Hookworm Infections

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An overview

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1. Introduction

Human hookworms are clearly fascinating organisms whose biology is so closely linked to that of man that despite nearly a century of research and of concerted attempts to bring about their eradication, the organisms still parasitize nearly one in four of the human race and are likely to continue doing so for many years to come (Gilman, 1982; Keymer and Bundy, 1989; Schad and Warren (Eds.) 1990).

The major reasons why hookworms have been so neglected in recent times are firstly, the mistaken belief that everything about them had already been learned through the intensive studies undertaken in the first half of this century and, secondly, their rigid host specificity with consequent problems of access to the human parasites in laboratories situated in non-endemic areas. Although the development of novel strategies for the control of human hookworms must rank as a vital objective in future research, it should by no means be the only goal. Hookworms have more often than not been lumped together as a single species of pathogen and yet it is abundantly clear that the biology of *N. americanus* is quite distinct from that of *A. duodenale* in a number of important details and both show major differences from the canine species (Behnke, 1990; Pritchard et al., 1990a). So little is known about some aspects of the biology of human hookworms, e.g., their biochemistry, antigens, genetics, host/parasite relationships etc., that research into these topics may bring with it discoveries with wider ranging beneficial applications than merely control and eradication. The development and exploitation of new models for laboratory experimentation must be considered high on the list of priorities, as success in this area has already opened the doors to the application of contemporary experimental techniques on hitherto rarely accessible stages of the parasites. Nevertheless, there is still much to accomplish before we can truly claim to have an accurate and totally comprehensive understanding of all aspects of the biology of human hookworms.

2. Models for human hookworm infection

The most thoroughly studied model of hookworm infection is *Ancylostoma caninum*, a parasite which is clearly related to *A. duodenale* and is morphologically very similar. Several authors have emphasized the eminent suitability of *A. caninum* as a model for its human counterpart and yet there are differences in the epidemiology, biology and immunology of the two species which warrant caution in extrapolating to *A. duodenale*, and even more so to *N. americanus*, conclusions based on research with the canine species.

An important aspect of the host/parasite relationship of *A. caninum* is age resistance (Sarles, 1929b; Foster, 1935; Otto and Kerr, 1939; Miller, 1965c). Thus by 11 months after birth, dogs resist infection with *A. caninum*, resistance acting at an as yet unidentified stage between exposure to infective larvae and patency (Miller, 1965c) and as a consequence canine hookworm disease is essentially a threat to pups and less so to mature dogs. Comparable experiments are unlikely ever to be carried out in man, but from limited

studies with adult European volunteers (Palmer, 1955; Ball and Bartlett, 1969; Ogilvie et al., 1978; Maxwell et al., 1987) and with Indian prisoners in Madras Penitentiary (Kendrick, 1934) it is clear that neither *A. duodenale* nor *N. americanus* experience major difficulties in infecting adult humans. Epidemiological and clinical studies are consistent with these observations in that older age groups are generally revealed as carrying the heaviest parasite burdens (see Chapters 5 and 6).

The human and canine species also differ with respect to their longevity following a single administration of larvae or under conditions of continuous reinfection. The life span of *N. americanus* may be in excess of 15–17 years (Palmer, 1955; Beaver, 1988) and that of *A. duodenale* 5–6 years (Kendrick, 1934) whereas the maximal duration of infection with *A. caninum* in dogs was recorded by Sarles (1929a) as 100 weeks. Under endemic conditions, where transmission with *N. americanus* and *A. duodenale* may be seasonal, but predictable from year to year, indigenous populations harbour worms throughout life (Hsieh, 1970; Udonsi, 1984). In contrast, repeated exposure of dogs to *A. caninum* elicits a spontaneous self-cure, during which most worms are expelled, leaving a small residual worm burden which continues to produce eggs for a few weeks longer (McCoy, 1931). Although not total, immunity to *A. caninum* is detectable after a single primary infection (Miller, 1966) and may become pronounced in repeatedly infected animals (Otto and Kerr, 1939). Effectively, dogs become immune to infection in early adulthood and no longer susceptible to hookworm disease. The rapid onset of age resistance and acquired immunity in *A. caninum* infection distinguish this species from *N. americanus* and *A. duodenale* and the knowledge gained from experiments with the canine hookworms may not be directly applicable to the human parasites.

