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A REVIEW ON HOOKWORM DISEASE: HISTORICAL, VIRULENCE, IMMUNOMODULATION AND IMMUNE ESCAPE STRATEGIES

By

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Abstract

Hookworms are one of the most prevalent soil-transmitted helminths (STH) worldwide, they are ubiquitous in impoverished nations with limited resources, where polyparasitism is prevalent and vaccine cold chain logistics are complicated. Over two billion disability-adjusted life years are lost due to hookworms, which infect around half a billion individuals globally.

Humans contract infection by ingesting the infective third-stage larvae (iL3) or skin penetration. Both *Ancylostoma duodenale* and *Necator americanus* were thought to be the most common species worldwide, but in certain regions, *Ancylostoma ceylanicum* emerged as a significant parasite.

Key words: Hookworms, Virulence, Immunopathology, Immunoregulation, A review.

Introduction

In Egypt hookworm infection was disease described in the Ebers papyrus (Inpankaew *et al*, 2014), and was the classic home of hookworm disease to understand this illness in modern times (Scott, 1937). Hookworm infects humans are *Acylostoma duodenale* (Dubini, 1843), or Old World, *Necator americanus* (Stiles, 1902), or New World and *A. ceylanicum* is zoonotic, with candies; main reservoir for zoonosis mainly in Asia (Traub, 2013). Man is accidentally infected by dog or cat hookworm or *A. caninium* causing only cutaneous larva migrans (creeping eruptions) or serpiginous tunnels in skin (Bahgat *et al*, 1999). Looss (1901) clarified the life cycle of *A. duodenale* and *N. americanus* was found in the Western Hemisphere.

The miner's anemia in Italy and extended across Europe was caused *A. duodenale* was the cause (Hotez *et al*, 2005). Infection with *A. duodenale* was linked to a higher prevalence of iron deficiency than infection with *N. americanus*, indicating that blood loss differes by species (Brooker *et al*, 2004). Consequently, the hookworm was eradicated from many tropical and sub-tropical areas, including the Caribbean and Latin America (Brooker *et al*, 2006). Tetrachloroethylene was administered as 3-4ml while fasting and followed by 30-45g of sodium sulfate, gave 80% cure rate for *Necator* infections, and only 25% cure rate for *Ancylostoma* infections, necessitating retreatment (Young *et al*, 1960). In Egypt, a single 2.5-gram Alcopar® dose (Bephenium hydroxynaphthoate base) was used to treat *A. duodenale* infections (Farid and Miale, 1962). Also, ancylostomiasis, in the country's first nationwide deworming campaign for soil-transmitted helminthiasis, organized by the Ministry of Health and Population with the WHO Country Office in Egypt among all school-age children were treated by Albendazole® twice, separated by two weeks (WHO, 2016).

Review, discussion and conclusion

In fact, hookworm infection is the second most significant parasitosis (after malaria) as the most significant neglected tropical disease (Zawawi and Else, 2020). There was geographic overlap between hookworms and malaria both co-infections are common, especially in sub-Saharan Africa. Besides, between 25% and 33% of the African pregnant women have hookworm infections suffered from severe anemia, higher rates of maternal morbidity and mortality, and fetal death or preterm birth (Freeman *et al*, 2019). Also, long-term hookworm infection was a risky factor for HIV/AIDS, TB, and other tropical diseases with an estimated $11 to $138 billion was lost in productivity annual globally due to hookworms (Uzoechi *et al*, 2023).

There were between 406 and 480 million

hookworm infections, which resulted in the loss of roughly 2.1 million disability-adjusted life years (CDC, 2024). In endemic regions, hookworm infection poses a risk to the health of both mothers and children. It causes maternal morbidity and death, 24 million severe anemia cases annually, with possibility of fetal loss or premature birth due to high iron demand (Sarkar *et al*, 2024).

Life cycle: Adult females live in the small intestine lay eggs that pass with patient's feces, hatch in the soil into rhabditiform larva (L1). Larvae molt to reach the infective filariform larvae (iL3), which infect man by skin penetration. They were carried by circulatory system to the lungs, molted into L4 in the alveolar spaces and move up to trachea by coughing, they were transferred to mouth and ingested into gastrointestinal tract. In the duodenum, they mature into adult male and female feed on patients' blood (Mortimer *et al*, 2006). But, *N. americanus* only infects man by skin penetration, shed off sheath and mature in the intestine without circulatory migration (Ferreira *et al*, 2004).

