selkirk, 2005) and nucleoside

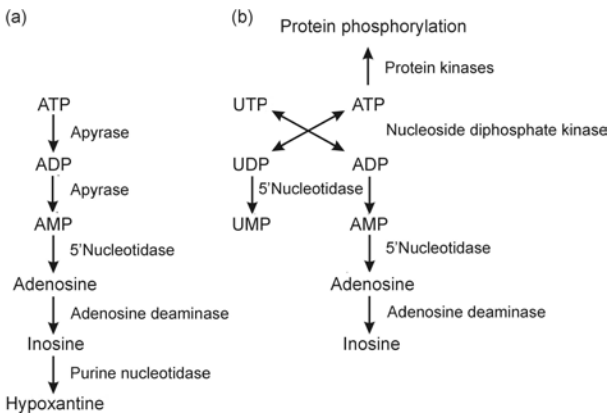

Figure 4. Cascades of enzymes metabolizing nucleotides. (a) The saliva of hematophagous insects; (b) excretory/secretory products of *Trichinella spiralis* (modified from Gounaris & Selkirk, 2005).

diphosphate kinase was found in the secreted products of *Haemonchus contortus* (Yatsuda *et al.,* 2003). It therefore appears likely that secretion of nucleotide-metabolizing enzymes is exhibited by a variety of nematode species.

Both adenosine and inosine exhibit potent immunomodulatory effects (reviewed by Gounaris & Selkirk, 2005). Metabolism to inosine might be advantageous to an invasive organism because it would shorten the action of adenosine, which apart from inhibition of platelet aggregation and vasodilatory activity, which is of benefit to hematophagous species (Biaggioni, 2004), promotes chemotaxis, activation, and degranulation of mast cells (McCloskey *et al.,* 1999; Linden, 2001). These cells are important gastrointestinal effectors against intestinal nematodes (Knight *et al.,* 2000; McDermott *et al.,* 2003). A major difference between the nucleotide-metabolizing enzymes secreted by hematophagous arthropods and those of *T. spiralis* is the absence of ATP-ase in *T. spiralis*. This is significant as, in addition to proinflammatory effects, mast cell degranulation (Di Virgilio *et al.,* 2001), ATP, UTP and UDP stimulate chloride secretion in epithelial cells, and mucus secretion from goblet cells (Bucheimer & Linden, 2004). This type of response is unfavorable for intestinal parasite, as epithelial chloride flux drives net fluid secretion, which clears the intestinal tract (Cooke *et al.,* 2003). However, *T. spiralis* secretes a nucleoside diphosphate kinase (Gounaris *et al.,* 2001) which would convert ATP to ADP or UTP to UDP in the presence of suitable nucleoside phosphate acceptors. Inactivation of UDP by the nucleotidase would act against fluid secretion contributing to parasite expulsion.

As it appears, secretion of nucleotide-metabolizing enzymes by both ecto- and endoparasites would be advantageous not only to hematophagous species, but also to those species that through tissue damage or residence in the vascular system might promote platelet aggregation and subsequent release of proinflammatory nucleotides. This might therefore prove to be a conserved and underappreciated feature of many infectious organisms, including bacteria (Zaborina *et al.,* 1999), with important consequences for pathogenicity.

PROTEINS SECRETED BY THE INTRACELLULAR METAZOAN PARASITE *TRICHINELLA SPIRALIS*

Trichinella spiralis infective larvae secrete many enzymes of poorly characterized functional properties. *T. spiralis* is an intracellular parasitic nematode capable of transforming striated muscles of mammals into a new syncytium known as a nurse cell complex. Besides its multinucleated structure, the nurse cell bears no morphological or biochemical similarity to the uninfected muscle (Stewart, 1983). Infection of skeletal muscle cells leads to the cell cycle re-entry and arrest at the G2/M phase boundary, and to a novel cellular phenotype (Jasmer, 1993; 1995). The structural and regulatory muscle genes are down regulated at the transcriptional level and the muscle differentiation program is inactivated in the nurse cell (Jasmer, 1993). However, the molecular mechanisms involved in host cell reorganization are unknown. Excretory/secretory products of infective larvae have been detected in the cytosol and nuclei of infected muscle cells (Despommier *et al.,* 1990; Lee *et al.,* 1991), and are also secreted into epithelial cells of intestinal mucosa (Capo *et al.,* 1986) where the nematode is believed to moult, mature and reproduce (Gardiner, 1976). Thus the products secreted by *T. spiralis* infective larvae might also play a role in the process of infection of enterocytes. The mechanism of cellular invasion by *T. spiralis* is still unknown, and the role of secreted or surface bound proteins with respect to invasion and survival of the parasite in either skeletal muscle or intestinal epithelia remains largely uninvestigated.