In contrast to *A. caninum*, the host-parasite relationships of *A. ceylanicum*, *A. braziliense* and *A. tubaeforme* have received very little attention. In general these species are less pathogenic than *A. caninum* or the human parasites and recent work with *A. ceylanicum* in dogs has demonstrated that acquired immunity is readily generated by primary infection or vaccination with adult worm antigens (Carroll and Grove, 1985a,b). Other models of hookworm infection are less satisfactory, reflecting abnormal host/parasite relationships, but they do offer some advantages over the canine models (e.g., cost, convenience and large sample size) and may indeed reflect accurately some aspects of the host-parasite relationship of the human species. Furthermore, rodent models, in particular, serve an important role in providing hitherto inaccessible stages of the human parasites, such as the lung stages of *N. americanus* (Behnke et al., 1986), for antigenic and biochemical analysis. In the absence of an ideal rodent model for hookworm disease, the murine and hamster systems fill an important gap and should enable some

basic questions to be answered. Perhaps the most pertinent task is to identify the parasite's points of susceptibility. Can adult worms be evicted from the host intestine and if so what sort of host response is required to bring about expulsion? What specific targets (antigens) should a host-protective immune response be directed against? Are responses against the invasive and tissue migratory stages of hookworms host protective? How do the parasites resist potentially aggressive responses by the host and can their evasive strategies be neutralized making the worms more susceptible to immune effector mechanisms?

3. The parasites

3.1. Nutrition and the biochemistry of metabolism

The biochemistry of hookworm metabolism is a uniquely neglected area of their biology. Amino acid metabolism has been studied in *A. ceylanicum* (Singh et al., 1987) and carbohydrate metabolism in hamsters infected with *N. americanus* (Kaul et al., 1982), but there are still enormous gaps to be filled before we have a comprehensive picture of metabolic pathways and species-specific differences among hookworms and their infected hosts.

Hookworms bite deep into the intestinal mucosa and are believed to feed on mucosal tissue and plasma constituents (Wells, 1931; Perez-Gimenez et al., 1967; Kalkofen, 1970), but the details of their food requirements are unknown. Some authors have concluded that erythrocytes pass unharmed through the nematode's gut (Wells, 1931; Roche and Martinez-Torres, 1960). Others have demonstrated lysis of cells in the buccal capsule and intestinal lumen of hookworms (Carroll et al., 1984; Kalkofen, 1970) and hence presumably utilization of the cellular constituents within the food bolus. In vitro studies have shown that *A. caninum* can take up glucose which may be subsequently converted to glycogen (Fernando-Gimenez et al., 1967). *A. caninum* can consume oxygen under both aerobic and anaerobic conditions, but produces proprionate as an end product of metabolism. Warren and Poole (1970) concluded that the glucose metabolism of *A. caninum* was intermediate between that observed for aerobes and for helminth anaerobes. To date most of what is known about hookworm metabolism is based almost exclusively on *A. caninum* and in total the subject has hardly been tackled. Here then is a clear area for future research. The provision of adult *N. americanus* and *A. duodenale*, through the recently developed laboratory models (Sen, 1972; Behnke et al., 1986; Schad, 1979) should broaden the scope for research in this area. The discovery of new,

specific metabolic pathways may point the way to targets for selective chemotherapy or immune intervention and hence to novel approaches for control of hookworms.

