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REVIEW ON ROLE OF ENZYMES AND IMMUNE RESPONSES IN HOOKWORM INFECTIONS

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ABSTRACT

The present review reveals that the enzymatic activities and immune response in hookworm infections (Ancylostomiasis). The term Ancylostomiasis is also known as miner's anaemia, tunnel disease, brickmaker's anaemia and Egyptian chlorosis. Helminthiasis may also refer to Ancylostomiasis, but this term also refers to all other parasitic worm diseases as well. Enzymatic studies provide an important pathological changes, cellular activities and thus helps to know the physiological changes of the host. This article reveals the physiological disturbances of the hosts are due to the synchronized activities of the parasite on various vital organs and tissues. The pathological effects of hookworms are manifested in the picture of blood, serum and in the lesions of liver, heart and skeletal muscles of the host. Thus enzymes plays a major role in Ancylostomiasis. This article may helpful to several authors to understand the Heatshok proteins (HSPs), Glutathione-S transferases (GSTs), Phosphotases, Proteases, Aspartic Proteases, Metallo proteases, Superdoxide and Calretialin. The abnormality of body physiology can be detected by estimation of enzymes. There was a temporal relationship between infection and enzymatic levels, since enzymatic changes associated with the immunological effects. The lifespan of adult hookworms is more likely determined by the genetics of the parasite than by host immunity. There seems to be Advances in molecular biotechnology should enable the identification and characterization of an increasing number of parasite molecules, improving our detailed understanding of the protective and pathogenic mechanisms involved in hookworm infections.

KEYWORDS: Ancylostomiasis, Hookworm, Enzymatic studies, Immune response.

BACKGROUND

Ancylostomiasis disease is estimated to cause about 65,000 global deaths annually (WHO 2002). However, the long-term sequelae of hookworm-associated anemia and malnutrition may account for losing up to 22 million disability-adjusted life-years annually (Diemert *et al*. 2008). Necator americanus and Ancylostoma duodenale can only infect humans, while Ancylostoma ceylanicum and Ancylostoma caninum are zoonotic species that can infect dogs, cats as well as humans(Aula *et al*. 2020). Studies have shown that Ancylostoma ceylanicum has become the second-largest hookworm infecting people in Southeast Asian areas (Traub *et al*. 2013), causing abdominal pain, diarrhea, malnutrition, and iron-deficiency anemia (Sungkar *et al*. 2019).

Hookworm infections are primarily due to high-medication administration, with annual doses of anthelmintics such as albendazole and mebendazole. However, a recent systematic analysis found that anthelmintic treatment with mebendazole was ineffective in improving anemia in hookworm-infected areas and was associated with lower overall cure rates. Therefore, vaccine development has emerged as a practical and viable alternative technology to control hookworm infections or to complement anthelmintic treatment. Human hookworm vaccines are considered inexpensive and cost-effective compared to high-dose drugs. Ancylostoma species as a model, which infects both animals (dogs, hamsters, etc.) and humans (focus areas in Malaysia and other regions of Southeast Asia), has proteins with high homology to two large human hookworms, N americanus and A. is a hookworm that expresses duodenale, which infects only humans and cannot adequately infect laboratory animals without the use of immunosuppressants. The complete transcriptome of his adult A. ceylanicum intestine was sequenced and analyzed. The results reveal several new macromolecules that are likely involved in parasite survival in mammalian hosts and may serve as future vaccine candidates or drug targets to combat hookworm infections. (Hotez *et al*., 2016; Smith and Brooker,2010; Keiser and Utzinger, 2008; De Clercq *et al*., 1997; Soukhathammavong *et al*., 2012; Hotez *et al*., 2013; Schwarz *et al*., 2015).

