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Brooke Wynalda
Seattle University

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Parasite Hospitality: How Parasitic Helminth Worms Help Researchers Prevent Type 1 Diabetes

Brooke Wynalda, Biology

Faculty Mentor: Carolyn Stenbak, PhD, Biology

Faculty Content Editor: Brett Kaiser, PhD, Biology

Student Editor: Lee Sasaki

Abstract

The hygiene hypothesis describes how human exposure to parasitic helminth worms (and other microorganisms) impacts immune system functions. The potential impacts include protection against autoimmune and allergic disease. Epidemiological disease mapping reveals cases of allergic and autoimmune disease are disproportionately concentrated in urban, developed countries where there is less risk of parasitic infection. This suggests an inverse correlation between autoimmune diseases and likelihood of helminth infection. These observations have led researchers to posit that helminth worms have coevolved alongside their human host. This hypothesis is supported by helminth antagonization of human anti-inflammatory responses. There is some evidence to suggest that helminth worms manipulate an anti-inflammatory cytokine, interleukin-10. This manipulation of IL-10, which is regulated by T helper cells, helps to maintain low levels of inflammation in the gut, and, as a byproduct, reduces risk of autoimmune disease. For this reason, similar modulation of the IL-10 pathway via parasitic induction or drug development could be useful for treating and even preventing certain diseases. This paper will discuss the hygiene hypothesis and its relevance to allergic and autoimmune disease research, emphasizing how helminth worms are currently being used to prevent and treat type 1 diabetes in diabetic mice models. Furthermore, this review provides a table to organize the following: various helminth worms; the worms' host environments; the autoimmune diseases in which the helminth worm has been found to be immunoregulating; and the specific proteins and immunomodulatory molecules secreted by the species of worm.

Introduction

Throughout its 30-year history, the hygiene hypothesis has proven to be a flexible and adaptable model for understanding human diseases in the context of chronic exposure to environmental pathogens. First introduced in the late 1980s by Dr. David Strachan and his work on hay fever in farm families, the hygiene hypothesis is now, in part, understood as the decreased susceptibility to disease due to early childhood exposure to less sanitary conditions. Currently, epidemiological evidence and the growing basis of knowledge on environmental effects on human genotypes continues to help researchers hypothesize the broader relationship between microorganisms and human disease. Research on T helper 2 cell behavior (even in the context of recent discoveries of more complex immune pathways) proceeds to shape how we interpret and use the hygiene hypothesis to understand and treat disease in humans. Both the hygiene hypothesis and the research that it informs are in constant revision and reinterpretation alongside challenging scientific developments. This review seeks to describe what the hygiene hypothesis is and its current utility in certain disease models.

This review will cover the following major themes in the listed order:

1. The hygiene hypothesis explains the potential protection parasitic helminth worms and other microorganisms provide against autoimmune and allergic disease in hosts, via host immune system regulation.
2. Parasitic helminth worms have a complex life cycle that is adapted to parasitic-symbiotic relationships with pre-industrial and rural human populations.
3. Human-helminth interactions persisted for thousands of years before the advent of strict sanitation, and consequently, there is a complex biological relationship involving immune system actors and helminths.
4. Some helminth worms manipulate a cytokine proliferation pathway via a molecule called Interleukin-10 in order to antagonize an anti-inflammatory state in the host gut.
5. Since both autoimmune and allergic diseases are caused by overactive pro-inflammatory immune cells, researchers have been able to utilize parasitic worms and IL-10 manipulating strategies to design treatments for, and in some cases prevent, autoimmune disease. The case of type 1 diabetes in mouse models is examined in detail.

Major Themes

The Hygiene Hypothesis

The hygiene hypothesis is useful for understanding global patterns of autoimmune and allergic disease in humans. The hygiene hypothesis can be summarized as the protection human hosts receive against autoimmune disease from moderate exposure to microorganisms (including parasitic helminth worms and gut flora) (Gale 2002; Yazdanbakhsh et al. 2002). "Hygiene" can be somewhat of a misnomer as the hygiene hypothesis does not refer to personal hygiene but, instead, the cultural shift towards higher levels of sanitation within communal settings (i.e. the shift towards plumbing and sewage treatment of human excrement and away from digging latrines or outhouses) (Gale 2002). This is because parasitic worms often live and mature in worm-infested fecal matter in soil (Jourdan 2018).