3.2. Excretory secretory products

Hookworms secrete a variety of excretory secretory (ES) products some of which are now well recognized (AChE, proteinase enzymes, anticoagulants) and at least one of which has been cloned using recombinant DNA technology (Hotez et al., 1986). Nevertheless, there are still great gaps in our knowledge and appreciation of the function of specific ES products. Perhaps the best known is acetylcholinesterase (AChE) which *N. americanus* secretes in copious amounts (Burt and Ogilvie, 1975). By contrast, *A. duodenale* (Wang Feng-lin et al., 1983) produces only 12% of the amount and *A. tubaeforme* and *A. ceylanicum* negligible amounts. One outstanding question to resolve is why *N. americanus* needs to sustain the intense metabolic effort which must be required to synthesize supplies of the enzyme daily, whilst other species can do without. A possible explanation may reside in the different maintenance behaviours employed by the species, as indicated by the contrasting morphology of their mouthparts. *Ancylostoma* species generally penetrate deeper into the mucosal tissues than *Necator* (Bonne, 1942) and it may be that the latter species depends on the additional holdfast provided by the enzyme through interference with local peristaltic activity of intestinal muscles (Yeates and Ogilvie, 1976).

The proteinase secreted by *A. caninum* has been characterized and similar studies on the human parasites have also revealed a spectrum of different enzymes (Pritchard et al., 1990b) some of which are believed to be species specific. The role of these enzymes will be elucidated over the next few years as pure enzymes become available through recombinant DNA techniques and their properties are better understood. It will be particularly interesting to determine whether any can be neutralized by host immune effectors to the detriment of parasite survival.

The excretion of acetic, propionic and isovaleric acids by *A. caninum* has been reported (Warren and Poole, 1970), but again no information is available for *A. duodenale* and *N. americanus*. The ES products of hookworms make intimate contact with the mucosal tissues and indeed are probably injected into the circulation. The activity of some of these products is gradually being unravelled as cloned gene products become available and this should be a particularly fruitful area for study now that the pioneering studies of Hotez et al. (1985) and Pritchard et al. (1990b) have paved the way.

3.3. Hookworm antigens

Very little is known about the antigens of hookworms although the advent of new analytical techniques with improved sensitivity has rejuvenated interest in this area (Hotez et al., 1985; Carr and Pritchard, 1986; Pritchard et al., 1986, 1990a,b). The molecules comprising the cuticular surface of hookworms, representing the interface between host and parasite, may be critically important in enabling the organisms to interact with and to survive in contact with the mucosa. It has been suggested that specific antibodies directed at epitopes in the surface coat of *N. americanus* may strip some of the components rendering the cuticle susceptible to the proteolytic action of mast cell proteinases (Pritchard et al., 1988; McKean and Pritchard, 1989). Thus the host response is likely to be highly intricate, involving several interacting components which need to be synchronized in a precise way to bring about worm expulsion. The processes involved need to be identified and the details defined to an extent which would permit their replication through vaccination.

The parasite's sensory organs presumably have a vital role to play in maintaining the worms in an optimal environment in the host (McLaren, 1976; Smith, 1974) and it is conceivable that immune responses directed against molecular constituents of the sensory apparatus may also have some host protective value. Receptor molecules in sensory organs are probably seldom exposed to the immune system and therefore remain unrecognized immunologically in infected individuals. However, were the receptor molecules to be isolated and purified, shown to be specific to hookworms and to possess immunogenic properties, then vaccination with the aim of disorientating the parasites through blocking their sensory input may become a viable proposition.

Hidden (cryptic or concealed) epitopes, not normally revealed to the host's immune system, have been exploited to develop vaccines against ticks (Willadsen and Kemp, 1988; Brown, 1988) and even nematodes. An antigen (contortin) on the brush border of the intestinal lining of *Haemonchus contortus* has been found to be immunogenic and to induce protective immunity (Munn et al., 1987). The endotube-brush border complex of *A. caninum* has a number of similarities (Munn and Greenwood, 1983) and it will be extremely interesting to extend these studies to *N. americanus* and *A. duodenale*. Whether associated with the parasite's gut or sensory organs, hidden epitopes could conceivably be utilized to render protection against hookworms and studies in this direction are currently in progress. A possible reservation about a vaccine exploiting hidden epitopes would be that subsequent natural challenge would not provide a boost to the host's immune status in the field. It may be that several booster injections will be required,

spaced over a period of years, as for example in the case of a number of well known classical, non-proliferating, killed vaccines against micro-organisms.