Protein kinases and phosphatases

These enzymes are known to affect a variety of cellular signaling processes (Redegeld *et al.,* 1999). The vast majority of protein kinases are intracellular; extracellular protein kinases are quite rare (Ehrlich & Kornecki, 1987) but ecto-protein kinase and phosphatase activities have been identified on a variety of cells (Walter *et al.,* 1996; Petitfrere *et al.,* 1996) and parasitic protozoans *Leishmania* and *Trypanosoma* spp. Infective larvae of *T. spiralis* have externally oriented enzymes catalyzing reversible protein phosphorylation on their surface (Smith *et al.,* 2000). Both serine/threonine and tyrosine phosphorylation and dephos-

phorylation of secreted and membrane bound parasite proteins was reported to take place at the infective larvae surface. Among phosphorylated proteins a major species of 45 kDa was observed only after bile treatment (Smith *et al.,* 2000). Thus, it is believed that this protein may be involved in some recognition of the activation process in the enteral phase of infection. An integral membrane protein with serine/threonine kinase activity has been detected on the surface of the trematode *S. mansoni*, although its catalytic domain is predicted to be cytoplasmic. As the protein is homologous to the TGF-β receptor family, it presumably acts to transduce signals by an unidentified ligand (Davies & Pearce, 1995). Apart from these ecto-enzymes, *T. spiralis* larvae secrete at least two distinct serine/threonine protein kinases of 70 and 135 kDa, which almost exclusively phosphorylate a protein doublet at 50–55 kDa from ES products (Arden *et al.,* 1997). These protein kinases could not be readily assigned to any of the major documented subfamilies of serine/threonine protein kinases. In connection with externally occurring protein phosphorylation, the earlier mentioned lack of ATPase in ES products of *T. spiralis* might therefore be essential for the efficient activity of protein kinases (Smith *et al.,* 2000). Of the proteins possibly important for muscle cells, MyoD, one of the family of muscle-specific transcriptional regulators (Weintraub, 1993) is phosphorylated by *T. spiralis* protein kinases *in vitro*, but according to Arden *et al.* (1997) it is premature to assign any physiological significance to this observation.

Endonucleases

The ES products of *Trichinella spiralis* contain double-stranded endonuclease which can recognize host muscle cells (Mak & Ko, 1999). Similar to the *T. spiralis* enzyme, endonucleases have been characterized from the slime mould *Dictyostelium discoideum* and the insectivorous plant *Drosera adelae* (Guyer *et al.,* 1985; Okabe *et al.,* 1997). However, the biological significance of both endonucleases is still not known, thus the role of the *T. spiralis* endonuclease for the parasite is speculative (Mak & Ko, 1999). As double-stranded endonuclease was detected only in *T. spiralis* and not in *T. pseudospiralis,* this may be correlated with the difference in pathogenesis caused by the two worms: *T. spiralis* causes a drastic reorganization of muscle cells leading to the establishment of the nurse cell, whereas *T. pseudospiralis* does not. Mak and Ko (1999) believe that the occurrence of the endonuclease only in the worm which can form nurse cells may suggest that the molecule plays a crucial role in host cell recognition. Further, there are numerous reports showing that persistent double stranded breaks in chromosomal DNA would not only destabilize DNA (accounting for genomic changes), but could also lead to the arrest of the cell cycle at the G2 phase, and eventually cell death (Bennett *et al.,* 1997; ap-Rhys & Bohr, 1996). Possibly, the secreted endonuclease may be involved in the arrest of *T. spiralis* infected muscles at the G2/M phase of the cell cycle (Jasmer, 1993). Therefore, there is good likelihood that one of the major functions of the endonuclease of *T. spiralis* is to control the cell cycle (Mak & Ko, 1999).