A parasite is an organism that lives on or in another organism (a "host") from which it obtains its nourishment at the expense of that organism. Humans are host to three different parasites that cause disease: protozoa, ectoparasites, and the key player in this review, helminths (Lindquist and Cross 2018). A "helminth" simply refers to a worm that can be parasitic and is often transmitted through worm-infected soil, ultimately infecting the host's gastrointestinal tract (Lindquist and Cross 2018).

Researchers hypothesize that the relatively low standards of sanitation for almost the entirety of mammalian evolutionary history led to ongoing cohabitation between humans and microorganisms (Helmby 2015). This constant parasite-host interaction has instigated the human gut to, in some ways, become codependent on the immune regulatory behavior of these microorganisms (Yazdanbakhsh et al. 2002; Jackson 2009; Helmby 2015). In some cases, these microorganisms act to balance inflammatory responses, and, in their absence, inflammation and destruction of healthy gut tissues take place (otherwise known as allergic or autoimmune disease) (Jackson 2009; Helmby 2015). These immune pathways will be covered in detail later in this review.

Much of the evidence in favor of the hygiene hypothesis comes from global human disease mapping of autoimmune and allergic diseases. Cases of allergic and autoimmune diseases are rising across the globe, but distribution of these cases shows they are concentrated in metropolitan, urban areas (Lerner 2015). For example, cases of allergic diseases are lower in developing countries with fewer urban hubs, whereas diseases such as rhinoconjunctivitis (also known as seasonal allergies), asthma, and eczema are significantly higher in countries with greater (in both size and quantity) industrialized regions (Yemaneberhan et al. 1997). There are even studies, including one study surveying populations of rural and urban Ethiopia and another surveying farmers in two Bavarian cities, that show fewer cases of allergic and autoimmune disease in rural areas than in urbanized areas of the same country (Yemaneberhan et al. 1997; von Ehrenstein et al. 2000).

Similar urban and rural trends are shown in the cases of type 1 diabetes. In a longitudinal study tracking incidence of type 1 diabetes from 1982 to 2010 in Canada, Serbia, Israel, and all European nations, researchers reported at least a 4.5% increase per year in instance of type 1 diabetes in all countries surveyed (Lerner 2015). Africa and South America are the continents with the highest risk of parasitic helminth infection (Yazdanbakhsh et al. 2002; Helmby 2015; WHO 2017; Jourdan 2018). North America and Europe have a significantly lower risk of helminth infection (Yazdanbakhsh et al. 2002; WHO, 2017). In a meta-analysis of studies conducted from 1980 to 2018, researchers found that within populations of Africa, the incidence of type 1 diabetes was 8 per 100,000 population ($P < 0.001$); whereas, among individuals of North America, the incidence of type 1 diabetes was 20 per 100,000 population ($P < 0.001$) (Mobasseri et al. 2020). Overall, researchers found that the global incidence of type 1 diabetes was 15 per 100,000 population ($P<0.001$) (Mobasseri et al. 2020). Evolutionary biologists and disease ecologists believe the sudden, extreme increase in autoimmune diseases among human populations has occurred too quickly to be a result of genetic divergence and is likely the result of environmental factors (Yazdanbakhsh et al. 2002; Helmby 2015).

Additional evidence in favor of the hygiene hypothesis comes from understanding T helper cell behaviors. T cells are lymphocytes that mature in the thymus (hence "T") and express a T-cell receptor: either CD3 and CD4, or CD8. T helper cells are a subset of T cells that are stimulated by antigens to provide specific signals and promote accurate immune responses. This includes, but is not limited to, initiating proliferation of cytokines. Cytokines, and particularly a cytokine called Interleukin-10, will be covered in greater detail later in the review.

There are two types of T helper cells, T helper 1 (TH1) cells and T helper 2 (TH2) cells. Under normal conditions, TH1 cells restrain the production of TH2 cells. If there are too few or too many TH1 cells, immune homeostasis is disrupted, resulting in allergic or autoimmune disease. TH2 cells are important for responding to pathogens that do not directly infect cells (such as helminth parasites) (Maizels and McSorely 2016). Low levels of TH1 cells, which result from fewer childhood infections, shifts the immune system into a proallergic state (Yazdanbakhsh et al. 2001; de Jong 2002). Lower childhood infection rates produce lower levels of TH1. Because there are fewer TH1, TH2 cells are not regulated properly within the immune system. These higher-than-normal levels of TH2 cells exhibit unrestricted behavior toward benign allergens, producing the side effects of allergic disease (Braat et al. 2010). However, research also shows that TH1 cell-related autoimmune diseases are increasing in prevalence. This trend does not support the hypothesis that a decrease in childhood infections is the only reason cases of allergic disease are rising (Yazdanbakhsh et al. 2002). Therefore, a plausible explanation for increases in both autoimmune and allergic disease incidences must consider changes in both TH2 and TH1 cells equally.