Pathogenesis and clinical manifestations: Hookworm severity and morbidity are directly associated with a higher parasite burden (Magalhães *et al*, 2021). Infective larvae penetrate the skin causing local itch inflammation with erythematous lesion or ground itch, and when they enter bloodstream to the lungs via heart evade the immune system (Hawdon and Hotez, 1996). Infection by the oral route (Wakana syndrome), patient may experience nausea, vomit- ting, pharyngeal irritation, cough, dyspnea, and hoarseness (CDC, 2020).

Larvae in the small intestine develop into adults, by their buccal capsule; teeth or cutting plates adhere to the intestinal mucosa, and excrete hyaluronic acid enzyme to erode blood vessels and supplies blood for adults, leading to chronic intestinal bleeding and iron deficiency anemia (Jourdan *et al*, 2018). Severe infections may cause hypoproteinemia, edema, or anasarca (White and Artavanis 2012).

Helminthic multicellular parasites use diffent mechanisms to invade the host ensuring long stay in, and causing more pathogenesis (Cassar and Dagenais, 2023). The factors of hookworms are 1- specific buccal permanent attached to mucosa for consume of blood by secreting proteolytic enzymes (Pearson *et al*, 2012) and plasminogen-binding surface proteins for the fibrin breakdown (Jiang *et al*, 2019), 2.- Serpins prevented coagulation by promoting human and mouse B & T cell responses (Bobardt *et al*, 2020), 3- Extracellular vesicles (EVs) to traffic the virulence components required for feeding, cytoadherence, invasion, infection, cytotoxicity, and immune system evasion (Khosravi *et al*, 2020). These were detected in helminthic E/S products (Tritten and Geary, 2018), as potential immunomodulatory functions (Drurey *et al*, 2021), 4- Immune evasion as hookworms' ability to suppress or elude host's immune system by the release of immunomodulatory proteins was partly linked to their long-term persistence in their host. For example, cystatins (CYS) are endogenously expressed as inhibitors of cysteine protease (CPs) that control the CPs expression. This proteolytic activity breaks down cellular membranes, and contributes to the egress cascade (Friedrich *et al*, 2012), which was closely linked to processes of disease dissemination, transmission, and inflammation. As a result, molecules involved in one or more egress mechanisms are regarded as important stages in infection and transmission; in other words, they are referred to as indirect virulence factors (Abaza, 2020). Besides, some molecules suppress inflammatory reactions by interfering with cytokine signaling and proteins to prevent T-cell proliferation (Eichenberger *et al*, 2018), and 5- Molecular mimicry by mimicking host proteins or generating miRNAs that target host gene expression, hookworms can control immune cell activity by manufacturing molecules homologous to host molecules (Johnston *et al*, 2017). The virulence factors harm host tissue and are crucial for the parasite's ability to spread and to develop

inside, by imitating host molecules, glycoproteins, and surface antigens to conceal parasite from being immune detected (Bobardt *et al*, 2020).

Immune response and immunomodulatory effects: Hookworm immune response is a complex interaction between host's immune system and its ability to evade immune defenses as the following.

Innate Immune Response: Hookworm larvae penetrate the skin during infection, triggering a localized immune response. Upon penetration, the majority of larvae shed their outer sheath as it is immunogenic, redirecting the immune response away from the larvae (Loukas and Prociv, 2001). This includes activation of keratinocytes, mast cells, and macrophages. These cells release cytokines like IL-1, IL-6, and TNF-α, which recruit other immune cells to the site of infection (Eichenberger *et al*, 2018). Toll-like receptors (TLRs) on innate immune cells recognize hookworm antigens, initiating signaling pathways enhanced the inflammation. Moreover, eosinophilia is a hallmark of helminth infections. Eosinophils release toxic granules and reactive oxygen species that target larval and adult hookworms. Hookworms elicit a highly polarized T helper type 2 (Th2) responses in the skin, lungs, and intestinal mucosa (Nair and Herbert, 2016) which includes $CD4^+$ T cell-dependent IgE production, eosinophilia, mastocytosis, and mucus formation. This response occurs after infectious larvae (L3) migrate through human tissues. To prevent potentially harmful pathology, hookworm infections are also characterized by the production of an immunoregulatory environment with regulatory T cells, type 2 innate lymphoid cells, tolerogenic dendritic cells, & M2 macro-phages, as well as the anti-inflammatory cytokines IL-10 and TGFβ (Faniyi *et al*, 2020).

