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Parasite role reversal: worms on trial

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Asthma has reached epidemic proportions globally. This has been attributed by many to improved hygiene. The frequent failure of conventional pharmaceuticals to manage the disease has led to the introduction of parasites as a potential alternative therapy for asthma and other immunological diseases. In this article, we briefly review the immunological rationale underpinning therapeutic parasitic infection, describe recently initiated trials, and highlight potential risks and benefits of introducing parasites into patient cohorts.

Worms and the hygiene hypothesis

By definition, parasites survive at the expense of their hosts. However, while many researchers strive to obtain a full molecular understanding of the immunological relationships that exist between humans and their parasites so that they can use this information to develop vaccines to control infection, there is a current drive to reverse this trend by reintroducing parasites into non-infected cohorts of patients for potential therapeutic benefit. In this article, we describe the scientific basis underlying two current trials in which *Trichuris suis* (the pig whipworm) and *Necator americanus* (the human hookworm) are being used in an attempt to attenuate inflammatory bowel disease and asthma.

Parasites have co-evolved with their hosts for many generations. Many parasites are, therefore, finely tuned to their specific hosts, resulting in minimization of damage and prolonged survival, despite their inherent antigenicity. By contrast, zoonotic infections often cause damaging pathology, as seen, for example, in eosinophilic enteritis [1] and anisakiasis [2].

Thus, studying how host-adapted helminthic parasites successfully downregulate, deviate or subvert the immune response of the host gives rise to knowledge that can be used for the identification of effective anti-helminthic vaccine targets and that might also provide novel and, possibly, selective immune suppressants for the treatment of diseases characterized by an unbalanced immune response, such as allergy and autoimmune diseases (e.g. psoriasis, inflammatory bowel disease and type I diabetes).

Parasites are, without exception, antigenic to their hosts. In the case of worm infection, the immunological response tends to be of a phenotype termed T helper (Th)₂, which is an immunological phenotype also associated with allergic disease and asthma, although there might be a stage dependency of the immunological phenotype induced. For example, different stages of the schistosome life cycle are known to engage Th1 (schistosomula) or Th2 (ova) responses [3].

Paradoxically, worm infections might protect against the development of allergic diseases. Early anecdotal evidence [4] was incorporated by epidemiological support [5–7] into the ‘hygiene hypothesis’ (Box 1), which, in turn, has been explored in further detail by several research groups [8–15]. Importantly, repeated removal of parasites by chemotherapy results in increased skin-test reactivity to house dust mites [16,17]. The anti-allergic, protective effect of helminth infection also receives substantial support from animal models [18–22].

The mechanisms underlying the hygiene hypothesis are still insufficiently explained, but current thinking points to the central importance of a regulatory cytokine network (for review, see Refs [23,24]) that is characterized, at least in part, by the production of interleukin-10, transforming growth factor- β and regulatory T cells.

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Box 1. The hygiene hypothesis

The hygiene hypothesis states that a reduced exposure to microbes and an absence of the ensuing immune response to these microbes are responsible for the increased prevalence of allergic disease, and is based on the original observation that allergic disease is less common in people with a large number of siblings [39]. This has been explained mechanistically by suggesting that a cytokine imbalance is responsible for the clinical effects seen following removal of the immune-stimulatory effects of microbes and parasites. However, the hygiene hypothesis has been questioned recently because there is evidence that some microbial infections might exacerbate, rather than alleviate, allergic asthma [40,41]. These findings, rather than disprove the hygiene hypothesis, could indicate that the Th1–Th2 paradigm is oversimplified and of limited value in understanding the complex underlying immunological processes [12]. Nevertheless, the suggestion that helminth parasite infection might protect against allergy [6,7,42] pre-dates the hygiene hypothesis [39] and, in fact, the Th1–Th2 paradigm [43].

The hygiene hypothesis, as far as the therapeutic use of worms is concerned, is based on several assumptions: (i) that an entrenched cytokine milieu in allergic and autoimmune diseases can be converted by therapeutic infection into a protective immunological phenotype; (ii) that bolus, abbreviated or repetitive therapeutic parasitic infection will mimic the protective effects recorded in parasitized communities; and (iii) that the immunological mechanisms underlying allergic and autoimmune diseases are fully understood. Notwithstanding these assumptions, clinicians have been sufficiently stimulated by the scientific developments so far to contemplate in some cases, and to begin in others, clinical trials of worm infections against immunological disease.

Worms on trial

Resolution of the issues highlighted will come from carefully controlled trials of parasitic infection superimposed on immunologically relevant diseases. Any future therapeutic application must also consider the potential hazards of such a procedure. Certainly, the choice of parasite is of paramount importance. The parasites used in the studies detailed later illustrate this point. *T. suis* (Figure 1) cannot establish itself in the human host because it is rapidly cleared [25], thus reducing the risk of adverse effects. However, it might be present long enough to induce a protective immunological phenotype. The downside of this is that the treatment must be repeated several times over a period of several months. By contrast, *N. americanus* can successfully colonize the human gut and persist for several years. Thus, a single treatment might be sufficient, although this remains to be established. In terms of potential pathology, *N. americanus* infections are relatively asymptomatic [26], provided that the dosage is carefully regulated. Certainly, other parasites such as *Schistosoma mansoni* that are also characterized by their longevity in humans are less amenable to a therapeutic use because the pathology would outweigh the potential benefits. Importantly, in terms of safety, it is unlikely that hookworm infections will be transmitted from patients in the trial to the general population because hookworm larvae require an incubation period of ~10 days to become infective following faecal

deposition. Current hygiene precludes this possibility. There is limited evidence that *T. suis* might synergize with other pathogens such as *Campylobacter jejuni* [27,28] but this potential risk must be evaluated and compared with the serious adverse effects of current therapies for inflammatory bowel diseases (long-term use of steroids and immunosuppressants). The effects of superimposing parasitic infections on chemically immunosuppressed patients should also be taken into consideration. With these caveats in mind, two trials have been undertaken.

