Raising Awareness of Neglected Tropical Diseases and the Prospects of Chronic Helminth Infections to Suppress Host Immune Response

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Abstract

This thesis will delve into the background knowledge of parasitic diseases, explore the current research in creating anti-helminthic therapeutics and vaccines, and describe how these diseases are preventable and treatable with proper infrastructure. Current research in the field of parasitology is focused on the correlation between chronic helminth infections and the disruption of autoimmune inflammatory responses. With this knowledge, anti-helminthic vaccines and therapeutics could be developed against autoimmune and inflammatory diseases. My thesis will explore why parasitic diseases affect tropical regions and why this issue is important. My thesis will also explore the field of immunology, in particular the T helper cell type 2 inflammatory responses, and the discovery of why parasites trigger this pathway. My goal is to provide the audience with an understanding of parasitic diseases, raise awareness of the poverty and devastation they bring to those in the tropics and bring attention to the current research in creating therapeutics for autoimmune and inflammatory disease.

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List of Abbreviations

FOX_p3	Forkhead Box P3
GALT	Gut-Associated Lymphoid Tissues
IFN_γ	Interferon Gamma
Ig	Immunoglobulin
IL	Interleukin
MALT	Mucosa-Associated Lymphoid Tissues
NTD	Neglected Tropical Disease
TCR	T-Cell Receptor
TGF-β	Transforming Growth Factor-Beta
Th1	T Helper Type 1 Immune Response
Th2	T Helper Type 2 Immune Response
Th17	T Helper Type 17 Immune Response
Tregs	T Regulatory Cells

Introduction

Medical parasitology is the study of pathogens that parasitize humans.[1] By definition, parasitism is a relationship where the parasite benefits and the host is harmed.[2] The variation of harm can vary greatly according to the host's immune system, age, and type of parasitic infection. To understand the pathogenicity of a parasite, it is important to know the life cycles that they exhibit. Life cycles can include both direct and indirect cycles.[1] A direct life cycle only requires one host, whereas an indirect life cycle requires an additional intermediate host.[1] An example of a direct life cycle is a nematode (roundworm) that penetrates the human as larvae and matures inside the host.[4] The mature adults will excrete eggs that are passed in the feces and turn into infective larvae in the environment that will infect another host.[4] An example of an indirect life cycle is *Plasmodium* species, which causes the disease malaria.[5] In order for *Plasmodium* species to infect humans it must first reproduce sexually inside a female *Anopheles* mosquito to produce the infective sporozoite stage that is injected into the human host during its blood meal.[5] The life cycle is important because it provides insights regarding the geographic distribution, how parasitic infections spread, the types of disease it can cause, and how to prevent or reduce further infections.

Background

Parasites can be classified into three categories: protozoa, helminths, and arthropods. Protozoa are unicellular eukaryotes that have complex internal structures and metabolic activities.[2] They have the ability to multiply inside the human host and some can live freely in the environment. Common protozoa include: amoeba, flagellates, ciliates, and sporozoa.[6] The life cycle of some protozoans includes a cyst stage and trophozoite stage.[6] Cysts are the protective and inactive forms of the parasite that can survive harsh conditions in the environment such as temperature, pH, and lack of nutrients.[6] If ingested through the fecal-oral route the cysts will transform into trophozoites in the human intestinal tract which will feed and multiply in the host.[6] In hemoflagellates,the life stages are classified as amastigote, promastigote, epimastigote, and trypomastigote by the absence or presence of flagella and kinetoplast.[6] Other protozoa such as the apicomplexa, have structures known as merozoites, gametocytes and gametes.[6]

Helminths are multicellular parasitic worms that can be free living or dependent on a host.[7] They grow from egg, larval, and adult stages. Over time, helminths have adapted to survive in the human host. These adaptations include specialized mouthparts, enzymes, and cuticle coverings to protect the parasite from recognition by the host immune system.[8] It is important to note that there are many types of helminths. The three types of helminths include nematodes (roundworms), trematodes (flukes), or cestodes (tapeworms).[4] Nematodes are cylindrical in shape and have separate male and female forms.[9] Trematodes are flat leaf-like structures that usually occupy the intestines, lungs, and liver.[4] Cestodes or tapeworms can live in the intestines or tissues of the host.[5] The cestode adults have a complex structure including a

scolex, neck, and proglottids for reproduction.[7] This thesis paper will focus on the soil transmitted helminths due to their contribution in the neglected tropical diseases.