Besides the double-stranded endonuclease, a non-specific, single-stranded endonuclease in the ES products of *T. spiralis* and *T. pseudospiralis* was found (Mak *et al.,* 2000). The biochemical properties of the single-stranded endonuclease of *Trichinella* differ from those of other well-characterized endonucleases (Mak *et al.,* 2000). Significant differences (especially in molecular mass, cation dependence, and species-specific expression) occur between the single and double-stranded endonucleases of *Trichinella*. According to the authors' suggestion, the two endonucleases may play a distinct role in the reorganization of host tissues or in pathogenesis. Moreover, the expression pattern of the single-stranded endonuclease suggests that it may have a more general role as in viruses and bacteria (discussed in Mak *et al.,* 2000). In *Mycoplasma penetrants* single-stranded endonuclease degrades host nucleic acids to acquire the precursors for nucleic acid biosynthesis, leading to chromosomal alterations (Bendjennat *et al.,* 1997).

DNA-binding activity

A DNA-binding peptide of 30 kDa was documented in the excretory/secretory products of the infective-stage larvae of *T. pseudospiralis* (Mak & Ko, 2001). This peptide could bind to the target DNA as a dimer, tetramer or multiples of tetramers with a low specificity. Similar activities were also observed in the ES products of *T. spiralis*. Since a DNA-binding protein has not been reported previously in parasitic organisms, one can only speculate on its possible functions. The occurrence of a DNA-binding peptide in both *T. spiralis and T. pseudospiralis* may point to its crucial role in the reorganization of host cells by trichinellids. The lack of specificity of the protein may facilitate the binding to DNA of myonuclei of all mammalian or avian species (Mak & Ko, 2001). In the case of an intracellular protozoan parasite, *Theileria annulata*, a putative parasite-encoded factor, *Tash AT2*, which modulates the host gene expression, has been identified. The protein contains three AT hook DNA-binding domains and is located in the host cell nucleus (Swan *et al.,* 1999). This provides the important evidence that factors can be transported from the parasite to the host nucleus

and bind host DNA. A similar event can also occur in trichinellosis (Mak & Ko, 2001).

Saposins

Saposins or sphingolipid activator proteins (SAPs) function as non-enzymatic cofactors for the hydrolysis of sphingolipids in lysosomes. Four of the five known members of this class (SAP-A to SAP-D), originally defined in vertebrates, are synthesized as a precursor termed prosaposin, which is proteolytically cleaved to generate four homologous glycoproteins having the molecular mass of approx. 11 kDa (Schuette *et al.,* 2001). Saposins facilitate the interaction between water-soluble hydrolytic enzymes and their membrane-bound substrates. The function of the mammalian secreted prosaposin has not been established, although it retains most of the activating properties of the mature saposins (Kishimoto *et al.,* 1992). Moreover, prosaposin binds several glycolipids and can facilitate their insertion into membranes (Soeda *et al.,* 1993).

Recently, it has been shown that infective larvae and adult specimens of *Trichinella spiralis* secrete a full-length prosaposin in an unprocessed form (Selkirk *et al.,* 2004). The secreted protein most probably originates from stichocytes or intestinal cells. It is possible that the parasite prosaposin facilitates invasion of intestinal epithelial cells and subsequent migration through this cell layer. The saposin-like protein family comprises pore-forming peptides, which have been identified in a variety of organisms. Among parasitic metazoans pore forming peptides have been found to be expressed stage specifically in juvenile and adult flukes of *Fasciola hepatica* and *Clonorchis sinensis* (Reed *et al.,* 2000; Lee *et al.,* 2002) and localized in the intestinal epithelium of adult flukes (Lee *et al.,* 2002). Hemolytic activity has been found in extracts from two blood-feeding nematodes *Haemonchus contortus* and *Ancylostoma caninum* (Fetterer & Rhoads, 1997a; Don *et al.,* 2004). It is tempting to suggest that the secreted peptide or protein hemolyzes the host blood cells ingested by the parasites and releases the host cellular components into the parasite intestinal lumen, making them accessible to digestive enzymes of those worms (cf. Fig. 1).

ANTIOXIDANT ENZYMES SECRETED BY HELMINTHS

All aerobic organisms require protection mechanisms that limit molecular damage caused by reactive oxygen species (ROS) such as superoxide anion radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^\bullet$). ROS are always generated during cellular metabolism. In addition, parasitic organisms need protection against ROS that arise from infection-stimulated host phagocytes (neutrophils and macrophages) (Cross & Jones, 1991) as a part of innate mechanisms of defense.