Some species of zoonotic hookworm (i.e., cat and dog hookworms) include: Ancylostoma braziliense, Ancylostoma caninum, Ancylostoma ceylanicum and Uncinaria stenocephala. Among these most commonly encountered hookwormis Ancylostoma braziliense. It causes cutaneous larva migrans (creeping eruption) generated by the larva migrating through the epidermis. This lesion is self-limiting, characterized by the erythematous

serpiginous lesions. Ancylostoma ceylanicum is the only species that develops to adult in humans, and causes enteric hookworm infection. Ancylostoma caninum occasionally reaches adulthood in humans, and causes eosinophilic enteritis (Bowman *et al*., 2010; Guill and Odom, 1978; Tu *et al*., 2008; Landmann and Prociv, 2003). The present review mainly focused on enzymatic and immune response of Hookworms.

Heat shock proteins (HSPs)

Several HSPs have been chosen as vaccine candidates and the protective efficacy of HSP vaccinations has been shown against various parasitic infections including *Plasmodium yoelii*, *Brugia malayi*, *Leishmania donovani*, and *Trichinella spiralis*. The other studies reveals that the 8 HSPs within the 100 most abundant proteins expressed in hookworm intestine, including HSP20, 40 and 70. The another reports expressed high expression in intestine and conserved functions for worm's survival suggest these hookworm intestine-expressed HSPs could be the targets for preventive vaccines or pharmaceutical drugs (Sanchez *et al*., 2001; Dakshinamoorthy *et al*., 2012; Kaur *et al*., 2011; Fang *et al*., 2014). The estimation approximately 5000 sterilized L3 were heat-shocked in 0.5 ml RPMI-c at 42 °C for 15, 30, and 60 min in individual wells of a 24-well cell culture plate. After 60 minutes of heat shock, L3 was attenuated to room temperature for 15, 30, and 60 minutes. The same treatment was done at 37°C and then attenuated to 22°C. After processing, L3 was harvested, immediately snap-frozen, and stored at −80 °C until RNA isolation. The present HSB proteins have a characteristic coiled-coil structural motif consisting of two interacting alpha helices, each containing a heptad repeat of hydrophobic amino acids (termed abcdefg). Normally, the first and her fourth residues (a and d) are located at the surface of the helix interface and interact with the corresponding residues of the opposite helix. Stability is determined by interactions between a and d residues, whereas specificity depends on interactions between e and g residues (Tai *et al*., 2002; Fu *et al*., 2006).

Glutathione-S transferases (GSTs)

Various authors describes the important enzyme Glutathione S-transferases (GSTs) are a detoxifying enzyme family that is essential for parasite blood-feeding and survival, and represent potential targets for hookworm vaccine development. Multiple GST-encoding complementary DNAs (cDNAs) have been cloned from Ancylostoma caninum and Necator americanus, but there are no reports about the cloning of this enzyme from Ancylostoma ceylanicum, the animal-derived zoonotic hookworm. And these Hookworms have evolved a

mechanism to detoxify free heme by homodimerizing two GST molecules to express nematode-specific Nu-class GSTs with higher-affinity heme-binding sites. Because of the importance of hookworm GSTs involved in heme binding and detoxification, vaccination with hookworm GSTs induces protective immunity in experimental animals against hookworm larval challenge, and Na-GST-1 from N. It has become the leading vaccine candidate of choice for hookworm infections. Immunolocalization revealed that natural Ace-GST is mainly located in the epidermis, muscle and intestine of the adult. To date, three of her GSTs have been cloned from N. americanus adults (Deponte *et al*., 2005; Zhan *et al*., 2005; Zhan *et al*., 2010).

