International Journal for Parasitology Vol. 22, No. 7, pp. 861–907, 1992
Printed in Great Britain

0020–7519/92 $5.00 + 0.00
Pergamon Press Ltd

INVITED REVIEW ARTICLE

UNDERSTANDING CHRONIC NEMATODE INFECTIONS: EVOLUTIONARY CONSIDERATIONS, CURRENT HYPOTHESES AND THE WAY FORWARD

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(*Received* 13 *February* 1992; *accepted* 1 *May* 1992)

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INDEX KEY WORDS: Nematoda; filariae; hookworms; gastro-intestinal nematodes; immunity; evasion of immunity; chronic infections; genetic control of immunity; MHC genes; background genes; immunomodulation; ecdysteroids, oxygen scavenging or antioxidant enzymes; host–parasite arms races; optimal regulatory responses; vaccination; surface antigens; shedding antigens; molecular mimicry; camouflage with host antigens; prostaglandins; eicosanoids; cytokines.

1. INTRODUCTION

MANY of the important nematode parasites of man and his domestic animals cause long-term infections which do not seem overtly to be regulated by host resistance on primary exposure and which are slow to elicit acquired resistance on repeated exposure. Parasitologists refer to such infections as chronic (Behnke, 1987). Among species affecting man, *Necator americanus* is known to live for 15–17 years following a single inoculation of infective larvae and *Onchocerca volvulus* takes 18 years to disappear from human populations in endemic regions, when transmission has been abolished through the elimination of insect vectors (Roberts, Neumann, Gockel & Highton, 1967; Plaisier, van Oortmarssen, Remme & Habbema, 1991). In fact many filarial parasites have the capacity to survive for long periods as adults (see Behnke, 1987 for earlier review; *Dracunculus insignis*—200 days+, Eberhard, Ruiz-Tiben & Wallace, 1988; *Acanthocheilonema viteae*—2 years, Johnson, Orihel & Beaver, 1974; *Breinlia booliati*—17 months+, Ho, Chew, Yap & Singh, 1987; Singh, Yap, Ho, Kang, Lim & Lim, 1976; *Loa loa*—9 years in patas monkeys, Orihel & Eberhard, 1985) and as microfilariae (*Dipetalonema gracile*—60–100 weeks, Eberhard, 1986; *Monanema globulosa*—350 days+, Bianco, 1984). In domestic animals, *Haemonchus contortus* causes infections which last for 10 months and longer. *Trichostrongylus tenuis* lives for 2–3 years in infected grouse, *Lagopus lagopus scoticus* (see Shaw & Moss, 1989a) and birds subjected to trickle infection regimes (Shaw & Moss, 1989b) or acquiring parasites naturally under field conditions (Hudson, 1986) accumulate worm burdens with little indication of immunity (Fig. 1).

There is a pressing need to understand the mechanisms of parasite survival, particularly because of the implications of evasive strategies for vaccination. Conventional vaccines (anti-infection) aim to exploit natural host resistance to parasitic infection by providing the vaccinee with immunological experience of relevant antigens and hence enabling rapid acquired immune responses to be elicited, limiting parasite survival and opportunity for the development of the associated disease. However, this ideal may not prove to be feasible in the case of nematodes. Under natural conditions acquired immunity, on which most conventional vaccines depend, is weak and unlikely to achieve the high levels of efficacy which would be desirable for commercial (and medical) success. As we will show later (see section 2.3), host immune defences may well have evolved to restrict infections within tolerable boundaries rather than to totally eliminate offending pathogens.

As in malaria, alternative strategies may need to be considered including anti-disease vaccines (aiming to curtail the success of the disease-causing stages of the parasite and/or limiting host immunopathological responses) and anti-transmission vaccines (anti-fecundity, aiming to limit the production of transmission stages of the parasite within the vertebrate host; Mitchell, 1984). One further approach which may be applicable to all three of the above categories has focused on so-called cryptic (also known as hidden or concealed) antigens (Willadsen & Kemp, 1988; Munn, Greenwood & Coadwell, 1987) in the hope that artificially induced responses against functionally critical parasite molecules, not normally exposed during infection, may provide an Achilles' heal through which immunity might be achieved. This latter approach aims to exploit the potential of the immune system to improve on naturally acquired immunity and if successful, should produce vaccines which would be independent of the limitations imposed on natural responses by selection (see section 2.3). However, such vaccines would be limited by the absence of natural reinforcement, since by definition they target antigens which the host does not normally experience during infection.

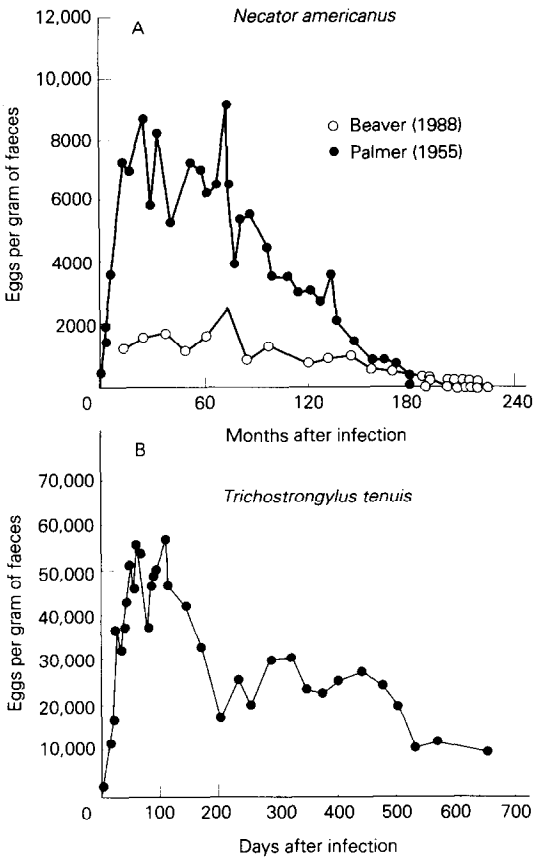

FIG. 1. Examples of chronic nematode infections. A. Longevity of *Necator americanus* in two cases of self infection (data redrawn from Palmer, 1955 and Beaver, 1988). B. The course of infection with *Trichostrongylus tenuis* in grouse, as monitored through faecal egg counts (data redrawn from Shaw & Moss, 1989a).

Two further reasons for understanding the mechanisms of chronic infections are apparent. Firstly vaccines based on defined antigens with proven laboratory efficacy may never work under field conditions since parasite evasive strategies, if not foreseen, may prove to be superior to the immunity induced through vaccination. In fact susceptibility to infection rather than resistance may be increased when inappropriate antigens are used (Harn, Cianci & Caulfield, 1989). Secondly, and optimistically, by identifying from the outset the precise mechanisms of parasite survival it may be possible to develop quite simple procedures to improve on natural immunogenicity in the face of parasite evasion and to allow host-protective responses to develop where they would not do so normally (see section 6).

The potential chronicity of nematode infections is not disputed, but the means through which chronic infections are achieved remain controversial and the last 5 years have seen slow progress in this area. The purpose of this article is to provide a background to understanding the significance of evasive strategies employed by nematodes especially in the light of evolutionary considerations which are often neglected by 'bench-bound' parasite immunologists. Thus we begin by reviewing evolution of the phylum Nematoda and consider the origin of the host–parasite arms race in terms of selection pressures operating on each side. We conclude that the failure of vertebrate hosts to have evolved natural sterilizing immunity against nematode parasites is predictable in terms of optimal resource allocation *vis-à-vis* host fitness and that this in turn may modulate the selection pressure for novel evasion strategies by parasites. We next review the evasive strategies adopted by nematodes and show that these can be understood in an evolutionary context. Finally we predict that there are many strategies remaining to be discovered, none of which are likely to be totally efficient in protecting parasites but which provide through an understanding of the mechanisms involved, the possibility of altering the balance of the host–parasite relationship in favour of the host. We make no apology for some of the speculative suggestions throughout this paper, perhaps exceeding current evidence on occasion: such was our brief. If these serve to rekindle interest in the subject, even if the hypotheses eventually prove unfounded, our objective will have been achieved.

2. EVOLUTIONARY CONSIDERATIONS

2.1. *The Nematoda*

The phylum Nematoda probably has its origins in the marine environment, although not all nematologists support this view. The phylum is divided into two classes, the Adenophorea and the Secernentea, and within each class parasitic forms arose independently (Anderson, 1988a), probably several times (Inglis, 1965; Adamson, 1986, 1987) although the exact number of occasions on which this may have occurred is by no means certain. However, parasitism of animals [some 40% of the 16,000 recognized species are animal parasites (Anderson, 1988a)] is thought to have been adopted only after the terrestrial environment had been colonized since all parasitic forms can be traced to terrestrial ancestors (Anderson, 1984, 1988b). At most it can be concluded, with relative certainty, that present day nematode parasites of vertebrates arose from several independent parasitic lineages.

The parasitic Secernentea are represented by the rhabditid-line and include taxa such as the Spirurida (Filariae), Ascaridida, Strongylida, Oxyurida and

Rhabditida which collectively comprise some 92% of the nematodes parasitizing man and other vertebrates. The rhabditid-line probably evolved from free-living soil forms feeding on bacteria which gave rise to lumen-dwelling organisms feeding on the host's microflora before speciating further and adopting more complex life cycles and preferred tissue sites (Adamson, 1986). Parasitism may have evolved on as many as four separate occasions (Inglis, 1965), although there is little certainty here. The Ascaridida and Spirurida may have had a common ancestor, which began as a parasite of the intestine of terrestrial vertebrates, before radiating into two branches one of which, the Ascaridida, exploited other vertebrates whilst the other, the Spirurida, arthropods for intermediate hosts (Anderson, 1984). The Ascaridida continued primarily as parasites of the small intestine of vertebrates, taking advantage of food-chains to provide suitable intermediate hosts and developing more elaborate life cycles, including migration through host tissues, later, possibly when intermediate hosts were dropped by genera such as *Ascaris*. Predecessors of the Rhabditida, Oxyurida and Strongylida all adopted parasitism separately on land and continued thereafter as parasites of terrestrial invertebrates and vertebrates. The Oxyurida arose as parasites of invertebrates whilst the Strongylida may have commenced as parasites of reptiles (Inglis, 1965). The latter taxon is still similar biologically and morphologically to the primitive rhabditoids and may have been the last of the nematode lineages to take up parasitism or alternatively may have retained some of the primitive features of free-living predecessors. The ancestors of all the Secernentea would have been preadapted to parasitism by the possession of a resistant third larval stage, the dauer larva, which became modified into a skin-penetrating or oral invasive stage (Anderson, 1984) and probably secondarily invaded tissues after initially establishing as intestinal species.

The Adenophorea are subdivided into two major groups, the marine Chromadorida and the Enoplida. The latter is further subdivided into two lineages, the Enoplina (which are predominantly freshwater and marine) and the Dorylaimina (which include many soil-dwelling species that gave rise to four parasitic lines, the mermithoids, the trichinelloids, dioctophymatoids and muspiceoids). Familiar taxa such as *Capillaria, Trichuris* and *Trichinella* are all grouped in the Trichinelloidea. The parasitic taxa are believed to have arisen from terrestrial predatory or plant parasitic, stylet-bearing forms such as the Dorylaimids (Steiner, 1917; Adamson, 1986), in which invasion of animal hosts may have been aided by a modified stylet apparatus. These taxa, originating from ancestors in moist soil environments which were relatively stable, did not have dauer larvae and there was no convenient stage to modify into an invasive larva. Thus, in contrast to the Secernentea, there is no dependence on infective L3 in the parasitic Adenophorea. For example in the trichinelloids the L1 stages became the infective larvae although different larval stages are used by other Dorylaimids. Since none of the parasitic species in this taxa live entirely within the gut lumen, it is suggested that initially they invaded tissues, subsequently coming to be secondarily associated with the intestinal mucosa (Adamson, 1986).

2.2. *The host–parasite arms race*

Evasion strategies appear to have evolved as a result of selection pressure provided by the host's immune system; organisms with better evasion strategies are likely to have had a fitness advantage over those with inferior mechanisms and, through their ability to survive longer, would have contributed their alleles to subsequent generations with greater frequency than organisms contributing alleles for alternative strategies which succumbed earlier to the immune system. Thus the interaction between the immune system, parasites and parasite evasive strategies parallels to some extent that between environmental stressors, free-living animals and their adaptations for life in a particular niche. It is essentially an ecological relationship in which the principal environmental stressor is the host's immune system. In each case, relevant strategies evolved sequentially as animals competed for survival, diversified into distinct genetic lineages and refined their adaptations in the face of continuing competition. The adaptability of parasites to changing circumstances is all too familiar to parasitologists and is clearly reflected in such phenomena as the onset of resistance to anthelmintics (Smith, 1990) and the incorporation of new hosts into parasite life cycles when infective stages are carried to non-endemic regions, populated by suitable alternative hosts (e.g. introduction of *Fasciola hepatica* to Australia; *Schistosoma mansoni* and *Onchocerca volvulus* to South and Central America).

As the ancestors of modern parasites adopted a parasitic existence they would have experienced primitive immune systems. Modern day cestodes, for example, are believed to have evolved from scavenging free-living Platyhelminthes which initially became micro-predators on primitive chordates and then endoparasites (Llewellyn, 1965). As hosts diversified so also parasites speciated in parallel, giving rise to contemporary phenomena such as the existence of closely related cestode groups among closely related vertebrate hosts and heirloom parasites in relict host species. Along with morphological and physiological

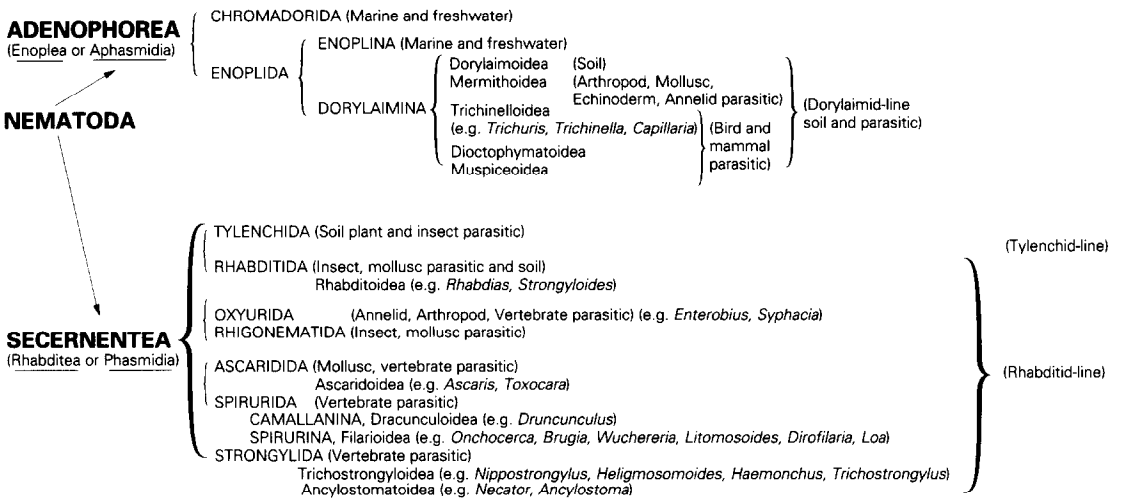

FIG. 2. The relationships between the higher taxa of the Nematoda. The figure is based on Anderson (1984, 1988a), the CIH keys to the nematode parasites of vetebrates (see Anderson, 1988a and Adamson, 1987). The position of genera referred to in the text is highlighted in italics. Alternative names to taxa are underlined in brackets. Note that the list of Secernentean superfamilies is not comprehensive. Only those of relevance to this article have been listed.

changes taking place in both hosts and parasites, strategies for avoiding recognition by the immune system would have been evolved by parasites as the vertebrate immune system itself became more refined in response to the threat posed by diverse micro-organisms as well as parasites. Thus the evolving vertebrate immune system presented an enormous challenge to parasitic organisms but, like many other environmental stressors, it proved susceptible to circumvention and exploitation. In nematodes the distinct parasitic lineages described above would have had some intralineage similarities following evolution of evasive strategies among parasitic ancestors, but contrasting lineage-specific evasive strategies resulting from different solutions among independent parasitic lineages for evading host defences. It is perhaps too early yet to test this prediction rigorously since we have no clear concept of how any single nematode evasive strategy operates but this review will attempt to piece together the existing data and an overall comparison of lineage-specific as well as lineage-shared strategies will be presented. The essential comparisons will be based on the subdivisions of the phylum Nematoda as presented in Fig. 2. In time, as further and perhaps novel strategies are unearthed, it will be necessary to modify the scenario which we present.

It is thus apparent that an arms race exists in which hosts and their parasites are involved in a series of escalating mutual counter-adaptations to exploit or inhibit exploitation (Behnke & Barnard, 1990). Each newly evolved host defence mechanism is likely to be overcome sooner or later by an appropriate parasite strategy reducing its overall contribution to protection of the host. On the other side of the coin, selection will act on hosts refining their defensive mechanisms further, maintaining fitness in the face of novel evasive strategies and exploitation by parasites. Microbiologists who have succeeded in relating distinct mutations in plants and fungi to this arms race refer to the phenomenon as the gene-for-gene theory (Flor, 1971).