Soil transmitted helminths are intestinal worms that spend a part of their life cycle in soil. These are more commonly found in geographical regions where there is a warm and moist climate. The four major soil transmitted helminths are *Ascaris lumbricoides*, *Trichuris trichiura* (whipworm), *Ancylostoma duodenale and Necator americanus* (hookworms).[4] These infections are common in places with poor sanitation and hygiene due to improper disposal of human waste. *Ascaris* and whipworm eggs mature in the soil into infective forms that are ingested; whereas, hookworm eggs hatch in the soil to form larvae that penetrate human skin.[4] *Ascaris lumbricoides* infections are initiated when a human ingests a fertilized egg that is found in soil. The larvae will hatch and migrate to the lungs where they are coughed up and swallowed, making their way to the intestinal tract where maturation will occur.[10] The adult worms lay eggs which are shed into the environment. With the proper disposal of human waste these parasites would not be able to infiltrate the soil or contaminate produce that are grown in soil.[4]

Ectoparasites are parasites that live on the skin of the human host.[1] These parasites survive outside the body and are common transmitters of disease. Arthropods are examples of ectoparasites. Arthropods come from the phylum *Arthropoda* and are a type of insect that have an exoskeleton.[1] Some of the medically important arthropods include sand flies, black flies, deer flies, tsetse flies, biting midges, anopheles mosquitoes, reduviid bugs, copepods, and other various mosquito species.[1] An example of an arthropod is the female *Anopheles* mosquito, that when infected with *Plasmodium* species, transmits the disease malaria to humans.[5]

Neglected Tropical Diseases

Neglected tropical diseases (NTD) are diseases found in places of poverty and with poor infrastructure which is defined as a lack of buildings, roads, power, water and sanitation supplies.[9] In developing nations, NTD's have contributed to the overall health and disability of those affected.[10] Physical disability leads to the inability to work which exacerbates poverty. Over one billion people are affected by NTD's and a majority can be treated and eliminated with medications, controlling transmitters of the disease, providing safe drinking water, and proper disposal of human waste.[13]

The life cycle of soil-transmitted helminths begins with ingesting helminth eggs via the fecal-oral route, meaning that the parasitic eggs are being ingested by the human host from the environment that is contaminated by human feces. Once ingested, the helminth will mature into an adult worm and colonize the small intestine. An example of a soil transmitted helminth is *Trichuriasis trichiura* also known as whipworm, (see Appendix A). Those infected with whipworm will have varying symptoms based on if they have a heavy or light infection.[14] Heavy infections can lead to painful bowel movements and rectal prolapse.[14] Whipworm infections in children can lead to severe anemia and cognitive impairments.[14]

Infections with NTDs not only affect the health of the individual but also their ability to work further creating a cycle of poverty and disability. According to Hannah Kuper, an example of an NTD related disability is *Lymphatic filariasis,* also known as elephantiasis. *Lymphatic filariasis* can cause lymphedema (swelling in the tissues), scrotal hydrocele (swelling in the scrotum), rheumatic and respiratory problems.[12] Infections with this parasite can lead to

difficulty walking and the inability to work, creating an economic loss.[12] It is important to raise awareness about these NTDs because with proper infrastructure, medications, and controlling the transmitters of disease, individuals will have a better quality of life.