Understanding, treating, and curing these diseases is of increasing urgency in the United States. The National Institutes of Health (NIH) estimates that 23.5 million people in the United States have an autoimmune disease (Julian 2014). According to a report by the National Institutes of Allergy and Infectious Disease, the cost of treating autoimmune disease in the United States exceeds $100 billion annually—a figure which is most likely downplayed (Julian 2014). Analysis of parasite-human interactions explain both the distribution and rise in allergic and autoimmune diseases globally, while simultaneously pointing to research studies of helminth worms to potentially treat these diseases (Burbank 2018).

What the Helminth?

"Helminth" is often used to refer to a parasitic worm that infects a human host (Lindquist and Cross 2018). There are three groups of helminths that are parasitic to humans: flatworms (platyhelminths), thorny-head worms (acanthocephalans) and roundworms (nematodes) (Lindquist and Cross 2018). Helminth parasites infect about 1.5 billion people worldwide (WHO 2022).

Thousands of years of parasite-human host relationships have allowed parasites to coevolve alongside the human gut microbiome. The human gut microbiome refers to the diversity of bacteria (and other small microorganisms), which symbiotically inhabit the gastrointestinal tract of humans (Kho 2018). The earliest evidence of parasite-human interaction was helminth lung fluke eggs found in fossilized feces dating back to 5900 BC (Cox 2002). Hookworm eggs and roundworm eggs have also been found in South America as early as 5000 BC (Cox 2002). Helminth worms have three life stages (eggs, larvae, and adults), but only late-stage larvae and adults can live inside the host, which is why helminth eggs have been the only evidence of helminth infections historically (Lindquist and Cross 2018). While infecting the human gut, parasitic helminths feed on the blood of the host—often times causing anemia, malnutrition, and in childhood cases, stunted growth (McKenna 2017).

