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Nematodes and the spleen: an immunological relationship

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Abstract. Despite being a major organ of the immune system, the spleen's role in resisting, controlling or simply ameliorating nematode infections has been neglected. A review of both filarial and gastrointestinal nematodes suggests that though it is difficult to fully assess or quantify the organ's importance in vivo, the spleen is prominent in acting against nematode parasites in mammals. One manifestation of this is that transfer of lymphocytes from the spleen of immunised individuals can protect recipients against the disease. Expansion of splenic lymphoid tissue also alludes to its activity during nematode infection. There is a considerable need for investigation of the spleen under natural conditions as well as much more rigorously controlled experiments even in mammals besides birds and other vertebrates.
Key words. Splenectomy; splenomegaly; granulomata; microfilariae; white pulp; eosinophils; mucosal mast cells; IgE antibody.

Introduction

Barring work by certain pioneers (e.g. Perla and Marmorston[48] and Tischendorf[62]), the functions of the spleen have received remarkably little attention for a major vertebrate organ; moreover these have been controversial[34,40]. In the last decade, this circumstance has been improved significantly with publication of a series of papers and books devoted to the spleen[8,40,51,63,67,74]. Much research has been done on the physiological aspects of the organ's haematological functions, and to a somewhat lesser extent its immunological activities (e.g. refs. 8, 57, 66) but study of the spleen's role in resisting or controlling specific types of disease has been patchy – malaria and babesia being two notable exceptions[4,75].

Recently John[34] conducted comparative analyses (to be reported elsewhere; for preliminary results see ref. 29) of the avian spleen with parasites using the technique of phylogenetic regression[24]. The weight of the spleen relative to body weight was found to be significantly larger in species in which many individuals were infected with haematozoa relative to the number of individuals examined for the parasites. This is not unexpected given the extensive knowledge of splenic involvement in destroying blood protozoa. But it is perhaps rather more intriguing to find that the same holds for nematodes. One possible interpretation is that bird species that face much nematode parasitism need to have more immunologically active splenic tissue (see John[34] for discussion). Unfortunately, there has been very little work done on immunity to helminths in birds[13,19]. Turning to mammals, the evidence for splenic participation is better and seems deserving of a review – especially as some early studies either reported little splenic involvement or simply noted histological changes[44,48]. One study found no role for the spleen in destroying microfilariae[2]. Pathological responses by the spleen have been observed with nematodes[36] as with other diseases, but here I direct attention mainly at the possibility of beneficial splenic activities. It is not the intention of this review to examine every study of immunity to nematodes where, say, the spleen is used as a source of T lymphoctyes, but rather to draw attention to the spleen for its own sake, and the plurality of its activities, by presenting a cross section of results.

It is advantageous – since the immunology of the gut differs in many ways from the immunology of internal tissues – to classify nematodes into three groups: 1) 'solely' intestinal, 2) intestinal but with stages of the lifecycle that migrate through and/or reside in the body of the host, and 3) filarial (for an introduction see refs 6, 45, 49, 69, 70).

Filarial nematodes

Splenectomy

Unsurprisingly the relationship between nematodes and the spleen has been most frequently investigated with filaria and their blood-borne microfilaria, and most of the few helminth splenectomy studies have been done with them. Duke[16] studied the course of infections in the drill (*Mandrillus leucophaeus*), experimentally infected with one of two strains of *Loa* (one of which was natural and nocturnally periodic, while the other was a diurnally periodic human strain): microfilarial density in the peripheral blood rose to a peak following a prepatent period of roughly 140 and 153 days, respectively, but then fell rapidly to low levels which persisted for the duration of the experiment. If the primates are splenectomised at this stage a greatly increased and persistent density of microfilariae results, with both

strains. Thus the spleen suppresses the microfilaria in the peripheral circulation[16]. However, the numbers of microfilariae (produced by the adult worms in the tissues) passing through the lymphatic system or existing in the lung-blood reservoir are apparently not reduced[16].

Intraperitoneal transplants of adult *Litomosoides carinii* into normal, and splenectomised, naive multimammate rats, *Mastomys natalensis*, revealed that the former but not the latter rejected the transplanted worms[73]. It was concluded that the spleen is necessary for rejection. However, the exact relevance of this to natural parasites of *Mastromys natalensis* is less clear. Likewise, the finding that two of 12 splenectomised laboratory rats but none of 23 intact rats could be experimentally infected with subperiodic *Brugia malayi*[44] is also difficult to interpret. Similarly, Hawking[28] noted that *Loa* may not be adapted to reside in the primates studied by Duke[16,17], in view of the low level of microfilaremia in the nonsplenectomised individuals.

