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The hookworm pharmacopoeia for inflammatory diseases

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ABSTRACT

In the developed world, declining prevalence of parasitic infections correlates with increased incidence of allergic and autoimmune disorders. Current treatments for these chronic inflammatory conditions have little to no effect on their prevalence and are referred to as "controllers" rather than cures. There has been limited success in therapeutically targeting allergic and autoimmune pathways, leaving an unmet need for development of effective anti-inflammatories. We discuss the benefit of hookworm infections and the parasite's ability to condition the immune system to prevent allergic asthma and inflammatory bowel diseases. We then examine the immunomodulatory properties of selected hookworm-derived proteins in these two models of inflammation. While hookworm protein therapy has yet to be fully exploited, the identification of these proteins and the mechanisms by which they skew the immune system will provide new avenues for controlling and optimally reversing key pathological processes important in allergic and inflammatory bowel diseases.

1. Ancient cures for modern diseases

Allergies and autoimmune disorders, considered together as chronic inflammatory diseases, are on the rise in the developed world (Prescott and Allen, 2011; Molodecky et al., 2012). One proposed explanation is that as populations become more hygienic with a lower exposure to pathogens (including parasites) during childhood, there is a concurrent increase in immune disorders resulting in increased incidence of chronic inflammatory conditions (Weinstock and Elliott, 2009). For the purposes of this Current Opinion we will focus on inflammatory bowel diseases (IBD) and asthma as examples of autoimmune and allergic diseases, respectively.

While both IBD and asthma are the result of inappropriate immune responses, they could not be more different in terms of the phenotype of the response. Allergic asthma has been widely described as an aberrant T helper 2 (Th2) immune response characterised by airway eosinophilia, the production of IL-4, IL-5, IL-9, IL-10 and IL-13 cytokines, elevated antigen-specific IgE levels, increased mucus production and structural remodelling leading to airway obstruction and hyperreactivity (Holgate, 2012). On the other hand, IBD, defined by two immunologically distinct conditions: Crohn's disease and ulcerative colitis, is a chronic idiopathic inflammation of the gastrointestinal tract (Monitoring, 2011). While Crohn's disease appears to be mainly a Th1-mediated disease, with an overproduction of IL-12 and IFN-γ, ulcerative colitis however seems to be more of a Th2-mediated pathology. It is characterised by the production of IL-4 and IL-5 and is associated with the production of various autoantibodies (Monitoring, 2011). A critical role for Th17 cells and the secretion of IL-17A, IL-17F, IL-21, IL-22 and IL-23 have been reported in IBD (Kanai et al., 2012). Other cytokines such as IL-15, IL-16 and IL-18 have also been observed in both conditions, which emphasise the distinct immunological basis of IBD and asthma (Pages et al., 2001; Nowak and Schror, 2007; Broadhurst et al., 2010). However, both IBD and asthma seem to evolve from an imbalance between effector T cells and regulatory T cells that results in the overwhelming inflammatory cascade (Akdis et al., 2004; Kanai et al., 2012). Finally, both chronic conditions are of multifactorial aetiology, where genetic factors and environmental factors such as the gut microbiota all contribute to the pathogenesis (Maloy and Powrie, 2011; Geremia and Jewell, 2012).

Helminth infections, including hookworm, are universally associated with responses characterised by Th2 cytokines, high levels of IgE, eosinophilia and mastocytosis (Yazdanbakhsh et al., 2001). Interestingly, this same immune signature is seen in allergies and asthma. So why aren't the billions of people infected with parasitic helminths showing classic signs of hypersensitivity and developing potentially fatal allergies to their parasites? Even more striking is the observation of an inverse association between the prevalence of certain microorganisms (including helminths) and allergic and autoimmune inflammatory diseases (Rook, 2012). There are different interpretations on the mechanisms and microorganisms at

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play, and these are well summarised by the "hygiene hypothesis" and the "old friends hypothesis" (Strachan, 1989; Rook and Brunet, 2005).

In terms of asthma, a condition that has reached "epidemic" proportions in the authors' country of residence (Australian Centre for Asthma Monitoring, 2011), immunoepidemiological studies highlight an inverse relationship between helminths and asthma (Lynch et al., 1987; Nyan et al., 2001; Scrivener et al., 2001; Yazdanbakhsh et al., 2001; Huang et al., 2002; Cooper et al., 2003; Hotez et al., 2005; Patel et al., 2008). Moreover, anthelminthic treatment of chronically infected children induces significant atopic reactivity and sensitisation to newly acquired allergens (Lynch et al., 1993; Borkow et al., 2000; van den Biggelaar et al., 2004). Importantly from the perspective of this article, the collection of reports on the beneficial effects of helminth infections on chronic inflammatory diseases, such as asthma, implicate hookworms more than any other species in protection against an inflammatory phenotype (Scrivener et al., 2001; Leonardi-Bee et al., 2006).

2. Is hookworm an "ideal parasite"?

The "ideal parasite" can be considered as possessing the following attributes. It is:

1. long-lived
2. highly fecund
3. easily transmissible
4. of limited pathogenicity/immunogenicity.