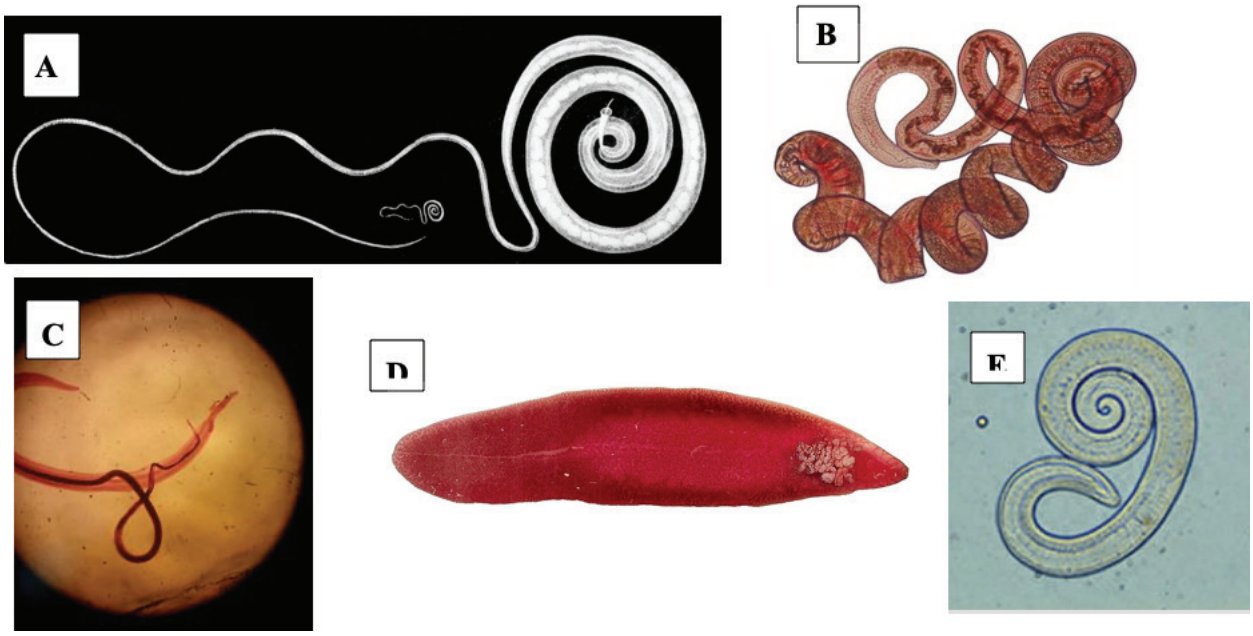

Figure 1 Depicted are images of important helminth worms involved in regulating, preventing, and potentially curing type 1 diabetes. A. Male *Trichocephalus trichiuris*, or whipworm, is depicted. B. *Heligmosomoides polygyrus* roundworm is commonly found in the intestinal tract of rodents. C. *Schistosoma mansoni* are water-borne parasites also known as blood flukes. The image shows a male and female pair. D. *Fasciola hepatica*, more commonly known as the common liver fluke, is depicted. E. *Trichinella spiralis* is an intestinal tapeworm most commonly found in pigs and rodents but sometimes found in humans.

Helminths cannot reproduce and mature their eggs inside the host; the host must expel the eggs and allow them to mature outside the host in the environment (Lindquist and Cross 2018). Most helminth worms are hermaphroditic (roundworms and blood flukes being the exception), and all helminths produce eggs for reproduction (Lindquist and Cross 2018). Adult female helminth worms can burrow into the gut wall and lay up to 50 eggs daily (Jourdan 2018). These eggs make their way out of the host via feces and need to be transferred to a wet, warm soil bed to mature into larvae (Jourdan 2018; Lindquist and Cross 2018). Eggs can reach soil when fecal wastewater, sewage, or human feces are repurposed as fertilizer—often referred to as "night soil" (Carlton 2014). Thus, the risk of ingesting or becoming infected with parasites is high in less industrialized agricultural regions and low in areas with developed sanitation surrounding agricultural practices. Once a worm is outside of the host, it can mature to the larval stage 3 in the soil and eventually reinfect a human host through host contact with parasite infested soil (Jourdan 2018). Contact includes ingestion of worm-infected soil or skin-to-soil contact (usually on the hosts' foot) that allows the worm to burrow into the host (Gale 2002). About 24% of the world's population is infected with soil transmitted worms, and despite their benefits to autoimmune regulation, they remain among the leading causes of death in developing countries (WHO 2022).

As sanitation has increased alongside urbanization and globalization, helminth worms have decreased in prevalence in industrialized areas. It is hypothesized that the increase in global sanitation (e.g. fewer latrines and exposure to soil transmitted helminths) rapidly and drastically changed the environmental conditions from which the human gut biome evolved (Jackson 2009).

This is Your Gut on Parasites

Once a parasitic helminth worm burrows into a host, a successful infection requires the parasite to survive through reproduction. Although the physiological and mechanical means of parasitic infection and reproduction in the host gut are an important element of the parasitic life cycle, the key to understanding parasitic infection, in light of the hygiene hypothesis, lies in how helminth worms inside the gut wall avoid eradication by the host immune system until reproduction is possible.

As may be expected, human hosts have evolved systems to fight off parasites that are part of the baseline human immune response. When a helminth parasite infects a human host, the host will send a molecule called immunoglobulin E to try to expel it (Kindt et al. 2007). Immunoglobulin E is a type of antibody (i.e. a protein that responds to foreign substances called antigens) mediated by cells called T-helper cells (Kindt et al. 2007). The tail end of an immunoglobulin E molecule will bind to receptors on eosinophils and mast cells (Kindt et al. 2007). Eosinophils are a type of disease fighting white blood cell that have molecular mediators, which can potentially kill helminth parasites as well as host cells (Kindt et al. 2007). Some of these molecular mediators are called cytokines, which are a broad group of cell-signaling proteins that, under normal conditions, help fight off the infections (Kindt et al. 2007). Mast cells release histamine and other substances (like cytokines) during inflammatory and allergic reactions to both harm the helminths and induce intestinal contractions to expel the parasites from the host's gut (Kindt et al. 2007). The combined responses of eosinophils and mast cells help a healthy host expel helminths from the body.