Humoral immune response: Patients with *N. americanus* produce all immunoglobulins, with high IgA levels and specific IgE, protection against hookworm infections. All decreased after treatment, but IgA & IgD continue to increase over 2 years later, with antibody response to L3 larvae and adults. IgE interacts with mast cells, basophils, and hookworm antigens to cause degranulation and histamine release, aiding in parasitic expulsion (Bąska *et al*, 2022).

Cellular immune response: Hookworms typically induce a strong Th2 immune response, characterized by production of cytokines like IL-4, IL-5, IL-10, &IL-13 (Colombo and Grencis, 2020). An early hookworm infection causes inflammation by upregulating pro- inflammatory cytokines and triggering a temporary TH1 response. During larval migration, inflammatory response persists, and during patency (adult hookworm phase), the immune system shifts to a Th2-predominant response. Tissue damage that occurs during larval migration and adult worm establishment is most likely the cause of this temporary Th1 reaction (Mendez *et al*, 2005). Human infection showed mucosal expression of Th1 cytokines, such as IFNγ, IL-2, & IL-15, and Th2 cytokines, such as IL-4, IL-5, & IL-13 (Wen *et al*, 2021). The Th2 cytokines, including IL-5, IL-13, & IL-9, are produced by innate lymphoid cells (ILC), particularly ILC2. Both IL-5 & IgE degranulate eosinophils and basophils/mast cells, respectively, causing histamine release and worms expulsion. Parasite-specific IL-5 levels positively correlated with resistance to re-infection after anthelminthic therapy (Quinnell *et al*, 2004).

By initiating a weep and sweep response that is mediated by goblet cell hyperplasia, mucus hypersecretion, and smooth muscle contraction, and enteric nerve stimulation, IL-13 speeds up the evacuation of worms (Nair and Herbert, 2016). By secreting TGF-β, IL-10, & IL-6, Tregs prevent Th2 cells from functioning. These cytokines work in concert with IL-4, IL-5, & IL-13 to reduce inflammation and tissue damage, treat disorders caused by Th1 & Th17 creating a balance to help parasite in avoiding immune system (Wiedemann and Voehringer, 2020). In secondary infection, IL-4 & IL-13, and mast

cells & basophils destroyed larvae lung migration before adults were established in gut (Langeland *et al*, 2024). Rather than overwhelming and killing host, hookworms and humans have coevolved to achieve an immunological status quo where Th2 responses probably keep worm burdens in check (Eichenberger *et al*, 2018).

Hookworm induced immune modulation: The substances released by hookworms are responsible for the immunomodulatory milieu they create. Excretory/Secretory products are a broad category of structurally and functionally unique macromolecules that are secreted and excreted by the adult hookworm stage. The E/S is primarily composed of arrays of proteins that are thought to interact with host tissues and promote parasite survival (Maizels *et al*, 2018). Along with the proteins, E/S also includes lipids and carbohydrates, and the parasite's exterior is continuously pumped with extracellular vesicles that carry nucleic acids (Uzoechi *et al*, 2023). An E/S product plays a key role in host-parasite interactions that mediate parasite survival in the small intestine and aid in hookworm infection, which directly and indirectly alter the immune system (Logan *et al*, 2020).

Direct effect: Hookworms strongly stimulate Tregs, and immunological tolerance markers like Foxp3^{+}, PD-1, LAG-3, & CTLA-4 were exhibited by patients with increased expression. A significant rise in IL-10 prod uction in hookworm patients showed that microRNAs encoding IL-10, TGFβ, & IL-22 were upregulated, elevated expression of genes in retinoic acid pathways, suggested tolerogenic dendritic cells presence, and that apoptosis induction a recognized immunomodulatory mechanism (Gaze *et al*, 2012). Also, the pro-apoptotic genes were upregulated, and TNF receptor family members (de ath receptors) were downregulated (Gazzinelli-Guimarães *et al*, 2013). Macrophage polarization response is another character of the immunomodulatory milieu in hookworm infection. To differentiate the macrophages, concept of M1 & M2 types was introduced, with IL-4 & IL-13 production resulted in alternatively activated macrophages (M2 phenotype), which induce arginase activity to aid in healing. Additionally, M2 macrophages were implicated as effector cells that aid in ejecting of the worms and offered defense against reinfection (Chen *et al*, 2012).