2.3. *Elimination or regulation: optimal regulatory responses*

Parasite immunologists, particularly those working on helminths, are often concerned with the need to develop sterilizing immunity in immunoprophylactic treatments both for treatment of the individual and for control of parasites at the population level. This objective is seldom achieved (Abraham, Grieve, Mika-Grieve & Seibert, 1988) and may be beyond the scope of existing vertebrate immune systems as designed by nature to cope with natural exposure to parasites. The drive for sterilizing immunity has arisen because parasitic helminths such as nematodes are considerably more obvious than viruses or bacteria and even a few persisting individuals are often very apparent and likely to be interpreted as indicating the failure of treatment. For this reason, if anti-nematode vaccines are to succeed in medicine they will need to induce sterilizing immunity, so that the efficacy of treatment

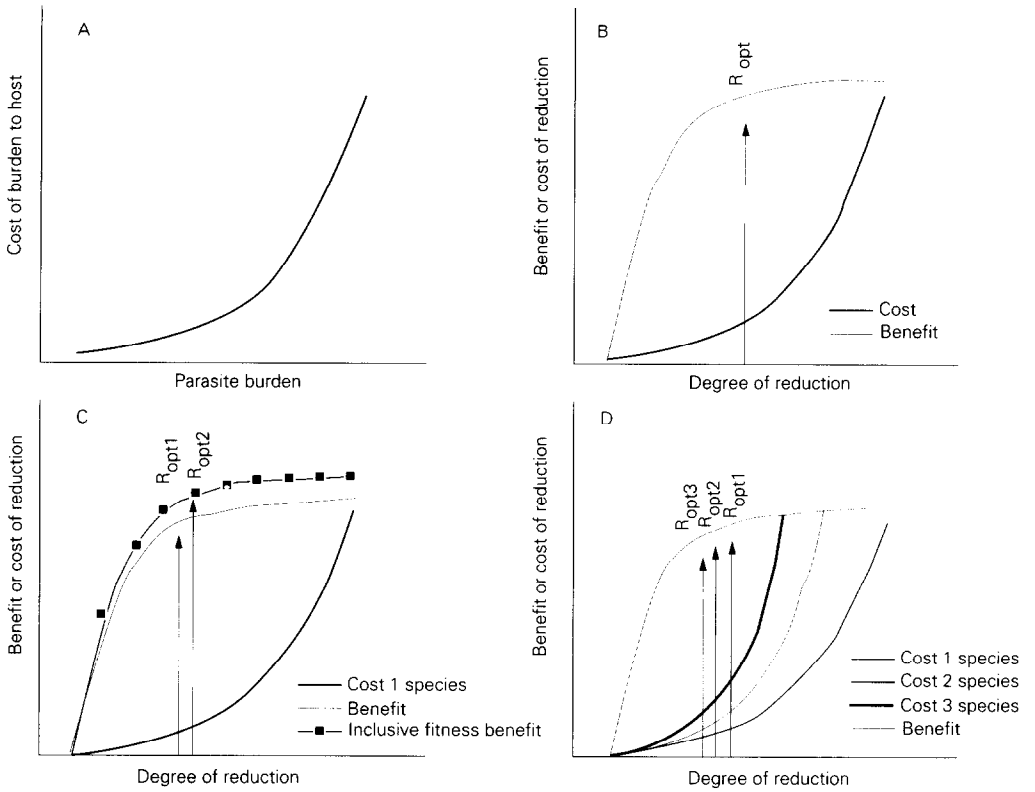

FIG. 3. A. A hypothetical cost function curve for increasing parasite burden. The cost to the host is assumed to increase disproportionately with increasing burden (see text). B. Hypothetical costs and benefits accruing to the host from different degrees of reduction in parasite burden. R_{opt}, degree of reduction conferring maximum benefit: cost ratio. C. as B but allowing for inclusive fitness advantages in reducing reservoirs of infection (see text). D. as B but taking into account effects of reducing the burden of one parasite on the ability to combat others (see text).

could not be misinterpreted by patients or health care personnel. The failure of the vaccine against canine hookworm disease is a case in point (Miller, 1971). Even if a vaccine were to cause significant reduction in morbidity, nothing less than total parasite eradication is likely to convince sceptics that such treatment has a role to play in human and even veterinary medicine.

This may be a vain objective, however, because the natural immune response on which vaccines depend may not be designed to produce sterilizing immunity. Indeed, the scarcity of sterilizing immunity among host species in nature suggests that it is rarely favoured by natural selection. At first sight this may seem surprising since we might expect the host's survivorship and reproductive potential to increase in relation to the degree of elimination of a parasite. This is likely to be a naive expectation, however, because it ignores both the cost of elimination to the host and the fact that the cost of infection and thus the benefit of attacking the parasite may not be a simple linear function of parasite burden.

In malaria, especially in *P. falciparum*, it is now recognized that much of the pathology is generated through overzealous production by the host of particular cytokines, whose primary objective is to mediate protective immunity (Clark, Hunt & Cowden, 1986; Clark & Howell, 1990; Clark, Rockett & Cowden, 1991). The acute intestinal response to nematode infections also involves major alterations in organ function (Castro, 1990; Castro & Bullick, 1983) and temporary disturbances which can be life threatening. Rats infected with *Nippostrongylus brasiliensis* or *Trichinella spiralis* may show inappetence (Crompton, Walters & Arnold, 1981; Castro, Copeland, Dudrick & Ramaswamy, 1979; Ovington, 1987) and consequent weight loss (Crompton *et al.*, 1981; Zohar & Rau, 1984), become less active and lethargic, and in extreme cases may die during the period when the intestinal inflammatory response is at its peak. In the wild, this would have important consequences for

survival: host-protective immunity may be costly.

In regions where Bancroftian filariasis is endemic, sensitivity to parasite antigens in a minority of cases is associated with pathology, notably elephantiasis (Ottesen, 1984). Such patients are almost invariably amicrofilaraemic and both microfilariae and adult worms are hampered if not destroyed by a combination of antibody and cell-mediated responses (Connor, Palmieri & Gibson, 1986; Nutman, 1989). It would appear, therefore, that in filarial infections, invoking a host-protective response to limit parasite development is costly in terms of the pathology generated by relevant host effector mechanisms. However, many indigenous people remain clinically asymptomatic with minimal detectable parasite-specific antibody and cellular activity despite the presence of adult worms and associated microfilariaemia (Ottesen, Weller & Heck, 1977; Ottesen, Weller, Lunde & Hussein, 1982; Piessens, McGreevy, Ratiwayanto, McGreevy, Piessens, Koiman, Saroso & Dennis, 1980). Tolerance induced prenatally or as a result of antigens in breast milk (Petralanda, Yarzabal & Piessens, 1988) as well as parasite-mediated immunodepression may play their roles in down-regulating host responses in such patients (Nutman, Kumaraswami & Ottesen, 1987) but, from the evolutionary perspective, it is conceivable that the cost to the individual of responding to infection, particularly in terms of impaired reproductive potential, may be sufficient to confer a selective advantage on those who moderate their response with the resultant spread of alleles for poor responsiveness and/or susceptibility to tolerance induction among local populations. It is pertinent that few immigrants from non-endemic regions, who would not have been subject to comparable selection pressure for poor responsiveness to filarial antigens, show microfilariaemia even after prolonged exposure but many develop elephantiasis (Beaver, 1970; Trent, 1963; Connor *et al.*, 1986).

If we consider the cost of infection by a given parasite to be a reduced probability of the host reproducing due to the loss of metabolic resources and parasite-induced pathology, it is reasonable to suppose that, in many cases, the cost will be an accelerating curvilinear function of parasite burden (Fig. 3a). Increases in burden when the overall burden is low make little difference to the host's prospects of reproducing. As the burden increases, however, resource loss and/or parasite-induced pathology mean that further increases are likely to be very detrimental to the host. Cost functions of this form mean that a law of diminishing returns is likely to operate on the benefits of attacking the parasite (Fig. 3b). Thus at high burdens, when the cost of infection is high, the benefit of a given reduction in burden is correspondingly high so that the slope of the benefit curve is initially steep. As the burden, and thus the cost to the host, decreases, the benefit accruing from the same degree of reduction also decreases and the slope of the benefit curve begins to decline.

Simply looking at the benefit to the host of reducing its parasite burden, however, ignores the cost to the host of the reduction effort itself. There may be several components to the cost of reducing a given parasite burden. Here, we shall assume expenditure of metabolic resources, host-induced pathology and compromised response to other parasite species. The cost curve in Fig. 3b assumes that the cost in these terms of a small reduction in burden is low but increases with the degree of elimination. The ratio of the benefit and cost curves at any point determines the net benefit to the host of different degrees of reduction in the burden. Since natural selection will favour the degree of reduction that maximizes net benefit, the optimal response may turn out to be moderate rather than total elimination (Fig. 3b). Changes in the form of the benefit and/or cost curves will shift the optimal response towards greater or lesser reduction.

Several factors, of course, may affect the form of the cost and benefit curves. The simplistic example discussed above considered benefits and costs only from the point of view of the reproductive success of the infected individual. While incomplete elimination may be the optimal response in these terms, such a strategy leaves a residual infection which may be transmissible to other potential hosts. If vulnerable hosts include relatives of the infected individual, inclusive fitness (Hamilton 1964a,b) benefits may accrue from reduction beyond the individual host's optimum, resulting in an increase in the optimal response (Fig. 3c). Two points thus emerge here. Firstly, optimal responses for individual hosts may fall far short of elimination and thus maintain transmissible reservoirs of infection within populations. Secondly, this reservoir may be reduced if there are inclusive fitness advantages to reducing the risks to uninfected conspecifics.

As we have already indicated, the cost of reducing the burden of one parasite may include a compromised ability to respond to others. The resources available to a host to combat parasites are likely to be limited, resulting in an adaptation budget (Dawkins & Krebs, 1979) which has to be allocated according to competing demands from different parasite species (Behnke & Barnard, 1990). Increasing expenditure against one species is thus likely to detract from expenditure against others, potentially increasing the cost to the host in terms of reduced reproductive success. The cost of reducing the burden of any given

parasite is therefore likely to increase with the number of other parasites exerting demands on the response budget (Fig. 3d). Furthermore, different individuals are likely to have different R_{opt} points for any given parasite because the costs and benefits of responding will vary with, for example, sex, age, social status, other parasites, etc. Variation in parasite burdens is thus predictable in terms of phenotype-limited optimal responses as well as other classical mechanisms (variation in exposure, genetic variation in innate and acquired resistance, etc.).

The above argument suggests that a limited response to infection may arise because hosts trade off the costs and benefits to themselves of different degrees of response. It may be, however, that a limit is imposed not by the relative costs and benefits to the host, but by the inability of the host to reject the parasite even though it would benefit by doing so. As Behnke & Barnard (1990) point out, the arms race between host and parasite is an asymmetric one (Dawkins & Krebs, 1979) in which there may frequently be a built-in advantage, for instance through shorter generation times, to the parasite. As a result, parasites may experience lengthy periods of grace before hosts counter-adapt, causing departure from the optimum host response. Attempts to stimulate natural immunity by vaccination during such periods of grace may well be fruitless.

It is therefore evident that it may be beneficial to have a selection of available defensive strategies at a low degree of alert which nevertheless maximize reproductive output despite the penalty of carrying some parasites and associated loss of metabolic resources. By aiming to limit pathology rather than to invoke mechanisms capable of sterilizing immunity, the energy budget of the host can be distributed more widely leaving fewer holes in the overall repertoire of defence. It is this important interpretation of the role of the immune system *vis-à-vis* host fitness which is frequently misunderstood by those seeking to develop vaccines against helminths. The vaccine against *Ancylostoma caninum* was totally effective in eliminating the principal pathological consequences, haemorrhagic anaemia, of canine hookworm infection but was unsuccessful commercially because it was non-sterilizing, vaccinated animals continuing to carry a few worms and pass eggs. This was erroneously interpreted as indicating its failure to control the disease (Miller, 1971). It is important to stress here that while the lack of sterilizing immunity as a yardstick for failure of a vaccine can be criticized on the basis of evolutionary arguments, this has most force in disease management at the individual level. However, if part of the goal of the vaccine is eradication of disease at the host population level, then non-sterilizing immunity, or at least reduction of parasite burdens below the threshold needed to maintain transmissibility, may be a legitimate criterion.

2.4. *Evolution of evasive strategies*

The pressures for the evolution of evasive strategies can be compared to the development of anthelmintic resistance, a topic which has received considerable attention in recent years because of its importance to both human and veterinary medicine (Smith, 1990). As with drug resistance it is assumed that evasive strategies are costly to the parasite, since they are likely to divert available resources away from reproduction. The expression of alleles encoding evasive devices is therefore likely to be governed by the overall fitness advantage they confer and, in the absence of a threat from the immune system, their frequency in the population would decline. The rate at which the frequency of alleles for resistance to anthelmintics (or in this case evasion strategies) increases in the population is known to increase as the frequency of treatment and the proportion of the target population exposed to the anthelmintic increase. Thus in relation to evasion strategies, the stronger the immune response the more likely alleles for evasion will appear sooner and spread throughout the population. It follows from this that an inefficient immune system will be less encouraging for the evolution of evasive strategies than a more efficient system and therefore the larger the proportion of non-responders in the population (see later) the less the pressure for evasive strategies. The same relationship should hold true for tolerance of increasing residual worm burdens.

Another conclusion from studies of drug resistance may also be applicable here. It appears that drug resistance develops sooner in trichostrongylid nematodes, which have both free-living and parasitic stages in the life cycle, when the proportion of the worms outside the host is low. Unfortunately, no drugs with high efficacy have been available until recently for treatment of filarial nematodes which have no external free-living phase and there is little evidence for drug resistance. However, the recent introduction of ivermectin will predictably result in the rapid generation of resistance among filariae, more so than among other species, because there is no free-living reservoir of infection in this group. It is likewise tempting to speculate that evasive strategies should be more efficient in this group for the same reasons and indeed this generalization does appear to hold true: the filariae are renowned for the chronicity of the infections they cause and for the success of their survival strategies in the face of host resistance.

3. MECHANISMS EMPLOYED BY NEMATODE PARASITES FOR EVADING HOST IMMUNITY

Whilst a number of potential evasive strategies have been described for nematode parasites there is still no irrefutable evidence that any, apart from immunomodulation, play a significant role in evading host immunity.

3.1. *Shedding surface components of the cuticle*

With the application of radio-iodination to the study of surface antigens of nematodes it has become evident that the cuticle is not entirely inert as previously believed (Philipp & Rumjanek, 1984). Surface components are shed when labelled parasites are maintained *in vitro* and if these constitute targets for specific antibody through which cellular cytotoxic mechanisms may surround and destroy parasites, their potential role in aiding survival is not difficult to appreciate. However, if such a mechanism is to function *in vivo*, it requires continuous synthesis, transportation, expression on the surface and shedding. If this cycle were to be inhibited or merely slowed down, the worms should become susceptible to host effectors. However, it is not at all clear from published work on nematodes whether shed antigens are replaced after shedding (Table 1). A comparable mechanism is known in *S. mansoni* where initial *in vitro* estimates of the half life of surface tegumental proteins were considerably shorter (Wilson & Barnes, 1977) than those indicated from *in vivo* studies based on the period necessary to allow worms to adapt to new hosts following transplantation (Smithers & Terry, 1976). However, experimental studies which allowed accurate quantification of shedding of isotopically labelled surface proteins *in vivo*, subsequently confirmed that indeed the half life of surface tegumental proteins was of the order of 5.4 days (Saunders, Wilson & Coulson, 1987), considerably longer than intuitively would seem to be compatible with tegumental turnover as a principal defence against offensive cellular activity.

3.1.1. *Intestinal species.* Surprisingly few nematode parasites have been examined with respect to shedding of cuticular antigens. The initial studies on *T. spiralis* (see Philipp, Parkhouse & Ogilvie, 1980), *N. brasiliensis* (see Maizels, Meghji & Ogilvie, 1983) and *Toxocara canis* (see Smith, Quinn, Kusel & Girdwood, 1981; Maizels, Desavigny & Ogilvie, 1984; Badley, Grieve, Rockey & Glickman, 1987) demonstrated loss of surface label *in vitro* over the course of some 20 h but whether surface proteins are replaced *in vivo* in these species is still not known. Moreover, the relevance of surface antigen shedding in lumen-dwelling species is questionable. Such a mechanism can conceivably serve a purpose in removing anti-cuticular antibodies, but there is no evidence that surface-specific antibodies pose a threat to worms such as *N. brasiliensis* or *H. polygyrus*, which as adults live entirely within the gut lumen.

However, two recent studies provide support for a role of antibody in the gut lumen in host protective immunity. In a comparison of immunoglobulin class-specific antibody responses to *T. spiralis* antigens, a prominent serum IgA response to surface antigens was shown to correlate with the onset of worm expulsion (Almond & Parkhouse, 1986). The extent to which parasite-specific serum IgA participates in luminal events is not clear, but *T. spiralis* is partially intracellular, living within tunnels created in a syncytium of cytoplasm surrounding the parasites following local breakdown of enterocyte cell boundaries. It is possible that serum antibody therefore has some direct access to worms, without the need to recirculate through the hepatobiliary system in order to gain access from the lumen. Secondly it has also been shown that mucosal mast cell proteases can digest nematode collagens (McKean & Pritchard, 1989). On this basis it has been proposed that parasite-surface-specific antibody may encourage the loss of the outermost cuticular components revealing unprotected deeper underlying elements now susceptible to proteolytic damage (Pritchard, McKean & Rogan, 1988).