In order for a parasitic helminth to survive within the host, it must avoid detection from the host's immune attack—in this case, TH1 and TH2 cells that mediate immunoglobulin. There are many ways helminths can passively avoid detection from the host immune system, but one major group of immune evasion mechanisms involves active interference with the host's immune responses (Hewitson et al. 2009; Everett et al. 2017; Zakeri et al. 2018). Parasites commonly interfere with the regulatory network that orchestrates the various arms of the immune defense—including inducing cytokine defense pathways that eventually produce anti-inflammatory responses (Zakeri et al. 2018). For example, all parasites secrete proteins through the outermost, membranous part of their body (Hewitson et al. 2009). Some research points to these proteins being key to manipulating T cells, B cells, macrophages, and T

memory cells into restricting pathogen degradation and inflammatory response, as well as taking advantage of anti-inflammatory cell pathways to artificially bring the immune system back into balance (Cooke 2009; Hewitson et al. 2009; Zakeri et al. 2018). Most important for understanding the role of parasites' relationship to autoimmune diseases and to type 1 diabetes, is the parasitic manipulation of T cells (Cooke 2009). Parasitic worms can deploy their counterattack by modulating the proliferation of T helper cells, which triggers the release of a molecule called Interleukin-10 (IL-10). For this reason, the IL-10 pathway is a source of immunomodulatory utility in treating human diseases, such as rheumatoid arthritis, irritable bowel disease, and type 1 diabetes (Zaccone and Cooke 2013; Maizels and McSorely 2016).

Interleukins are found in patients with allergic and autoimmune diseases *and* patients who are infected with parasites. Interleukins are cytokines secreted by white blood cells (Kindt et al. 2007). Cytokines are a class of proteins that regulate the intensity and duration of the immune response by exerting a variety of effects on lymphocytes and other immune cells (Kindt et al. 2007). Cytokines can either be pro-inflammatory (produce inflammation) or anti-inflammatory (reduce inflammation) (Kindt et al. 2007). Additionally, cytokines are part of the innate immunity—innate immunity refers to the non-specific defense mechanisms implicit within a host as the basic form of immunity in the evolutionary makeup of all vertebrates and invertebrates (Kindt et al. 2007). Unlike adaptive immunity, humans have innate immunity without prior exposure to specific pathogens (Kindt et al. 2007).

Interleukins are secreted by leukocytes that primarily affect the growth and differentiation of various hematopoietic and immune system cells (Kindt et al. 2007). Interleukin-10 (IL-10) is the anti-inflammatory cytokine that is secreted by TH2 to inhibit the synthesis of TH1 products, such as pro-inflammatory cytokines. (Kindt et al. 2007). This counteracts the hyperactive immune response of the human body and rebalances the system (under normal conditions) to a non-allergic, non-autoimmune state (Kindt et al. 2007). Specifically, IL-10 stimulates or enhances the proliferation of B cells, thymocytes, and mast cells. IL-10 has two primary activities: Immunoglobulin A synthesis resulting in secretion of human B cells and TH1 cell generation antagonism (Kindt et al; 2007).

Interleukin-10 and Helminth Worms

Interleukin-10 has strong anti-inflammatory properties. The primary characteristics of IL-10 are: (1) IL-10 is an anti-inflammatory cytokine. (2) IL-10 is secreted by T helper 2 cells as a way offset the pro-inflammatory responses of T helper 1 cells. (3) Helminth worms can also modulate IL-10 because some helminths can modulate T helper 2 cells.

IL-10 inhibits the host immune response to pathogens (like helminth worms), and thus prevents and mitigates damage to the host tissues. When IL-10 executes its counterattack of pro-inflammatory molecules within the hosts immune system, immune responders can

continue to respond to non-threatening pathogens (e.g. any foreign foods, pollen, pet dander) without causing unnecessary carnage to the host's gut. When insufficient IL-10 is present to balance the immune response, immune molecules can wreak havoc on the host. Since some helminth worms can instigate the proliferation of IL-10, they effectively evade the immune responders which seek to eradicate it from the host's gut. It is only secondarily that helminths prevent the immune system from exerting an overactive response to non-threatening allergens, thereby stopping pro-inflammatory disease from occurring.

Researchers believe that helminths primarily regulate the downstream effects of T helper cells via the secretions from their outermost membranes of their body. For example, parasitic worms such as *Nippostrongylus brasiliensis* (an intestinal roundworm) and *Litomosoides sigmodontis* (a nematode in rat hosts) produce cystatin proteins through their tegmentum or cuticle (outermost layer of "skin"). Cystatin (and other proteins) are recognized by toll-like receptors (TLRs), a family of cell-surface receptors in the protein that recognizes molecules from various pathogens. TLRs are an innate part of immunoregulation; however, they can have downstream impacts related to adaptive immunity. These downstream impacts include B cell and T cell production, and, in the case of the cystatins from helminth worms, influence cytokine production. Cystatins are recognized by TLRs and lead to the eventual promotion IL-10 in both mice and humans (Hartmann et al. 1997; Pfaff et al. 2002; Hartmann et al. 2003). While the cystatin protein superfamily is currently being studied in relationship to many human diseases (mostly related to kidney dysfunction), the exact pathway by which the cystatin protein—found in helminths like *Nippostrongylus brasiliensis* and *Litomosoides sigmodontis*—induces this immune response is still unknown. Yet, understanding how these worms manipulate an IL-10 pathway may be of great utility to immunologists studying autoimmune disease pathways.

