



Intestinal allergy expels hookworms: seeing is believing

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It is unclear how immunity limits hookworm infection. Australian researchers, using capsule and conventional gastrointestinal endoscopy in volunteers inoculated with *Necator americanus*, have reported that virtually all larvae reach the intestine within six weeks. Unlike the neutral response surrounding resident hookworms, newly arrived adults provoke an eosinophilic enteropathy. This allergic reaction curtails the attachment of hookworms and accompanies the passage of additional worms as they are expelled from the proximal small intestine.

A fortuitous insight

After commencing a proof-of-concept study to establish colonies of *Necator americanus* in Crohn's disease patients, Australian researchers were inoculated to provide reservoirs from which to harvest larvae [1]. Using gastrointestinal endoscopy, the intestinal dynamic between parasite and host was examined. The migration of hookworms to the intestine seemed to be unimpeded and not, as previously suspected, subject to depletion during passage through the dermis and lungs [2]. In the intestine, *N. americanus* provoked an allergic response that was sometimes subtle but sufficient to expel additional parasites. Mature hookworms survived by protecting the integrity of the host mucosa. Allergic inflammation in the intestine was suggested to provide an effective immunity against hookworms [2]. Here, we discuss a new paradigm of human immunity that accommodates a sustainable hookworm infection.

Necator americanus: pathogen or commensal?

Hookworm infection is regarded as an important tropical disease in humans [3], although most infections are sub-clinical. A hookworm will survive, on average, for five years in the intestine, where it feeds efficiently [4,5]. The *N. americanus*–human relationship has evolved beyond the need for the parasite to establish a complex life-cycle or undergo a protective hibernation; instead, a mutually symbiotic relationship has been suggested [6].

Autoimmunity, hygiene and parasites

Hookworms are so resilient that it has been argued that they occupy an immune-protected niche [5,7]. However, immune protection is, at best, a defence that is balanced

against the collateral damage caused when foreign antigens are appropriately engaged and the necessity to avoid autoimmune pathology, which occurs when self-antigens are inappropriately engaged [8]. Adaptive immunity comprises T helper (Th)1 cells, which secrete interferon- γ (IFN- γ), Th2 cells, which secrete interleukin-5 (IL-5), and regulatory T (Treg) cells. These systems are induced when antigens engage T-cell receptors, with the final immune outcome reflecting a collective and integrated response.

In the developed world, an increasing incidence of autoimmune diseases has occurred, which has been linked to improved hygiene [9–11]. Nematode infections seem to protect against this trend [12]. Some autoimmune diseases are associated with a biased Th1 (e.g. Crohn's disease) or Th2 (e.g. asthma) response, or a failure of the Treg process (e.g. ulcerative colitis) [7]. In most cases, protection against intestinal nematodes relies on Th2 responsiveness [13] and, as indicated in nematode–mouse models, chronic infections depend on the ability of the parasite to induce Th1 and Treg immunity [7,14].

Based on the premise that chronic inflammatory bowel disease might be inhibited by stimulating a Th2 immune environment, Summers *et al.* modified disease pathology in 29 Crohn's disease and 54 ulcerative colitis patients by inoculating them with the porcine whipworm *Trichuris suis* [15,16]. In a similar vein, Crohn's disease patients were inoculated with *N. americanus* [1], with healthy volunteers used as donor reservoirs. The evaluation of these subjects included endoscopy [1,2].

Gastrointestinal endoscopy

Capsule endoscopy is a recently developed technology that was designed to investigate the human small intestine using a wireless endoscope of similar size and shape to an encapsulated medication [17]. After being swallowed, it relies on intestinal motility for propulsion. From within the capsule, a battery-powered light flashes to enable the capture of an image that is developed by a microprocessor. Images are taken at nearly two frames per second over an eight-hour passage, transmitted by the capsule to antenna pads fastened to the abdomen and collated into a video format. Investigations are typically performed on ambulant patients. The quality of the image is equivalent to that from conventional gastrointestinal endoscopy (Figure 1) but the completeness of each study is subject to the unpredictable presence of opaque intestinal fluid and the random transit and orientation of the capsule.