Enzymes dealing with superoxide anion radical, superoxide dismutases (Fig. 5), which catalyze the dismutation of superoxide radicals into hydrogen peroxide and oxygen have been described in every species of parasitic helminths examined (reviewed in James, 1994), whereas enzymes that deal with hydrogen peroxide have been difficult to identify. The control of H_2O_2 levels is essential, as H_2O_2 leads to the formation of $OH^\bullet$ for which no specific scavenger exists, due to its extreme reactivity. Therefore, the best way to control the $OH^\bullet$ levels is the defense against H_2O_2 and $O_2^{-\bullet}$. One class of enzymes capable of this function in mammals are selenium-containing glutathione peroxidases. These enzymes catalyze the reduction of H_2O_2 and organic hydroperoxides along with oxidation of glutathione (GSH) to glutathione disulfide (Henkle-Dührsen & Kampkötter, 2001). However, they appear to be absent in parasitic nematodes (Callahan *et al.,* 1988), although a selenium-independent glutathione peroxidase family exists. These selenium-independent enzymes have low or no activity with hydrogen peroxide. A second class, catalases metabolizing hydrogen peroxide into molecular oxygen and water, have been detected with low activity in most parasitic species but sequences encoding a typical catalase polypeptide have only been identified in a few species of nematodes such as *Haemonchus contortus*

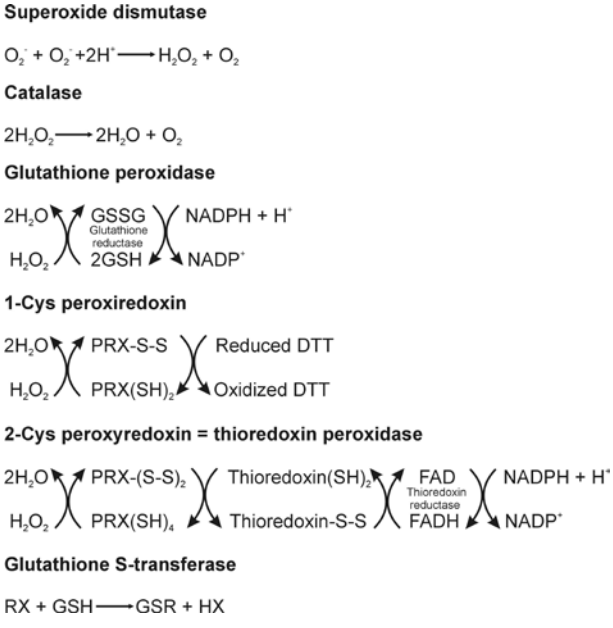

Figure 5. Antioxidant enzymes secreted by helminths. GSSG, oxidized glutathione; GSH, reduced glutathione; DTT, dithiothreitol; PRX, peroxiredoxin; RX, xenobiotics, electrophiles.

(Kotze & McClure, 2001) or *Ascaris suum* (Eckelt *et al.*, 1998), and a high level of catalase activity was documented in somatic extracts of *B. malayi* (Ou *et al.*, 1995).

However, a new family of enzymes has recently been described, the peroxiredoxins (Chae *et al.*, 1994b) (Fig. 5) that are probably the major H_2O_2-detoxifying enzyme in parasitic nematodes as well as in many other pathogens (McGonigle *et al.*, 1998). In parasitic nematodes, peroxiredoxins may play a pivotal role, since they appear to be both a major antioxidant enzyme and could also be involved in cellular signaling processes. The next paragraph will focus on the occurrence and localization of these new antioxidant enzymes in parasitic helminths.

Peroxiredoxins

The discovery of this enzyme system represents a major advance towards the understanding of how parasitic nematodes deal with both internal and environmental oxidative stress. Peroxiredoxins (PRXs) exist as homodimers. They share the property of reducing hydrogen peroxide to water and alkyl hydroperoxides to the corresponding alcohols and have been classified into two families: the 1-Cys and 2-Cys peroxiredoxins according to the presence of one or two highly conserved cysteine residues (Chae *et al.*, 1994c).