In some cases, helminth eggs have also been effective in modulating immune responses. For example, *Schistosoma mansoni* eggs seem to mediate transducers and activators of transcription 3, a JAK/STAT3 pathway (Yang et al. 2017). JAK/STAT, or Janus kinase (JAK) signal transducer and activator of transcription (STAT), is another critical conductor in cytokine proliferation and modulation of T helper cells (Seif 2017). The JAK/STAT pathways utilize suppressors of cytokine signaling and protein inhibitors to determine the start, length, and finish of the signaling pathways that lead to IL-10 production (Seif 2017). For this reason, when the JAK/STAT pathway is disrupted or left unregulated, the downstream effects on T helper cell count can cause autoimmune disease (Seif 2017). It is thought that some of the proteins on the membranous parts of the *Schistosoma mansoni* eggs can trigger the JAK/STAT pathway, providing balance in T helper cell interactions and reversing symptoms of autoimmune disease (Yang et al. 2017).

Researchers Manipulate IL-10 to Treat Type 1 Diabetes in Mice Models

In order to understand how IL-10 has become useful in research for type 1 diabetes prevention, a brief analysis of type 1 diabetes is necessary. Type 1 diabetes is one of the few remaining autoimmune diseases without any approved immunological treatment—that is to say, direct insulin induction and self-regulation are the only treatments for patients experiencing type 1 diabetes. This could be, in part, because type 1 diabetes is frequently categorized as a "childhood" disease, as it is commonly diagnosed in children and teens; although, type 1 diabetes can develop at any age and cause extreme financial burden well into adulthood through the duration of the disease (Sussman et al. 2020). While all autoimmune diseases have steep costs for care, folks seeking care for type 1 diabetes and, more explicitly, in need of insulin to correct hyperglycemic episodes, spend upwards of $2,500 per year in out-of-pocket costs for necessary life sustaining treatment (Sussman et al. 2020).

Type 1 diabetes is categorized by the loss of insulin-producing β-cells in pancreatic islets in genetically susceptible subjects. The key function of β-cells is to produce insulin, a hormone that regulates the amount of glucose (a type of sugar) in the blood. T cells (both CD4 and CD8) have been implicated as active players in β-cell destruction, and thus results in the onset of the pro-inflammatory consequences (Knip and Silijander 2008). The resulting state is called hyperglycemia, or colloquially, high blood sugar. In humans, the onset of type 1 diabetes manifests when 70% of β-cell mass is destroyed, creating an environment that is insufficient for maintaining glucose homeostasis because there is not enough insulin being produced (Cooke 2009). This is why insulin injections are required for survival; the subject cannot create insulin on their own without it being targeted for destruction. Additionally, this also serves as an explanation for the substantial and continuous efforts by researchers to prevent, and even reverse, the effects of type 1 diabetes (particularly those containing autoimmune origins).

Since type 1 diabetes results from the destruction and inflammation of islet cells in the pancreas, regulating anti-inflammatory cytokines, like IL-10, could mitigate islet cell damage. Researchers found that using both *Litomosoides signmodontis* antigen and a biosynthetic precursor to insulin called proinsuli prevented type 1 diabetes in nonobese diabetic (NOD) mice after insulitis started (Ajendra et al. 2016). Insulitis is a biomarker for type 1 diabetes; it is categorized as a disease of the pancreas that is characterized by the infiltration of lymphocytes. This is the first demonstration of a helminth-based therapy successfully treating type 1 diabetes, even after islet cells became inflamed (an indicator that the onset of type 1 diabetes has occurred) (Ajendra et al. 2016). A different study showed that infection by the helminth *Schistosoma mansoni* can prevent diabetes onset in NOD mice (Zaccone et al. 2003). This study led to a survey of other parasites' potency in preventing and treating type 1 diabetes in NOD mice—namely *Trichinella spiralis, Heligmosomoides polygyrus, Litomosoides sigmodontis, and Dirofilaria immitis* (a dog heartworm rarely found in humans) (Zaccone et al. 2003). Overall,

it was concluded that *Schistosoma mansoni* continues to show the most promise in preventing inflammation of islet cells in NOD mice.

