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Note added in Proof

Articles on *Leishmania* histone H3 genes⁴⁴, *Crithidia* histone H2A genes⁴⁵, and *Leishmania* histone H1 (Ref. 46) have recently been published. Histone H4 genes from *Leishmania* (C. Alonso, pers. commun.) and from *Trypanosoma* (L. Aslund, pers. commun.) have been cloned and sequenced.

Focus

Hookworm-provoked IgE-mediated Pathology: Capricious Damage or Remarkable Strategy?

J. Croese

The existence of the canine hookworm Ancylostoma caninum in the human gut seems to be curtailed by an effective IgE-mediated inflammatory response against the helminth's feeding secretions, which, by damaging the host, compromises the parasite's feeding opportunities. In contrast, immunity does not develop against adapted, anthropophilic hookworm species, and it is suggested this relates to modulation of the inflammatory response by hookworm secretions. Here, John Croese discusses this hypothesis and its implications for the development of drugs to suppress allergy.

Eosinophilic enteritis is a descriptive diagnosis encompassing several, usually rare, conditions that are restricted to the gut and share a common histological appearance¹. Allergic eosinophilic gastroenteritis, perhaps the best characterized of these, is identified by eosinophilic inflammation of the stomach and proximal duodenum, and has been linked to food allergy². As with other allergic diseases, the pathology is thought to develop capriciously in predisposed individuals, and as a consequence of inflammatory mediators triggered by essentially innocuous antigens^{2,3}. In northern Australia, eosinophilic enteritis is remarkably common as a result of infection by a canine hookworm, *Ancylostoma caninum*^{4,5}. Unlike that occurring with allergy, inflammation here is purposeful and of benefit to the host.

Human infection by *Ancylostoma caninum*

An epidemic of eosinophilic enteritis was reported in Townsville in northern Australia (latitude 19° south) in 1988 (Ref. 4). The suggestion that this was caused by a zoonotic hookworm was confirmed by the retrieval of single worms of *A. caninum* sp. from several patients undergoing laparotomy or colonoscopy^{4–9}. In contrast, the anthropophilic hookworm species *A. duodenale* and *Necator americanus* have not been endemic for over 50 years¹⁰. Most of the patients had painful enteric syndromes, sometimes mimicking acute appendicitis, or

with a gut obstruction, and these had resulted from an eosinophilic enteritis^{4–9}.

Five percent of Townsville people undergoing colonoscopy, including those with symptoms unrelated to hookworm presence, were found to have tiny white (aphthous) ulcers with predominantly eosinophilic histology in the ileum and/or caecum, and these were assumed to relate to prior feeding attachments⁸. The presence of ulcers was linked to the season in a manner similar to the prevalence of eosinophilic enteritis: both conditions are more common in the wet monsoon months, which accords with hookworm transmission between hosts^{8,11,12}. The ulcers were further linked to *A. caninum* by serological testing^{8,13}.

Hookworm ova have not been found in this geographical region, except for cases that can be linked clearly to importation, despite examination of over two thousand human fecal specimens annually (J. Croese, unpublished). In addition, hookworms have not been reported postmortem, and in the cases referred to above, never has more than a single worm been found. Thus, in contrast to the situation with *A. duodenale*, colonization clearly does not occur. Given the apparent prevalence of infection, it must be that individual *A. caninum* manage to persist for only a brief period in the human gut – much less than the duration of a monsoon season, which is regarded as the life span of *A. duodenale*¹², or the 30–36 week life-span of *A. caninum*¹⁴. The hookworms retrieved from humans were small but otherwise morphologically normal; the average length was 8 mm versus the 12–16 mm found in dogs¹⁵. Those worms observed colonoscopically were attached, feeding vigorously, and apparently healthy.

Parasitism by *A. caninum*

Hookworms feed by attachment to the gut. In the dog, *A. caninum* changes attachment sites each 4–6 h¹⁵. A tiny lesion develops at the bite, along with an inflammatory infiltrate, but repair is rapid¹⁵. It has been proposed that: 'parasites ... change their feeding sites and may thus avoid potential damage which could be inflicted by inflammatory cells'¹⁶. It is also possible

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that the hookworm can no longer feed effectively at a site degraded by inflammation.