3.4. Evasion of immunity: immunomodulatory factors (IMF) and resistance to immune effectors

The existence of IMF in hookworms is still largely speculative and is based, in part, on the observation that the worms cause chronic infections and therefore, presumably employ some strategies for avoiding host protective responses in the gut. Both *N. americanus* and *A. ceylanicum* have now been shown to cause non-specific immunodepression of responses to heterologous antigens in infected hamsters and both parasites have a potent depressive effect on the proliferative response of lymphocytes from infected animals following stimulation by concanavalin A in vitro (unpublished observations and McKean, 1989; Garside, 1989). If hookworm survival is shown to depend on the manipulation of the intestinal immune system through immunomodulatory molecules, as is believed to occur in *Heligmosomoides polygyrus* infection in mice (Behnke 1987; Monroy et al., 1989), the isolation and characterization of the factors involved would rank as a top priority for research. Two major applications of such molecules are immediately obvious. Chemotherapeutic and immunological interference with the activity of these molecules could be an important prophylactic measure; neutralization of the in vivo activity of immunomodulatory factors may enable host protective responses to occur unhindered. Secondly, the isolation and identification of such molecules may facilitate their synthesis for therapeutic use; locally acting immunomodulatory drugs may be extremely useful in medicine, for example in the treatment of food allergies and other intestinal disorders.

There is also the possibility that hookworms resist immune effectors through other mechanisms. Recent work has suggested a principal role for oxygen radicals (Hughes, 1988) in mediating the expulsion of gastrointestinal parasites, such as *Nippostrongylus brasiliensis* from the parasitized rodent intestine (Smith and Bryant, 1989). Host tissues are generally protected from the consequences of the release of radicals by oxygen scavenging enzymes which ensure that free radicals are short lived. However, parasites have also evolved methods for blocking or overcoming oxidant-mediated host effector mechanisms (Callan et al., 1988) and it is conceivable that hookworms employ such a strategy. The murine parasite *Heligmosomoides polygyrus*, which is not expelled by mice and which causes chronic infections, has 2-times the level of superoxide dismutase, 3-times the level of catalase and 4-times the level of glutathione reductase compared to *N. brasiliensis* (which is rejected by rats) (Smith and Bryant 1986). The inference

from these studies is that the higher content of oxygen scavenging enzymes in *H. polygyrus* enables host effectors of immunity to be resisted. By contrast nothing is known about the oxygen scavenging enzymes of hookworms. This aspect deserves urgent attention and may well provide new insights into the underlying causes of the chronicity of hookworm infections in man and new possibilities for manipulating the parasites. At the very least one conceivable cause of chronicity will have been eliminated if the content of oxygen scavenging enzymes in hookworms proves to be unimpressive.

3.5. The migration and normal/arrested development of hookworm larvae in host tissues

The migration of hookworm larvae through the somatic tissues of the host is yet another fascinating aspect of their biology, which has received some considerable attention over the years particularly by Japanese workers (Komiya and Yasuraoka, 1966), but which has still left unanswered a number of crucially important issues. How do the organisms locate particular organ systems? Are active (Crompton, 1976) or passive (Croll, Matthews and Smith, 1975; Croll and Ma, 1978) processes involved? The mechanisms used by larvae to identify their environment, the triggers and stimuli used to evoke growth and development or particular patterns of behaviour, are areas about which little is known. Canine *Ancylostoma* species adopt arrested development in rodent hosts, such as mice and this enables the parasites to exploit paratenic hosts and to eventually enter the definitive host via the food chain (Behnke, 1990). Even *A. duodenale* can exploit paratenic hosts (Schad et al., 1984), but more interestingly the parasite also undergoes arrested development in man during seasons, such as the hot and arid period in between monsoons, when environmental conditions are detrimental to the development of infective larvae (Schad et al., 1973). Little is known about the mechanisms of arrested development, the signals used by parasites to identify the need to arrest and those to continue normal development. It is also apparent that *N. americanus* does not undergo arrested development, an important point of distinction between the human parasites (Hoagland and Schad, 1978), but with potentially important, although as yet undefined, consequences for the epidemiology of hookworms.