Indirect effect: By altering the host microbiome, hookworm infection can also indirectly affect the immune response. The immune system and gut microbiota have an inherent link that has emerged as a crucial aspect of maintaining homeostasis and overall health. Hookworm infection changed the gut microbiota, and anti-microbial peptides generation can be enhanced by IL-22 increasing hookworm infection (Mourão Dias Magalhães *et al*, 2021). Helminths can directly be interacted with intestinal flora by releasing antimicrobial peptides. By altering the host's physiology, intestinal epithelium, and immunology, the parasites may also indirectly modify the microbiota. Additionally, the hookworm's capacity to modify microbiota may go beyond the stomach, as changes may occur during skin penetration and larval migration (Mladineo *et al,* 2023).

Hookworm therapy: A comprehensive approach combining medical treatment, nutritional support, and preventive measures can significantly reduce the burden of this parasitic infection. Effective treatment involves eliminating parasites, alleviating symptoms, and addressing complications like anemia.

Antiparasitic medications, which eradicate or kill the worms from the intestines, are the mainstay of treatment. Mebendazole®, albendazole®, and Pyrantel pamoate were examples of Benzimidazole® drugs that were frequently prescribed; however, their efficacy in treating human hookworm infection varied greatly depending on factors such as country, worm burden, age, therapeutic protocol (single or multiple doses), and pre-treatment (Soukhathammavong *et al*, 2012). Several lines of evidence indicated that a single nucleotide polymorphism in the β-tubulin

gene at codon 198 is most likely the mechanism behind hookworm resistance to benzimidazoles (Keiser *et al*, 2008). Moreover, Emodepside® proved effective in treating all human STH infections, including hookworm disease in regions where resistance to Ivermectin®, Levamisole®, and Benzimidazoles® were identified (Sarkar *et al*, 2024).

Immunotherapy: The concept of using the live worms or E/S chemicals as an immunotherapy was prompted by hookworms' remarkable capacity to distort the immune response. Hookworms and their hosts primarily communicate via E/S products. Both larvae and adult hookworms release E/S products, which were made up of a mixture of the proteins, glycoproteins, and tiny molecular weight compounds crucially for hookworm invasion, migration, and survival in the host (Abuzeid *et al*, 2020).

The cysteine-rich glycoprotein known as neutrophil inhibitory factor (NIF), secreted by adult *A. caninum* hookworms, prevented activated human neutrophils from adhering to vascular endothelial cells and preventing them from releasing H_2O_2. Besides, in mice, NIF inhibited neutrophil activation, migration, and sequestration, which inhibited lung damage and tissue inflammation (Schnyder-Candrian *et al*, 2012). The primary proteins released by mature hookworms (6% of all E/S products) are tissue inhibitors of metalloproteases (TIMPs). The TIMPs released by hookworms were used to treat autoimmune and allergy disorders and were involved in the immunomodulation of the host immune system by cleaving eotaxin, a strong eosinophil chemo-attractant furcated by CCR3, and expressed on the eosinophil, basophils, mast cells, and Th2 T cells, metalloproteases can function as immunomodulators in vitro (Shalash *et al*, 2021). Calreticulin was also found in E/S products from adult worms and was first recognized as an allergen in *N. americanus* patients. In hookworm, calreticulin inhibits T lymphocytes and natural killer cells, and production of performing pores, tumor growth, angiogenesis, and C1q-dependent complement activty (Logan *et al*, 2020).

Hookworm E/S peptides study was a promising area led to development of the novel, more manageable treatments for metabolic and inflammatory disorders instead of live worm therapy. Also, a deeper proteins comp rehension of the involved in infection yielded novel targets for the hookworm to develop vaccine (Cobos *et al,* 2022).

Vaccine candidates: The effective vaccine would minimize transmission, decrease pathology, and be inexpensive to prepare. The vaccine of hookworm would be a transformative tool in reducing the hookworm disease burden globally, and offers hope for a sustainable and cost-effective solution to improve the health and well-being of millions living in the endemic regions (Hotez *et al*, 2018).

Undoubtedly, helminths are complex multicellular parasites with complex genomes. In several ways, creating a vaccine against the hookworms was much more difficult than creating vaccines against unicellular parasites as caused by protozoa. Also, another obstacle to the identification and development of a vaccine was absence of an animal model that could replicate human hookworm infections (Hoogerwerf *et al,* 2023).

