The cost and potential benefit of hookworm infection
Can parasites be good for you?

Is the human-infecting parasitic hookworm *Necator americanus* the ‘American murderer’ or a therapeutic agent? The following article describes current thinking around this interesting infection. At high infection intensities, the worm is undoubtedly pathogenic, and must be treated by de-worming or, in the future, by vaccination. Conversely, low doses of this immune-suppressive infection could be beneficial to the treatment of immunological diseases such as allergy and auto-immunity. The challenge currently facing scientists is to find a safe dose of sufficient intensity to provide benefit without causing distress to the patient. This will be an interesting immunological journey, and early indications suggest that therapeutic dosing regimes can be developed.

The hookworm *Necator americanus* has, like many parasites, an intriguing life cycle. Fertilized eggs are laid by adult females following insemination by males in the lumen of the duodenum. The worms are, at this time, attached to the intestinal wall, essentially acting as micro-syringes, sucking blood from the capillary bed of the intestine. If the worm burden is too great, the ensuing blood loss leads to iron-deficiency anaemia. The relationship between worm burden and plasma ferritin levels has been plotted (Figure 1) to indicate when the burden becomes biochemically intolerable.

Fertilized eggs pass with faecal matter on to the soil at locations where sanitation is minimal, and the climatic conditions favour further development of the life cycle (Figure 2). The infective L₃ larva, having gained sustenance and developed in the faecal mass, is then ready to infect the next person in the chain. This is achieved through percutaneous penetration, probably involving the secretion of a combination of tissue-degradative enzymes. Infective larvae in due course enter the circulation, traverse the lungs and negotiate the trachea vertically, to be swallowed and returned to the gut. This completes one cycle. In infected populations, it has been proven that infection is cumulative throughout a lifetime of exposure to infective larvae, indicating that *N. americanus* is continually cycling through the bodies of people in the tropics.

The clinical cost of hookworm infection and the Hookworm Vaccine Initiative

The health implications of hookworm infection can be significant, with blood loss to worms in the gut, and during systemic migration causing the aforementioned iron-deficiency anaemia, which results in lassitude, and cognitive impairment in children. For this reason, the Gates-funded Hookworm Vaccine Initiative is rightly searching for a vaccine (Figure 3) to reduce the pathogenesis of hookworm infection (www.sabin.org). Vaccines will provide long-term protection; worm-expulsion chemotherapy is effective, but is regarded as a short-term measure, as the worm-paralysing compounds tend to act predominantly against adult worms in the gut, allowing repeated infection by larvae. Larvicidal compounds such as albendazole are also available, but would need to be administered with a logistically difficult regularity to populations which are not always accessible or compliant. Vaccines offer a better long-term solution.
The immunobiology of hookworm infection

Any rational search for a vaccine will take into account evidence of naturally occurring protective immune responses to *N. americanus* and exploit these responses by vaccination to expel the adult parasite or prevent skin penetration by infective larvae.

Our work in Papua New Guinea points to the fact that individuals with high levels of what is termed a T-helper 2 response to the worm, characterized by heightened IgE and eosinophilia in the circulation, harbour smaller and less fecund worms. Furthermore, worm expulsion restored the some cytokine responses in the host, indicating a degree of parasite-induced immune suppression in the host. Taken together, one interpretation of these data is that an immunogenic parasite, in close contact with the immune system throughout its life cycle, is partially controlled by heightened immune reactivity in some. However, the parasite, in order to propagate the species, actively suppresses the immune system to stay ahead.

This infection-associated immune suppression may explain why hookworms and other parasites have been associated with the reduced incidence of immunological diseases such as asthma, Crohn's disease (www.broadfoundation.org) and multiple sclerosis.

These associations are undoubtedly compelling, and have led to a common belief that increased hygiene, together with the loss of parasite infection, may have led to an increased incidence of immunological disease. The hygiene hypothesis, if applicable to necatoriasis, can only be tested through clinical trial, and we have recently completed a number of such trials in Nottingham, where worms from Papua New Guinea have been deliberately introduced into patient populations.