Immunology

The immune system has mechanisms for preventing foreign pathogens from entering the body and causing an infection. Humans were born with a natural immunity which is a part of the innate immune response. The innate immune response is immediate and non-specific. Innate immunity includes natural barriers such as skin and mucous membranes and chemicals in our sweat that are antimicrobial.[1] Also, innate immunity includes neutrophils and macrophages which are phagocytes that can engulf and destroy pathogens. Neutrophils and macrophages can be found widely throughout the body and are part of the immediate innate immune response. There are also cells such as cytotoxic T cells, natural killer cells, and activated macrophages that kill virus-infected cells. Other key players in the immune response are mast cells that respond to antihistamines during allergic responses and lymphoid cells with broad specificity.

In contrast to innate immunity which is non-specific, adaptive immunity is highly specific. Our adaptive immune response is facilitated by B cells and T cells. Every cell expresses molecules or epitopes on its surface including proteins, polysaccharides or macromolecules that are considered antigens.[1] The adaptive immune response includes cells that have receptors specific for antigens and those antigens only. There is a process called clonal selection where upon exposure to a foreign antigen, a B cell or a T cell will create clones of itself, fight the foreign invader, and create memory cells so that in the future**,** that same antigen

can be recognized.[1] In a normal immune response, foreign antigens are attacked while self-antigens are not.[1] This is called self-tolerance. Without the ability to distinguish between foreign antigens and self-antigens**,** an autoimmune disease will develop. There are two types of tolerance: central and peripheral. Central tolerance occurs in the primary lymphoid organs which includes the thymus and bone marrow. Immature lymphocytes that can bind to self-antigens are prevented from maturing further by apoptosis (cell death) and receptor editing. If the lymphocytes follow the CD4 lineage and react to self antigens, they may differentiate into Tregs that are specific to that antigen. Peripheral tolerance occurs in the secondary lymphoid organs such as the tonsils, lymph nodes, spleen, peyer's patch, appendix, mucosa-associated lymphoid tissues (MALT), and gut-associated lymphoid tissue (GALT).[1] In these peripheral tissues, mature lymphocytes that bind to self-antigens will die by apoptosis or become anergic (unresponsive to antigenic stimuli).[15]

Inflammation normally occurs through the Th1/Th17 pathway. During the immune response, leukocytes infiltrate tissues, cause edema, and increase body temperature and metabolism.[16] Local inflammation involves the skin, lungs, and intestines, whereas systemic inflammation is anaphylactic and life threatening. In contrast, the Type 2 Helper T Cell (Th2) immune response is triggered by allergens and helminths. The cells involved include eosinophils, basophils, and mast cells. These cells play an important role in the resolution of inflammation, tissue remodeling, and restoration.[16] Mast cells are involved in allergic responses and release proteases and histamines. It is thought that mast cells can be beneficial to the host by possibly forming granulomas around the larvae stages of helminths to trap them, or by degrading them by proteases. Mast cells also release the cytokines IL-4 and IL-13 which could activate

fibroblasts to promote collagen deposition, mucus production from goblet cells, activate smooth muscle and stimulate release of effector molecules from intestinal epithelial cells. All these actions would make the intestinal environment challenging for helminth survival.

Cytokines

Cytokines are proteins that regulate and coordinate a wide variety of activities in the cell during innate and adaptive immune responses.[15] There are many cytokines that are important in the activation of the Th2 response (see Appendix B). Interleukin-25 (IL-25) and Interleukin-33 (IL-33) are released from epithelial cells during both allergic responses and helminth infections.[16] (pg 475) IL-25 and IL-33 promote the expansion and activation of type 2 innate lymphoid cells and are a major source of IL-5, IL-13 and IL-4. IL-4 stimulates the production of IgE antibodies which bind to the Fcε receptors on eosinophils and mast cells. IL-13 stimulates mucus production in the airway epithelial cells.[15] IL-5 activates eosinophils to release lysosomal hydrolases and eosinophil peroxidase from their granules that are toxic to helminths and bacteria.[15] The Th2 response causes the intestine to expel the parasite and is mediated by IgE antibodies and mast cells.[15] The Th2 response also promotes tissue repair through the alternative activation of macrophages by promoting collagen synthesis and fibrosis. Alternatively**,** activated macrophages also produce cytokines that stop inflammation and initiate tissue repair.[15] Current research is focused on using parasites to treat autoimmune disease, due to their ability to suppress the Th2 response, stop inflammation, and promote tissue repair.