There is conjecture as to whether or not nutritional elements that might be peculiar to an individual host species determine the specificity of hookworm infections¹⁴, although there is *in vitro* evidence to indicate that these could be a consideration¹⁷. The confusion reflects the poor understanding of the crucial elements of the hookworm diet. Worms bite into, and ingest, a plug of mucosa. It is not clear if this contributes to nutrition, or is simply a means to access a blood supply. Blood is then voraciously, seemingly wastefully, sucked through the worm, and probably provides the major elements of the diet¹⁵. The rapid transit would restrict the possibilities for the digestion and absorption of complex constituents, and it is probable that hookworms rely on an abundant supply of simple sugars, peptides and oxygen^{15,18}. *Ancylostoma duodenale* has been established in immunosuppressed dogs, and there are many other examples of hookworm species being able to establish feeding (and fecundity) in a range of hosts and *in vitro*¹⁴. Thus, it seems unlikely that critical nutritional adaptations are an essential criterion for successful colonization.

Attached worms inoculate the host with a wide range of excretory/secretory molecules¹⁹. These molecules, and their structures and functions, are only just beginning to be researched. Two broad functional categories can be recognized: certain proteinases that digest tissue at the bite and another group that appears to regulate the host response. Within the latter group, two proteins have been described recently in some detail: Cappello and colleagues have identified a peptide that inhibits factor Xa and is a potent anticoagulant²⁰ and Moyle and colleagues have sequenced and produced a neutrophil inhibitory factor (NIF)²¹. NIF is a ligand of integrin CD11b/CD18 that blocks neutrophils adhering to endothelium²¹. Sawangjaroen, using monoclonal antibodies, has localized NIF to the excretory/secretory glands (pers. commun.). Given that these structures deliver their contents externally, this would suggest that the biological actions of NIF are exerted at the bite, rather than internally (for example, to protect the hookworm's gut). There are many other excretory/secretory proteins yet to be defined²², and presumably some of these also act to block inflammatory mediators.

The host response to *Ancylostoma* spp

Immunological recognition of these feeding proteins occurs in both anthropophilic and *A. caninum* human hookworm infections^{7,9,13,23,24}. In experimental volunteer infections and in naive non-human hosts, IgM and IgG antibodies develop against excretory/secretory antigens as the hookworm begins feeding in the gut^{25,26}. An IgE response is detected by enzyme-linked immunosorbent assay (ELISA) approximately four weeks after the development of IgG antibodies, but given that eosinophilia and a positive skin test (Prausnitz-Küstner reaction) occur much earlier, it seems likely that re-aginic immunity is established at the outset²⁷⁻²⁹. Loukas and colleagues have established IgG, IgG4, IgM and IgE antibody responses against hookworm feeding proteins in patients with either proven *A. caninum* infections or eosinophilic enteritis^{13,23}. Paradoxically, they found that these responses were quantitatively less than those in *A. duodenale*-infected controls.

There is clear evidence that IgE and eosinophils are able to participate in cytotoxic reactions against some helminths, particularly during larval migration through tissue²⁸. *In vitro*, *Schistosoma mansoni* larvae are susceptible to eosinophil-mediated cytotoxicity via activation of a high-affinity cell-surface IgE receptor [Fc(R1)]²⁸. Hypo-eosinophilic animals have compromised immunity to migrating larvae of *Strongyloides venezuelensis* and *Trichinella spiralis*, but not to gut-living stages, 'demonstrating that protective mechanisms in this locale are ... distinct'²⁸. There is little evidence that inflammatory processes damage feeding hookworms directly.