It is easier to appreciate the significance of cuticular shedding in the biology of tissue migratory stages of nematodes. Hookworm larvae are often quoted as having the capacity to shed surface antigens, but to date only one species, *Ancylostoma caninum* has been examined in this context. Antibodies to the surface determinants of *A. caninum* L3 bind to frozen worms but not to live parasites. However, binding can be demonstrated on live worms which have been inhibited by azide (Vetter & Klaver-Wesseling, 1978). Although adherence of inflammatory cells to L3 has been demonstrated *in vitro*, there is considerable doubt whether the latter actually play a significant role in protection *in vivo*. Live, exsheathed larvae activate complement by the alternative pathway and bind C3 to their surface (Klaver-Wesseling & Vetter, 1979). Leukocytes are attracted to C3 covered larvae and adhere to their surface but cellular binding is considerably enhanced in the presence of antibody. This interaction between C3 and antibody is synergistic because neither component alone is capable of mediating as intense cytoadherence as when both are present (Klaver-Wesseling, Vetter & Schoeman, 1982). It is also likely that C3 somehow stabilize the adherence of antibody to the cuticular surface since antibody alone will not bind to live worms. Under optimal conditions *in vitro*, several layers of leukocytes will accumulate around larvae, but the worms remain active for 5 h or more, indicating that even when surrounded with adherent effector cells, they still remain resilient enough to resist lethal damage. These *in vitro* studies have to be considered in the light of what is likely to take place *in vivo*. *A. caninum* larvae migrate through the tissues to the intestine and may arrive there as soon as 5–6 h after penetration. On route they pass through blood capillaries attracting

TABLE 1—SPECIES OF NEMATODE PARASITES IN WHICH SHEDDING OF SURFACE CUTICULAR ANTIGENS AND/OR SURFACE ADHERENT SPECIFIC OR ADSORBED NON-SPECIFIC IMMUNOGLOBULINS HAS BEEN DESCRIBED

Species	Microfilariae	L2	L3	L4	Adult worms	References
SECERNENTEA						
SPIRURIDA						
Mf without sheaths						
Onchocerca cervicalis	Yes(Sp.Ig)‡	NA				Edwards *et al.*, 1990
Acanthocheilonema viteae	Yes (Sp.Ig)‡	NA				Gatrill *et al.*, 1991
	Yes(14.5 & 30 kDa)	NA				Apfel & Meyer, 1990
Dirofilaria immitis		NA	Yes (6 & 35 kDa)†			Ibrahim *et al.*, 1989; Scott, Ibrahim & Tamashiro, 1990;
	Yes (Non-Sp.Ig)‡ No (Sp.Ig)	NA				Hammerberg *et al.*, 1984
					Yes*	Scott *et al.*, 1988
Mf with sheaths						
Brugia malayi	Yes (30, 55, 150 kDa)	NA				Egwang & Kazura, 1987
		NA	Yes (Sp.Ig)‡			Carlow *et al.*, 1987
		NA			Yes (29 kDa)	Maizels *et al.*, 1989; Selkirk *et al.*, 1990
		NA			Yes (15, 29, 51 kDa)	Kwan-Lim *et al.*, 1989
Brugia pahangi		NA	Yes (67 & 94 kDa)	No	No (30 kDa)	Marshall & Howells, 1986
		NA			Yes (30 kDa)*	Devaney, 1988
	No (Sp.Ig)	NA				Hammerberg *et al.*, 1984
Wuchereria bancrofti	Yes (17 kDa but also secreted)	NA	Yes (17 kDa)			Maizels *et al.*, 1986
Litomosoides carinii	Possibly (55 kDa Ag is also secreted)					Philipp *et al.*, 1984
ASCARIDIDA						
Toxocara canis	NA	Yes‡				Smith *et al.*, 1981; Maizels, Desavigny & Ogilvie, 1984; Smith, Kusel & Girdwood, 1983
STRONGYLIDA						
Nippostrongylus brasiliensis	NA	NA			Yes (70–300 kDa)	Maizels *et al.*, 1983
Heligmosomoides polygyrus	NA	NA			Yes	Pritchard, Crawford, Duce & Behnke, 1985
Ancylostoma caninum	NA	NA	Yes‡			Vetter & Klaver-Wesseling, 1978
Angiostrongylus cantonensis	NA	NA	Yes		Yes*	Shih & Chen, 1990
Haemonchus contortus	NA	NA	No		Yes (143, 30, 58, & 81 kDa)	Rhoads & Fetterer, 1990

RHABDITIDA					
Strongyloides ratti	NA	NA	Yes—ensheathed infective L3		
			No—parasitic L3		Murrell & Graham, 1983
Strongyloides stercoralis	NA	NA	Yes— ensheathed infective L3 (12–150 kDa)		Brindley, Gam, Pearce, Poindexter & Neva, 1988
ADENOPHOREA		L1			
Trichinella spiralis	NA	Yes (55, 105 kDa)		Yes (20, 33, 40, 56 kDa)	Philipp *et al.*, 1980
Trichuris muris	NA			Yes	Roach, 1986 (unpublished PhD thesis, University of Nottingham)

*Slow release, over several days.
Non-Sp.Ig, non-parasite surface-specific immunoglobulin; Sp.Ig, parasite surface-specific antibodies.
†Once lost, not replaced.
‡Rapid release, in hours, major loss under a day.

the response described above, but as parasites move through fine capillaries and force their way out of blood vessels in the lungs, the consequent abrasion is likely to dislodge adherent cells although the degree to which this could occur has not been evaluated. Thus the necessity of stabilizing surface antigen via C3 in order to allow antibody-dependent cellular adhesion and the intense activity of the migrating worms probably combine to make the response described *in vitro* ineffective in destroying all but a few larvae, possibly those which have run out of energy reserves and become exhausted before they could recommence feeding (Behnke, 1990). Once the worms pass out into the trachea and migrate to the gut lumen they are likely to be safe from the potentially damaging effects of adherent cells.

These *in vitro* studies of cytoadherence to *A. caninum* larvae have not been repeated on other hookworm species. Cytoadherence has, however, been described between human peripheral blood eosinophils and exsheathed larvae of *Necator americanus* (Desakorn, Suntharasamai, Pukrittayakamee, Migasena & Bunnag, 1987). Here adherence appears to be solely C3 mediated, since heat-inactivated sera were unable to mediate significant adherence. Experiments using immune sera taken from mice exposed to *N. americanus* have extended these observations. Peritoneal leukocytes adhered to ensheathed larvae of *N.americanus* and complement was considered to be more important than antibody in mediating cytoadherence (Wells, 1987, unpublished PhD thesis, University of Nottingham). Studies are currently under way to examine other species of hookworms, since it is important to know which of the above is more representative of hookworms in general.

Among the most impressive changes in surface structure are those described for the tissue-resident L2 stages of *Toxocara canis* (see Grieve, 1990). These larvae can secrete a cuticular coat of glycoconjugate which is continually synthesized and lost from the surface (Badley *et al.*, 1987). Therefore antibody specific for this surface binds only to frozen or azide-inhibited larvae (Smith *et al.*, 1981). On exposure to host leukocytes, whole sections of surface coat may be shed (Rockey, John, Donnelly, McKenzie, Stromberg & Soulsby, 1983) and in the case of eosinophils, despite degranulation on the parasite surface, cells eventually drop off leaving L2 apparently unharmed (Fattah, Maizels, McLaren & Spry, 1986). The *in vivo* success of the evasion strategy of *T. canis* is attested by the ability of larvae to migrate out of granulomatous murine livers (Abo-Shehada, Al-Zubaidy & Herbert, 1991).

3.1.2. *Tissue inhabiting species.* Loss of surface cuticular antigens has also been reported in filariasis. Surface components are now known to be released from the cuticle of several species (Table 1) and there are similarities as well as clear differences within the group of the spectrum of antigens and their properties.

3.1.2.1. L3 and L4 surface antigens. A number of elegant studies have shown that the mammalian infective stages of filarial parasites undergo radical changes within days of entry into the host and that these are associated with changes in their susceptibility to host effectors of resistance.

Marked differences in the surface properties of *D. immitis* L3 and L4 have been described by Abraham, Grieve & Mika-Grieve (1988) who proposed that this change in the antigenicity of the parasite surface serves to delay potentially destructive immune responses (Grieve, 1990). Infective L3 shed antigens but these were not resynthesized and following experimental infection of rats 10–20% of the labelled molecules were shed per day so that by the time of the L3–L4 moult, little of the original label remained. Since the 35 kDa antigen was the dominant surface protein and effectively represented the only antigen recognized by immune sera, the larvae gradually lost antigenicity as they grew and prepared for the next developmental stage. After moulting a new set of antigenically distinct molecules was presented to the host (Ibrahim, Tamashiro, Moraga & Scott, 1989). Not surprisingly L3 larvae of this species did not absorb canine albumin or immunoglobulins onto the surface. However, despite the labile surface, mouse peritoneal exudate cells were attracted to L3 in culture and adhered to the cuticle but no killing was detected and most larvae survived long enough to moult to the L4 stage which no longer attracted cells (Abraham, Grieve & Mika-Grieve, 1988). Once the moult had been completed, L4 reacted strongly with anti-canine Ig and anti-albumin, suggesting that host molecules were adsorbed onto the surface or that host-like epitopes were expressed by this stage. It would appear then that *D. immitis* L3 have a dominant surface antigen which attracts antibody and leukocyte activity but which is shed over the course of 48 h, presumably preventing the cells from invoking their cidal mechanisms long enough to impart irreversible damage and providing the parasite with an opportunity to grow into the next developmental stage. Once this crucial stage has been successfully negotiated the evasive strategies switch to dependence on host-like epitopes and camouflage.

Interestingly, similar observations have been made on *O. volvulus*. Although the surface of L3 is thought to be poorly immunogenic in humans despite seven detectable cuticular antigens (Titanji & Mbacham, 1990; Taylor, Goddard & McMahon, 1986), *in vitro* eosinophils adhered to and killed L3 but not L4 and in

cultures containing both larvae only the former attracted eosinophils (Strote, Brattig & Tischendorf, 1990). To date no evasion strategy has been described for L3/L4 stages of *O. volvulus* but the above findings suggest that a radical change in the antigenicity and/or susceptibility to host cellular attack, such as occurs in *D. immitis* takes place here also. In fact major differences in structure and chemical composition of the cuticle of L3 and L4 stages have been described (Lustigman, Huima, Brotman, Miller & Prince, 1990) but how these relate to greater resistance to host effectors is not known.

The infective stages of other filariae also shed surface antigens in preparation for the L4 moult. The L3 of *B. malayi* shed surface-bound immunoglobulin, detected through immunofluorescence, within 3 h when cultured at 37°C (Carlow, Perrone, Spielman & Philipp, 1987). Loss of surface label was believed to parallel loss of a surface epitope confined exclusively to the late L2 and L3 stages and *in vivo* this was shed within 2 days of infection of mammalian hosts (Carlow *et al.*, 1987). However attempts to immunize with this antigen have been unsuccessful (Carlow, Busto, Storey & Philipp, 1990).

B. pahangi L3 lost 90% of the original radioiodinated surface label *in vivo* in the period between infection and the L4 moult. In contrast surface-labelled L4 and adult *B. pahangi* appeared to have relatively stable surface components and no loss of label was detected over a period of 7–8 days following implantation into recipient jirds (Marshall & Howells, 1986). The infective larvae of *W. bancrofti* shed a 17 kDa antigen (Maizels, Burke, Sutanto, Purnomo & Partono, 1986), but less vigorously than the *Brugia* species and consequently are easily surrounded by human eosinophils *in vitro* which bind through antibody (IgG)- or complement-dependent mechanisms (Higashi & Chowdhury, 1970). Freeze-killed or formaldehyde-treated larvae had lower adhesion scores suggesting that actively secreted antigens were responsible, but treatment with azide had no effect and despite metabolic inhibition, sufficient material was still present to facilitate cytoadhesion. If the 17 kDa antigen was involved in cytoadherence, turnover must have been sufficiently slow and the residence of the antigen on the cuticular surface must have been sufficiently firm to enable effector cells to bind.

In contrast to the above the L3 of *A. viteae* have capacity to resist host effector mechanisms. When implanted into immune jirds in diffusion chambers no killing of L3 was observed until day 10 post-implantation. *In vitro* in the presence of peritoneal exudate cells, no adherence was observed on fresh L3 and not until 4 days in culture. Abraham, Weiner & Farrell (1986) concluded that some developmental changes were required before the larvae became susceptible to killing. Their culture system permitted L3 to develop into advanced L3s by 7–11 days but no further. However, *in vivo* in jirds, *A. viteae* moults by day 7 (Johnson *et al.*, 1974) and it is conceivable that L3 of this species are resistant to cellular killing until changes associated with preparation for this moult are initiated. If so, the relative susceptibility of L3 and L4 stages of *A. viteae* appears to be exactly the opposite of *Onchocerca* and *Brugia* species as described above. In an unrestrained environment *in vivo*, where the worms migrate subcutaneously, the initial resistance of L3 to host effectors, providing protection for a few days after infection, may be all that is required to enable the worms to grow and complete the L3–L4 moult. Worms are active in the subcutaneous site (Court, Stables, Lees, Martin-Short & Rankin, 1988) and host cells would have to adhere tightly enough to prevent them being detached through abrasion as the worms migrate. In contrast in culture or in chambers implanted *in vivo* parasite mobility is greatly restricted and consequently cytoadherence is more efficient. The initial resistance of L3 is intriguing and not yet understood but surface antigen shedding, disguise with host molecules or secretion of immunomodulatory factors may all account for the reported observations.

3.1.2.2. Adult worms. In contrast to the L3 and microfilariae (mf) of some species, the surface of adult filarial parasites appears to be more stable. Scott, Diala, Moraga, Ibrahim, Redding & Tamashiro (1988) found that *D. immitis* released only 0.1% of total surface ^{125}I label in 8 h of incubation. Initial studies on adult *B. pahangi* suggested that surface antigens were stable (Marshall & Howells, 1986), but subsequent work revealed that the 30 kDa surface antigen of adult worms was released albeit relatively slowly (Devaney, 1988). A similar molecule on *B. malayi* which is also slowly released in culture (Maizels, Gregory, Kwan-Lim & Selkirk, 1989) and shared with L4 (Selkirk, Gregory, Yazdanbakhsh, Jenkins & Maizels, 1990) has 42% homology with mammalian glutathione peroxidase (Cookson, E., Baxter, M. & Selkirk, M. E., unpublished data presented at the Spring Meeting of the British Society for Parasitology, 1991, in Liverpool) and may therefore function as an oxygen radical scavenger. The slow release of this molecule may hence relate to its protective role against radical-mediated attack and not antigen shedding as an evasive strategy.

The adults of *D. immitis* and *Brugia* seem to shed surface material very slowly, do not employ masking by host antigens nor do they express host-like antigens

and yet in all cases worms live for a long time. It may be that immunomodulatory strategies are involved or as yet undefined characteristics of surface lipids (Kennedy, Foley, Kuo, Kusel & Garland, 1987). Scott *et al.* (1988) showed that labelled surface peptides were only degraded by proteases after the surface had been treated with chloroform with consequent removal of glycolipids from the surface.

Another tissue nematode *Angiostrongylus cantonensis*, not closely related to the filariae, lives in lung capillaries of rats and this parasite also sheds its surface at a relatively slow rate. Scanning electron microscopy revealed sections of the epicuticle being sloughed off by preadults (Shih & Chen, 1990). However, the rate of release was estimated at only 6% per day, again raising doubts about the usefulness of such a response in the face of a concerted host cellular offence.

3.1.2.3. Microfilarial surface antigens. The mf of *L. carinii* express only one dominant surface antigen with a M_r of 55 kDa (Philipp, Worms, McLaren, Ogilvie, Parkhouse & Taylor, 1984) which is immunogenic in cotton rats following immunization with live subcutaneously administered mf but does not induce a specific serum antibody response in most normally infected patent animals (Lawson, Wenk & Storey, 1989). This molecule may be shed, although this is not clear at present, but a molecule of identical size is actively excreted/secreted by mf and other stages (Philipp *et al.*, 1984). The host may therefore have an opportunity to recognize the antigen or cross-reacting epitopes from earlier developmental stages as well as during the patent period. Its failure to respond normally with circulating mf-surface-specific antibodies may reflect some downregulation of the immune response or alternatively the efficient removal of such antibodies by the enormous quantity of new mf produced daily in the tissue and organ sites such as the lungs (Lawson *et al.*, 1989). During the patent period, mf in the circulation did not bind significant quantities of antibodies, i.e. considerably less than mf within organs such as the spleen, lungs, liver and kidneys (Muller-Kehrmann, 1988).

One explanation for this may be the adsorbed host albumin on their surface (see Table 3) which probably interferes with antibody binding (Court & Storey, 1981; Philipp *et al.*, 1984). It is conceivable that the mf of this species, which are short-lived, depend initially on host albumin to camouflage the dominant surface 55 kDa antigen, but this may be a temporary and unstable association (but note that Court & Storey, 1981 found that mf surface-bound host components were not removed following seven washes). Their survival may also be facilitated by downregulatory mechanisms, which are not yet clear, as well as removal of anti-surface antibodies by massive overproduction of mf stages. Mf with surface camouflage which has deteriorated, and others which were recognized by antibodies before managing to become camouflaged, expose cuticular antigens which facilitate sequestration and destruction in organ sites.

The mf of *O. cervicalis* express a number of low molecular weight surface molecules, some of which are involved in anti-surface immune responses and can be shed together with bound antibody. Using a fluorescent antibody technique Edwards, Busto, James, Carlow & Philipp (1990) showed that loss of surface-bound immunoglobulin was temperature dependent. At 4°C there was little loss of surface immunofluorescence over the course of an hour, but at 37°C most of the label was lost within 30 min.

When *B. pahangi* or *D. immitis* mf were cultured *in vitro* in the presence of non-specific immunoglobulin (i.e. sera from uninfected dogs), temporary attachment was observed only with *D. immitis*. *B. pahangi* mf bound only trace quantities. However, both species reacted with sera from hyperimmunized animals indicating that specific antibodies were capable of making more stable bonds with cuticular antigens (Hammerberg, Rikihisa & King, 1984). Rzepczyk, Bishop & Atwell (1986) observed that when *D. immitis* mf were co-incubated with neutrophils, some mf always remained free of cells as if there were differences in some respect antigenically or possibly through better evasion mechanisms such as more efficient shedding of surface antigens. Interestingly, the mf of neither species had detectable immunoglobulin on the surface when freshly examined from dogs, although *D. immitis* mf were shown to have surface immunoglobulins when examined in saline containing azide, suggesting that in this species non-specific immunoglobulins are temporarily adsorbed and then shed through an active metabolically dependent process (Hammerberg *et al.*, 1984). Perhaps surprisingly Rzepczyk, Bishop & Atwell (1986) found no evidence for adsorbed albumin and Tamashiro, Ehrenberg, Levy & Scott (1986) observed that less than 10% of radioactivity was spontaneously released in a 30 min period of incubation following surface labelling of mf with ^{125}I. It thus appears that the mf of *D. immitis* have a slow surface turnover, do not bind albumin, but bind some non-specific immunoglobulin transiently and still some fail to attract neutrophils in culture. Taken together these observations imply that as yet unknown mechanisms protect the surface of the mf of this species.