The microfilaria counts increased in some cases following splenectomy in dogs, cotton rats (*Sigmodon hispidus*) and jirds (*Meriones unguiculatus*) infected with, respectively, *Dirofilaria immitis* and *D. repens*, *L. carinii*, and *Dipetalonema witei*[28]. The results were inconclusive; for example, there was no dramatic change in the density of microfilaria when the spleen was removed from two dogs relatively late in the infection (microfilariae had been patent in the blood for 16 and 26 months), but after the splenectomy of a third dog, possessing an infection which had been patent for only 2.5 months, the microfilariae count increased quickly by at least three-fold[28]. However, in the experiments with dogs there were no control animals. Although a sudden increase in microfilarial density immediately after splenectomy is highly suggestive, spontaneous changes may occur[28]. With jirds, microfilarial density decreased in the two controls (briefly in one case) while the three splenectomised individuals yielded a large rise, a moderate rise, and a small decrease respectively. In a comparison of the *D. immitis* microfilaremia in splenectomised and non-splenectomised dogs[77], splenectomy of two microfilaremic dogs had little obvious effect. But a single uninfected dog that was splenectomised prior to being given a transfusion of blood containing microfilariae produced an immediate increase in the percentage of recoverable microfilariae, though the period of microfilaremia was no more prolonged than that in the non-splenectomised control individual[77]. Wesley[76] reported that splenectomy did not modify the resistance of female jirds to *Brugia pahangi* infection.

In short, the evidence from experimental splenectomy for the spleen's role in filarial infections is suggestive but equivocal. This is in part because 1) the studies adopted small samples, and, with one exception, none offered statistical analyses, 2) because of the difficulty of interpreting results from unnatural host-parasite combinations, and 3) because of the potential for other tissues to compensate for the lack of a spleen.

Granulomata

Has histological research been more revealing? In the drill, various histological changes in the spleen associated with the destruction of microfilariae have been recorded[17] – numerous subcapsular granulomata, through which blood percolated, developed during the infection, and microfilariae were destroyed within them by macrophages and giant cells; infiltration by eosinophils was also observed. Duke[17] noted that previous studies had found fibrotic nodules in human spleen but these had been interpreted differently – those in patients infected with *Loa loa* contained numerous eosinophils but the microfilariae within showed no overt degeneration, and Klotz[38] suggested that these nodules simply arose from irritation by microfilariae. Nodules which lack microfilariae have also been described in areas where loiasis was absent, provoking the hypothesis that microfilariae are coincidentally trapped in nodules, in geographic areas where *Loa loa* occurs[52].

The splenic changes in the drill[17] were not observed in various other filarial infections investigated by Hawking[28], who also stated that post-mortem examination of human patients infected with *Wuchereria bancrofti* usually reveals no evidence that many microfilariae are destroyed in the spleen. Other authors, however, have suggested that the splenic granulomatous reaction aids the destruction of the microfilaria (including *W. bancrofti*) though they noted that such lesions are found infrequently[14,50].

Prominent granulomatous lesions occur in the spleens of jirds subcutaneously inoculated with *B. pahangi*; these granulomas are in the red pulp and also involve infiltration by eosinophils and Langhans' multinucleate giant cells[68]. This inflammation of the splenic red pulp is strongly associated with the presence of microfilariae, but, although it leads to the destruction of some parasites, Vincent and Ash[68] concluded that the role of this activity in the regulation of microfilaremia remains difficult to assess.

Granulomata can undoubtedly be pathological; for example, the eggs released during schistosomiasis induce granuloma formation in the liver, blocking blood vessels, and leading to portal hypertension and the concomitant enlargement of the liver and the spleen. However, granulomata may serve a useful function even in this disease[69]. Formation of granulomata is mediated by T cells through the release of lymphokines, leading to the attraction and stimulation of eosinophils which can be cytotoxic to the eggs. Mice which have been immune suppressed or deprived of T cells, display reduced granuloma formation but suffer higher mortality[69].

There is evidence that granulomata-associated pathology stems from an over-reaction and that, as the infection progresses, this is modulated by T suppressor cells and/or antibodies leading to a reduction in the volume of granulomata[69], suggesting a continuing effort to balance the benefits and costs of granulomata. In mice infected with *Schistosoma mansoni*, splenectomy at the peak of the granulomatous response causes marked enlargement of liver granulomata, and the spleen (presumably through the activities of its T lymphocytes) may regulate the specific granulomatous inflammatory response to the eggs of this digenean, these mice appearing not to benefit from removal of the spleen[32]. Most people infected with schistosomes (more than 90%, for *Schistosoma mansoni*) do not develop clinically serious liver fibrosis, without which most patients are asymptomatic[78]. Perhaps fibrogenesis is normally down-regulated along with antischistosomal T cell responses in chronic infections, this failing to take place in individuals that develop severe fibrosis[78]; such persistent immunoresponsiveness by the T cells might possibly stem from immunoregulatory gene differences[78] (see also Stadecker and Colley[56]).