Regulatory T Cells

Regulatory T cells (Tregs) control the activation of the Th2 response which can damage the host through inflammation and fibrosis[16]. By increasing Tregs the Th2 response is dampened and stops inflammation and fibrosis.[16] Helminth parasites will activate Tregs as a mechanism to prevent the host immune system from attacking it. In turn, this leads to a decreased ability of the body to attack self-antigens and is beneficial to those with autoimmune diseases.

Tregs play an important role in the dampening of the Th2 immune response. Their activation leads to the suppression of autoimmune cells which is an important part of preventing autoimmune disease. Research has been conducted which demonstrates that an absence of Tregs has severe consequences in the intestinal tract**,** pointing to the idea that they respond to commensal bacteria and food antigens.[16] An example of this consequence is ulcerative colitis which is the chronic form of inflammatory bowel disease and Crohn's disease.[16] Two suppressive cytokines that are released from regulatory T activation are TGF-β and IL-10.

TGF-β is a cytokine that induces cell proliferation, differentiation, migration, and survival. TGF-β is important for Treg development. The binding of TCR and TGF-β induces expression of FOXp3 in nonregulatory CD4+ cells and turns them into Tregs.[16] This means that CD4+ (helper T cells) will develop into Tregs by expression of FOXp3, a protein that is involved in the development and function of T regulatory cells.[17] In the presence of inflammatory cytokines, the conversion to regulatory cells can develop along the Th17 or Th9 pathways.

Another promising discovery is that TGF-β is involved in the tissue repair pathway. This cytokine has a wide variety of effects that requires a careful balance between cell proliferation, differentiation into Tregs and wound healing.

IL-10 is involved in decreasing the inflammation caused by the Th2 response by suppressing dendritic cells and granulocytes.[16] IL-10 also suppresses the Th1 and Th17 pathways, allowing for the Th2 immune response to occur. IL-10 promotes Th2 responsiveness by repressing the Th1 pathway and reducing IFN_{γ} levels.

Infections with helminths and ectoparasites induce a strong Th2 immune response. Studies have shown that mice infected with the intestinal nematodes *Heligmosomoides polygyrus* and *Strongyloides ratti* have increased $FOXp3^{+}$ Treg cells.[16] An increase in Tregs is beneficial to the parasite because it prevents the immune system from attacking and expelling it. Mice that are depleted of Tregs through anti-CD25 antibodies have shown reduced worm burden.[16] Current research is being conducted to harness the ability to activate Tregs for their use in preventing negative autoimmune responses. In other words, people that are suffering from parasite infections do not have autoimmune responses and can manage their symptoms better. Therefore, to limit the inflammation from an autoimmune response which can result in damage to the body, researchers have found that increasing Tregs is beneficial.

Vaccines and Therapeutics

As stated above, Tregs have an important role in controlling the Th2 immune response. During helminth infections, it is beneficial to dampen the immune response because it would ultimately lead to expulsion and death of the helminth. In research, mice with increased T regs

had a higher worm burden then those that were depleted of Tregs.[16] Current research focuses on generating antigen-specific regulatory T cells for those with autoimmune disease or allergies.[16] The goal is to reduce inflammation in those with overactive immune systems or prevent the allergic pathways. The approach to increasing Tregs is dependent on the disease. For example, exogenous Vitamin D proves beneficial in steroid-resistant asthma.[16] Other mechanisms on the basis of antigen-specificity could involve low dose exposure to induce Treg activity or conversion from effector cells to Tregs.[16]

The first anti-helminthic vaccine against cattle was created in the 1950s.[16] Three types of vaccines were used: attenuated, mixture of helminth somatic antigens, and purified recombinant helminth antigens.[16] Attenuated vaccines are injections of dead parasites which can induce immunity and a Th2 response.[18] Recombinant proteins are a mixture of antigens that will build antigen-specific immunity against helminths.[16] Purified recombinant vaccines or DNA based vaccines are now a possible option due to the availability of genomic sequences.[18] DNA vaccines would give a gene for a specific vaccine antigen and are controlled by promoters.[16] The goal of vaccines isn't to completely avoid helminth infections but rather lower the worm burden below transmittable status. It is important to create anti-helminth vaccines for livestock because of economic losses due to infections with tapeworm, ascaris blood-feeding nematodes, and trematodes.[14] We can also learn from animals to create human targeted anti-helminthic vaccines.