The PRXs are distinct from other peroxidases in that they have no cofactors, such as metals or prosthetic groups. Experiments with a human 1-Cys peroxiredoxin suggest that Cys-47-SH is the site of oxidation and that the oxidized product (probably Cys-SOH) can be reduced back to cysteine by dithiothreitol (Kang *et al.*, 1998), but the physiological donor has not been identified (Seo *et al.*, 2000). The 2-Cys peroxiredoxins are thioredoxin peroxidases, which have been shown to reduce H_2O_2 with the use of electrons from the thioredoxin system. Reduction of hydroperoxides by 2-Cys enzymes is accompanied by the formation of an intermolecular disulfide bond which is subsequently reduced by electrons donated by thioredoxin. Thioredoxin is regenerated by the system of thioredoxin reductase and NADPH (Chae *et al.*, 1994a) (Fig. 5).

It is worth to mention that the mammalian members of the PRX family are believed not to be simply back-up systems for glutathione peroxidase and catalase (Brigelius-Flohe, 1999) but in addition to eliminating potentially damaging ROS, they can play a role in signal transduction and gene expression due to alterations in ROS concentrations (Lim *et al.*, 1998). In this respect, a trematode, *S. mansoni*, was shown to possess both bacterial-like (resistant to oxidative inactivation, important in regulating cell signaling pathways), and mammalian-like sensitive peroxiredoxins (Sayed & Williams, 2004).

Peroxiredoxins in parasitic helminths

Antioxidant enzymes dealing with ROS originating from phagocytic cells must function near by host-pathogen interface, e.g., they must be secreted or presented on the surface of worms. The localization profiles of the peroxiredoxins indicate that these enzymes are active in dealing with both internal and external oxidative stress (reviewed in Henkle-Dührsen & Kampkötter, 2001).

Schistosomes lack catalase and have relatively low levels of glutathione peroxidase (Mkoji *et al.*, 1988; Mei & LoVerde, 1997) but peroxiredoxin plays a major role in the antioxidant defence in *Schistosoma mansoni* (Kwatia *et al.*, 2000). 2-Cys peroxiredoxin protein (thioredoxin peroxidase) was expressed in both male and female adult worms (Kwatia *et al.*, 2000). Genes encoding two 2-Cys peroxiredoxins were identified in the expressed sequence tag data base of *S. mansoni*. These 2-Cys peroxiredoxins efficiently utilize reducing equivalents from both the thioredoxin and glutathione systems. The ability of schistosome peroxiredoxins to use alternative electron donors may reflect their presence in different cellular sites and emphasizes the significant differences in overall redox balance mechanisms between the parasite and its mammalian host (Sayed & Williams, 2004). The role of peroxiredoxin in the antioxidant defense of *S. mansoni* is supported by the fact of thioredoxin expression by all mammalian stages of the schistosome life cycle. Thioredoxin is present in egg secretory products and may cooperate in facilitating the passage and survival of eggs across inflamed host tissues (Alger *et al.*, 2002). Similarly, another trematode *Fasciola hepatica* does not express a catalase and presents little glutathione peroxidase activity (McGonigle *et al.*, 1997), thus peroxiredoxin could be the major hydrogen peroxide-removing antioxidant in this parasite.

Peroxiredoxins have been found in several species of nematodes. The enzyme from the swine roundworm *Ascaris suum* was cloned and shown to be present in extracts of adult female worms, suggesting that the native *Ascaris* peroxiredoxin might act as a major antioxidant enzyme in *A. suum* (Tsuji & Kasuga-Aoki, 2001). In *Onchocerca volvulus* thioredoxin peroxidase was predominantly localized to the hypodermis and cuticle (Lu *et al.*, 1998). Peroxiredoxin was identified in parasite extracts and larval and adult excretory-secretory products of *Dirofilaria immitis*. The enzyme was localized to the lateral hypodermal chords of both female and male worms (Chandrashekar *et al.*, 2000). Similar localization was demonstrated with the thioredoxin peroxidase-1 of

Brugia malayi (Ghosh *et al.*, 1998). According to the opinion of these authors the apparent lack of an association of this enzyme with the surface or with excretory-secretory products of *B. malayi* does not preclude a role for thioredoxin peroxidase in countering radical attack from exogenous sources. Cuticle is easily permeable for radicals, so when they pass through, the first cells they would encounter would be those of the hypodermis-lateral chord. It is worth to note that one of the major surface-associated molecules of filarial nematodes is glutathione peroxidase (Cookson *et al.*, 1992), being however, a selenium-independent enzyme.