Recently two research groups have shown that the microfilariae (mf) of *Acanthocheilonema viteae* lose surface antigens. Apfel & Meyer (1990) found that ^{125}I

labelled surface proteins from microfilariae were shed *in vitro*. Four major surface-exposed proteins in the range 14.5–19 kDa were observed and one of these (14.5) together with a suspected dimer (30 kDa) was shed and replaced continuously on the cuticle of mf. A larger (40 kDa) molecule was also evident on the surface but was less abundant and not released in culture. Post-infection sera from chronically infected jirds did not recognize the surface of living mf, but bound to fixed or azide-inhibited parasites and reacted with shed antigen. Thus *A. viteae* mf appear to shed continuously the surface proteins against which antibody responses are directed, and replace shed material with new protein. In another study (Gatrill, Kee, Behnke & Wakelin, 1991) using immunofluorescence to demonstrate surface-bound antibody, it was shown that sera from C57BL/10 mice, which clear microfilariaemia rapidly, contained surface-specific IgM antibody which bound to the surface of mf, whereas BALB/c mice, which tolerate a chronic microfilaraemia, had surface-reactive IgM antibody which could be demonstrated on the mf surface only when mf had been treated with azide or frozen. At 37°C surface-bound antibody from the sera of BALB/c mice was shed faster than from sera of C57BL/10 mice suggesting that one explanation for the two contrasting response phenotypes of these strains was a difference in the avidity of their IgM responses to surface cuticular determinants. An alternative explanation may be that C57BL/10 mice recognize the more stable cuticular components whereas BALB/c mice respond only to the labile elements which are continuously shed.

3.1.3. *Overview*. The release of surface antigens by microfilariae is easy to appreciate as a very effective survival strategy. In long-lived mf such as those of *A. viteae*, replacement would have to be maintained continuously, but in addition to investing their progeny with evasive devices, female worms replace lost larvae daily and can sustain mf production for very long periods of time. This device, however, is not totally foolproof and specific antibodies can make more stable bonds with the cuticular surface despite shedding, at least in *D. immitis*. In *B. pahangi* non-specific immunoglobulins are not adsorbed and specific antibodies cannot be easily shed (Hammerberg *et al.*, 1984). In the case of L3 stages, antigen shedding allows the parasite a window in an otherwise hostile environment, providing temporary protection in the period to the L4 moult as in *D. immitis*. It is tempting to speculate that resistance to L3 invasion may depend on other antibodies, inhibiting growth and delaying the moult so that effector mechanisms have an opportunity to recognize the underlying, perhaps more stable antigens. However, the significance of antigen shedding by adult worms is less clear. Adult filariae are extremely long-lived and with the exception of *Loa loa* generally reside in the close confines of their specialized sites. For *W. bancrofti* and *Brugia* spp. these are lymphatics. Shed antigen will result in immune complexes accumulating and it is possible that these cause immunodepression locally. However, the secretion, replacement and shedding of the antigens in question would have to be maintained for months and years in the long-lived species and would have to be a fairly dynamic process since slow turnover, as in *S. mansoni*, would seem to be of little value as an evasive device. Whether this is the case or not remains to be determined; there are no data on replacement of surface antigens by adult worms but current studies suggest that the rate of loss of Gp29 from *Brugia* sp. is relatively slow (Selkirk *et al.*, 1990).

From the evolutionary perspective, surface antigen shedding has been reported in both the Secernentea and the Adenophorea, and within the former group in the Rhabditida, Spirurida & Ascaridida, and Strongylida. By comparison the Adenophorea have been poorly studied with information being available for only the two related genera, *Trichinella* and *Trichuris*. Nevertheless antigen shedding appears to be a feature of four nematode lineages in which parasitism has been adopted independently and therefore probably represents an adaptation of an ancestral mechanism evolved before parasitism or reflects a readily adaptable molecular feature of the nematode cuticle shared by all groups. However, because of the variation in experimental approaches adopted by research groups (antigen shedding has been inferred from studies using surface-radiolabelled antigens, loss of fluorescent-labelled specific antibody, loss of antibodies against host immunoglobulins, etc.) it is still not clear whether antigen shedding is a single mechanism or a range of related mechanisms. The situation will not be clarified until inter-species comparisons are completed utilizing each technique in turn. The most apparent common feature is that antigen shedding is frequently encountered in infective stages which soon moult to the L4, where other mechanisms take over, and in transmission stages such as mf, although in each case there are exceptions possibly indicating loss of the mechanism and adoption of alternative strategies.

3.2. *Antioxidant enzymes*

Oxygen radicals are produced within cells as part of normal oxidative metabolism and most aerobic organisms possess a range of oxygen-scavenging enzymes which protect tissues, organs and cells by removing potentially damaging radicals from sites where they could impair organ function. Additionally,

TABLE 2—ANTI-OXIDANT ENZYME CONTENT OF PARASITIC NEMATODES

Species	SOD	Catalase	GSH-px	GSH- tase	References
SECERNENTEA					
SPIRURIDA					
Spirurina, Mf without sheaths					
Onchocerca volvulus	Moderate				Henkle *et al.*, 1991
Onchocerca cervicalis					
Adults	High	Trace	Moderate		
Mf	High	Trace	Moderate		Callahan, James & Crouch, 1988
Onchocerca gutterosa				Present	Pemberton & Barrett, 1989
Dirofilaria immitis					
Adults	V. high	Trace	Moderate		Callahan, James & Crouch, 1988
				Moderate	Jaffe & Lambert, 1986
Mf	V. high	Trace	Moderate		Callahan, James & Crouch, 1988
Spirurina, Mf with sheaths					
Brugia malayi					
Adults	None			None	Kwan-Lim *et al.*, 1989,
			Present		Cookson *et al.*, unpublished (see text)
Brugia pahangi					
Adults				Moderate	Jaffe & Lambert, 1986
Litomosoides carinii					
Adults				Present	Bhargava, Le Trang, Cerami & Eaton, 1983
ASCARIDIDA					
Toxocara canis					
Adults	Present				Maizels & Page, 1990.
	High	None			Sanchez-Moreno *et al.*, 1987
Toxocara cati					
Adults	High	None			Sanchez-Moreno *et al.*, 1987
Toxocaris leonina					
Adults	High	None			Sanchez-Moreno *et al.*, 1987
Ascaris suum					
Adults				Present	Douch & Buchanan, 1978
	High	None			Sanchez-Moreno *et al.*, 1987

STRONGYLIDA					
Nippostrongylus brasiliensis					
Adults	Moderate	Moderate	High		Smith & Bryant, 1986
L3	V. high				Knox & Jones, 1992
Adults	Moderate				Knox & Jones, 1992
Nematodirus battus					
L3	V. high				Knox & Jones, 1992
Adults	Moderate				Knox & Jones, 1992
Ostertagia circumcincta					
L3	V. high				Knox & Jones, 1992
Adults	Moderate				Knox & Jones, 1992
Heligmosomoides polygyrus					
Adults	V. high	Moderate	High		Smith & Bryant, 1986
Haemonchus contortus					
L3	High				Knox & Jones, 1992
Adults	Low				Knox & Jones, 1992
Adults				Moderate	Kawalek, Rew & Heavner, 1984
Trichostrongylus colubriformis					
L3	High				Knox & Jones, 1992
Adults	Low				Knox & Jones, 1992
Trichostrongylus vitrinus					
L3	Moderate				Knox & Jones, 1992
L4	Moderate				Knox & Jones, 1992
Adults	Low				Knox & Jones, 1992
ADENOPHOREA					
Trichinella spiralis					
Newborn larvae	Low	None	None		Rhoads, 1983
Muscle larvae	V. high	None	V. high		
Adults	Moderate	None	Moderate		

Present—amount not reported.
SOD—Superoxide dismutase; GSH-tase—glutathione-S-transferase; GSH-px—glutathione peroxidase.
Unless a reference is given, data taken from Callaghan, Crouch and James, 1988.

free oxygen radicals are generated during the respiratory burst which follows ingestion of pathogens by phagocytic leukocytes and are released into their phagosomes. There is evidence for extracellular release and indeed some of the pathology associated with parasitic diseases is now recognized as being attributable to overexuberant release of oxygen radicals in tissue sites (Clark *et al.*, 1986). Free oxygen radicals damage cell membranes through lipid peroxidation and are believed to be responsible for intraerythrocytic crisis forms in malaria and *Babesia* infections (Clark & Howell, 1990).

Oxygen-scavenging enzymes therefore constitute a natural regulatory system enabling organisms to employ oxidative metabolism whilst being protected from the damaging effects of their by-products. Not surprisingly, pathogens have exploited the system to their own advantage. A range of parasitic organisms including nematodes (Table 2) are now known to possess relatively high amounts of these enzymes compared to host tissues suggesting that they may be employed additionally to protect against free oxygen radical release during host responses (Callahan, Crouch & James, 1988). The most widely studied enzyme is super oxide dismutase (SOD) which has been detected in Secernentean (Spirurida, Ascaridida and Strongylida, sometimes in extraordinarily high amounts; Knox & Jones, 1992; Sanchez-Moreno, Leon, Garcia-Ruiz & Monteoliva, 1987) and in Adenophorean groups (notably *T. spiralis*, see Rhoads, 1983).

Recently, evidence has been provided for a role for oxygen radicals in the gut lumen (Smith & Bryant, 1989a,b). The demonstration that free oxygen radicals may be released in mucosal sites and detected in the intestinal lumen provides yet another potential mechanism for damaging worms in the intestinal lumen prior to or as part of expulsion (Smith & Bryant, 1989a). Treatment of rats infected with *N. brasiliensis* with antioxidants reduced intestinal free oxygen release and inhibited expulsion of worms (Smith & Bryant, 1989b). In this context an interesting comparison has been made between the oxygen-scavenging enzymes of two intestinal nematodes, one of which is expelled by rodents (*N. brasiliensis*) whilst the other causes chronic infections (*H. polygyrus*). In contrast to the former *H. polygyrus* had twice the SOD, four times the glutathione reductase and three times the catalase levels on a per gram basis (Smith & Bryant, 1986) and was shown to be more resilient in the face of free oxygen radicals generated *in vitro* in culture. In view of the demonstration that free oxygen radicals are released in the intestinal tissues, it is conceivable that the resilience of *H. polygyrus* may be attributable to its ability to inactivate the radicals before tissue damage is sustained. *N. brasiliensis* which has a lower enzyme content and which *in vitro* is more susceptible to free oxygen attack may be unable to resist this response *in vivo*.

In a similar vein Knox & Jones (1992) have recently demonstrated that adapted *N. brasiliensis*, which are considerably more resilient in the face of host immunity than normal adults, have significantly higher levels of SOD (30.2 and 10.9 units mg^{-1} protein, respectively). Their study also included five species of ovine GI nematodes (Table 2) and perhaps surprisingly, the highest levels of SOD were observed in infective L3 stages (range 16.3 (*T. vitrinus*) – 79.4 (*O. circumcincta*) units mg^{-1} protein) rather than adults (range 6.1 (*T. vitrinus*) – 12.6 (*O. circumcincta*) units mg^{-1} protein). This finding was attributed to the closer contact with the host mucosa which the larval stages exhibit and the attendant greater risk of damage by host effectors. However, when worms (*T. vitrinus* and *O. circumcincta*) were assayed for release of SOD in culture, adults and L4 stages released significantly higher quantities than L3s, suggesting that parasites can release the enzyme to change the environment in which they live. Isoenzyme polymorphisms between species (see also Sanchez-Moreno, Monteoliva, Fatou & Garcia-Ruiz, 1988 for data on *Ascaris suum*) and in response to immunological stress as reflected in more isoenzymes in adapted adult *N. brasiliensis* indicated that the SOD enzyme system may be modulated by the parasites and may play a crucial role in survival. However, values for all adult worms studied were comparable or marginally lower to those reported earlier (15.3 units mg^{-1}) for *N. brasiliensis* (Smith & Bryant, 1986) and were all much lower than values for *H. polygyrus* (31.6 units mg^{-1}), only adapted *N. brasiliensis* approaching levels of the former species.

H. polygyrus is not totally resistant to host effectors and in mice concurrently infected with *T. spiralis*, adult *H. polygyrus* were expelled during the acute inflammatory phase to *T. spiralis* (Behnke, Cabaj & Wakelin, 1992). Expulsion was more thorough during secondary responses to *T. spiralis* and in fast responder mouse strains relative to slow responders. This observation serves to emphasize that *H. polygyrus* is probably more dependent on other mechanisms for its survival, notably the immunomodulatory strategy considered below. However *H. polygyrus* were seldom lost completely from concurrently infected mice, a few worms always persisted even after *T. spiralis* had been entirely cleared from the intestine. A proportion of *H. polygyrus* is therefore more resistant than *T. spiralis*. It would appear that whilst *H. polygyrus* clearly prevent the inflammatory response being generated in the first place, they also have a second line of defence against elicited host effectors and this may be based on oxygen-scavenging enzymes.

Among filarial nematodes *Onchocerca cervicalis* and *D. immitis* have high SOD levels but extremely low catalase and moderate glutathione peroxidase content in both adult and mf stages (Callahan, James & Crouch, 1988; Weller, Longworth & Jaffe, 1989). *O. volvulus* releases SOD into incubation medium and the gene encoding the enzyme has been cloned (Henkle, Liebau, Muller, Bergmann & Walter, 1991). Catalase and SOD are both required for protection against H_2O_2 and since *O. cervicalis* has only low levels of the former it remains sensitive to H_2O_2 as well as $.OH^-$ and O_2^- mediated damage. The mf of *D. immitis* (Rzepczyk & Bishop, 1984) are also sensitive to H_2O_2 but the picture has been somewhat confused by observations which have established that despite the oxygen burst, neutrophil-mediated killing is effected by a cidal mechanism independent of oxygen metabolites (Rzepczyk, Bishop, Cheung, Atwell & Ferrante, 1986). In support Hopper, Subrahmanyam, Gregory, Nelson, Rao & Chandrashekar (1984) concluded that oxygen metabolites played no role in macrophage- and neutrophil-mediated cytotoxicity to *B. pahangi* and *Litomosoides carinii*. No details of the oxygen-scavenging enzyme content of either of these latter species have been published. However, no evidence was found for either SOD or glutathione S transferase in *B. malayi* (see Kwan-Lim, Gregory, Selkirk, Portono & Maizels, 1989) but on the other hand the major surface antigen of adult worms is believed to be a glutathione peroxidase (see 3.1.2.2).

Apart from SOD, the oxygen scavenging enzyme content of the filarial worms which have been studied is unremarkable and since most appear to be sensitive to H_2O_2, it would appear that the chronicity of filarial infections is dependent on other strategies, perhaps preventing contact with leukocytes or preventing their activation, possibly through an immunomodulatory mechanism.

3.3. *Relocation in the tissues*

Where resistance is mediated locally, as for example at the site of attachment of a parasite, detachment and relocation to a new site may be a temporary solution to avoiding host resistance. The canine hookworm *Ancylostoma caninum* changes its site of attachment at regular intervals, perhaps as frequently as every 4–6 h (Kalkofen, 1970). Unpublished observations from our own studies on hookworms in hamsters confirm that there are considerably more lesions than worms, suggestive of regular reattachment to fresh feeding sites. By moving to a fresh site hookworms escape the local cellular response and the same mechanism probably operates in large ruminants affected with *Haemonchus* and *Ostertagia*. However, sooner or later the host response may develop into an acute involvement of a large proportion of the intestine and relocation in this situation would not provide a satisfactory escape route.

3.4. *Camouflage with molecules of host origin or expression of host-like molecules on the surface: molecular mimicry*

Sprent (1962) originally proposed, on purely hypothetical grounds, that parallel evolution of hosts and parasites should lead to an antigenic similarity between the two species, thereby minimizing antigenic disparity and overall immunogenicity and hence permitting parasites to cause chronic infections. In schistosomiasis two distinct mechanisms have been identified, one involving adsorption of a variety of host molecules on the tegumental surface, including blood group antigens and MHC molecules (Smithers & Terry, 1976), and the other expression on the parasite's surface of schistosome gene products with similarity to host molecules (Damian, 1987). Attempts to demonstrate similar mechanisms in the Nematoda have had mixed fortunes (McGreevy, Ismail, Philips & Denham, 1975). Whilst many studies suggest that host molecules are adsorbed onto the cuticular surface, none have demonstrated unequivocally that nematodes express host-like antigens. Support for the latter strategy requires demonstration of active synthesis of host-like molecules, through evidence for the presence of RNA message encoding relevant genes in nematode cells, as has been done in schistosomiasis.

Among the earliest studies in this area (actually preceding Sprent's hypothesis), were a series of papers by Oliver-González & Torregrosa (1944) and Oliver-González (1946) who found evidence in *T. spiralis*, *A. suum*, and *N. americanus* for the existence of antigens similar to the human blood group substance A. Essentially these studies employed parasite extracts, which were probably homogenates, and used these to neutralize anti-A agglutinins in human sera. Whilst they provide evidence for the existence of host-like molecules in the three species studied, there was no evidence that the molecules were external on the parasite surface. There remains the possibility that structural or other internal molecules provided cross-reacting epitopes which had no relevance for minimizing the antigenic disparity between the parasites and their hosts. Of the original species studied by Oliver-González, only *Ascaris* has subsequently been confirmed as expressing host molecules on its surface, namely albumin (Kennedy & Qureshi, 1986).