On the other hand, *N. americanus* undergoes obligatory development in the lungs en route to the intestine, whereas the *Ancylostoma* species merely pass through the lungs after percutaneous infection, without any noticeable development taking place. In contrast to *N. americanus*, the *Ancylostoma* sp. are equally capable of establishing in the intestine and of completing normal development locally after oral ingestion (Komiya and Yasuraoka,

1966; Hoagland and Schad, 1978; Behnke, 1990). A thorough understanding of the factors governing parasite activity in host tissues may enable the manipulation of their movement and residence in host tissues, through drugs providing confusing signals interfering with migratory/maintenance behaviour. In any case it will be important to have a full understanding of such aspects of hookworm biology if vaccination is to be effectively employed in the future. The differences between the species augur that attempts to develop a common vaccine will be fraught with difficulties, unless common and indispensible components of the developmental cycle of both species are identified and the effectors of host-protective immunity targetted against them.

3.6. Genetic heterogeneity of hookworms

In common with all organisms, including other nematodes, such as *Trichinella spiralis* (Flockhart, 1986; Pozio et al., 1989; James, 1989) hookworms undoubtedly show some intraspecific heterogeneity. At the most basic level differences in the size of adult worms and variation in other morphological characters are readily apparent (Rep, 1963). Whether such variation has a genetic basis or reflects phenotypic influences has not been determined, although there is evidence that overcrowding in heavy infections reduces parasite fecundity and growth (Krupp, 1961; Nagayuchi, 1956). Polymorphism of genes encoding enzymes remain unstudied and nothing is known about whether hookworms vary from one endemic region to another. Contemporary techniques, such as DNA fingerprinting and resolution of isoenzyme banding patterns on electrophoretic analysis should help to establish whether there are local variations including strains and substrains or whether hookworms have a common genetic repertoire around the globe. It is most unlikely that hookworms will be genetically identical within a species, so the degree of variation and the genetic composition of and differences between populations will need to be defined. The importance of initiating research programs in this area cannot be overstated because differences in antigenic composition, unless foreseen and catered for, may be crucial in limiting the scope of any future vaccines if critical antigens vary between strains and individuals within strains.

4. The host-parasite relationship

4.1. Molecular or defined antigen vaccines

Although a better understanding of parasite antigens would do much to accelerate the advent of a vaccine, there are still important gaps in our knowledge about the host/parasite interactions at the immunological and molecular levels. An effective vaccine, based on the use of irradiated larvae, was introduced to control canine hookworm disease (Miller, 1978), but a comparable product would be unacceptable and logistically impossible to implement for human use and hence the urgency in pursuing research which may ultimately lead to a molecular or defined antigen vaccine (Mitchell, 1984). When appropriate pure preparations of relevant antigens become available, their immunogenic properties will require careful appraisal. The specificity, site of action and the characteristics of the immune responses elicited will need to be examined and their efficiency in protecting against whole parasites evaluated. As was emphasized earlier, there is still no convincing evidence for host protective immunity against hookworms in man, and therefore the protection afforded by putative vaccines must be considerably better than any (if indeed any!) arising during normal exposure to infection, if they are to be successful in radically altering the balance of the host-parasite relationship in favour of man. The reasons for the lack of effective resistance following exposure to parasite antigens need to be thoroughly understood in order to enable suitable antigen engineering to improve on natural immunogenicity. Thus, if fundamental defects in antigen recognition and/or presentation are involved, structural alterations to parasite molecules, the use of synthetic analogues and modifications of the form in which antigens are presented to the immune system may selectively promote T helper function leading to protective immunity. If putative immunomodulatory factors are shown to be instrumental in enabling chronic hookworm infections to persist, structural modifications to these molecules may eliminate the immunodepressive epitopes, whilst enhancing overall immunogenicity, thereby permitting specific host responses to neutralize their effectiveness in vivo.