Level of antioxidant enzymes and parasite survival

Effective protection of an invading parasite from host-produced ROS would depend on levels of scavenger enzymes in the invasive forms of the parasite. Studies on *Nippostrongylus brasiliensis* infection showed that the upregulation of superoxide dismutase, catalase, and glutathione peroxidase is correlated with persistence in the host. On the other hand, increased ROS production by peritoneal leukocytes has been correlated with the rejection of *N. brasiliensis* (Smith & Bryant, 1989). The different degree of activities of anti-oxidant enzymes in *Nippostrongylus brasiliensis* and *Nematospiroides dubius* is correlated with the parasite's ability to survive in the host. *N. brasiliensis* adults are expelled from the small intestine in 10–12 days, whereas *N. dubius* adults persist for several months (Smith & Bryant, 1989). *N. dubius* has 2-fold higher superoxide dismutase activity, and about 4-fold higher catalase and glutathione reductase activity than *N. brasiliensis* (Smith & Bryant, 1989). It has been also demonstrated that *Brugia malayi* microfilariae and adults are relatively resistant to H_2O_2 (Ou *et al.*, 1995) compared with the related filarial species *Onchocerca cervicalis* (Callahan *et al.*, 1990) or *Dirofilaria immitis* (Rzepczyk & Bishop, 1984). The susceptibility to H_2O_2 of both adults and microfilariae of *O. cervicalis* is correlated with small amounts of catalase and peroxidase in this species (reviewed in Callahan *et al.*, 1988). Newly excysted juvenile flukes of *Fasciola hepatica* are relatively resistant to killing by free radicals in comparison to schistosomula of *Schistosoma mansoni.* This resistance could, in part, be due to the significant activity of oxidant-scavenging enzymes of newly excysted juvenile flukes (Piedrafita *et al.*, 2000).

Stage-specific expression of antioxidant enzymes and parasite survival

The importance of antioxidant enzymes to the survival of *T. spiralis* has also been studied (Bass & Szejda, 1979). It has been shown that superoxide dismutase is actively secreted by muscle larvae of *T. spiralis* cultured *in vitro* (Rhoads, 1983). Various developmental stages of *T. spiralis* contain different amounts of superoxide dismutase and glutathione peroxidase (Kazura & Meshnick, 1984), correlating with the sensitivity of the organism to oxidant-mediated killing. Oxidant-resistant adult worms and muscle larvae had several times more of glutathione peroxidase and about four times more of superoxide dismutase than the oxidant-sensitive newborn larvae. Newborn larvae were partially protected against oxidant damage when mixed with adult worms. Similarly, newly excysted juvenile flukes of *Fasciola hepatica* expressed 2.5–20-fold lower levels of superoxide dismutase and glutathione *S*-transferase activity relative to immature or adult parasites. Incubation of newly excysted juvenile flukes with inhibitors of peroxidases and inhibitors of glutathione metabolism increased their killing by LPS-stimulated rat phagocytes (Piedrafita *et al.*, 2000).

Studies of the developmental regulation of localization of glutathione peroxidase and superoxide dismutase in the trematode *Schistosoma mansoni* (Mei & LoVerde, 1997) showed that these enzymes were found to be associated only with the adult tegument and gut epithelium. In *Onchocerca volvulus*, expression of thioredoxin peroxidase increased during differentiation to the infective L3 larva. This enzyme was also detected in post-infective larvae and adult worms. Its highly upregulated expression in infective larvae may aid in parasite establishment following transmission to the definitive host (Lu *et al.*, 1998). In *Brugia malayi* glutathione peroxidase is expressed at negligible levels in mosquito-derived infective larvae, but after infection of the mammalian host, its synthesis is upregulated and the enzyme is transported to the cuticle (Devaney & Jecock, 1991).

ROS-induced changes in expression of helminth scavenger enzymes

Zelck and Von Janowsky (2004) have shown that stage-dependent expression of superoxide dismutase, glutathione peroxidase, and glutathione *S*-transferase in *Schistosoma mansoni* is regulated at the transcriptional level. Generation of ROS by xanthine/xanthine oxidase resulted in increased transcript levels for all three enzymes. They compared influence of phagocytic cells of snails (schistosome intermediate hosts), susceptible and resistant to infection, on the level of the scavenger enzymes. It appeared that hemocytes from susceptible hosts induced higher levels of these enzyme expression in schistosome sporocysts, compared to hemocytes from resistant hosts. These results indicate that phagocytic cells of resistant snails may

directly or indirectly downregulate schistosome antioxidant enzyme activity, thus facilitating killing of the parasite (Zelck & Von Janowsky, 2004). This suggests that beside induction of scavenger enzyme transcription, snail phagocytes are able to modulate parasite antioxidant enzyme expression.