Proteases

Proteases are instrumental in clotting (and anticlotting) cascades but also play immunoevasive roles in some parasitic infections. The first protease from hookworms to be described by mechanistic class was a zinc-dependent, elastin-cleaving metalloprotease from *A. caninum* (see "Anticoagulant peptides" above). Metalloproteases in ES products of adult *N. americanus* are important in immunomodulation by cleaving eotaxin and inhibiting eotaxin-mediated recruitment of eosinophils. Several authors reported the Proteases and in 2,971 FPKM of the entire worm, Proteases is the most advanced protein in the worm intestinal transcriptome. Using the RNA-SEQ to 8,495, using the RNA-SEQ using the consemic database. Identify the protein sequence and E -value less than 10-6. There were 3,473 hits, of which 674 were unique. Of these unique hits, 518 were classified as peptidase, of which 156 were classified as Peptidase inhibitors. If peptidase is further classified as peptidase, cysteine peptidase, metalo peptidase, serine peptidase, Sleonipeptidase, threadnin peptidase and unknown peptidase, the most common peptidase in the intestine is a cystone peptidase (2.6%of all FPKM). rice field. Continued Serinpeptidase (2.1%), Metallo Peptidase (1.4%), asparagin peptidase (0.7%), and Sleonin Peptidase (0.2%). Compared to the entire worm transfer, it indicates the possibility that cysteine peptidase, Serinpeptidase, asparagin peptidase, and threaded peptidase are priority in the intestine, and they may be involved in blood digestion. Proteases encompass a broad class of hydrolytic enzymes that play essential roles in cellular, developmental and digestive processes, blood coagulation, inflammation, wound healing and hormone processing. Parasite proteases, some of which are in the excretory–secretory (ES) products, facilitate the invasion of host tissues, aid in the digestion of host proteins, help parasites evade the host immune response and mediate molting in parasitic nematodes. As a result, parasite proteases are considered to be potential targets for

the development of novel immunotherapeutic, chemotherapeutic and serodiagnostic agents for the next generation of antiparasite interventions. (Tort *et al*., 1999; Dalton *et al*., 2003).

Aspartic proteases

The enzyme Aspartic proteases belonging to clan AA have two catalytic aspartic acid residues at their active site clefts. Aspartic proteases are considered to be the most conserved group of the four classes of proteases (consisting of aspartic, cysteine, serine and metallopro teases) and the most well known aspartic proteases belong to the A1 family of enzymes typified by the mammalian gastric enzymes pepsin and gastricsin. The A1 family also includes the lysosomal processing enzyme cathepsin D, and digestive enzymes from blood-feeding parasites including Plasmodium falciparum plasmepsins and schistosome cathepsins D. In both of these parasites, aspartic proteases are thought to be responsible for the first step in degradation of host Hb and, in Plasmodium at least, this step can be inhibited by addition of the aspartic protease inhibitor, pepstatin. However, there is controversy over the roles of Plasmodium aspartic and cysteine proteases and where each enzyme fits into the cascade of Hb proteolysis in vivo. Schistosoma cathepsin D is expressed in the gastric skin of adult parasites and overexpressed in female nematodes (presumably to meet higher metabolic requirements for oviposition) and digests Hb at acidic pH in vitro. (Brindley *et al*., 2001; Banerjee *et al*., 2002). Brindley, P.J. *et al*. (2001) Proteolysis of human hemoglobin by schistosome cathepsin D. Mol. Biochem. Parasitol. 112, 103–112.

Aspartic proteases are another class of proteolytic enzymes found in parasitic helminth secretions and intestines and are involved in hemoglobin digestion. Reports on Larval and adult N. americanus extracts contain aspartic protease activity, and an EST encoding a pepsinogen-like aspartic protease was recently discovered in adults. In addition, a cDNA encoding a cathepsin D-like aspartic protease was cloned from A. caninum. Computer modeling of this protein (AcASP1) predicted that it cleaves canine hemoglobin more efficiently than human hemoglobin. Recently, recombinant S. japonicum aspartic protease was shown to cleave Igs at the hinge region and complement protein C3 in vitro (Brown *et al*., 1995; Brown *et al*., 1999; Brinkworth *et al*., 2000; Verity *et al*., 2001; Harrop *et al*., 1996).

Metallo proteases

To infect a host, hookworm larvae must be excreted and migrate through connective tissue. Using a modified in vitro skin chamber, we showed that the human hookworm Ancylostoma

duodenale and the zoonotic canine hookworm Ancylostoma caninum penetrate the epidermis, basement membrane, and dermis in a similar manner. These similarities in tissue-invasive properties mirror the biochemical similarities observed in parasite protease composition. Larvae of both species contain protease activity that is inhibited by o-phenanthroline. This identifies the protease as a metalloprotease. Enzymatic activity exhibits an alkaline pH optimum between pH 9-10. During modified sodium dodecyl sulfate-polyacrylamide gel electrophoresis using protein substrates (either casein or gelatin), the protease activity was resolved into a major band of Mr 68,000 and a minor band of Mr 38,000. The proteases were released in vitro from living A. caninum larvae and cleaved purified, radiolabeled casein into smaller peptides. Agile hookworm larvae were incubated with in vitro purified and radiolabeled connective tissue macromolecules. Both Ancylostoma species degraded human fibronectin to his 60,000 Mr polypeptide intermediates, but were unable to degrade solubilized bovine elastin or human laminin. In contrast, the skin-penetrating obligate nematode C. elegans degraded all three substrates. This biochemical difference may explain some observed differences in invasiveness.