Conclusion

In conclusion, there are many types of parasites with different life cycles and geographic locations. In particular, soil-transmitted helminths are common in developing countries with

warm and moist climates. Due to poverty, these individuals are stuck in a negative cycle of infection due to factors that are out of their control. With proper infrastructure and medications, these individuals can have a better life. It has been noted that those with soil-transmitted infection in developing countries do not have as high of a rate of autoimmune disease. One theory is that those infected with soil-transmitted helminths have suppressed Treg activity and decreased inflammation. More research is needed because the pathways are complex, but possible treatment methods include antigen-specific Treg stimulation. On-going research focuses on creating anti-helminthic vaccines to reduce worm burdens below transmittable levels. It is more feasible to provide vaccines as opposed to changing an entire infrastructure in developing countries.

Biographical Note

Upon completing my first clinical rotation in nursing school, I discovered my passion for laboratory science. As a former nursing student, I learned the important role that medical laboratory scientists have in helping a patient receive the proper diagnosis and treatment. My nursing background has given me an appreciation for the underlying science behind medicine, which in turn led me to pursue my education in Medical Laboratory Science at Oakland University. After completing my education and internship, I would like to work full time as a generalist in a hospital laboratory. I think that it will be a valuable experience and strengthen my skills by gaining exposure to multiple areas of the lab. Once I have gained experience, I would be interested in working for the microbiology department and eventually becoming certified in that discipline. My thesis relates to my major because parasitology is a field of study in medical laboratory science.

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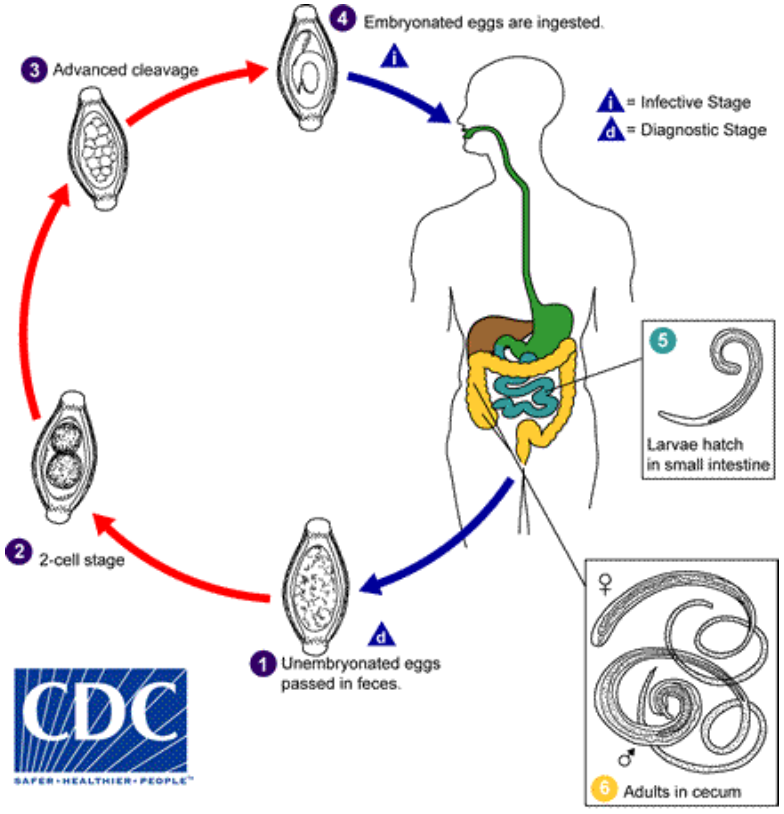
Appendix B - Gause, W. C., & Artis, D. (2016). *The th2 type immune response in health and disease: From host defense and allergy to metabolic homeostasis and beyond*. Springer. Page 8.