4.2. The site for vaccine-induced parasite attrition

From a purely theoretical viewpoint, immune responses may be directed against several different stages during in vivo development of hookworms. The infective larval stages of *N. americanus* stay at the skin site, in close proximity to the area penetrated for at least 2 days after infection (Tanabe,

1962; Ishikawa, 1966; Behnke et al., 1986) and in vaccinated hosts there would be ample time for an immune response to trap the worms by secondary cell-mediated immunity. Cutaneous reactions (called Koi Kabure in Japan) to penetrating larvae are common in individuals who have been repeatedly infected by hookworms (Matsusaki, 1966; Ball and Bartlett, 1969; Wijers and Smit, 1966; Areekul et al., 1970) and are unsightly and uncomfortable to the patient, but whether such reactions are associated with protective immunity is still unclear.

An important drawback of a vaccine aiming to induce skin entrapment of infective larvae would be possible circumvention by *A. duodenale*, which can establish equally well following oral ingestion. This is believed to be an important route of infection in some endemic areas (e.g., Japan), but overall the cutaneous route is probably the more common for both species. Thus a vaccine aiming to prevent successful skin entry into the host, may have an important role to play in the future, provided that exacerbation of the undesirable skin reaction to the invasive stages of hookworms was avoided, otherwise it is unlikely to be acceptable.

In order to facilitate the development of such a vaccine, considerably more information will be required on the penetration process adopted by hookworms, the signals recognized by infective larvae to trigger penetration activity, the enzymes employed to aid in passage through the skin, the antigens which may be targetted by host-protective effector mechanisms and the essential (protective) compared with non-essential (possibly harmful, undesirable) components of the host response. If protective immunity cannot be dissociated from overt inflammatory cutaneous reactions, such as dermatitis, the future for a vaccine targetting skin stages of hookworms would be bleak; it would be most unlikely to be approved for human use and unlikely to succeed in competition with anthelmintics.

Studies in animals have revealed that hookworm larvae may be trapped and killed in the lungs (Wilkinson et al., 1990). If the lung response were to be readily inducible and reliably host-protective, without causing excessive inflammation and pneumonia, a vaccine based on this mode of operation may prove to be useful. Whilst offering the advantage of preventing hookworm infection reaching the pathogenic, anemia inducing adult stage, a lung stage targetted vaccine is likely to leave individuals still susceptible to larval invasion and exposure to the soil-fecal contaminants which the larvae carry during the initial stages of infection. In the next phase of analysis of the mouse/*N. americanus* model it will be important to establish precisely how effective the pulmonary response is and how sensitive immune animals are at responding to the very small numbers of larvae that would be expected to enter the lungs during normal exposure patterns in endemic areas.

Finally, a vaccine directed at the intestinal stages of hookworms would

be highly desirable, but there are still important questions to be answered before the feasibility of such a product can be contemplated seriously. Most individuals in endemic areas harbour only a few worms (Croll and Ghadirian, 1981; Pritchard et al., 1990c) and whilst they are probably only marginally if at all disadvantaged, their parasites contaminate the environment and constitute a source of infection for the more susceptible proportion of the population. Hookworm infections in the field show relative stability from one year to the next at the population level (Chandler, 1935; Behnke, 1987), but little is known about the changes in worm burdens in individuals harboring low or high intensity infections, living under conditions where reinfection occurs continuously (Nawalinski et al., 1978). If low intensity infections are controlled through immunological mechanisms operating above a certain threshold, then conceivably the threshold may be lowered by vaccination to curtail worm survival totally. Vaccination, however, may be of little benefit to the 'wormy' proportion of the population if a genetically determined inability to recognize or respond to parasite antigens is responsible for their susceptibility to infection (Wakelin, 1985). Such a finding need not necessarily herald the end of the road for vaccination. There is much interest currently in the development of alternative vaccination strategies with the aim of eliciting immunity in otherwise 'non-responder' individuals. Exploiting hidden (crytic) epitopes may be one way forward, but novel adjuvants (Bomford, 1989) and unconventional vaccination protocols may also provide a solution. In *Schistosoma mansoni* infection in mice, injection of antigen in BCG via the intradermal route stimulated enhanced resistance to percutaneous challenge with cercariae (James, 1987) and since hookworms employ the same route of entry into the host, it is more than likely that common principles apply.