In recent years the most commonly used assays have been immunofluorescence, employing reagents against host serum molecules such as immunoglobulins and albumin, and surface radioiodination followed by precipitation with specific antisera against

TABLE 3—SPECIES OF NEMATODE PARASITES IN WHICH EVIDENCE HAS BEEN FOUND FOR THE ADSORBTION OF HOST MOLECULES OR EXPRESSION OF HOST-LIKE MOLECULES ON THE CUTICULAR SURFACE

Species	Microfilariae	L2	L3	L4	Adult worms	References
SECERNENTEA						
SPIRURIDA						
Mf without sheaths						
Onchocerca volvulus	No albumin					Taylor *et al.*, 1986
	Yes, type VI collagen		No, type VI collagen		No, type VI collagen No, albumin	Titanji & Mbacham, 1990
					No, Ig	Engelbrecht, Braun, Connor, Downham, Whitworth & Taylor, 1991
Onchocerca gibsoni	Yes, albumin	NA				Forsyth, Copeman & Mitchell, 1984
Onchocerca lienalis	Yes, albumin					Conraths *et al.*, 1991
Dirofilaria immitis	No, non-Sp.Ig*	NA				Hammerberg *et al.*, 1984
	No, albumin					Rzepczyk, Bishop & Atwell, 1986
			No, non-Sp.Ig	Yes, non-Sp.Ig Possibly albumin or host-like molecules		Abraham *et al.*, 1988
						Abraham, Grieve & Mika-Grieve, 1988
					No, albumin	
					No, Ig	Scott *et al.*, 1988
Mf with sheaths						
Brugia pahangi	Yes, ensheathed, serum proteins					
	No, exsheathed	NA				Sayers, Mackenzie & Denham, 1984
	No, non-Sp.Ig	NA				Hammerberg *et al.*, 1984
					No	McGreevy *et al.*, 1975
Brugia timori	No					Maizels, Philipp, Dasgupta & Partono, 1984
Wuchereria bancrofti	Yes, albumin	NA				Maizels *et al.*, 1984
	Yes					Ridley & Hedge, 1977
Litomosoides carinii	Yes, albumin	NA				Philipp *et al.*, 1984
	Yes					Muller-Kehrmann, 1988
	Yes	NA	No	No	Yes	Court & Storey, 1981
Loa loa	Yes	NA				Ridley & Hedge, 1977
ASCARIDIDA						
Ascaris suum	NA	Yes, albumin	Yes, albumin			Kennedy & Qureshi, 1986
Toxocara canis	NA	Yes, human A & B blood group like Ags				Smith *et al.*, 1983

*No immunoglobulin detected *in vivo* unless treated with azide. *In vitro* non-specific immunoglobulin bound to mf and was rapidly shed. Specific antibodies in hyperimmune sera bound more stably.

Non-Sp.Ig, non-parasite surface-specific immunoglobulin; Sp.Ig, parasite surface-specific antibodies.

host components. Since it is not easy to distinguish between the two mechanisms (adsorption of host or expression of host-like molecules) the following section considers these strategies collectively. The species for which relevant data are available are listed in Table 3 and it is readily apparent that these mechanisms have only been observed in the case of the Spirurida and Ascaridida, considered to be the most ancient of the Secernentean parasitic nematodes, sharing a common parasitic ancestor. It is thus tempting to propose that molecular mimicry had a common ancestry in these two groups and that other Secernentean and Adenophorean parasitic nematode lineages have not yet developed comparable strategies. However, a distinction has to be made between the two mechanisms of molecular mimicry because if adsorption of host components is involved, common ancestry, perhaps based on the electrostatic properties of cuticular components, is conceivable. On the other hand, if molecular mimicry involves expression of host-like molecules, based on parasite gene products, common ancestry would only be possible if all the hosts involved shared a crucial set of common epitopes, an unlikely situation. Having made this suggestion on the information which is available to us, we are nevertheless aware that comparable studies may not yet have been attempted on the other groups. Furthermore, workers may have attempted to assess other groups, failed to find appropriate evidence and subsequently not bothered to publish or had rejected what would have been taken for 'negative' findings. Thus the absence of published data for other groups constitutes an impediment to our hypothesis which will only be rectified when the appropriate studies have been completed or when those with 'negative' findings report their results. We hereby encourage workers with pertinent unpublished and presumably otherwise 'unpublishable' data to write to us to enable the present hypothesis to be updated.

In some studies host immunoglobulin has been demonstrated on the surface of parasites. This poses the problem as to whether surface-attached immunoglobulin represents an evasion strategy (possibly through Fc receptors) or a host response to surface determinants (binding through Fab). In this context an interesting comparison between the sheathed mf of *B. pahangi* and the non-sheathed mf of *D. immitis* led to the conclusion that only the latter bind non-specific immunoglobulin to their surface and only when metabolism has been inhibited by the presence of azide. There was no evidence of host albumin on live mf (Rzepczyk *et al.*, 1986) and non-specific immunoglobulin was shed within an hour (Hammerbert *et al.*, 1984), although the loss of radiolabelled surface antigens was considerably slower (Tamashiro *et al.*, 1986; see also 3.1.2.3). It is possible that *D. immitis* employs an active metabolically dependent process to ensure continuous turnover of surface molecules to which immunoglobulins might bind non-specifically (Table 1) but the rate of loss is slow. Interestingly, when mf were incubated in specific anti-mf serum, immunoglobulins bound in a considerably more stable union. Therefore, the device does not protect mf from specific antibodies in hyperimmunized animals, although it is likely that a state equivalent to experimental hyperimmunization seldom arises in nature. The value of shedding surface-bound antibody in this case may be to give mf a temporary window of security by delaying their susceptibility to existing host antibody soon after patency through minimization of the antigenic surface exposed to the host. This combined with continuous production of mf by long-lived adult worms ensures that some are always available in peripheral blood for transmission.

The adsorption of host albumin on the cuticular surface has been singled out for particular attention. Interestingly *Brugia* species do not appear to express host albumin and yet the very similar and closely related *Wuchereria* does (Maizels, Philipp, Dasgupta & Partono, 1984). Similarly *O. gibsoni* mf express host albumin as do *O. lienalis* (see Conraths, Worms, Preece, Harnett & Parkhouse, 1991) but *O. volvulus* do not (Table 3). These differences may be explained by a secondary loss of the mechanisms enabling adsorption in genera such as *Brugia* or alternatively changes in the surface properties of the species which adsorb host molecules but this would entail the same mechanism evolving on more than one occasion within the Spirurida.

A possible explanation for some species being able to adsorb host components and others not links this property to surface charge. *B. pahangi* which does not adsorb host albumin has a net negative surface charge whereas *D. immitis* which shows transient adsorption when metabolism has been inhibited, has a neutral surface charge (Abraham, Grieve, Mika-Grieve & Seibert, 1988; Hammerberg *et al.*, 1984). The infective larvae of some Rhabditid (*S. ratti*) and Adenophorean taxa (*T. spiralis*) also show a net negative surface charge (Murrell, Graham & McGreevy, 1983). Indeed, it has been suggested that a negatively charged cuticle may help nematodes resist desiccation by attracting moisture to the surface of the cuticle, as for example among free-living species, infective larvae on vegetation and even infective larvae of filariids released by insect intermediate hosts. Thus species which exploit host albumin on their cuticle may be species in which a common charged amino acid(s) comprising collagen or other surface components has been replaced by neutral amino acid(s). The existence of Spirurids with

neutral surfaces suggests that loss of surface charge may be an essential first step in adoption of adsorption of host molecules as an evasive strategy. Species such as *Brugia* spp. may have retained the ancestral condition of a negatively charged cuticle and antigen shedding, both of which we suggest originated earlier among free-living species from which parasitic lineages evolved. Certainly *Brugia* mf shed more antigenic components than *Wuchereria* (in *W. bancrofti* the 17 kDa surface antigen is also secreted and may in fact be shed only in minute quantities, Table 1), although no information is available for the *Onchocerca* species in question, only *O. cervicalis* having been shown unequivocally to shed surface antigens.

4. IMMUNOMODULATION

Without doubt the single evasive strategy which has received more attention than any other is immunomodulation in its various guises. Immunomodulation for the purpose of this article is defined as the activity of any parasite factor which reduces the effectiveness of host immunity or in some way downregulates the host's attempts to express a potentially protective response. This is a large topic which has been reviewed comprehensively in several publications in recent times and it is not our intention to go over old ground. Non-specific immunodepression is easily demonstrable across both classes of Nematoda although specific immunodepression of responses to the antigens of the infecting parasite is well documented only in the case of filarial species (Piessens, McGreevy, Piessens, McGreevy, Koiman, Saroso & Dennis, 1980a; Piessens, Ratiwayanto, Tuti, Palmieri, Piessens, Koiman & Dennis, 1980) but even here the story is not quite clear cut, since a proportion of microfilaraemic individuals may respond to filarial antigens (Lammie, Leiva, Ruff, Eberhard, Lowrie & Katz, 1988). Some animal-based studies have also revealed few differences in cellular and antibody responses between microfilaraemic and amicrofilaraemic hosts (e.g. ferrets infected with *B. malayi*, see Thompson, Crandall, Doyle, Hines & Crandall, 1986). On the other hand, in no species other than *H. polygyrus* has it been conclusively demonstrated that immunodepression of antiparasite responses is essential for the chronicity of infection and adult worm survival (for review see Behnke, 1987).

In most filarial infections, microfilaraemia is associated with the absence of antibodies specific for mf surface antigens (*B. malayi*, McGreevy, Ratiwayanto, Sekar, McGreevy & Dennis, 1980; *D. immitis*, Rzepczyk & Bishop, 1984; *A. viteae*, Weiss, 1978; *W. bancrofti*, Ravindran, Satapathy, Das, Pattnaik & Subramanyam, 1990). In cotton rats infected with *L. carinii*, circulating IgG and IgM antibodies for mf homogenate antigens decline in intensity at the onset of patency (Muller-Kehrmann, 1988). The most likely explanation for this latter observation is that antibody was mopped up by the antigens secreted and expressed by the large numbers of mf which probably never left the lungs. However, later on, as patency neared its end, antibody levels increased, either because they were no longer being removed by the presence of mf or because an 'immunomodulatory' influence on antibody synthesis was no longer present and synthesis was now unrestrained. In *B. pahangi*-infected cats, clearance of mf has been linked to the appearance of antibody to an 11–22 kDa adult worm antigen (Fletcher, Birch, Samad & Denham, 1986).

Numerous studies now indicate the existence of parasite-derived immunodepression in filarial infections but there have been few attempts to show that parasite molecules (as distinct from infection by parasites) are responsible (Table 4). In an unusual but fascinating approach Prince, Albiez & van de Ende (1985) observed that human *O. volvulus* nodule tissue containing adult worms survived longer on transplantation to chimpanzees than subcutaneous tissue transplanted without worms. Their results imply that parasite-derived factors were involved, supporting other studies which have shown immunodepression in patients with onchocerciasis (Kilian & Nielsen, 1989a,b).

Suppressor cell activity is now well recognized in a variety of chronic infections (Piessens, Ratiwayanto, Tuti, Palmieri, Piessens, Koiman & Dennis, 1980; Piessens, Partono, Hoffman, Ratiwayanto, Piessens, James, Palmieri, Koiman, Dennis & Carney, 1982) including bacterial (Petit, Richard, Burghoffer & Dauget, 1985; Jarrells, 1985; Tomai, Elmquist, Warmka & Fitzgerald, 1989) and several filarial systems (*B. pahangi*, see Portano, Britton & Ash, 1976; Lammie & Katz, 1983a,b, 1984a,b; *A. viteae*, see Weiss, 1970). It has been established that the failure of splenic T cells in jirds infected with *B. pahangi* to respond to infection is attributable to a nylon/plastic adherent cell population with histamine receptors, i.e. macrophages (Lammie & Katz, 1984a) and evidence has been presented showing that the effect of these cells is to downregulate IL-2 secretion by splenic lymphocytes (Leiva & Lammie, 1989). IL-1 secretion by splenic macrophages was normal and there was no evidence for a defect in T cells. Rather an active suppression of IL-2 secretion by both parasite-specific and heterologous antigen-specific T cells was maintained for as long as the accompanying suppressor cells were co-incubated with T cells. One explanation may be that the worms secrete prostaglandin-like factors through which IL-2 secretion is downregulated. Although to

our knowledge there are no data that the filariae can secrete prostaglandins a variety of other helminths are known to do so (see 4.1.1.3) and in *Treponema pallidum* suppressor cell activity has been linked to secretion of PGE2. However, in the closely related *B. malayi* immunosuppression has been linked to T lymphocytes (Lal, Kumaraswami, Steel & Nutman, 1990) and it is therefore necessary to consider the existence of more than one active mechanism or alternatively several routes leading to the same common outcome.

The literature is replete with reports of non-specific immunodepression during particular phases or throughout infection with a variety of different nematode species, ranging from laboratory studies involving rodents (*N. brasiliensis* to DNP, Haig, Lima & Mota, 1980 and heterologous antigens, McElroy, Szewczuk & Befus, 1983; *A. suum* to Con A, PHA & PWM, Barta, Stewart, Shaffer, Huang & Simmons, 1986), through large domestic animals such as cattle (*Ostertagia ostertagi, Trichostrongylus axei* to Con A & PHA, Snider, Williams, Karns, Romaire, Trammel & Kearney, 1986; reviewed by Klesius, 1988) to observations in man (*O. volvulus* to tetanus, BCG and other recall antigens, Kilian & Nielsen, 1989a,b). Besides demonstrating the universality of the phenomenon these studies have contributed little to our understanding of evasive strategies employed by nematodes. Almost all groups have failed to persist with the investigation of their model systems long enough to unravel the factors involved. In short the majority of such studies can be classified as superficial, descriptive of the phenomenon but little more. A crucial issue to answer here is whether the suppressor cell activity or generalized immunodepression are part of a concerted evasion strategy by the parasites involved aimed at reducing the effectiveness of host immunity. If so how are they mediated? Do parasites release factors such as PGE2 and if so precisely what molecules are involved and how do they act? Alternatively are we seeing here intentional downregulation by the host in an effort to minimize pathology? If so, what conditions are required to induce such responses? What are the qualities and characteristics of relevant parasite antigens capable of influencing the host response in this way? And perhaps most challenging of all, can the antigens employed to downregulate responses generating immunopathology be dissociated from those responsible for protection and can they be employed in vaccines?

4.1. *Characterization of immunomodulatory factors*

Despite the indirect evidence that immunomodulatory factors must exist, progress in isolating and analysing potential candidates has been extremely slow. There are several reasons for this. Firstly as has been argued above immunomodulatory factors are likely to be labile, components with short half-lives *in vivo* and their isolation from parasites, either extracted directly from hosts or maintained *in vitro*, is likely to be problematic.

4.1.1. *Known factors and their modes of action.* 4.1.1.1. Factors described to date. Table 4 summarizes studies in which extracts of parasites, their ES products or specific fractions of these have been assayed for their immunodepressive properties either *in vivo* or *in vitro*. One thing is very clear: there is still a long way to go before we have even a vague idea of the role of the molecules involved. The only common theme from the few studies in which fractions of parasite products have been investigated is that in general the molecules involved appear to be relatively small, <50 kDa (smaller than albumin, 67 kDa). *B. malayi* mf appear to be the exception, since Wadee, Vickery & Piessens (1987) found suppressive activity in fractions of ES in the molecular range from 50 to 190 kDa and it is likely that several molecules of different size were involved or alternatively that a relatively small molecule had the capacity to form multimeric units. However, the phosphocholine-bearing antigens of adult *B. malayi*, which are relatively large (>90 kDa, Maizels, Burke & Denham, 1987), are also known to be immunosuppressive as adjudged by their effect on PHA-stimulated proliferation of human lymphocytes *in vitro* (Lal *et al.*, 1990) and molecules with PC are released by *Brugia* and *Onchocerca* spp. *in vitro* and *in vivo* (Maizels *et al.*, 1987).

4.1.1.2. Smoke screen antigens / immune complexes. In culture all nematodes secrete or excrete an array of molecules some of which are potently immunogenic (Lightowlers & Rickard, 1988). It is conceivable that others act in a smoke screen capacity diverting the immune system into unnecessary expenditure of energy reserves in an effort to clear the circulation, through the formation of immune complexes which cause downregulation of the host's capacity to respond (Barnett, 1986) or through mitogenic properties leading to polyclonal activation of host cells and consequent immunodepression through exhaustion of reactive lymphocyte clones (Wadee & Piessens, 1986). Although there is circumstantial evidence to support such hypotheses, it is extraordinarily difficult to substantiate that relevant mechanisms may be beneficial for worm survival.

Many nematodes seem to reside in tissue sites surrounded by inflammatory cells but apparently unaffected by their presence. *O. volvulus* and *W. bancrofti* are prime examples. In the latter, inflamed

TABLE 4—IMMUNODEPRESSION BY EXTRACTS, ES PRODUCTS AND IMMUNOMODULATORY FACTORS OF NEMATODE PARASITES

Species	Immunodepression *in vivo*	IMF	Stage secreting IMF
SECERNENTEA			
SPIRURIDA			
Mf without sheaths			
Onchocerca volvulus		Yes	Mf or adults
			Adults
Onchocerca gibsoni		10 kDa	Mf
Mf with sheaths			
Brugia malayi		50–190 kDa	Mf
			Serum factors Mf/host derived?
			Adults
Brugia pahangi			Adults
ASCARIDIDA			
Ascaris suum	ES & Homogenate		Adult
Anisakis simplex & *Terranova* spp.		>10 kDa	L3
STRONGYLIDA			
Heligmosomoides polygyrus		<12 kDa	L4
		<25 kDa	Adult
	Homogenate		Adult
Oesophagostomum radiatum		25–35 kDa	L3 & L4
ADENOPHOREA			
Trichinella spiralis	Homogenate		Muscle larvae

TABLE 4—*Continued*

Effect observed	References
Inhibition of mixed lymphocyte response & PHA induced proliferation	Prince *et al.*, 1985
Suppression of response to PPD but not ConA	Luty, Downham, Whitworth, Morgan, McNicholas & Taylor, 1990
Suppression of ConA induced proliferation of bovine lymphocytes	Foo, Nowak, Copeman & McCabe, 1983
Suppression of ConA induced proliferation of lymphocytes.	
Suppression of IL-2 production by mitogen stimulated lymphocytes	Wadee *et al.*, 1987
Suppression of antigen induced lymphocyte proliferation *in vitro*	Piessens,Ratiwayanto, Tuti, Palmieri, Piessens, Koiman & Dennis, 1980
Suppression of PHA induced proliferation of lymphocytes	Lal *et al.*, 1990
Reduced lymphocyte responsiveness to antigens	Miller *et al.*, 1991
Suppression of reaginic & haemagglutinating response	Komatsu, Nishimura, Sano & Shinka, 1979
Suppression of mitogen induced proliferation of murine lymphocytes	Raybourne, Desowitz, Kliks Deardorff, 1983
Suppression of mitogen induced proliferation of murine lymphocytes	Losson, Lloyd & Soulsby, 1985
Suppression of mitogen induced proliferation of murine lymphocytes	Monroy, Dobson & Adams, 1989
Suppression of development and expression of homologous protective response in mice	Pritchard & Behnke, 1985
Suppression of mitogen & antigen induced proliferation of peripheral blood leukocytes at 50 ng/per culture (50%)	Gasbarre, Romanowski & Douvres, 1985
Suppression of response to SRBC	Barriga 1975, 1978a
Reduced response of cells to mitogens	Barriga, 1978b

lymph nodes may also show follicular atrophy (Connor *et al.*, 1986), although whether this is a consequence of the activities of the parasite or the host response to the presence of worms is not known. *T. canis* larvae not only reside within granulomatous murine livers but escape from these and accumulate in the brain, apparently shrugging off host inflammatory cells as posing no threat to them at all (Abo-Shehada *et al.*, 1991). Eosinophils will attach temporarily to *T. canis* larvae and degranulate but within 1–2 h the larvae have fought free from adherent cells, and having sloughed off their surface coat continue without loss of viability (Badley *et al.*, 1987).