Williamson *et al*., reported that the Metalloproteases are among the highly represented proteins in ES products of adult hookworms. Hookworm metalloproteases secreted as ES products mainly belong to astacin-like zinc-metalloprotease (MTP) class. The activated *Ancylostoma caninum* L3 secretes *Ac*-MTP-1 that aids in the larval tissue migration by hydrolyzing fibronectin, gelatin, laminin and collagen. *Ac*-MTP-1 was immunolocalized to glands in the esophagus and the channels connecting the esophagus to the cuticle, and its specific antisera inhibited larval migration (Williamson *et al*., 2006).

Zhan et al., stated that the Mammals secrete many matrix metalloproteases (MMPs) from immune cells and gut epithelium at the inflammation site. These metalloproteases help in remodeling extracellular matrix and are controlled by tissue inhibitors of metalloproteases (TIMPs). TIMPs are the major proteins secreted by adult hookworms, accounting for 6% of the total ES products (Zhan *et al*., 2002).

Superoxide dismutases

Parasites also produce antioxidant enzymes called superoxide dismutases (SODs), which catalyze the conversion of superoxide anions into hydrogen peroxide and molecular oxygen. The toxic hydrogen peroxide can be removed by several enzymes, including peroxidase, glutathione peroxidase and catalase, which were detected in adult *A. ceylanicum* extracts.

Complementary DNAs encoding SODs have been cloned from multiple parasitic nematodes, such as *Onchocerca volvulus*, *Haemonchus contortus Brugia* and *Toxocara canis*.

Superoxide is one of the main reactive oxygen species in the cell. As a consequence, SOD serves a key antioxidant role. The physiological importance of SODs is illustrated by the severe pathologies evident in mice genetically engineered to lack these enzymes. Mice lacking SOD2 die several days after birth, amid massive oxidative stress. Mice lacking SOD1 develop a wide range of pathologies, including hepatocellular carcinoma, an acceleration of age-related muscle mass loss, an earlier incidence of cataracts, and a reduced lifespan. Mice lacking SOD3 do not show any obvious defects and exhibit a normal lifespan, though they are more sensitive to hyperoxic injury. Knockout mice of any SOD enzyme are more sensitive to the lethal effects of superoxide-generating compounds, such as paraquat (Huang *et al*., 1995; Elchuri *et al*., 2005; Muller *et al*., 2006; Sentman *et al*., 2006).

Calreticulin

A multifunctional protein called calreticulin participates in all phases of parasite infection. Calreticulin can be secreted by parasites to control the host's immunological response, according to studies. We created a recombinant Ace-CRT eukaryotic expression plasmid in order to examine the immunogenicity of the eukaryotic expression plasmid of Ancylostoma ceylanicum calreticulin (Ace-CRT) (pEGFP-N3-Ace-CRT). Indirect immunofluorescence and Western blot analysis were used to confirm that the target protein was successfully expressed in Human Embryonic Kidney (HEK) 293 T cells. A plasmid containing pEGFP-N3-Ace-CRT was used to immunise BALB/c mice. The recombinant plasmid increased the formation of IgG antibodies in mice, as determined by ELISA measurements of IgG antibody levels in sera from immunised animals. Mice that had received the vaccinations had their spleens removed to analyse the proportion of T cell subsets and the levels of cytokine expression (Zhuang *et al*., 2022). Calreticulin is a highly conserved and multifunctional calcium-binding protein, which is present in all cells of higher creatures, except erythrocytes. Numerous calreticulin-associated functions in parasites were reviewed by Ferreira *et al*. These functions include calcium storage, chaperoning, integrin-mediated signaling and cell adhesion, modulating gene expression, as well as inhibiting T cells and natural killer cells perforin pore formation, tumor growth, angiogenesis, and C1q-dependent complement activation. Hookworm calreticulin was originally identified as a key allergen reacting with IgE from *N. americanus*-infected patients. Calreticulin (CRT) is a multifunctional protein that