The acceptability of a vaccine against hookworms would depend on the type of response induced and on the severity of pathological side effects. If the only way to expel worms from the intestinal lumen proves to be an overt, whole-organ inflammation of the gut, with accompanying enteritis, diarrhea and discomfort, the frequent induction of these symptoms by individual worms arriving in the gut at intervals in vaccinated persons, would doubtless not be appreciated. On the other hand, were a vaccine to accelerate local host protective responses against individual parasites in the intestine or enhance the secretion of host-protective antibody, preventing the establishment of L4 larvae in the mucosa, its acceptability would be assured provided no unforeseen side effects were to become manifest.

It is anticipated that a successful hookworm vaccine will aim to reduce migrating and developing larvae in the lungs and will impair L4 establishment in the intestine. In both sites specific, but local immune responses may prove to be the most suitable. Immunological studies have shown that

antigens presented via the alveolar epithelium in the lungs (Galvin et al., 1986) or via the lumen of the gut (Cox and Muench, 1984) initiate potent local antibody responses. There is no reason why parasite antigens, once characterized, should not be administered in the same way. Inhalation may prove to be problematic, especially if repeated daily exposure were to be required, but oral administration would be fairly routine. Chemical modification of antigens may reduce proteolytic breakdown and improve the efficiency of uptake across mucosal surfaces without loss of immunogenicity. Studies along the lines of those described by Lim and Rowley (1985) using labelled antigens may aid in tracing the route and subsequent metabolic processing of orally presented and inhaled antigens.

Ultimately, of course, the capacity of locally induced immune responses to protect the host would have to be evaluated. At present this is still a major problem because of the lack of a suitable model for the human hookworms. Nevertheless, some progress can be made using the animal models described earlier and it may be that human trials would have to be contemplated earlier than normal.

Finally it is important to remember that hookworms are encountered mainly among impoverished people with low socio-economic standing, living in environments with poor sanitary standards in tropical and semitropical regions. A successful vaccine would have to be cost effective and must be seen to be a useful addition to established methods of control. Chemoprophylaxis, environmental changes and simply wearing shoes have been recognized for some time as methods which could eradicate hookworm infection if thoroughly applied, but have done little to reduce hookworm disease on a global scale. Hence there may be a place for a novel, alternative strategy and an effective cheap vaccine may have a marked impact on transmission rates if used wisely alongside established measures rather than in direct competition with them.

5. The epidemiology and control of hookworm infections

The epidemiology of hookworm infection in man was a subject of intensive interest in the first half of this century (Chandler, 1935; Sawyer, 1925; Hill, 1926), but was then virtually ignored in the period 1940–1960, until a resurgence of interest in recent years (Yanagisawa, 1966: Banwell and Schad, 1978; Schad, 1978; Schad et al., 1983; Hsieh, 1970; Udonsi, 1984; Croll and Ghadirian, 1981; Haswell-Elkins et al., 1988; Pritchard et al., 1990c). The data collected in field surveys are extremely complex and subject to multifactorial variation which cannot be easily analysed. The devel-

opment of mathematical models and appropriate mathematical analytical techniques for processing the data has been a major and significant advance, facilitating a more comprehensive interpretation of the information which has been accumulated over the years (Anderson and May, 1982; Anderson, 1982; Anderson and Medley, 1985).