Sawangjaroen and colleagues have demonstrated *in vitro* that antibodies harvested from patients with eosinophilic enteritis target contents within the hookworm's excretory/secretory glands³⁰. However, immunological access to feeding proteins occurs only after they are inoculated into the mucosa; the glands are structurally isolated from contact with the host. The hookworm's intestine could be vulnerable to antibodies given the volume of blood passing through, but this did not appear to be the case³⁰; presumably the host is not exposed to, and immunized against, intestinal tissues.

Implications

How then to reconcile the immunological recognition of both *A. caninum* and anthropophilic hookworm infections with the effective human immunity seen against *A. caninum* versus the failed immunity that accommodates anthropophilic infections? The *A. caninum*-human relationship produces a more vigorous inflammation at attachment sites than that which develops in most colonizing infections⁵. As discussed above, *A. caninum* occasionally precipitates an overly vigorous response identified as eosinophilic enteritis, but more usually it seems to cause small ulcers that quickly evolve after a bite. It also seems that *A. caninum* may have a much curtailed or tenuous attachment in the human gut; the presence of aphthous ulcers in the ileum indicates the worm is vulnerable to being washed away by lavage taken preparatory to colonoscopy. It is possible that *A. caninum* in humans are smaller and survive a much shorter time than do counterparts dwelling in dogs because they provoke more damage to the host mucosa, and this damage reduces feeding opportunities. This proposal is akin to the military 'burnt earth' strategy; a localized and immediately effective IgE-mediated response, provoked by feeding proteins, damages the host to compromise feeding. By corollary, adapted species would appear to have evolved responses capable of delaying or blocking the effects of this allergic cascade and, by so doing, maintain feeding sites and colonize. As with *A. caninum* in humans, other hookworms are smaller than normal when found outside the specific host¹⁵, and quite possibly, this 'burnt earth' defense has a universal application. When hookworms are found in hosts to which they are not adapted specifically, including some earlier reports of *A. caninum* in humans⁷, they have been present as mixed infections of which an adapted species is dominant. Eosinophilic enteritis has not been a feature, and it may be that the adapted hookworm, by suppressing this response, protects the alien species. Such a phenomenon would help explain why eosinophilic enteritis has only recently been reported in northern Australia: it is now some time since the disappearance of anthropophilic hookworms.

Ancylostoma caninum and *A. duodenale* share a close evolutionary past, and this is reflected by many morphological and serological similarities. Excretory/secretory antigens from canine hookworms reacted identically in western blots incorporating sera from either patients with *A. caninum* infection or controls infected with *A. duodenale*¹³. Although NIF was isolated from *A. caninum*, it inhibits human neutrophils *in vitro*²¹. Similarly, anticoagulants sourced from zoonotic species (*A. caninum* and *A. ceylanicum*) function against human plasma^{20,31}. These functional relationships, however, may not be as effective *in vivo*. Clearly, if both species secreted totally identical feeding substances, then it would be expected that they would be equally adapted to both human and canine hosts. It seems probable that some excretory/secretory molecules are subtly and crucially unique, and that these differences determine host specificity. These differences might relate to their functional specificity or, alternatively, their immunogenic properties might allow one species an opportunity to evade recognition in the adapted host.

Conclusions

Observations on the defense mounted by hosts against adapted hookworm species have helped to entrench the view that immunity does not develop, or at best provides only a limited protection. This is certainly not the case for humans infected by *A. caninum*. In most, the parasite survives only a short time, and it has been proposed that this results from immunity mediated by IgE causing inflammation that is more directly damaging to the host than to the worm. As such, IgE-mediated damage is not simply capricious, but germane to its intended role as a defence against hookworms. Occasional individuals develop eosinophilic enteritis, and this exaggerated pathology is acceptable in evolutionary terms given the protection afforded to the community. There is still a great deal to learn about hookworms, particularly the subtle characteristics that govern the specificity of different species for a particular host. The prospect to develop anti-inflammatory (and possibly anti-allergy) drugs from hookworm feeding secretions is just being realized. Efforts so far have concentrated on material sourced from zoonotic species. If, indeed, host specificity is crucially linked to uniquely adapted feeding proteins, then it may become necessary to use material sourced from anthropophilic species.

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