Infectious larvae of *A. caninum* attenuated by radiation were used in the veterinary field to describe the first canine hookworm vaccination. L3 larvae exposed to radiation increased the generation of antibodies and prevented tissue penetration. Fujiwara *et al*. (2006) in vitro showed that dogs vaccinated with irradiated L3 also prevented tissue penetration. But, the drawbacks of live attenuated and/or un-attenuated hookworm vaccines, notwithstanding their potential impracticality for mass use, short vaccine shelf life, and high manufacturing costs (Tang *et al*, 2014).

These candidates contained two recombinant antigens; *N. americanus* glutathione-S-transferase-1 (Na-GST-1) and Aspartic Protease (Na-APR-1), formulated atop an adjuvant of AL hydrogel (aluminum hydroxide gel suspension) are both safe and immunogenic. The Phase I & II trials of Na-GST-1 &

Na-APR-1 showed safety profiles and induced immune responses in humans. Besides, vaccines against Na-APR-1 reduced anemia and the overall parasitic load (Zinsou *et al*, 2024). Vaccines produced notable IgG1 responded to both antigens were safe, and well tolerated when used separately or in combination (Adegnika *et al*, 2021). Recombinant vaccines showed several difficulties, including the need for an adjuvant, the need for many vaccinations and to account post-translational alterations (Nascimento and Leite, 2012). *Ancylostoma* secreted protein ASP-1, ASP-2, and the astacin-like metalloprotease (MTP-1) didn't express these compounds; only L3 does. ASP-2 is involved in the parasite's invasion of host tissues. Vaccines targeting this protein seeks to block the initial infection. ASP vaccinations, but troubling since hookworms have a propensity to cause the host immune system to develop IgE to these proteins leading to allergic reactions upon vaccination if the patient was previously exposed to the parasite (Fujiwara *et al*, 2005). The heme-detoxifying enzyme Na-GST-1 and hemoglobinase aspartic protease (Na-APR-1) of *N. americanus* have drawn attention as they are molecules that adult worm employ while the blood-feeding process, and vaccines targeting Na-GST-1 to reduce the parasite's survival and ability to thrive within the host (Diemert *et al*, 2018).

Extracellular vesicle (EV) surface proteins found in E/S products have recently attracted attention as a possible target avenue for vaccine development (Mekonnen *et al*, 2018). Since cathepsin B cysteine proteases of hookworms produce antibodies counteract the gut's catalytic activity of hookworm antigens, reducing the motility and viability of adults, by developing prophylaxis against soil-transmitted helminths (Noon *et al*, 2019). Cysteine proteases are also known for triggering type 2 immune responses, including cytokines IL-4, IL-5, IL-13, and IgG1-specific antibodies, and are important for both human and experimental hosts to develop immunity (Ferreira *et al*, 2013).

Pearlsand other problems: Years of use the anthelmintic mass drug administration for hookworms and other STH infections didn't stop the zoonotic spreading of these worms. This led to the drug resistance. The concern for novel approaches to treat *Ancylostoma* and other STH infections, particularly in end emic areas, is heightened by the rising rates of medication failure and the inability to offer sustained protection. Multiple *Ancylostoma* species antigen-targeting vaccines have been developed with the potential to provide long-term protection and stop the spread of illness. The availability of the vaccine will significantly help public health combat these illnesses (Ng'etich *et al*., 2023).

Conclusion

Hookworm is one of the most common soil-transmitted helminths (STH) in the world. Imidazole anthelmintic medications are currently approved to treat hookworm infections; however, their effectiveness in treating hookworm infections is limited. New and effective immunizations are needed to overcome the shortcomings of the current anthelmintic treatments to provide adequate protection against reinfection, lower the burden of infection, and halt the spread of disease. By targeting virulence factors and understanding the immune response and immunomodulatory pathways of hookworm infection, anthelminthics or vaccinations can be safely developed.