Through a series of in vitro experiments with *Schistosoma mansoni* antigens, researchers determined that T cells were the primary responders to *Schistosoma mansoni* (Zaccone et al. 2003). Ultimately, the induction of *Schistosoma mansoni* induced an anti-inflammatory response to the islet cells, resulting in a TH2-type response (Zaccone et al. 2003). This pathway also involved immunoregulation of IL-10 secretion by the *Schistosoma mansoni* parasite, leading the researchers to conclude that parasitic helminth regulation of TH2 cells and downstream regulation of IL-10 are responsible for preventing islet inflammation and, thus, type 1 diabetes in NOD mice. (Zaccone et al. 2003). Their research points to IL-10 being the starting point for further investigation as it may indicate the presence of regulatory T cells (Zaccone et al. 2003).

Additional studies have isolated the molecules that make up the mucosal layer of the parasite's body (i.e. the excretions and secretions through the cuticle or tegument of the helminth) and administered these molecules to NOD mice as treatment for type 1 diabetes. Namely, the secretions from helminth parasite *Fasciola hepatis* have been utilized as successful prevention techniques of type 1 diabetes in NOD mice when administered at the time of high T helper cell activity. Administering these secretions is thought to inhibit the initiation of T helper cell imbalance; preventing the perpetuation of T helper cell behaviors results in autoimmune disease (Lund et al. 2014). This evidence supports the notion that modulation of IL-10 (as a downstream consequence of production of T helper 2 cells) is the principal mechanism by which helminth parasites regulate host immunity (Lund et al. 2014).

While IL-10 may be the most promising of the anti-inflammatory interleukins being studied to prevent type 1 diabetes, researchers have also found evidence of other pro-inflammatory interleukins being useful in directly reversing the impacts of islet cell inflammation. For example, the gastrointestinal nematode *Heligmosomoides polygyrus* has been studied in treating streptozotocin-induced diabetes in mice. Unlike NOD mice that experience spontaneous development of insulitis, streptozotocin-induced diabetic mice are injected with the molecule streptozotocin (STZ), a compound that initiates the destruction of β-cells in the pancreas and thus mimics type 1 diabetes hyperglycemia in mice. When STZ-induced diabetic mice were given a series of low dosage injections of *Heligmosomoides polygyrus* derived compounds, like interleukin-23 (IL-23), diabetic mice experienced significant reduction of hyperglycemia (Osada et al. 2013). Other researchers have postulated that helminth worms like *Trichocephalus trichiuris* may increase induction of pro-inflammatory cytokines like IL-22, which may enhance the mucosal barrier in the host intestinal epithelium (Leung et al. 2013). This may allow for the proliferation of microorganisms in the gut and return the gut to net gastrointestinal homeostasis. While IL-22 from *Trichocephalus trichiuris* has been found to protect against autoimmune diseases like irritable bowel disease (IBD), it has not been proven

to be effective in treating type 1 diabetes. However, conditions that cause IBD are closely tied to an imbalance of intestinal gut microbiota (sometimes called dysbiosis), which is strongly associated with type 1 diabetes (Leung et al. 2013).

While there is promising research in mice models for the prevention of type 1 diabetes via helminth antigen treatment, there has been little success in translating these results to human clinical trials. First, there are limitations to using NOD and STZ-induced mice model organisms to study disease. Models using STZ-induced mice are problematic because the synthetic induction of type 1 diabetes means that mice do not share the same genetic biomarkers as human subjects suffering from type 1 diabetes (Al-Awar 2016). Some of those genetic differences could result in successful drug treatment in STZ-induced mice models and failed treatment in humans with type 1 diabetes (Al-Awar 2016). Although NOD mice share important genetic similarities to humans with type 1 diabetes, the time point of drug intervention during the progress of type 1 diabetes and the dosage of antigen in mice have proven to be difficult to translate to human subjects (Al-Awar 2016). Treatments for type 1 diabetes used in mice have not been used in human subjects. In large part, this is because of challenges in applying mice models to treatments of autoimmune diseases with helminth antigen induction in human systems (Helmby 2015). As of 2015, six clinical trials using *Trichuris suis* eggs, an intestinal nematode, to treat two irritable bowel diseases (Crohn's disease and ulcerative colitis) were ongoing or completed; however, at least three of these studies have not met phase two requirements for efficiency in human subjects and have been discontinued (Helmby 2015). One hypothesis for these results is that some prior exposure to helminth worms (before immune shift to a pro-inflammatory state and onset of autoimmune disease) is necessary in order for the reintroduction of helminth worms to be effective (Helmby 2015).