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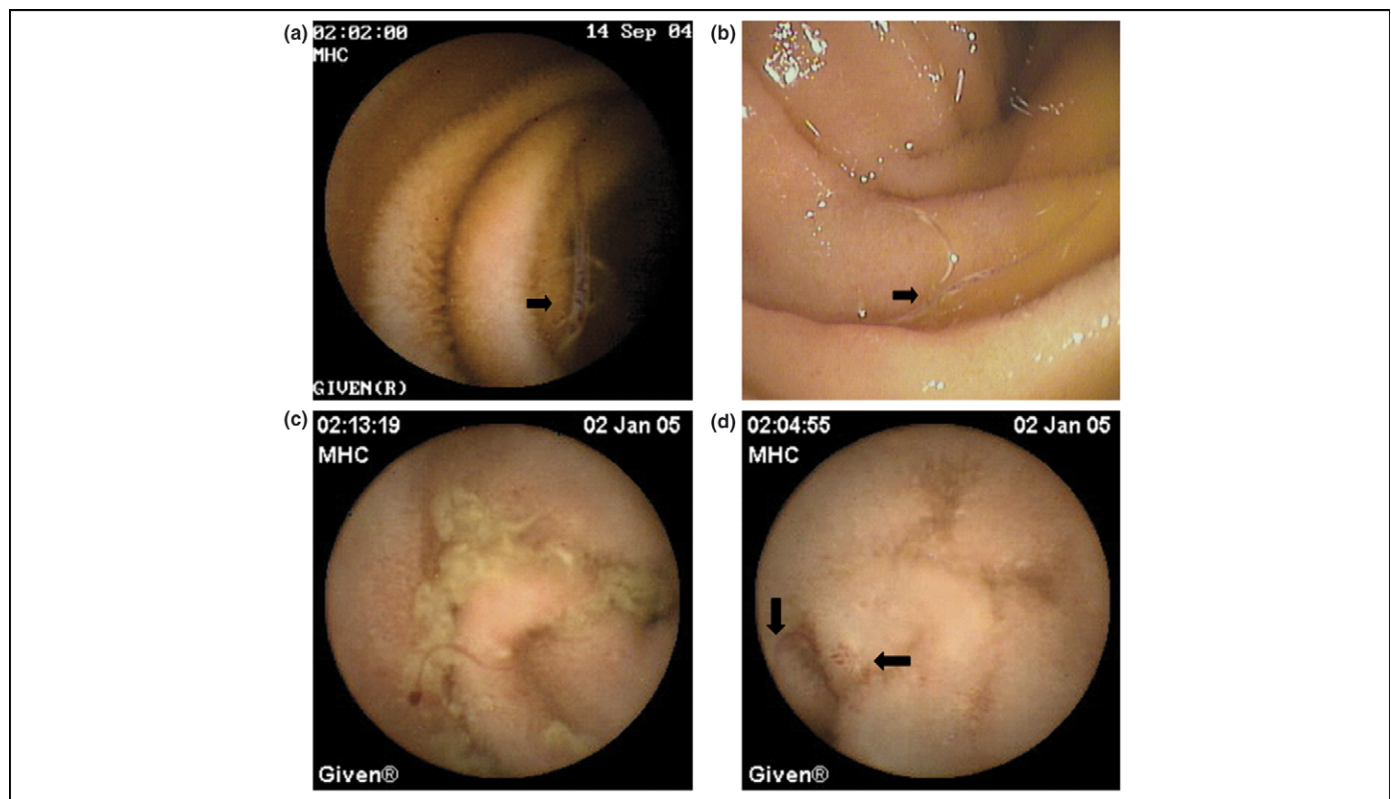