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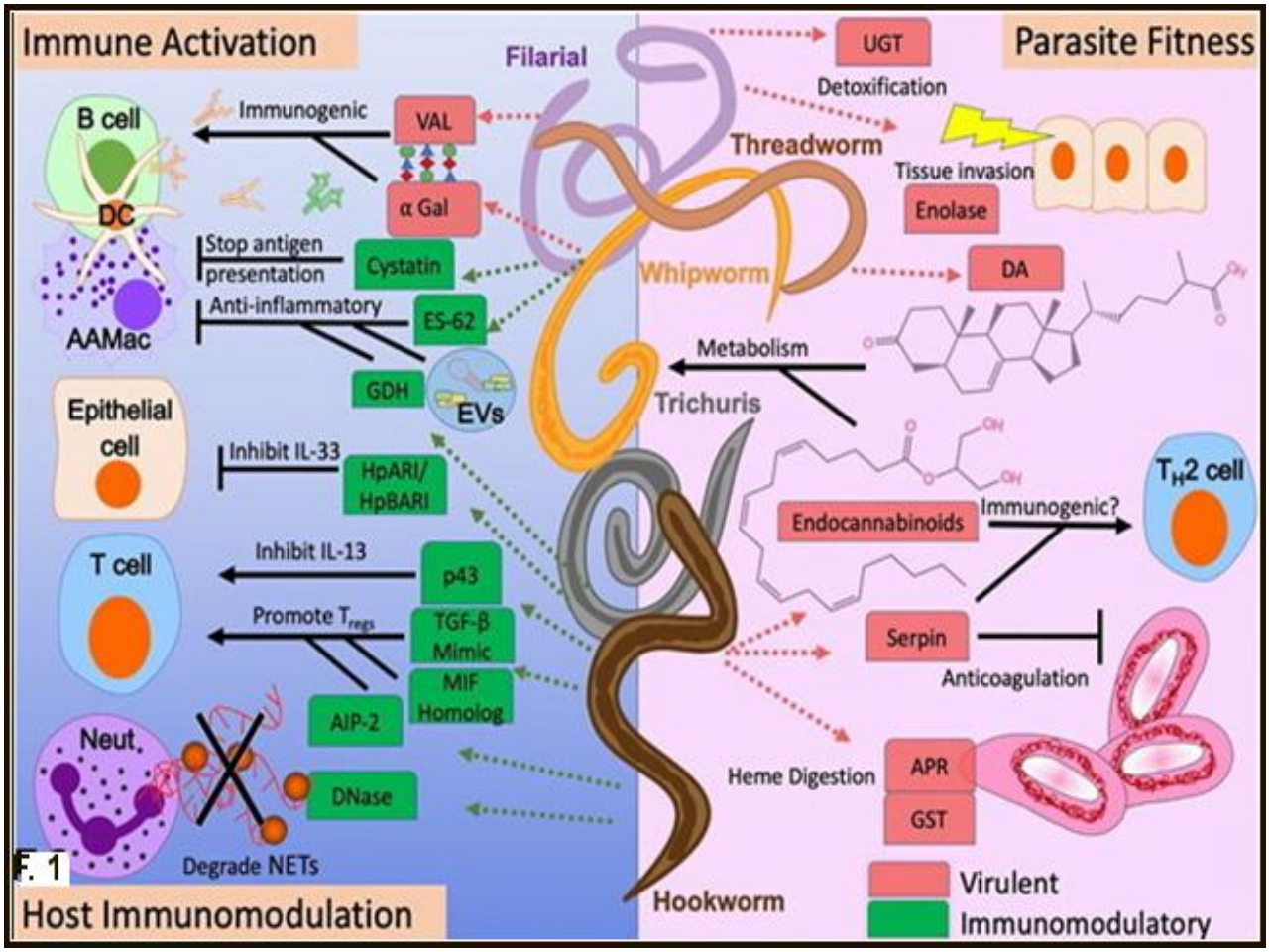
Explanation of figure

Fig. 1: Pleiotropic functions of nematode-derived molecules. Functions range from promoting or inhibiting host immune response (left), to providing essential physiological functions for nematode parasite (right). Understanding virulence (red) and immunomodulatory (green) potential for specific nematode-derived molecules allows us to determine their utility as vaccines, anthelminthics, or new therapeutics for allergic or inflammatory diseases (Bobardt *et al*, 2020).


Immune Activation
Filarial
Parasite Fitness
UGT
Detoxification
B cell
Immunogenic
VAL
α Gal
Threadworm
Tissue invasion
Enolase
DC
Stop antigen presentation
Cystatin
Whipworm
DA
AAMac
Anti-inflammatory
ES-62
Metabolism
GDH
EVs
Trichuris
Epithelial cell
Inhibit IL-33
HpARI/ HpBARI
Endocannabinoids
Immunogenic?
TH2 cell
T cell
Inhibit IL-13
p43
Promote Tregs
TGF-β Mimic
Serpin
Anticoagulation
MIF Homolog
AIP-2
Neut.
Heme Digestion
APR
GST
DNase
Degrade NETs
F. 1
Host Immunomodulation
Hookworm
Virulent
Immunomodulatory