A central issue in epidemiological research is still the question of predisposition to infection. Frequency distribution studies of hookworms in affected communities reveal an aggregated distribution of the parasites in people, with the majority of individuals harbouring light infections and a few carrying disproportionally heavier worm burdens (Croll and Ghadirian, 1981; Haswell-Elkins et al., 1988; Pritchard et al., 1990c). It is possible that the latter category are more prone to infection for immunological/ behavioural reasons, but evidence for a genetic basis to these observations is lacking, because in practice it is difficult to obtain, requiring careful monitoring of individuals, families and communities over several generations and analysis of MHC and other cell surface antigens. Various workers have monitored reinfection rates after chemotherapy in order to identify whether individuals heavily infected before treatment acquire heavy worm burdens afterwards. This is still a controversial area because some studies have found no evidence to support predisposition to heavy infection (Ashford and Barnish, 1989), whereas others have concluded that certain individuals identified as heavily infected before treatment, became heavily reinfected subsequently (Schad and Anderson, 1985; Nwosu and Anderson, in press; Croll and Ghadirian, 1981; Haswell-Elkins et al., 1988). The possible role of immunological phenomena in controlling infection with hookworms and the possibility that genetic factors may operate at the immunological level, has not yet been examined thoroughly in epidemiological studies. Immunological analyses of wormy/non-wormy individuals have only recently been initiated (Pritchard et al., 1990c) and the results to date indicate that antibodies against ES products of *N. americanus* and against cuticular antigens of the parasite increase with increasing parasite burden and age, respectively, and probably therefore reflect rather than determine parasite burden or history of exposure to hookworms. These and other studies in the next decade will attempt to focus on the immunological reactions to hookworm infections among people in endemic areas and will attempt to evaluate under field conditions, the relative contribution of host immune responses to the regulation of hookworm populations. It will be particularly important to establish whether heavily infected individuals have the capacity to control worm burdens immunologically, otherwise vaccination may have no significant impact upon transmission rates.

The two human species of hookworms often occur sympatrically and in fact mixed species infections are more common in epidemiological surveys

than single species (Hsieh, 1970; Nawalinski et al., 1978, Haswell-Elkins et al., 1988). Since the eggs of most hookworm species are virtually indistinguishable on morphological criteria under light microscopical examination of fecal smears, the different species are usually identified only after a proportion of the fecal samples have been cultured long enough to produce infective larvae (usually in Harada-Mori (1955) cultures). The relative proportion of each species in infected individuals can then be calculated taking into account the different fecundity of each species. More laboriously, the proportion of each species can be assessed after expulsion chemotherapy. Alternative methods (quicker, less labour intensive and simpler) for distinguishing the parasites might be useful in epidemiological studies. One approach may be to develop monoclonal antibody probes for egg surface antigens, which would then enable the proportion of eggs of each species to be measured directly on fecal smears, through fluorescent antibody tests, eliminating the usual two week waiting period when employing Harada-Mori cultures. Such an approach has been successfully adopted to distinguish taeniid eggs in fecal and soil samples from Kenya (Craig, 1988), and there is already evidence that hookworm eggs can be distinguished from other nematode eggs, but not from each other, using polyvalent sera raised in rabbits (Zaman and Sinh, 1965). Further developments in immuno-diagnosis may include serological tests for identifying the presence of or assessment of previous history of exposure to hookworms, particularly when dealing with human societies unwilling to provide fecal material for analysis.

Another area which is attracting attention is the relative value of targeted, selective or nondiscriminatory chemotherapy in hookworm control. Current epidemiological studies are supported by appropriate mathematical models which predict the proportion of the population in each age class and the number of individuals in the population who would need to be identified and treated in order to achieve the critical reduction in adult worms which would lead to a cessation of transmission (Anderson and May, 1982; Anderson and Medley, 1985). Current field studies comparing and contrasting the various approaches should provide a firm basis on which to select the most cost-effective treatment for hookworm eradication campaigns in the future.

As has already been emphasized, financial considerations are an important aspect of hookworm control. Even if an effective vaccine or the ideal anthelmintic were to become available tomorrow, it is extremely doubtful whether hookworm infection would be brought under control in the foreseeable future, unless appropriate therapeutic efforts were to be backed by a global campaign to improve the low socio-economic standards of the affected communities, a thorough educational campaign with administrative backup to ensure that all those at risk of exposure are contacted and an international effort to finance the entire package, but especially the

required treatment, so that negligible cost is incurred by indigenous people.

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