Figure 1. *Necator americanus* in the human host. (a) Capsule endoscopy provides 47 000 high-resolution images during transit but the view is subject to chance and the quality of the bowel preparation. (b) The conventional upper-gastrointestinal video endoscope enables the proximal margin of the hookworm colony to be accessed and biopsies to be taken. (a,b) Copulating *N. americanus* pairs are shown (indicated by arrows). The healthy integrity of the mucosa is obvious in both frames. (c) An embedded immature *N. americanus* hookworm is surrounded by a haemorrhagic spot, presumably the result of trauma and the injection of antiplatelet and anticoagulant ESMs. Feeding is evident from the blood that defines the gastrointestinal tract of the hookworm. (d) A red spot occurring independently (horizontal arrow) was interpreted as a recent feeding spot. A tiny aphthous ulcer has developed at this old attachment. A barely visible hookworm is nearby (vertical arrow). In most cases, the mucosa surrounding immature hookworms looked inflamed. Mature hookworms were distinguished from those inoculated within four to six weeks by the relative size and the improved clarity of their internal structures. Reproduced, with permission from the American Gastroenterological Association, from Ref. [2].

Using capsule and conventional endoscopy, the latter to biopsy tissue from mucosa adjacent to feeding hookworms, a new perspective on the dynamic between *N. americanus* and its human host has been reported [1,2].

Eosinophils expel *Necator americanus*: seeing is believing

Capsule endoscopy was performed serially on two healthy researchers with previously established stable hookworm infections immediately before and after inoculation with 50 additional larvae. By week six, virtually all larvae had reached the intestine but most of the newly arrived worms failed to attach and, together with a small number of mature worms, were expelled distally (Figure 2). By week 21, the colony in each host had been restored to the status quo.

When populated by immature hookworms, the mucosa was overtly inflamed compared with the normal appearance seen surrounding mature worms (Figure 1). All subjects developed eosinophilic enteritis after first contact with *N. americanus* but the histology of the mucosa adjacent to mature worms was normal [1,2]. The intensity of the eosinophilic response correlated with the number of worms expelled.

Constitution and tolerance

The duration of hookworm attachment was estimated by counting recently vacated feeding sites (Figure 1). Quali-

tative similarities between the two researchers included: (i) the failure of immature worms to attach proximally, with consequent expulsion; (ii) a shortened attachment time for immature hookworms compared with mature

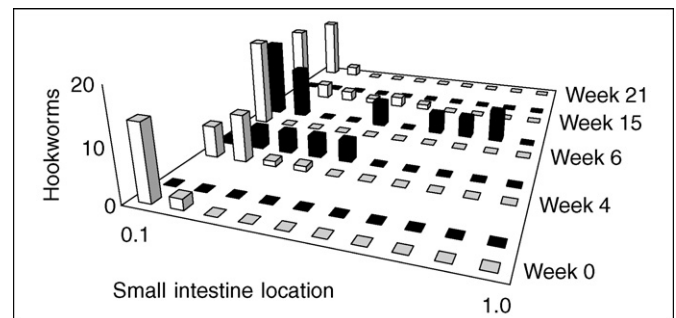


Figure 2. Immature hookworms fail to attach. The transit of the capsule through the small intestine of the volunteer (small intestine location) is represented as a time scale divided into ten equal periods, with 0 representing the start, or first duodenal image, and 1 the last image of the terminal ileum. Immature (filled boxes) and mature (open boxes) hookworms present in each segment were counted. In this volunteer, each of five capsule studies is identified by the number of weeks it was performed after skin contact with 50 infective larvae. At week 0, only mature worms were present because the colony was restricted to the proximal intestine. Immature worms could be recognized at weeks four and six after inoculation. The sequence demonstrates that mature worms mostly survive and newly arrived worms mostly fail because they are caught in a distal drift, leading to expulsion. At week four, some mature worms seemed to have been displaced by newly arrived worms. At week 21, the colony had been restored to the status quo. Reproduced, with permission from the American Gastroenterological Association, from Ref. [2].

worms; and (iii) a correlation between restricted attachment and allergic damage to the host mucosa. The volunteers were, however, quantitatively different, with one expelling immature worms more quickly and retaining a smaller colony than did the other, more tolerant, volunteer. Anecdotally, more eosinophils, more-debilitating enteritis, higher levels of total immunoglobulin E (IgE) and a stronger correlation between inflammation and shortened attachment time were evident in the more resistant host.