Another example is found in *H. polygyrus*, the L3 and L4 larvae of which develop in the *muscularis externa* of the mouse intestine for a period of 8–9 days before returning to the gut lumen as adults. In immune, challenged mice, these sites of development become surrounded by inflammatory cells and despite the intensity of local cellular activity, few if any worms are actually killed within such foci of cellular activity. There is a very obvious stunting of worms and their capacity to survive in the gut lumen is greatly impaired once they have completed development. However, larvae can survive in such granulomata for weeks and still succeed in returning to the gut lumen. Ey (1988) has suggested that larvae are protected, at least to a degree, through continuous secretion of antigens which mop up specific antibody and form immune complexes. The parasites also deplete complement locally. Soluble immune complexes containing C3 fragments would compete with cuticle-bound C3 and antibody for appropriate receptors on leukocytes. Ey has hypothesized that this may prevent attachment of effector cells to the parasite cuticle, thereby allowing worms sufficient time to complete development and eventually to escape from the granulomata despite the intense local accumulation of inflammatory cells.

Immune complexes are recognized as having the potential to cause downregulation, presumably through feedback circuits which turn off unnecessary antibody secretion (Barnett, 1986). In this context *W. bancrofti* is associated with immune complexes (Dasgupta, Bala & Dutta, 1987a; Prasad, Reddy & Harinath, 1983; Dissanayake, Galahitiyawa & Ismail, 1982) and a recent study has shown that the dominant antigen involved was the 200 kDa phosphorylcholine (PC)-bearing glycoprotein often detectable in the circulation of infected persons (Lunde, Paranjape, Lawley & Ottesen, 1988) and experimentally infected monkeys (Maizels, Morgan, Gregory, Selkirk, Purnomo, Sukartono & Partono, 1988). Immune complexes have also been observed in cats with *B. pahangi* and humans with *B. malayi* (see Au, Denham, Steward, Draper, Ismail, Rao & Mak, 1981). Functional studies on peripheral blood lymphocytes have indicated that cells from filariasis patients with circulating antigens are less responsive to stimulation by L3 antigens (Dasgupta, Bala & Dutta, 1987b) or mf ES antigens (Prasad & Harinath, 1987) than those from persons without circulating antigens. However, *B. malayi* mf also have mitogenic properties (Wadee & Piessens, 1986) and it could be that fewer reactive cells are present because they have been swamped by the proliferation of clones which do not recognize parasite antigens. However, Wadee & Piessens (1986) detected immunodepressive activity in lyophilized extracts of mf and there remains the possibility that internal molecules not normally released in the host were involved. In a later study they showed that mf release immunodepressive factors (see 4.1.1.1).

4.1.1.3. Prostaglandins and eicosanoids in nematodes. Some helminths have been found to contain large amounts of free fatty acids which could conceivably be used in the manufacture of lipid-derived mediators such as prostaglandins and leukotrienes, known to have potent immunomodulatory activity. Indeed, prostaglandins have been reported from a few helminths, e.g. *Taenia taeniaeformis* (see Leid & McConnell, 1983a,b). In *S. mansoni* prostaglandins have been shown to be necessary during cercarial penetration but there is no evidence that they are actually of parasite origin as distinct from exploited host-synthesized prostaglandins (Salafsky, Yu-Shang Wang, Fusco & Antonacci, 1984). In *F. hepatica* PGE and PGF had a marked effect on glycogen phosphorylase activity but again the PGs were not shown to be of parasite origin (Simonic, Sartorelli & Locatelli, 1983). Recent studies, however, have detected PG E2 and F2 in *S. ratti* (see Minematsu, Yamazaki, Ufi, Okabe, Korenaga & Tada, 1990) and PG A, B, C, D, E and F in *T. spiralis* and *T. pseudospiralis* with up to 39.28 and 41.8 $\mu g\ ml^{-1}$ of parasite homogenate, respectively (Hadas & Staude-Adamczewska, 1990). Furthermore, the L3 of *Necator americanus* are now known to secrete quantities of eicosanoids (including both prostaglandins and leukotriennes) during penetration of mammalian skin (Salafsky, Fusco & Siddiqui, 1990). *O. gibsoni* adults have a non-glycosylated lipid composition and a substantial repertoire of glycolipids including many with the characteristics of gangliosides (Maloney & Semprevivo, 1991). Adult filariae may release some of these lipid compounds and since it is known that glycosphingolipid and sphingolipid breakdown products including phospholipids can depress the host's immune response (Hannum & Bell, 1989), these molecules also have potential as immunomodulators.

In rabbits infected with *Treponema pallidum* PGE secretion by host macrophages 2 weeks after infection

has been implicated as responsible for inducing suppression of T cell activity. PGE is known to down-regulate IL-2 secretion and consequently blast cell activity and clonal expansion of potentially reactive T cells with resultant severe overall depression of host responsiveness (Salafsky & Fusco, 1987). Prostaglandins also have potent anti-inflammatory effects (Moqbel & MacDonald, 1990). Tomai *et al.* (1989) in fact suggested that this sequence of events may even be initiated by *T. pallidum* secreting PGE2 which in turn enhances PGE2 secretion by macrophages thereby mediating suppression. Clearly parasites which secrete PGE or similar molecules could also exploit these immunoregulatory pathways to tone down host protective responses. In support of this suggestion it has been shown that immunosuppression initiated by *Trypanosoma brucei* infection in mice is mediated through at least two mechanisms, one of which is prostaglandin-independent and accounts for down-regulation of IL-2 receptor expression and the other, involving prostaglandins causes suppression of IL-2 secretion (Sileghem, Darji & de Baetselier, 1991).

4.1.1.4. Ecdysteroids in nematodes. Some nematodes have been reported to contain and release ecdysteroids. Molecules resembling ecdysone or showing ecdysone-like activity have been found in larval (L3 of *H. contortus*, Dennis, 1977) and adult Sercernentea (adult Ascaridida, e.g. *Ascaris lumbricoides*, Horn, Wilkie & Thomson, 1974; Fleming, 1985; *Parascaris equorum*, O'Hanlon, Mercer & Rees, 1991 and *Anisakis simplex*, Evershed, Mercer & Rees, 1987; adult Spirurida, e.g. *D. immitis* and *B. pahangi*, Mendis, Rose, Rees & Goodwin, 1983; Barker, Mercer, Rees & Howells, 1991; larval Strongylida, e.g. L3 of *N. brasiliensis*, Bottjer, Whisenton & Weinstein, 1984) and also in the Adenophorea (L1 of *T. spiralis*, Hitcho & Thorson, 1971). *Parascaris equorum* released 20-hydroxyecdysone 25-glucoside (O'Hanlon *et al.*, 1991). Endogenous ecdysone and 20-hydroxyecdysone have been detected in *Ascaris suum* and *D. immitis* (Cleator, Delves, Howells & Rees, 1987). The function of steroid molecules in nematodes is not understood but members of the steroid family of molecules are important growth- and reproduction-modulating hormones in vertebrates and invertebrates (Beckage, 1991). Barker *et al.* (1991) concluded that ecdysone, an insect hormone, may play an important role in reproduction in filarial worms, by stimulating mf release in *B. pahangi* and by inducing meiotic reinitiation in oocytes in *D. immitis*. In comparison 20-hydroxyecdysone had little effect. However, attempts to demonstrate *de novo* synthesis of ecdysteroids from radiolabelled cholesterol in *B. pahangi* and *D. immitis* (Mercer, Barker, McCall, Howells & Rees, 1989) and *L. carinii* (Koolman, Walter & Zahner, 1984) have been largely unsuccessful. It is therefore likely that parasites exploit host-manufactured precursors further along the biosynthetic pathway towards ecdysone than cholesterol or alternatively that they employ synthetic pathways for ecdysteroids yet to be defined.

Overall, there is still too little relevant information for a comprehensive assessment of these molecules in nematodes and, as emphasized above, a central problem in most studies has been to demonstrate conclusively that the steroid molecules detected originated from the parasite's metabolic pathways and not those of the host (Barker, Chitwood & Rees, 1990). However, since there is evidence for the presence and release of ecdysteroids in both the parasitic Sercernentea and Adenophorea [and also among free living species (Dennis, 1977), where the evidence for endogenous synthesis of ecdysteroids in *Caenorhabditis elegans* has also been questioned (Barker *et al.*, 1990)], it is possible that the ability to exploit steroid molecules, whether *de novo* or xenobiotically synthesized, evolved prior to parasitism in the phylum. Consequently it would not be surprising to discover that some parasitic nematode lineages have evolved the pathways necessary to produce host-metabolism regulating molecules from steroids and/or other precursors. Lipid metabolism has been documented in *Trichuris globulosa* (see Sarwal, Sanyal & Khera, 1989) and *O. gibsoni* (see Maloney & Semprevivo, 1991) and shown to involve fast turnover of several key molecules including cholesterol. The potential importance of steroid-based molecules in affecting host immunoregulatory pathways must therefore be considered. In most mammals steroids and related molecules are potent immunosuppressants, agents such as cortisone and betamethasone having been used extensively in immunosuppressive therapy as well as experimental work. Furthermore, recent reports showing that ecdysone and 20-hydroxyecdysone have dose-dependent immunosuppressive activity in mice (Barker, Chitwood & Rees, 1990; Barker, Mercer, Rees & Howells, 1990), can be detected in nodule tissues immediately surrounding *O. gibsoni* and *O. volvulus* (Mercer, Barker, Howells & Rees, 1989) and that parasite-derived ecdysteroids (from *Loa loa* and *Mansonella perstans*) may be identified in the host's circulation emphasize that this is a realistic possibility (Lansoud-Soukate, Gharib, Baswaid, Capron & de Reggi, 1990). Finally, the association of high ecdysteroid levels in man with pathology suggests that host immunoregulation may indeed be perturbed by these molecules (Lansoud-Soukate *et al.*, 1990).

4.1.2. *Possible factors and likely modes of action.* 4.1.2.1. Cytokine-like molecules. In a recent paper Dopheide, Tachedjian, Phillips, Frenkel, Wagland &

Ward (1991) reported that they had cloned an 11 kDa molecule normally secreted by L4 and adult *T. colubriformis*. On sequencing, this factor showed striking homology with a human gamma interferon-induced protein. The latter is a molecule comprising 145 amino acids in a simple linear arrangement and is thought to be an intermediate of gamma interferon-induced activation (Blomstrom, Fahey, Kutny, Korant & Knight, 1986). It is conceivable (although we concede highly speculative at this stage) that nematode parasites have evolved similar molecules through which they might downregulate host immunity. In rodents, where $CD4^{+ve}$ T lymphocytes are divided into two subsets now referred to as Th1 and Th2 cells, the latter are believed to be instrumental in mediating the inflammatory response usually associated with worm expulsion (Mosmann & Coffman, 1987). Mouse strains which respond with Th1 cells fail to express good immunity (Else & Grencis, 1991). The Th1 response is associated with increased levels of gamma interferon and TNFβ in the circulation, both cytokines originating principally from the Th1 cells. Furthermore, IFN gamma is known to downregulate the Th2 response (Mosmann & Moore, 1991). Thus by secreting a molecule which functionally resembles the gamma interferon-induced protein, *T. colubriformis* may be committing the host to a non-protective Th1 response and thereby avoiding immunity.

Another molecule also cloned from *T. colubriformis*, 30 kDa in size, shows 28% sequence homology with a 25 amino acid peptide called valosin from the porcine upper intestine (Savin, Dopheide, Frenkel, Wagland, Grant & Ward, 1990). The latter is a modulator of acid and pepsin in the stomach and bicarbonate and protein in the pancreas, with additional electrophysiological effects including changes in gut motility. It has been proposed that the parasite molecule may mediate some of the pathophysiological consequences of infection with *T. colubriformis* (Savin *et al.*, 1990).

4.1.2.2. Other targets for parasite immunomodulatory factors. Besides the studies discussed above, there is little further information at present to indicate how nematode factors might interfere with host immunity. Part of the problem is still an incomplete understanding of what exactly is required in order to kill, eliminate or expel parasitic nematodes from the host. In bacterial, viral and plant parasitic fungi the evasive strategies of invasive organisms are much better comprehended because a detailed molecular description of host-protective resistance is available.

Perhaps among the most intriguing of relevant mechanisms are the so-called bacterial 'superantigens' such as the Staphylococcal enterotoxins. These molecules have affinity for class II MHC, to which they bind outside the conventional peptide-binding groove. Communication with T cells is effected through the MHC–T cell receptor interaction although the component of the TCR involved is also distinct from that involved in conventional recognition of MHC-presented peptide fragments. Thus MHC-bound superantigens interact with the V_β chain of the TCR, the exact V_β allele determining susceptibility to particular superantigens. Each superantigen therefore has the capacity to activate a particular panel of T cells depending on the V_β chain expressed. Superantigens are the most potent activators of human and murine lymphocytes yet described mediating activation at picomolar concentrations. Although the biological function of this interaction is not fully understood, it has been suggested that superantigens stimulate $CD4^{+ve}$ and $CD8^{+ve}$ T cells which proliferate and on differentiation into cytotoxic T cells proceed to kill MHC expressing antigen presenting cells (e.g. macrophages and B cells) as well as activated class II bearing autologous T cells. This results in the depletion of the cells necessary for the generation and expression of host protective mechanisms locally and thereby generates an opportunity for the bacteria secreting superantigens to proliferate (see Dohlsten, Hedlund & Kalland, 1991 for recent review).

We concede that to the best of our knowledge no such factors have as yet been described from parasitic nematodes although many helminth parasite antigen preparations have undefined mitogenicity. In a recent study Behnke & Wahid (1991) showed that mice bearing the MHC haplotype $H\text{-}2^b$ were particularly slow at expelling *H. polygyrus*. Moreover this haplotype produced the slowest antibody responses to the parasite and it was suggested that parasite factors preferentially impair $H\text{-}2^b$ bearing antigen presenting cells and/or homologous T cells through direct interactions. Although this interpretation is highly speculative, the precedent among bacterial superantigens shows that parasite factors can have affinity and specificity for host membrane receptors and there is no reason why factors capable of blocking or directly impairing such molecules should not have evolved. On an even more speculative note, we predict that future studies will reveal parasite factors which block cytokine receptors and others which mimic cytokines, thereby causing disregulation of normal pathways and circuits through which the immune response is orchestrated. Such mechanisms are especially appealing because communication via cytokines is extremely local, usually expressed at the cell–cell interface. Parasites which have evolved the ability to mimic or disrupt these channels of communication should impair host immune competence locally

and not systemically, enabling their own survival but leaving the host with the competence to respond to other infectious organisms.

4.2. *The lessons from host specificity*

Some parasites have a wide host specificity (e.g. *Trichinella spiralis*) but by far the majority depend for their survival in nature on only a single host species or at best several closely related hosts. Whilst physiological/ecological incompatibilities obviously rank as major impediments to the colonization of other hosts and hence serve to limit the host range in nature, the immune system in abnormal hosts may be an equally formidable obstacle. Even closely related hosts, occupying similar ecological niches whilst harbouring similar species of parasites, will nevertheless have species of their own and will share few identical species, e.g. sheep, cattle and deer grazing common pasture.

Whilst some evasive strategies, such as an acquired disguise of host molecules, may be equally effective in unrelated host species, in general immunomodulation is likely to be host-specific because of the degree of fine tuning necessary to disrupt immunoregulatory pathways. Examples of parasites which can be grown or even maintained in abnormal hosts treated with immunosuppresive drugs are too many to list. It is likely that immunomodulatory mechanisms function with optimum efficiency only in the hosts for which they evolved, although it is conceivable that some IMF may interfere with conserved host molecules.

Heligmosomoides polygyrus is but one species in a family of nematode parasites affecting rodents throughout the world. Several closely related parasites affect voles in Europe, Asia and North America. Experiments with the *H. polygyrus bakeri*, a species now maintained in laboratories around the globe in laboratory house mice, have established that it will not survive for long in the European field mouse *Apodemus sylvaticus* even though when immunosuppressed the parasites develop normally and grow into fecund adults (Quinnell, Behnke & Keymer, 1991). Essentially the only obstacle limiting the usefulness of *A. sylvaticus* as a host is the immune system. Thus the immunomodulatory strategy which is so successful in *Mus domesticus* does not work to the benefit of the parasite in field mice. Likewise *Heligmosomoides polygyrus polygyrus*, which affects *A. sylvaticus* in Europe and causes chronic infections in the field mouse (Gregory, Keymer & Clarke, 1990), is easily killed by *M. domesticus* during the tissue stage of development. Again the immunomodulatory strategy fails in a closely related but different host species.

The specificity of immunomodulatory mechanisms for definitive hosts suggests that the factors are intimately linked to the particular qualities of the relevant immune system. Apart from domestic animals, man and laboratory rodents few other animals have been studied in sufficient detail for molecular differences between their receptors and cytokines to be understood. However, it is likely that each parasite produces several molecules with greater or lesser specificity for the particular components of the host species which it has evolved to parasitize, i.e. the host with which it has been involved in an arms race.