mainly regulates calcium ions homeostasis, intracellular calcium concentration, and calcium storage in the endoplasmic reticulum (Krause and Michalak 1997). Calreticulin also functions as an intracellular chaperone for glycoproteins, engaging carbohydrate side chains via well-defined lectin domains (Coppolino *et al*. 1997). In addition, CRT can also participate in signal transduction and cell adhesion, regulating gene expression and other functions (Johnson *et al*. 2001).

Immune Responses to Hookworm Infection

Suitable animal models for human hookworm infection are severely lacking, and extrapolation of immunological findings in aberrant hosts may be unreliable. For example, N. americanus matures in hamsters, but only if the puppies are infested with larvae between 1 and 3 days of age. Older animals quickly acquire resistance and do not develop infections. A. duodenale is also a fastidious dog, surviving to adulthood only in immunocompromised dogs. Moreover, laboratory-selected and bred parasites may represent unusual strains with atypical characteristics.

A review article by author Behnke, indicates that the major contributions to hookworm immunology come from immuno-epidemiological studies in human populations. Although these studies provide interesting insights and raise new questions, they may be related to undetermined past and current co-infections (with other helminths, protozoa and microbes), unknown exposure and treatment history of the individual, diet., and genetics. A pilot project using expressed sequence tags (ESTs) has discovered a large number of gene families encoding hookworm proteins with potential immuno modulatory properties, providing new information and potential novelties for use in controlling hookworm infection. We provide targets.

The complexity of the hookworm life cycle provides many opportunities for the parasite and host to interact at the molecular level. In addition, natural abrasion of larvae occurs at critical barriers. B. During skin entry and passage through lung tissue, and arrival in the intestine and entry into its mucosa, the host faces a variety of antigenic challenges, immune stimulation, and possibly immune modulation. Experimental analyzes of this complex interaction have only touched on some of its individual components (Behnke, 1987; Rajasekariah 1985, Schad, 1979).

Hookworm disease is iron deficiency anemia, a direct outcome of adult parasite feeding activity (especially in *A. duodenale* infection) in a host with deficient iron and protein intake. However, gastrointestinal disturbances are also a feature, especially in the earlier stages of infection, and seem to reflect intestinal inflammation.

DISCUSSION

The enzymatic content was significantly attend in mice treated with single doses in the earlier study (Vardhini, 2001). The immune system may be sensitive target for lead the aqny metals can induce enzymatic changes not only when given alone as reported by others (Sharme et al., 1998, Vardhini 2001), but also along with A. canium infection.

There is direct correlation between the retained worm load and enzyme levels. Increased larval rretention may be due to decreased globulin production by antibody producing cells (Agarwal et al., 1989).

The other reports in phosphatases and creatinine in tissues indicated necrosis of cells/ proteolysis in tissues which forms amino acids and are used in TCA cyde for energy production during stress conditions(Griggs, 1964). The enzymatic variations are tissue specific and hence are useful as markers of heavy metal toxicity phosphatases and creatinine studies helps in evaluating the host response in conditions of physiological stress especially due to parasitism and toxicity thus the enzymatic values are important in dices of infection or disease or abnormal body function.

Jana and Bandyophyay (1987) Lomte *et al*.,(2000) reported a significant fall in total protein content of tissues in channa punctatus and in a fresh water bivalve parreysia cylindrical exposed to lead. Over the past decade, the molecular basis of this blood supply process and digestion has been partially identified as a cascade of hemoglobin-degrading enzymes. After the worm ingests blood into the intestine, red blood cells are lysed by hemolysin, releasing hemoglobin. Released hemoglobin is degraded and digested by a series of hemoglobin-degrading enzymes that initiate the cleavage of hemoglobin molecules by aspartic proteases (APR), followed by several cysteine proteases and Further digestion with metalloproteinases follows . These proteases are expressed in the intestinal brush border membrane of the parasite (Ranjit *et al*., 2009; Hotez *et al*., 2010; Jones *et al*., 2002; Loukas *et al*., 2005).