Allergy: capricious or purposeful?

In Australia, painful eosinophilic enteritis occurs in humans infected with *Ancylostoma caninum*, which is a hookworm of canines [18]. Most infections are subclinical and are found during colonoscopy performed for unrelated reasons [19]. The allergic response caused by this zoonosis was argued to be an immune defence [20]. It now seems that anthropophilic hookworms that exceed constitutive tolerance are similarly managed.

Parasite survival, at least in some nematode–mouse models, is determined by the balance between host-protective Th2 (mediated by IL-5) and parasite-protective Th1 (mediated by IFN- γ) responses [14]. In the human intestine, however, the only visible evidence of immunological interaction between host and hookworm is allergic damage that is inconsistently provoked by hookworm-derived excretory–secretory molecules (ESMs) after they have left the parasite and interacted with the host.

When first infected with hookworms, volunteers experience painful eosinophilic enteritis almost immediately, before an adaptive response could have developed [5]. Falcone *et al.* [21] argue that lectins, ESMs from feeding hookworms, generate an innate response by cross-linking IgE independently of antigenic binding, thereby activating mast cells and basophils to secrete Th2 cytokines. In mouse–nematode models, Th2 cytokines enhance motility and induce intestinal goblet cells to secrete more and qualitatively different mucus – physiological responses to trap and expel parasites (although this process is also Th1 restricted) [22]. Functionally, ESMs have evolved to facilitate feeding; for example, one ESM peptide described by Hsieh *et al.* [23] stimulates natural killer cells to secrete IFN- γ , conceivably to promote the Th1 inhibition of Th2 immunity.

Nutrition and restricted access

Despite being recognized by T cells, mature hookworms attach to healthy mucosa and are seemingly surrounded by a zone of ‘immune privilege’ [2,5]. In a community in Papua New Guinea (PNG) in which *N. americanus* is endemic, total IgE levels positively correlated with the pre-treatment hookworm burden (as measured by faecal egg counts) but the correlation was negative for IFN- γ [24]. Although it is easy to appreciate the correlation between IgE and ESM stimulus, it is counterintuitive that an immunoglobulin associated with host protection reflects rather than manages the worm burden. Paradoxically, IgE correlated negatively with the fecundity and size of individual worms [25]. The researchers identified IL-5 as the cytokine linked most strongly to protection against reinfection, an

association that is intuitively appropriate given the important defensive role attributed to eosinophils [24].

These characteristics of *N. americanus* infection in PNG reflect a cross-sectional analysis, so the sequential relationship of key elements can only be guessed. IgE seems to enhance allergic damage when a colony exceeds the size that is constitutively appropriate for the host. The declining level of IFN- γ , which relates to the number of hookworms, mirrors failure of the Th1 response, which might otherwise protect the mucosa against allergic damage. Croese *et al.* [2] argue that a point is reached at which the speed of mucosal degradation forces hookworms to change attachments so often that a negative nutritional balance occurs (i.e. individual parasites lose weight and levels of egg production decrease). Healthy and mature parasites can, presumably, deliver more ESMs than can immature or nutritionally stressed hookworms. Therefore, the colony size is set when the parasite load drives the allergic response past the level that immature and stressed hookworms can tolerate. Anthelmintic therapy is associated with a rebound in circulating IFN- γ , which might explain why usually vulnerable immature hookworms so easily re-establish when cognate immunity is at its theoretical peak [24].

An enlightened future

Although mature hookworms are separated from the host, they are still subject to immunity, albeit indirectly. Gastrointestinal endoscopy and the safe access to tissue that it provides, and new technologies such as microarrays have enormous potential to increase the understanding of hookworm infection. Has a mutually beneficial relationship been established? A causal association between increasing autoimmunity and decreasing parasitization is far from proven but it is an intriguing and worthy hypothesis.

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