Appendix A


4 Embryonated eggs are ingested.
i
i = Infective Stage
d = Diagnostic Stage
3 Advanced cleavage
5
Larvae hatch
in small intestine
2 2-cell stage
d
1 Unembryonated eggs
passed in feces.
♀
♂
6 Adults in cecum
CDC
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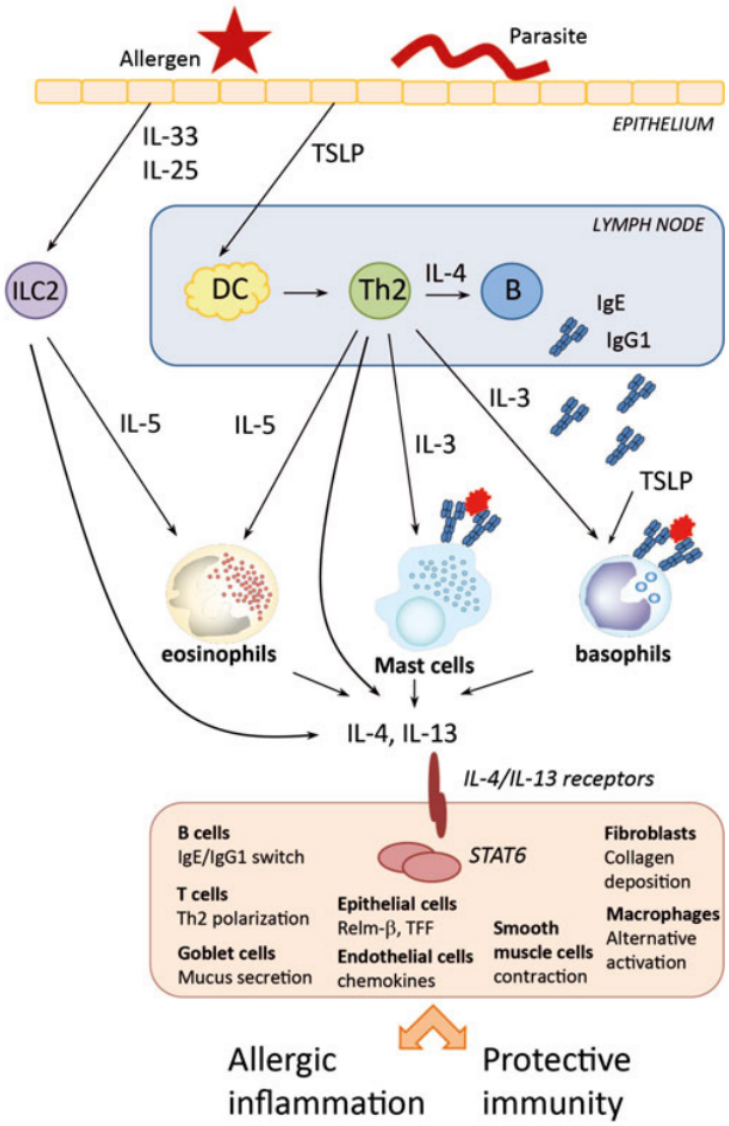
Appendix B


Allergen
Parasite
EPITHELIUM
IL-33
IL-25
TSLP
LYMPH NODE
ILC2
DC
Th2
IL-4
B
IgE
IgG1
IL-5
IL-5
IL-3
IL-3
TSLP
eosinophils
Mast cells
basophils
IL-4, IL-13
IL-4/IL-13 receptors
B cells
IgE/IgG1 switch
T cells
Th2 polarization
Goblet cells
Mucus secretion
STAT6
Epithelial cells
Relm-β, TFF
Endothelial cells
chemokines
Smooth muscle cells
contraction
Fibroblasts
Collagen deposition
Macrophages
Alternative activation
Allergic inflammation
Protective immunity