Conclusion

A Parasitologist and a Rheumatologist Walk into a Bar…

Research using parasitic helminths as a model for understanding how interleukin-10 manipulates the immune system is optimistic at best. Current studies are building mechanisms to induce IL-10 immunomodulation in mice with type 1 diabetes and other autoimmune diseases with some success. However, variability in success rates seems somewhat related to the lack of conclusive molecular research being conducted on how individual parasites co-opt host immune responses. While it is known that inducing parasites as a therapeutic treatment for autoimmune disease can lead to minimization of symptoms and, in some cases, prevention of disease, the mechanism by which parasites regulate IL-10 and, thus produce a favorable patient outcome, is unknown and hardly even hypothesized about in the current literature.

Table 1 This table synthesizes a subset of helminth worms' key worm-protein secretions with their impact on type 1 diabetes modulation and their relationship to IL-10 pathways. This summary captures several different helminths and their utility in modulating (via restriction, symptom suppression or prevention) type 1 diabetes. This is a non-exhaustive list. Please see reference Cooke 2008 for information not directly related to IL-10 pathways.

Parasite	Protein Secretions	Type 1 diabetes result	IL-10 pathway	Citation
Litomosoides sigmodontis	Multiple Cystatin Molecules	Antigen and intranasal pro-insulin combination prevented type 1 diabetes in NOD mice after insulitis started while individually administered antigens did not.	CD8+ and CD4+ manipulation via TGF β IL-10 TLR targeting	Ajendra et al. 2016
Fasciola hepatis	FhES	Secretions at a time coincident with T cell priming events inhibits the initiation and perpetuation of autoimmune damage, preventing type 1 diabetes.	IL-10 secreting β-cells were increased (using a TGF β pathway)	Lund et al. 2014
Heligmosomoides polygyrus	Unknown	Multiple low dosage strategies using Hp significantly reduces hyperglycemia for STZ-induced type 1 diabetic mice.	IL-10 independent pathway (IL-22 and IL-13-closely related to IL-10)	Osada et al. 2013 Leung et al. 2013 Zaccone et al. 2003
Schistosoma mansoni	Cystatins, VALs	*S. mansoni* antigens in vitro and were responsible for IL-10 secretion, and thus reduce β cell destruction. *S. mansoni* eggs into NOD mice prevent type 1 diabetes via shift to the TH2 response.	CD8+ andCD4+ manipulation via TGF β and IL-10 TLR targeting	Zaconne and Cooke 2013

However, viruses—a parasite of sorts—are well known to co-opt the hosts molecular pathways. Over the course of coevolution with their hosts, viruses have captured genes from their hosts to produce molecules that disarm host immunity. These genes code for "natural" host molecules that regulate the host's immune response (host cytokines becoming virokines) or host molecules that act as decoy receptors, thus impeding the immune response. Cystatins and JAK/STAT pathway manipulation might provide possible avenues for immunotherapies to explore. Perhaps understanding how other parasites modulate their hosts can serve as a starting point to form future research. Similarly, studies in successful mice models need to be ethically approved for clinical trial in human subjects.

Additionally, it may be particularly useful to focus on the enzymatic and molecular secretions of the different species of helminth worms, the different levels of interleukins these

secretions produce, and the variability in T helper cell imbalance the host experiences. Through the range of studies evaluated in this review, it is evident that the spontaneous development of diabetes in non-obese diabetic mice can be inhibited by several different infectious parasitic agents but not by all infections in the same way. The timing of infection plays an important role in the success of treatment and prevention; this is because infections have been able to inhibit the onset of diabetes only if they occur before there is significant pancreatic infiltration. Still, if proteins secreted by parasites are found to not be useful in identifying and providing treatment for type 1 diabetes, these findings can contribute to treatment plans and drug development for other autoimmune diseases. By identifying the ways in which parasites explicitly manipulate the host response and inhibit negative autoimmune response, it will become possible to develop novel therapeutic approaches that may more exclusively target specific molecules and inhibit the hyperglycemic response. It is my hope that this initial analysis and synthesis of parasitic proteins and disease outcomes is a starting block for future hypotheses.

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