Hookworms possess all of these attributes, explaining their ubiquitous tropical distribution (in humans and animals). They are long-lived – hookworms live upwards of 10 years in their human hosts (Palmer, 1955). Female worms lay thousands of eggs per day, which pass into the environment through the host's faeces and hatch to release larvae that molt in the faeces-contaminated soil and become directly infective to the next human host. Much of their long existence can be attributed to the masterful immuno-evasive strategies that hookworms have evolved to ensure their long-term survival and propagation (McSorley and Loukas, 2010).

A successful parasite–host relationship is one that borders on commensalism, where the parasite causes little to no overt damage or loss of fitness to its host, thus ensuring its continued transmission. It is therefore not unreasonable to suggest that the ultimate host-parasite relationship is one that approaches mutualism, where the host actually derives some benefit from the parasite (Pritchard and Brown, 2001). It is important to note that some anthropophilic helminths induce little pathology in small numbers, particularly during carefully controlled experimental infections in healthy volunteers, and these are now being considered in a therapeutic light (Blount et al., 2009; Daveson et al., 2011). There is perhaps no better example of such a therapeutic parasite than the hookworm, *Necator americanus* (Pritchard et al., 2012). We base our opinion on the following salient points:

1. Low dose human experimental infections with *N. americanus* are safe with no documented serious adverse events (Wright and Bickle, 2005; Mortimer et al., 2006; Geiger et al., 2008; Blount et al., 2009; Feary et al., 2009; Daveson et al., 2011).
2. *Necator americanus* migration in humans is predictable, via the lungs (larvae) to the gastrointestinal tract (adults worms).
3. *Necator americanus* does not replicate within the host.
4. Adult *N. americanus* can live for many years in the gut (Palmer, 1955).
5. Immunoepidemiological data supports a protective role for *N. americanus* against asthma (Scrivener et al., 2001).
6. Hookworm excretory/secretory proteins suppress inflammation in animal models of disease (Ruyssers et al., 2009; Cancado et al., 2011).
7. Two recombinant hookworm proteins have already been tested in clinical trials and shown to be safe, highlighting the potential for discovery and testing of new therapeutic molecules.

Key to the longevity of human hookworms is the absence of any form of robust protective immunity in infected populations. There are reports of reduced faecal egg counts associated with elevated levels of IL-5 and IgE responses against defined L3 antigens, but in general it is the elderly who harbour the heaviest infections (Quinnell et al., 2004a; Bethony et al., 2005, 2006). While *N. americanus* infections exhibit some of the hallmark features of protective Th2 immune responses, including IgE and local and systemic eosinophilia, these immune responses clearly fail to protect most infected people. The reason for the observed failure of humans to mount effective anti-hookworm responses is clearly multifactorial, but one mechanism by which hookworms skew the host immune response in the worm's favour is through the production of excretory/secretory (ES) proteins (Moyle et al., 1994; Hsieh et al., 2004; Cuellar et al., 2009). We will delve further into this topic later in the article.

3. Hookworm infection to treat inflammatory gut disorders

Summers and colleagues (2003, 2005) provided the first evidence from clinical trials that helminth therapy was an efficacious in treating IBD. They showed that experimental infection with eggs of the zoonotic pig whipworm, *Trichuris suis*, reduced the activity of both Crohn's disease and ulcerative colitis (Summers et al., 2003, 2005). While the successful amelioration of disease in these trials is unquestionable, we envisage some drawbacks with the use of *T. suis* egg therapy: (1) infection is short-lived because hatched larvae of this zoonotic parasite do not mature in the human host, thus requiring constant dosing; (2) the parasite is zoonotic so wayward migration to various organs in the human host cannot be discounted; (3) the financial cost of such frequent dosing is high; (3) difficulty in standardising (and obtaining federal approval in different countries) Current Good Manufacturing Practices for production of eggs. The hookworm, *N. americanus*, however, is a specialist gastrointestinal pathogen that infects over 500 million people. Contrary to most teaching, infection with *N. americanus* is generally benign once adult worms are established in the gut unless infection intensity is heavy or if iron status is compromised (Hotez et al., 2004; Blount et al., 2009; Daveson et al., 2011). In hookworm-endemic populations the parasite induces a mixed peripheral blood T helper cell response but Th2 and regulatory cytokines such as IL-10 and TGF-β are dominant (Quinnell et al., 2004a; Geiger et al., 2007). The mucosal response to hookworms in the gut of previously hookworm-naïve, experimentally infected individuals living in a non-endemic area was recently characterised, revealing that the same cytokine responses detected in the blood were also present in the mucosa, including elevated expression of regulatory and Th2 cytokines and diminished expression of Th17/23 cytokines (Gaze et al., 2012).

Over the last 7 years the safety and therapeutic effect of low dose *N. americanus* infection has been assessed for a number of inflammatory diseases. Croese (2006) showed that *N. americanus* reduced the Crohn's Disease Activity Index (CDAI, histological marker of pathology in the gut) in most patients with active disease by