It is regrettable (but easily appreciated why) that most laboratories concentrate their activities on one chosen host and parasite system. Comparative studies of parasites in different host species as well as in host strains of varying response phenotype can yield interesting data which may help to explain immunomodulation. In *Mastomys natalensis*, the pattern of primary microfilaraemia was much the same for *L. carinii*, *A. viteae*, *B. pahangi* and *B. malayi*, all four species generating microfilaraemia which lasted for well over 250 and even 350 days (Sanger, Lammler & Kimmig, 1981). Thus whatever the evasive strategies of these parasites, the differences in immunodepression or accompanying alternative mechanisms, all four were successful. Perhaps these results indicate that *M. natalensis* are unable to respond with appropriate host-protective immunity against filariae in general or they may reflect a mechanism targeting conserved rodent molecules as explained above.

When eosinophilia was studied in three groups of rodents infected with *B. pahangi*, an interesting contrast emerged. Thus in resistant mice (BALB/c) where no microfilaraemia was induced, eosinophilia was confined to the first 4 weeks of infection and was probably linked to the response against L3 and L4 stages. In susceptible jirds, eosinophilia was observed only in the first 8 weeks of infection and declined with the onset of microfilaraemia (Nakanishi, Horii, Fujita, Terashima, Ueda & Kurokawa, 1987), perhaps indicating that some form of immunomodulatory influence was exerting a downregulation of the Th2–IL-5 axis. Consistent with this suggestion is the recognized suppressor cell activity in jirds infected with *B. pahangi* (see earlier section 4) and subsequent work by the authors showing that jirds with chronic microfilaraemia had markedly depressed eosinophil responses to homologous but not heterologous challenge (Horii, Nakanishi, Mori, Zaitsu, Ueda, Kurokawa, Oda & Fujita, 1989). However, in rats which are also susceptible, eosinophilia remained high throughout patency and there was no evidence of a decline until mf were eliminated by anthelmintic treatment. One conclusion from this study was that eosinophilia played little role in resistance, at least in

rats. In this example, does the immunomodulatory mechanism fail in rats thereby allowing eosinophilia to persist? If the immunomodulatory mechanism is successful in jirds (and other studies suggest that indeed it is, Lammie & Katz, 1983a,b), does this offer an opportunity to identify the immunological target through comparative across-species studies? *In vitro* experiments using leukocytes from both species may identify the point of resistance to immunomodulation in rats and thus provide clear indication of the target of parasite immunomodulatory factor (IMF).

4.3. *One system or more?*

Clearly, there are several potential evasion strategies open to parasites. The question thus arises as to which strategy to adopt and whether to specialize or generalize. Choice of strategy will be determined by the feasible option that most reduces the host's impact on the parasite's survival and reproductive success relative to the cost of the strategy to the parasite. Whether to specialize or generalize will depend on the relative advantage of each approach to the parasite in the host/parasite arms race.

Specializing has the advantage that more resources can be invested in the chosen strategy which may thus afford a longer period of grace before the host counter-adapts, but the disadvantage that the host can also concentrate its resources. However, if the host is threatened by a range of parasites, it may not have the resources to concentrate. The effectiveness of specialization is also likely to depend on the scope for counter-adaptive refinement. An immunomodulatory strategy, for example, could be escalated by secreting different, preferably unrelated, molecules to impair the various cytokine-mediated pathways which regulate the immune system. Each step in this complexity is open to manipulation. A parasite molecule resembling a particular cytokine may block, impair or compete with a corresponding cytokine or in turn may target the corresponding cell receptor. Parasite immunomodulatory molecules may have wide-spectrum efficacy, blocking more than one vital interaction between host components or may target key cytokines influencing divergent pathways in the existing hierarchical structure, or there may be a panel of molecules with individual targets. Which ever way, there would be scope for evolution of novel factors in response to refinement in the host's defences. Such a strategy could be pursued for several rounds of the arms race before the parasite wins, succumbs or switches to a different strategy (e.g. camouflage).

Generalizing to two or more evasion strategies spreads both host and parasite resources more thinly. However, by reducing the selection pressure against each strategy, it may increase the efficacy of strategies with limited scope for escalation as a specialism. In the context of the host's entire parasite community, generalizing adds a tier of within-species diversity that may compound the host's difficulties in responding to parasite counter-adaptations.

4.4. *Stability or lability?*

Despite considerable efforts and clear evidence that parasite immunomodulatory factors must exist, there is still no single immunomodulatory molecule from any nematode which has been identified, its structure elucidated and its biological properties understood. The simplest explanation for this lack of progress is that the factors concerned are difficult to isolate, possibly because they may be short-lived or involve complex interactions not yet understood. Thus IMF may be intrinsically labile or, like cytokines, they may operate most efficiently when several component factors can synergize. They may be rapidly metabolized and probably act at short distances (i.e. membrane to membrane). If indeed parasite strategies prove to be dependent on many distinct IMFs, then the isolation of any one such molecule would represent only the start. As with cytokine research in recent years the full complexity of the interactions involved would only be understood when all the components had been identified and their interrelationships appreciated.

From the evolutionary perspective, it would be expected that parasite IMFs have short half-lives. If the purpose of IMFs is to extend parasite survival to maximize the production of transmission stages through which subsequent generations of genes might be perpetuated, it follows that the extent to which the host should be suppressed becomes a dilemma of some importance. Long-lived factors would accumulate in the host as the parasite survived and if released systemically would have a progressively increasing deleterious overall effect on the host, weakening its immune system. It would hardly be of benefit to a chronic species to create a space for itself initially and then to lose it as the host dies from other infectious diseases against which it cannot respond through severe immunodepression. If such factors could be confined locally, however, a safe haven from the immune system might be created, but such a solution would require the parasite to have the ability to modulate the release of IMF as and when required in the parasite's immediate vicinity. A far simpler solution would be for IMFs to have short half-lives and to be continuously secreted, the net outcome of which would also be to produce local immunodepression confined to the immediate microhabitat of the parasite in question, much like the manner in which cytokines are now known to operate *in vivo*.

4.5. *Consequences of immunomodulation*

Whilst immunomodulatory strategies are likely to be finely tuned to the particular characteristics of the definitive host, their mechanisms of action are likely to be non-specific. A prediction of this hypothesis is that other parasites living in proximity to the immunomodulatory species will be able to benefit from the local downregulation of host immunity.

Earlier literature has already been reviewed (Behnke, 1987) but in recent years interactions of this type have been described for bovine species (Kloosterman & Frankena, 1988). Thus cattle infected with both *Ostertagia ostertagi* and *Cooperia oncophora* are more susceptible to infection with the lungworm *Dictyocaulus viviparus* and develop larger and more fecund female lungworms (Kloosterman, Frankena & Ploeger, 1989). This effect appears to be associated with experience of combined infections with both of the intestinal species but is not dependent on their presence since increased susceptibility is still apparent when the gut worms are removed by anthelmintics (Kloosterman, Ploeger & Frankena, 1990). The mechanism through which this is achieved is unknown but the observations could be explained by immunomodulatory activity of the intestinal worms affecting components of the mucosal system and consequently downregulating resistance in the lungs. However, in this case a long lasting effect has been observed and it may be that another explanation will be found but it is conceivable that a persistent immunodepression follows experience of infection with both *O. ostertagi* and *C. oncophora* through the synergistic interaction of their individual mechanisms. It is pertinent that neither species alone was capable of enhancing the establishment and growth of lungworms. Other examples include enhanced establishment of *A. viteae* in jirds concurrently infected with *B.malayi* (Court *et al.*, 1988) and increased establishment of *Ostertagia ostertagi* and *O. leptospicularis* in mixed infections compared with single species infections (Al Saqur, Armour, Bairden, Dunn, Jennings & Murray, 1984) (see also Behnke, 1987 and Christensen, Nansen, Fagbemi & Monrad, 1987).

Another consequence may be that parasite species accumulate in sites within hosts which they transform into safe havens from the host response through their collective immunomodulatory activity. Examples of parasites aggregating within infected hosts are again only too familiar. There are of course other benefits such as the availability of the opposite sex in dioecious species but often the numbers involved vastly exceed the requirement for one representative of each sex. In rats infected with *Litomosoides carinii*, many worms become entangled in knots within the pleural cavity. *O. volvulus* likewise accumulate two to four in a nodule but often many more. *B. pahangi* aggregate in lymphatic vessels of dogs and a recent study has shown that under infection intensities such as those normally encountered in the field, there may be local immunodepression in popliteal lymph node cells to parasite antigens whilst peripheral blood lymphocytes remain responsive (Miller, Schreuer & Hammerberg, 1991). Gastrointestinal species are seldom uniformly distributed along the gut. Often the majority of worms may be found in only a few centimetres in large hosts and less than a centimetre of the small intestine in rodents. It is particularly noticeable that the degree of aggregation increases as the worms come under stress from the immune response. This has been observed in *H. polygyrus, T. colubriformis* and in *T. vitrinus*. In the latter two species, 'fingerprint' lesions can be detected in the late stages of infection. Most of the pathology is confined to these lesions and most of the worms are located in them. Neighbouring sections of the gut may have quite normal morphology and may be totally free of worms (Jackson, Angus & Coop, 1983; Angus & Coop, 1984).

5. GENETIC CONSIDERATIONS

Each host–parasite association is the product of a complex interrelationship, the outcome of which is decided by an interaction between the genetically determined characteristics of the two species concerned. In the examples considered here, i.e. 'natural' host–parasite relationships, the nematode is preadapted to the morphological and physiological characteristics of the host and is therefore readily able to establish in the host. No evidence is available for genetic variation in host molecules that might act as ligands for parasite receptors and which, by analogy with the situations known to exist in relation to infection with *Escherichia coli* (see Sellwood, Gibbons, Jones & Rutter, 1975) and *Plasmodium vivax* (see Miller, Mason, Clyde & McGinniss, 1976) might prevent establishment in otherwise permissive hosts, although these might well exist. Once established, the worm will survive for a shorter or longer period, the greatest threat to long-term survival being the host's adaptive immune response. The duration of survival could be determined at one extreme, in the absence of effective immunity, by the genetically programmed life span of the parasite or, at the other, by the strength and speed of the host immune response. In the simplest terms it might therefore be considered that the prime evolutionary considerations are for the parasite to achieve the maximum possible survival by avoiding or downregulating the host's immune response, whatever the cost, and for the host to minimize parasite survival by mounting the strongest possible response, again whatever the cost. However, as this review makes

clear, such a simple view is misleading because it fails to take account of the need for each partner to maximize fitness, which inevitably involves cost–benefit trade-offs in relation to subverting or increasing immunity. Evaluation of these trade-offs is difficult, not least because comparatively little is known about the theoretical maximum life-spans of parasitic nematodes; it is not possible in *in vivo* studies to exclude all influences of host origin, even in immunologically anergic animals, and *in vitro* studies are inappropriate. The data of Robinson, Wahid, Behnke & Gilbert (1989) on survival of *H. polygyrus* infections in mice provide one of the few detailed and well-controlled studies in this area, showing clearly how life-span in this species is largely host-determined. Conversely, although many parameters of the host response can be used to measure the speed and strength of response to infection, it is difficult *in vivo* to monitor the subtle degrees of difference in parasite-derived antigenic stimulation or, more importantly, in immunomodulation, that ultimately determine these parameters. Immunoparasitologists interested in the biology of chronic infections must therefore interpret the data provided by experimental approaches with considerable caution if they are to reach valid conclusions about the determinants of parasite survival.

One particular area in which this caution has to be exercised concerns the immunogenetics of host–parasite relationships. There is now a substantial body of information concerning host genetic influences upon immune and inflammatory responses to nematode infection (Wakelin, 1988) and it is universally accepted that there is considerable variation within outbred populations in ability to respond immunologically to, and thus control, infection. It must also be the case that there will be considerable genetically determined variation within parasite populations in ability to elicit or to modulate host responses.

5.1. *Parasite genes influencing host responses*

Host responses to nematode infection are elicited by two subsets of molecules, those expressed at the cuticular surfaces and those released (excreted/secreted) into the immediate environment of the worm (E/S molecules). Both include protein, glycoprotein and carbohydrate components, and it is now well-established that, in many species, the two sets have components in common. The biological functions of the majority of these molecules are unknown, except where enzymatic properties have been defined, but it is clear that many play an important role in eliciting or modifying host responses (Almond & Parkhouse, 1985; Lightowlers & Rickard, 1988). Among the immunologically active components will be molecules acting as antigens and allergens, and immunomodulatory molecules acting as factors regulating host immune and inflammatory responses. Other molecules may be involved in camouflaging the exposed cuticular surface. All of these molecules are gene products and it is well known that there can be developmental regulation of their expression and release, i.e. the molecules may show stage specificity. Equally, as there undoubtedly is genetic polymorphism within all populations of worms, there will be genetically determined variation in the structure of these molecules, some of which is likely to influence both their biological and their immunological activity. At the present time there are data relating to molecular variation within parasitic nematodes, albeit largely at a descriptive level, and a little is known about the influence of such variation on the host response, but almost nothing is known of the genetic basis for this variation. This is one area in which the research carried out with the model nematode *Caenorhabditis* will provide valuable pointers.

The belief that the molecular variation existing in nematodes will be reflected in significant differences in host response, and therefore in parasite survival, is supported by the observation that relatively few of the many molecules exposed to the host appear to function as immunodominant antigens. Given it is likely that host recognition of these antigens is directed against a limited number of epitopes, it can be assumed that quite small changes in molecular structure could result in significant differences in host recognition and response. If such differences resulted in enhanced parasite survival or reproduction there would be a positive selection to maintain the highly variable molecules in the population. Maintenance of variable immunologically active molecules in the parasite population can be seen as an evolutionary strategy designed to counter host-population heterogeneity in immune responsiveness and, while not the primary selection pressure, the effect would be to reduce the effectiveness of long-term host immunity to a particular species (Allison, 1982). If it is the case that nematodes release molecules whose primary function is to interact with and modulate host responses, then it is clear that molecular variations leading to more effective modulation will be strongly selected, polymorphism in this case having less selective value. A similar argument applies to variation in molecules involved in parasite camouflage, although in both cases, the fitness costs of synthesizing and releasing these molecules have to be taken into account.

Evidence that intraspecific parasite variation can influence the outcome of infection is limited to a few nematodes and some of this evidence is observational rather than experimental. Perhaps the best known observational data concern the variations in the

immunology and immunopathology of onchocerciasis between the forest and savanna zones (Piessens & McKenzie, 1984). It has been proposed that these variations reflect molecular and other biological differences between populations of *O. volvulus*, but, although these are known to exist, there are few data available directly to support this correlation. Experimental studies of intraspecific variations influencing immunological aspects of infection have been carried out using isolates of *Trichinella*. This genus is, perhaps, the least host-specific of all nematodes, and to some extent this lack of specificity (compounded by a basic morphological similarity) has led to an uncertain taxonomy. At the present time there is a view that the genus contains a number of distinct gene pools, some of which are agreed to represent full species (Pozio, 1987). Several studies have shown that when different *Trichinella* isolates are used to infect particular strains of laboratory hosts, there can be substantial variation in parameters of infection (Dick, 1983). Among the most significant variables are the length of survival of adult worms in the intestine and the level of worm reproduction. Some isolates survive for a comparatively long period in the intestine (i.e. are relatively chronic) and generate large numbers of muscle larvae, others have a short survival time and produce relatively few larvae. Some workers have attributed variation between different isolates purely to genetically determined differences in parasite life-span and fecundity (Dick, 1983), however, both of these parameters are known to be significantly influenced by the host's immune response (Wakelin & Denham, 1983) and could therefore reflect not variation in programmed capacity for worm survival and reproduction, but variation in immunogenicity. Experimental support for this view has been obtained by comparing the outcome of infections with different isolates in inbred mice of different response phenotype, as well as intact and immune-suppressed animals (Bolas-Fernandez & Wakelin, 1989). Although differences in immunologically recognized molecules between *Trichinella* isolates are well known, we are still a long way from being able to correlate these differences with functional host responses, although such work is being undertaken (Wassom, Dougherty & Dick, 1988; Bolas-Fernandez & Wakelin, 1990). Given the progress now being made in analysis of *Trichinella* antigens at the immunochemical and molecular level, and our present understanding of the initiation, regulation and expression of anti-*Trichinella* immune responses, we can be optimistic that this system has the potential to provide a real insight into the role of genetically determined parasite variation in determining the outcome of infection and thus contribute to our understanding of chronicity of infection at this level.

5.2. *Host genes controlling protective responses*

It has been known empirically for many years, and is now universally accepted, that the interaction of hosts with infectious organisms is profoundly influenced by genetic factors. This is most clearly demonstrable when inbred strains of laboratory mice are exposed to standardized inocula under uniform experimental conditions, but is equally evident when detailed studies are made of outbred populations (both human and animal) under natural conditions of exposure to infection. There is now an enormous literature describing both the genetics of this intraspecific variation and the components of host defensive responses through which variation is expressed (Wakelin & Blackwell, 1988). In a small proportion of cases the genes involved have been identified and sequenced, and the functional significance of their gene products determined, but in the majority of cases analysis has not proceeded quite so far, and this is certainly true of host–parasite relationships involving nematodes.

It is true to say that the existence of such extensive variations in ability to respond to infection has usually been interpreted in terms of the genetic diversity necessary for maintaining high levels of host population resistance in the face of constantly changing populations of pathogens. In this view, evolution is seen to act by selecting the genotypes associated with the strongest and most efficient immune responses. It is our contention that it is equally valid to see both host and parasite variation as providing the evolutionary material for the selection of host–parasite relationships that confer optimal fitness on both partners. Selection may well, therefore, be for less than maximal host responsiveness and less than maximal parasite evasive or immunomodulatory capacities, in order to produce an interaction in which both partners can survive and reproduce.