Glutathione *S*-transferase

This enzyme is involved in xenobiotic metabolism, intracellular binding, and biosynthesis of endogenous substrates, such as prostaglandins and leukotrienes (Boyer, 1989). Glutathione *S*-transferase isoenzymes which have very little homology with those from mammalian tissues have been found in helminths, such as trematodes and nematodes (reviewed in Rao *et al.*, 2000). Immunohistochemical studies revealed that glutathione *S*-transferases in worms are predominantly associated with metabolic and reproductive sites of the organisms (reviewed in Rao *et al.*, 2000). Glutathione *S*-transferases may potentially favor parasite survival by neutralizing the toxins acting against them and may repair host-induced damage (Mitchell, 1989). In this respect *T. spiralis* glutathione transferase which functions as a selenium-independent glutathione peroxidase was detected by electron microscopy in the secretory organs (stichocytes) of L1 larvae. This enzyme seems to play a protective role against lipid peroxidation (Rojas *et al.*, 1997).

In filarial nematodes the presence of selenium-independent glutathione peroxidase activity associated with nematode glutathione *S*-transferase has been described in *D. immitis* (Jaffe & Lambert, 1986). In adult *O. volvulus*, one of the two identified isoenzymes of glutathione *S*-transferase has been found to be released by the worms (Liebau *et al.*, 1994). Although glutathione *S*-transferase is ubiquitous in all stages of *Brugia pahangi* and *B. malayi* life cycle, a high activity of this enzyme was observed in L3 and L4 larvae (Rao *et al.*, 2000). The elevated levels of glutathione *S*-transferase protein expression in *Brugia* larvae may be involved in the parasite's strategy for evasion, aimed at a host's early defense mechanisms, and may also assist in larval migration to the site of predilection (Rao *et al.*, 2000). Glutathione *S*-transferase has been reported to be present on the body surface of the trematodes *S. mansoni* (Taylor *et al.*, 1988) and *F. hepatica* (Wijffels *et al.*, 1992).

Although the presented data points to the importance of antioxidant enzymes for the survival of some parasitic helminths, the role of the antioxidant enzymes in the host-parasite relationship may still be controversial for many other infectious metazoan species. It is obvious that those parasitic helminths which are able to utilize oxygen are absolutely dependent on their antioxidant defense system alone to deal with endogenously generated ROS. In essentially anaerobic tapeworms, such as adult *H. diminuta* and adult *Moniezia expansa* (the common tapeworm of sheep) no enzyme capable of metabolizing H_2O_2 such as catalase, lipoxygenase, glutathione peroxidase, NADH peroxidase, or NADPH peroxidase has been detected (reviewed in Callahan *et al.*, 1988).

CONCLUSIONS

This review presents evidence that worms use many strategies to colonize a host and to stay in its organism. They secrete anti-oxidant enzymes which combat reactive oxygen species produced by the host phagocytic cells in response to parasitic infection. Nucleotide-metabolizing enzymes found in excretory/secretory products of nematodes could prevent platelet aggregation and the release of proinflammatory nucleotides. Secreted proteinases serve for digestion of the meal as well as for extracellular matrix degradation, which is necessary for tissue invasion. Proteinase inhibitors control proteinase activity. Secretion of proteinase inhibitors has a dual function. First, an inhibition of the host proteinase activity in order to defend against proteolysis by the host enzymes. Second, both proteinases and their inhibitors could take part in subverting the host immunological defense and polarizing the immunological response towards anti-inflammatory Th2. Although this response favors expulsion of gastrointestinal worms from the gut, generally parasites take advantages of the polarized Th2 response. Helminths produce homologues of cytokines possibly to influence the host defense. Some molecules such as phosphorylcholine containing ones, or cysteine proteinase inhibitors (cystatins), have been shown to interfere with the host immune cell signaling pathways. Different properties of cystatins from parasitic nematodes in respect to their free-living relatives suggest that they have acquired anti-inflammatory properties during co-evolution of the parasites and their hosts. The majority of molecules secreted by metazoan parasites have their homologues in other animals. These are universal molecules of crucial role in developing strategies of host invasion by parasites.

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