Whipworms

The largest trial to date, based on the positive outcome of a small-scale pilot study [25,29] and on extensive epidemiological data [30], has been started by Joel Weinstock (Iowa University, USA). In these studies, the effects of infection with *T. suis* on the clinical course of two inflammatory bowel diseases, ulcerative colitis and Crohn's disease, are being assessed.

Weinstock reports that 'the first was a double-blind study in ulcerative colitis. The study included 54 patients randomized to either *T. suis* ova or placebo. They received ~2500 ova orally every two weeks. The trial duration was 12 weeks. The overall major response rate in the treatment group was ~50%, with placebo responses of



Figure 1. Worms on trial: the protagonists whipworm and hookworm. Shown here are (a) an adult worm and ovum of the pig whipworm *Trichuris suis* and (b) the human hookworm *Necator americanus*, attached as an adult to the gut wall. Image in (a) courtesy of Joel Weinstock, Iowa (USA). Image in (b) courtesy of Alan Brown, Nottingham (UK).

~16%. This difference was highly significant. The time to response was ~6 weeks.

The Crohn's disease study was a 29-patient open label trial of 24-week duration. Patients received 2500 ova orally every three weeks. In this study, the remission rate was 72.4% and the response rate was 79.3%.

We have treated more than 110 patients, some out beyond four years. It is our impression that this agent can maintain remission. To date, we have not witnessed side-effects or complications' (J. Weinstock, personal communication). The results of the studies of Crohn's disease and ulcerative colitis are soon to be published [31,32].

Hookworms

Another trial has been initiated in Nottingham (UK), involving our participation, to assess the effects on asthma of infection with *N. americanus*. The human hookworm (Figure 1) was chosen for this trial because of the available epidemiological data supporting its protective effects against the development of respiratory wheeze [10] and because of its well-documented immune-modulatory capacity [26,33]. The Nottingham trial consists of three phases and has received full ethical approval.

Phase 1

The aim of this phase was to determine which dose does not reduce lung function, yet produces EPG (eggs per gram of faeces) levels associated with protection against respiratory wheeze [10]. Consenting volunteers with no evidence of airway hyperresponsiveness were infected with a dose range of third-stage larvae. Stool samples, haemoglobin levels, plasma ferritin levels and clinical indications were assessed for 12 weeks. This phase was recently completed, and full clinical and immunological details of this study will be published elsewhere.

Phase 2

This phase is a study of the effect of hookworm infection on airway responsiveness in non-asthmatic allergic subjects. Healthy adults with skin-test positivity to *Dermatophagoides pteronyssinus*, cat fur or grass pollen, and measurable airway response to methacholine will receive larvae or histamine placebo (double blind). The primary outcome will be the measurement of airway responsiveness; the secondary outcome will be the change in skin sensitivity to allergens.

Phase 3

This phase is a placebo-controlled study of patients with asthma. Should the trial proceed to phase 3 and show benefit to patients, the introduction of therapeutic parasitic infection will be seriously considered.

Drugs from bugs

These examples illustrate the current level of interest in parasites as potential therapeutics. Should the trials continue to provide optimism, it is highly likely that the parasites responsible for the beneficial effects will, in turn, be mined for immune-suppressive molecules. The pharmaceutical industry would certainly feel more comfortable if parasitologists and chemists could isolate, identify and

then synthesize small molecules from the parasites responsible for the immune-modulatory effects seen. The establishment of appropriate sustained delivery formulations through mucosal surfaces might be needed to mimic successfully the immunological responses induced in the context of a natural infection.

The fact that the compounds from parasites might have evolved to unlock the human immune system selectively will also result in less attrition when compounds are tested for safety and adverse activity profiles.

Furthermore, the diligent sourcing of organisms for the isolation of immune-modulatory compounds might lead to the discovery of disease-selective therapies. Recent work detailing the isolation and synthesis of immune-suppressive compounds from *Pseudomonas aeruginosa* serves to illustrate this point. Some of these compounds seem to have a degree of selectivity in suppressing Th1 responses [34]. It is possible that nematodes – considering the role of Th2 responses in host protection, as suggested by both field studies and murine-model data [35–37] – might have evolved to modulate the Th2 response locally. Therefore, it might follow that helminths will provide anti-allergic therapeutics and bacteria therapeutics for autoimmune diseases, although the Th1–Th2 demarcation in immunity to these respective classes of pathogen might be blurred in many cases, particularly when considering the impact of populations of regulatory T cells [38].

Role reversal

Thus, although much of the effort and research funding in our research community is aimed at defeating helminth diseases, some clinicians are striving to reintroduce these parasites into a diseased, yet parasite-free population. Assuming that this therapeutic use delivers the expected benefits, it could lead researchers to rethink whether these organisms might not rightfully claim a new status as healers rather than pathogens, or even symbionts rather than parasites, provided that dosage is carefully regulated.

Acknowledgements

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Letter

Human balantidiasis in Iran: an unresolved enigma?

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Balantidium coli (Malmsten, 1857) (Ciliophora: Balantiidae) is the largest protozoan and the only ciliate parasite to infect humans. Infection with this parasite is not common in humans, and only 1000 cases had been reported before 1980.

Its presence has been reported in parts of the world as far north as Sweden, Finland and Northern Russia, and the highest prevalence rates are in tropical and subtropical regions. Estimates of worldwide prevalence are usually <1%, although higher rates are reported in hyperendemic areas and in some residential institutions [1]. The presence of *B. coli* is frequently reported in Central and South America

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