Genetic variation in response to nematodes can be measured by a number of parameters, including prevalence and duration of infection. Differences in prevalence in populations known, or suspected, to be exposed uniformly to infection can be assumed to reflect differences in duration of parasite survival resulting from differences in the degree of host-protective responses. This assumption can only be tested in studies where sequential time course assays of parasite survival are possible. Data from studies involving human hosts, and many of those on domestic or wild animals, provide only prevalence data, time course data come largely from experimental systems and particularly those involving mice. In some systems (e.g. with *Trichinella spiralis*) differences between individual mice or between strains of inbred mice may be quite small, high responders retaining infection for only a few days to a week or so more than low-

responders (Wakelin, 1980). In other systems differences may be very large and, on occasion absolute. For example, in some inbred strains of mice infections with *H. polygyrus* or *Trichuris muris* are controlled immunologically and the worms expelled, in others, equally 'normal' as far as conventional immune responses are concerned, the infection may persist until worm senility intervenes (Robinson *et al.*, 1989; Wahid, Robinson & Behnke, 1989; Else & Wakelin, 1988). With the latter species such differences can also be seen between individuals within strains, both outbred and inbred. Similar data are available for filarial infections in mice. Infections with the jird species *Acanthocheilonema viteae* can be established by direct transfer of female worms. In some strains this produces a long-term, high-level microfilaraemia, in others microfilaraemia is of low level and short lived (Haque, Worms, Ogilvie & Capron, 1980; Storey, Wakelin & Behnke, 1985). Strains of mice also differ in the ability to sustain infections with *Brugia* spp., whether these are initiated by injection of infective larvae or microfilariae, or by transfer of adults (Howells, Devaney, Smith & Hedges, 1983; Fanning & Kazura, 1983; Suswillo, Doenhoff & Denham, 1981). Collectively these experimental data provide support for the view that genetically determined differences underlie a significant proportion of the variation in levels of infection apparent when prevalence data relating to gastro-intestinal and filarial nematodes are collected in the field.

In terms of the major gastro-intestinal nematodes of man (*Ascaris*, hookworm, *Trichuris*) there is now a consensus that infections always show aggregated patterns of distribution in the host population, a minority of individuals being heavily and continuously infected, the majority being only lightly infected or free of worms (Bundy, 1988). Although it is clear that many behavioural and environmental factors can contribute to this aggregation, it is difficult to believe that an absence or low level of infection in an endemic area can be wholly explained by lack of exposure. If it is accepted that variation in protective responses can determine whether these worms do or do not survive, then it can be assumed that the worm burdens in the chronically infected individuals arise in one of two ways. They may result from infections in which worm life span is prolonged, because of ineffective or strategically reduced immunity, or from infections where the worm population shows a regular turnover, individual parasites having short life spans, but weak immunity allowing frequent and successful reinfection to take place. Both of course may occur simultaneously, but the net result is a chronic infection. For some of these gastro-intestinal species there are data showing that intrinsic parasite longevity is long. For example hookworm infections in man have been studied through several volunteer exposures and these have indicated a life-span of 15–17 years in the case of *N. americanus* (see Beaver, 1988). However, all the trials with *N. americanus* have been carried out on Caucasian volunteers. There are data from the time when hookworm infection was widely distributed in the southern states of the U.S.A. (Smilie & Augustine, 1925; Leathers, Keller & Wyman, 1936), that the disease and consequently heavy infections were more common among the Caucasian inhabitants of these regions compared with the sector of African origin in the population. It may be, therefore, that races which have lived in endemic regions for a long time have evolved resistance mechanisms, but to date these have not been identified and there is still no clear evidence that humans as a whole have any protective device to curtail hookworms (Behnke, 1991). Data from work with domestic animals are more clear cut. Many authors have shown genetically determined differences within and between breeds of sheep in ability to resist infections with gastro-intestinal trichostrongyles, and there is no doubt about the role played by immune responsiveness in this resistance (Windon, 1991). Low responder sheep sustain heavy and chronic infections with these worms, high responders can exert a substantial degree of control. The genetic basis for differential resistance is still largely unknown, although a single dominant gene has been proposed for high response to *Haemonchus contortus* (see Gray, 1987) and detailed breeding studies have been undertaken to analyse inheritance of resistance to *Trichostrongylus colubriformis* (see Gray, 1991).

One of the intriguing facets of human filarial infections, both those involving the lymphatic species and those involving *Onchocerca*, is the broad spectrum of clinical manifestations and infection levels seen in endemic areas, where challenge from the bites of infective vectors is likely to be common to all members of the community (Piessens & McKenzie, 1982; Piessens, McGreevy, Piessens, McGreevy, Koiman, Sarosa & Dennis, 1980; Piessens, Ratiwayanto, Tuti, Palmieri, Piessens, Koiman & Dennis, 1980; Piessens, McGreevy, Ratiwayanto, McGreevy, Piessens, Koiman, Saroso & Dennis, 1980; Ottesen, 1984; Nutman, 1989). At one extreme individuals may show chronic infections, with persistent microfilarial burdens, at the other individuals may be parasitologically negative, despite immunological evidence of exposure to infection. Some of the parasitologically negative individuals will suffer marked pathology (e.g. obstructive conditions such as elephantiasis and hydrocoele, or severe skin conditions), others will show no pathology. Pathological conditions may be equally variable among those who are positive for microfilaria. Many studies have identified significant differences in

immunological responsiveness between these various groups (Piessens & MacKenzie, 1982; Ottesen, 1984; Nutman, 1989) implying a correlation between immune response capacity and the outcome of infection. Chronicity of infection here may again result from defective responsiveness.

Although many associations have been made in humans and in domestic animals between specific immune components and high or low responsiveness to infection, detailed analysis of the immunogenetic basis of these differences is possible only using defined experimental models. Some of these models allow identification of defects in particular protective responses as the cause of the low-responder phenotype, in others no direct correlation is yet possible. For example, low responsiveness to *T. spiralis* appears to arise from an inherent inability to generate effective intestinal inflammatory responses, reflected in levels of mastocytosis and eosinophilia (Tuohy, Lammas, Wakelin, Huntley, Newlands & Miller, 1990; Lammas, Mitchell, Tuohy & Wakelin, in press). Mice capable of mounting early immunity to *T. muris* make earlier and greater specific IgG1 responses (Else & Wakelin, 1989). Low responsiveness to filarial infections is associated with defective IgM production and possibly with low antibody affinity (Gattrill, Kee, Behnke & Wakelin, 1991). In contrast, low responsiveness and chronic infection in mice infected with *H. polygyrus* appear to reflect genetically determined host susceptibility to parasite immunomodulation (Behnke & Wahid, 1991), although the critical protective components that are suppressed are not yet known in detail.

All of these models, and many others, have been used for intense genetic analysis of response phenotype and particular attention has been paid to the relative roles of major histocompatibility complex (MHC)-linked and non-MHC-linked genes. The reasons for this focus are three-fold: resistance to certain infectious diseases is known to show MHC linkage; MHC-linked genes control the basic process of antigen recognition; MHC gene products provide convenient markers for population studies concerned with differential resistance to infection. In general, as far as nematodes are concerned, MHC-linked genes do not exert an overriding influence on resistance *per se*, which appears to be most strongly influenced by background (non-MHC) genes. Different MHC haplotypes do have a significant effect on the outcome of infection, however, when background genes are held constant (Wassom, Brooks & Cypess, 1983; Else & Wakelin, 1988; Behnke & Wahid, 1991) suggesting that MHC-linked genes do play a role in regulating expression of responses necessary for effective resistance, the absolute levels of which are determined elsewhere in the genome; what this role is remains uncertain. There are now several examples [in *Ascaris suum, B. malayi, H. polygyrus, T. spiralis* and *T. muris* (see Kennedy, 1989)] of MHC-linked differences in the patterns of serologically detectable antigen recognition and it is possible that differential antibody responses may contribute to differential protective immunity. Individual variation in antigen-recognition patterns have been described in many human nematode infections, and MHC-linked influences on antibody-mediated responses that might be protective against *Trichuris* in humans have also been proposed (Bundy, 1988). However, it seems more likely that the most important consequences of MHC-linked genes may be their influence on the pattern of initial T cell responses to parasite antigens and thus the cytokine profile generated. In this way MHC genes would control the range of effector mechanisms ultimately available for interaction with the parasite, although these would still be regulated by non-MHC genes, as outlined earlier. This aspect of immunoparasitology is now a very active area of research (Finkelman, Pearce, Urban & Sher, 1991) and one that should contribute very greatly to our understanding of the determinants of host resistance and parasite survival. In the mouse, where there is convincing evidence for the existence of defined T cell subsets and subset-restricted cytokine production (Mosmann & Coffman, 1987) it is striking that, in most cases, nematode infections selectively promote T helper subset 2 (Th2) responses. Th2 cells release the cytokines Il-3, 4, 5, 9 and 10, which are involved in the downregulation of Th1 cytokine production and in the promotion of eosinophilia, mastocytosis and IgE synthesis, all hallmarks of nematode infections. It has been suggested that Th2 responses may be deliberately induced by parasites as a strategy for downregulating host-protective Th1 responses, but the evidence that the latter are significantly involved in resistance to nematodes is equivocal. Although this interpretation is supported by the work of Pond, Wassom & Hayes (1989) on *T. spiralis*, other data from work with this species show that both high and low responder mice produce Th2-pattern responses when infected (Grencis, Hultner & Else, 1991). Additionally, work with *Trichuris muris* shows that low responsiveness is associated with Th1 responses, high responsiveness with Th2 (Else & Grencis 1991). These latter data are significant in the context of this review, because mice that are low responders to *T. muris* not only fail to expel the worms early in infection, but appear to become 'tolerized' by exposure to later stages of the parasite and lose their ability to expel both the existing and subsequent infections (Else, Wakelin & Roach, 1989). The possible role of parasite-secreted cytokine-like molecules is discussed in section 4.1.2.1.

The major roles played by non-MHC genes in determining overall levels of susceptibility and resistance to infection are still undetermined in the majority of cases. As with MHC-linked genes there is evidence of an effect upon the specificity, isotype and kinetics of antibody responses made to infection (Almond & Parkhouse, 1986; Tomlinson, Christie, Fraser, McLaughlin McIntosh & Kennedy, 1989; Else, Wakelin, Wassom & Hauda, 1990b) and these may of course influence worm survival. Background gene effects upon inflammatory response capacity have been clearly defined in mice infected with *T. spiralis* and a good correlation established with protective immunity to the intestinal stages (Wakelin, 1985). There is suggestive evidence that similar correlations may exist with high and low responsiveness to trichostrongyle infections in sheep (Windon, 1991).

5.3. *Host genes providing resistance to parasite immunomodulation*

The emphasis in the above section has been on host genes that may directly control elements of the protective response to nematode infection and therefore influence parasite survival. It has already been pointed out, however, that the outcome of host–parasite relationships depends upon an interaction involving both host and parasite. Parasitic nematodes, like all parasites, are not simply passive vehicles for antigen presentation, many of them are known to interfere actively with the host response, using a variety of strategies (section 3). An important component of these strategies is the release of immunomodulatory molecules, which downregulate, divert or interfere with host-protective responses. The production of these molecules is likely to be variable within parasite populations (see above) and the degree to which the host is susceptible to their effects is also likely to be variable within host populations. Hence some hosts may be susceptible to infection, fail to mount rapid host-protective immune responses, and support chronic infections, not because they lack the genes necessary for the expression of an appropriate effector response, but because they are susceptible to the immunomodulatory products of the worms themselves. This aspect of resistance and susceptibility has so far received comparatively little attention, but it is clear from experimental studies with *H. polygyrus* and with *T. muris* (Behnke & Wahid, 1991; Else *et al.*, 1989) not only that it exists, but that it can be an important determinant of the outcome of infection. Evolutionary considerations would predict that hosts would be under selective pressure to develop resistance to parasite immunomodulation, either by modifying the molecules that are the targets for immunomodulatory factors, or by developing counter-measures, such as the ability to make antibodies that neutralize the activity of these factors. These aspects of the host–parasite relationship are, of course, another facet of the continuing arms race between host and parasite, but they are of considerable practical importance. Identification of the immunomodulatory factors used by parasites and of the mechanisms involved in host counter-measures could make a substantial contribution to the rational control of many chronic nematode infections.

6. THE WAY FORWARD

Throughout this paper we have attempted to identify common principles which apply across species, genera and higher taxa as well as points of distinction between them with respect to their evasive strategies. It is still early to synthesize a coherent picture, but the process has been initiated and already it is evident that there exist significant differences in survival strategies even between closely related taxa. These do not bode well for future immunotherapy.

The chronic survival of adult nematodes and the associated extensive patent period must be important in enabling the generation of the quantities of transmission stages required to ensure successful transmission, otherwise they would not have evolved. In filariae, the persistence of mf would maximize the likelihood of encountering a suitable vector and would achieve the same objective (Bayer & Wenk, 1988). However, quite different strategies may be envisaged for achieving ultimately these same objectives. For example *L. carinii* has mf which are relatively short-lived, surviving perhaps only 2–14 days at most (Wegerhof & Wenk, 1979). Here the emphasis appears to be on overwhelming the host's ability to clear mf with minimum protection for individual transmission stages mediated primarily through adsorbed host albumin. The daily output from one female *L. carinii* in cotton rats was estimated by Haas & Wenk (1981) to be 20,000 200,000 mf although later Mossinger & Wenk (1986) concluded that 22,000 was a more realistic figure. Mf are coated with host albumin and there is accompanying immunodepression but large numbers of mf are successfully sequestered and destroyed in various organs. The daily output of *Loa loa* is estimated at 10–20,000 (Eberhard & Orihel, 1986) and there is evidence that the mf of this species, like those of *L. carinii*, express host-like or adsorbed host antigens (Ridley & Hedge, 1977) but in this case mf are long-lived surviving 6–12 months (Duke, 1960). *A. viteae* (7000 mf per female per day, Mossinger & Barthold, 1988), *D. perstans* and *Onchocerca volvulus* (1–4000 per female per day, Engelbrecht & Schulz-Key, 1984; Schulz-Key & Karam, 1986) produce few, relatively long-lived mf daily which can survive for the

best part of the year and more (see reviews by Behnke, 1987; Duke, 1968). In these latter species the emphasis appears to be on antigen shedding as a protective device ensuring the survival of individual mf.

It follows from this that the precise significance of each survival strategy must be evaluated in order to allow immunological intervention a chance to succeed. A particular strategy may be quite crucial with others contributing to but not solely capable of enabling survival. This is eloquently illustrated by studies with *H. polygyrus*. Mice maintained under trickle infection regimes and subjected to regular doses of *H. polygyrus* take 8–12 weeks to develop immunity which expels adult worms and ensures protection against challenge larvae (Brailsford & Behnke, 1992). The chronicity of infection is essentially determined by IMF from adult worms. Once this was realized, short-term immunizing infections, abbreviated with anthelmintics before the larvae had developed to the adult stage, were explored and found to induce potent acquired immunity (Behnke & Robinson, 1985). Even non-responder mice such as C57BL/10 and CBA can be made totally resistant to the parasite by a 6-day primary infection. Under trickle-infection regimes both of these latter strains accumulate worms and die (Brailsford & Behnke, 1992). Thus mouse strains which previously could not be made immune were found to express strong resistance to challenge so long as they did not experience adult worms during the immunization period. The lesson from these findings is that non-responsive animals and hopefully patients as well, can be made resistant, once the interrelationships between host resistance and parasite evasive strategies are understood and taken into account when designing immunization procedures.

If immunomodulatory mechanisms prove to be host-specific, then factors produced by parasites affecting non-primates may well have little relevance for medicine, except perhaps in enabling us to comprehend the general principles involved. The search for immunomodulatory molecules of potential value in medicine should concentrate on specific parasites causing chronic infections in man because it is these which are most likely to target human cell receptors and/or immunoregulatory molecules. Here the paradox: host-specific organisms affecting man are extremely difficult to study because they cannot be easily maintained in laboratory animals. Some of the most widespread species affecting man, e.g. *Ascaris lumbricoides, Ancylostoma duodenale, Trichuris trichiura* and filarial species such as *Wuchereria bancrofti* and *Onchocerca volvulus* still cannot be maintained easily in laboratory animals, other than through the use of powerful immunosuppressive agents or by exploitation of exotic host species. There is therefore an urgent need to find *in vitro* ways for their maintenance or adaptation to more readily available experimental animals.

Despite the difficulties, there must be a concerted effort to isolate, characterize and to understand the mode of action of human nematode IMFs. Animal models such as *H. polygyrus* offer the advantage of convenience but in the longer term what is learned through animal models will have to be used to provide the basis for the exploration of factors from human nematodes. It is likely that the active molecules are to be encountered in the secretory/excretory products of nematodes and it is here that the search must continue. Whilst molecular techniques may be one solution, until we have some idea of what it is we are looking for, it is difficult to imagine how the relevant factors might be identified from gene libraries, other than through the meticulous screening of the entire genome, each fusion product in turn. One approach worth exploring would be to try using DNA sequences for cytokines and vertebrate immunoregulatory molecules, many of which have now been cloned, to identify complementary sequences in the nematode genome.

In recent years studies on parasite-induced immunodepression seem to have lost their appeal and are no longer in vogue. Loss of interest in this aspect of nematode biology stems primarily from the picture emerging in the last two decades which essentially indicated that virtually all species could cause immunodepression and there was little relationship between immunodepression and resistance to infection. Much has changed in the last decade. We hope to have shown here that indeed patterns are emerging and that these may be linked to the evolutionary biology of the Nematoda. There is still a long way to go and much of the road will involve repetitive studies on the species which have not yet been examined. Ultimately we believe that the rewards will be valuable indeed. A thorough understanding of the scope of the mechanisms evolved by nematodes to counteract the principal stressor to which they are exposed in vertebrate hosts may provide us with a direction which will lead to successful vaccination and hopefully new agents for the treatment of immunological complications and disorders in man and animals. We believe that this road is worth following and encourage others not to be dissuaded by the difficulties ahead.

Acknowledgements—We are grateful to Prof. P. N. R. Usherwood for the facilities provided for our research groups in the Department of Life Science (formally Zoology) at Nottingham University. JMB would like to thank the Trustees of the Colt Foundation, the Sir Halley Stewart Trust and the Medical Research Council for financial support provided in recent years. We are also grateful to Prof. R. C. Anderson, W. Piessens, H. H. Rees, Dr A. Pike and Dr E. R.

James for their advice, provision of reprints and comments on the manuscript.

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