As an interesting aside, worms can be brought back from Papua New Guinea in two ways, internally and externally. I preferred the external route, with explanations ready for the airport screening personnel as to the contents of my vials of precious liquid. One of my colleagues, Dr Alan Brown, preferred the internal route, and carries a healthy population of Papuan hookworms to this day. His faecal cultures provide the ‘snek bilong bel’ or snakes that live in your belly, for today’s trials.

The potential benefits: worms on trial

Safety Trial 1

To ascertain the potential benefit of hookworm infection, it was first necessary to determine a dose of infection tolerable to the target patient populations. This involved the recruitment of ten volunteers on campus, who were then infected with ten, 25, 50 or 100 infective larvae. These were administered topically via a sticking plaster, and subsequent symptomology was recorded.

Once the intense itching had subsided, caused possibly by vasoactive amine release while larvae entered the dermal vasculature, ten and 25 larvae were well tolerated, with 50 larvae proving problematic in some (the author), but 100 larvae were definitely not well tolerated, causing severe gastrointestinal disturbance and vomiting.

On a positive note, the well-tolerated level of infection
Features
Parasites

Parasites produced an infection intensity, assessed by faecal egg counts, which equated to the level of infection associated with reduced respiratory wheeze to environmental allergens in a field study of the effect of hookworm infection on asthma. The tolerable levels of infection were also recognized by the immune system, causing a peripheral eosinophilia and inducing antibody responses to antigens secreted by the adult parasite. This is an important point, as worm recognition will be an essential early component of any future regulatory event which might alleviate allergic disease. In short, regulatory events usually supersede immunological activation.

Safety Trial 2
Having safely determined a tolerable yet immunogenic infection dose, ten infective larvae were then compared with an equally itchy histamine placebo in allergic rhinitis patients already exhibiting heightened bronchial reactivity to challenge by AMP. This trial sector was designed to demonstrate that infection with a lung migratory phase to its life cycle...
did not adversely affect bronchial disease. The trial was not empowered to determine clinical benefit, although clinical scores were included as secondary outcomes.

A degree of inherent risk was associated with this stage of the proceedings, in that infection with a murine trichostongyle parasite, *Nippostongylus brasiliensis*, used as a model system for necatoriasis, can potentiate immunological responses to allergens if infection precedes allergen challenge. Given that the trial participants were already showing signs of bronchial hyper-responsiveness, as recruitment took place predominantly during the hayfever season, there was an inherent risk of increasing lung reactivity.

Significantly, infection with ten larvae did not heighten bronchial reactivity and did not potentiate IgE responses to the allergens to which the patients were sensitized. Immunologically, infected patients responded as in the first trial, and in addition indicated early signs of an onset of immune suppression, although circulating levels of natural regulatory T-cells were not increased by infection. It is on this T-cell phenotype that researchers are now focusing in the context of the control of immunological disease.

Regulatory T-cell numbers are significantly increased and activated by high levels of worm infections in murine models, and have an effect in moderating immunological disease. However, the infection of a 20 g mouse with 200 worms equates to in excess of 100 000 worms in a 70 kg human, which would clearly be a lethal dose.

The future challenge

The challenge for those of us using necessarily lower levels of worm infection in humans is to mimic the positive effects seen in animal models using alternative and inherently safe strategies, such as boosting with low-level 'trickle infection', as is likely to occur in the tropics. The mean worm burden in our study population in Papua New Guinea was 23 adults, with worms likely to be entering and leaving the body throughout life. This is the scenario that we may need to replicate in patient populations in order to fully ascertain the potential for benefit. Alternatively, adjuvants based on entities such as 'Tregitopes' could be used to boost the therapeutic potential of low-level infection.

In this context, we now have clearance to attempt in treating multiple sclerosis in Nottingham with *N. americus*, and this is thanks in large part to the generosity of the MS Society. The MS Society agreed to fund a three-year study to look at the potential benefits of using hookworms in this way. The MS Society has said that it is particularly keen to support such a study among its many varied research grants as there are limited treatment options currently available for MS and the development of hookworms as a potential therapy could lead to a cheap and relatively safe treatment for the often debilitating condition.

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References