Vaccine development strategies focused on interfering with hookworm blood supply processes are underway. It targets an enzyme-containing protein found in the gut of Necator americanus, a large human hookworm involved in the breakdown of hemoglobin and the detoxification of the breakdown products of the process. Vaccination may induce anti-enzyme antibodies, which enter the hookworm gut via the blood supply and subsequently inactivate target enzymes in the hookworm's digestive tract and other organs (Williamson *et al.*, 2003; Williamson *et al*., 2004; Ranjit *et al*., 2008).

The author Joseph (2012) reported the HSPs, the transcription factor HSF-1, together with DAF-16, plays a central role in regulating C. elegans developmental arrest, and our data suggest a similar function for these transcription factors in hookworm development. increase, hookworm L3 arrest recovery and transition to parasitism are regulated by an unknown transcription factor, TFX, which may be DAF-16, HSF-1, and DAF-12. DAF-16 and HSF-1 target genes are thought to be involved in maintaining developmental arrest by positively regulating developmental arrest-associated genes and repressing developmental genes. Some of these target genes may be co-regulated by both transcription factors, as in C. elegans (Hsu et al., 2003). Thus, in locked L3, DAF-16 and HSF-1 occupy promoters and regulate these lock-associated genes. When caninum L3s are orally introduced into a permissive host, they encounter developmental signals that stimulate ILS in the gut, resulting in the negative regulation of DAF-16. Our data show that a simultaneous shift to physiological temperature induces HSB-1 expression and negatively regulates HSF-1.

CONCLUSION

The hookworm gut expresses various proteins or enzymes involved in blood supply and homeostasis between the parasite and its host. Functional antigens expressed in the hookworm gut have become important targets for vaccine and drug development. To discover additional antigens for hookworm vaccines, the intestinal transcriptome of A. ceylanicum was generated and analyzed. Ancilostoma seylanicum was chosen for vaccine testing in a hamster experimental animal model due to its relative convenience. Analysis of the complete transcriptome of adult male A. ceylanicum intestines shows that two categories of proteins are highly expressed in the hookworm intestine. proteolytic enzymes involved in blood digestion and transport proteins involved in the absorption of nutrient metabolites and maintenance of homeostasis. Involved parasites and their environment. Glutathione S-transferase, which is involved in binding and detoxifying oxidative heme from blood intake,

was also highly expressed in the gut. In addition, other proteins including the Cysteine-rich/antigen 5/pathogenesis-associated protein (CAP) family. C-type lectins; and heat shock proteins are all also upregulated in the hookworm gut and may contribute to nematode survival within the host. Thus, although at least two of his classes of potential hookworm antigens are related to adult blood nutrition, the functions of his CAP proteins, C-type lectins, and heat shock proteins in the host–parasite relationship are still unclear it is in under investigation.

Proteins are the most abundant nutrients of the blood and, therefore, the major digestive enzymes in haematophagous parasites and blood-sucking insects are thought to be proteases. The blocking of proteolysis of host Hb with protease inhibitors results in significant antiparasitic and antipathology effects in schistosomiasis. However, the identity and roles of the schistosome enzymes that participate in the degradation of host Hb have yet to be fully elucidated (Wasilewski *et al*., 1996; Bogitsh *et al*., 1992; Brindley *et al*., 2001). Our previous re[orts on absence of any significant effect of lead on phosphatases/ creatinine levels at three exposure times indicates that this heavy metal did not induce non-specific chemical stress which could contribute to this observed immuno suppressive effect. There was a temporal relationship between infection and enzymatic levels, since enzymatic changes were associated with the immunological effects. Ours results indicate that lead suppress natural immunity in a time and dose dependent manner and suggest a significant susceptibility.

Conflict of interest

The author declare that there is no conflict of interest regarding the publication of this review article.

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