Conceivably, the balance between the benefits and costs of granulomata may be delicate, requiring close and continuous evolutionary association between host and parasite. If so, then special care must be taken when interpreting the pathology of this tissue response in unnatural host-parasite combinations. Microfilaria granulomata do occur, however, in the spleens of wild animals. Tissue responses to malaria in platyrrhine primates have been studied, granulomas being noted in the red pulp of a few old individuals e.g. some spider monkeys (*Ateles* spp.) which had been shot in the wild[59]. These eosinophilic granulomas invariably contained microfilaria, but Taliaferro and Cannon[59] observed that filariasis is practically universal among adult spider monkeys and therefore hesitated to ascribe the granulomas to filaria. Perhaps as animals age they become less able to sustain an intricate balance between immune responses, with the result that granulomata become most evident in this age class. Note that the low incidence of the observed lesion could be due to reversibility[50]; thus it may be that the process does exist in younger animals though it is, perhaps, more likely to be seen in older animals because of its misbehaviour in these individuals. This would not preclude in some host individuals a genetic propensity to produce suboptimal immune responses, as mentioned in the schistosome context[78] (See Hamilton et al.[27] for an evolutionary perspective on the notion of sustained interplay between coevolving hosts and parasites – interestingly, schistosome production of neuropeptides that induced changes in host immune activity has been cited as an example of the adaptive mimicry of host mediators by pathogens[47]).

In summary, the status of microfilarial granulomata, particularly under natural conditions, is obscure. There seem to be two contending ideas. Granuloma production could be a common (though only briefly apparent) process that normally aids the disintegration of microfilariae but which occasionally fails to do so, thereby producing chronic lesions, at least in some individuals, that are more likely to be detected. Alternatively, perhaps granulomata exist solely in individuals that are unable to efficiently clear microfilariae by other 'normal' means, and in the event of this failure act to contain the microfilaria even though they cannot be quickly destroyed.

Other tissue responses including splenomegaly

Granulomata are by no means the only splenic response to filaria; they are more moderate in jirds infected with *B. patei*, and minimal or absent in individuals infected with *B. malayi* – the levels of microfilaremia are also lower in these infections compared with *B. pahangi* infections[68]. Yet, all three *Brugia* spp. produced intense follicular activity, expansion of red pulp and increased spleen size coinciding with the onset of microfilaremia. The positive correlations between spleen size (in proportion to b.w.) and mean microfilaremia were significant for males infected with *B. malayi* or *B. patei*, but not for *B. malayi*-infected females, which had much lower microfilarial densities than corresponding males, or males infected with *B. pahangi*[68].

Similarly, in a study of experimental infections (using either subcutaneous injection or infected ticks) of inbred Mongolian gerbils (jirds) with infective larvae of the filarial parasite, *Dipetalonema viteae*, which is a natural parasite of some gerbil species, Abraham et al.[1] did not mention the presence of granulomata in histological sections; moreover, Hawking[28] stated that studies with jirds infected with *D. witei* did not yield any histological evidence that appreciable numbers of microfilariae are destroyed in the spleen. Yet experimental infections lead to an increase in spleen weight, due predominantly to hyperplasia of the white pulp but also the expansion of the red pulp[1]. This splenomegaly is distinctive even during the prepatent period, in contrast to infections with *Brugia* spp. in gerbils during which splenomegaly roughly coincides with the appearance of microfilaremia[68]. The first detection of *D. viteae* antigen-reactive cells takes place at the onset of white pulp expansion[1]. These findings are particularly relevant because it is known that jirds with prepatent *D. viteae* infections develop acquired immunity. A close association between the immune status of *Mastomys natalensis* during *Acanthocheilonema viteae* (*Dipetalonema viteae*) infection (brought about relatively naturally through infective bites of tick vectors) and the level of γ-glutamyl-transpeptidase in lymphoid tissues, including the spleen, has been recorded; this activity possibly

reflects lymphocyte replication and synthesis of monokines and immunoglobulins[54].

In an attempt to separate the effects of microfilaria from those of adults, mice have been injected with *B. malayi* (mice are unnatural hosts in which the adult worms do not develop)[25]. Large numbers of microfilariae were seen in the small vessels of the lungs but none were observed in the spleen. Following the injection of microfilariae labelled with ^{51}Cr, most radioactivity occurred in lungs (57%), while the next highest levels were in the liver (8.5%) and spleen (2.9%). There was, however, little reaction by the host's tissues to the parasites. The spleen was the exception, and its weight increased quickly, peaking on the fourth day of infection with an average weight that represented a 2.5 fold increase (as a percentage of b.w.), and then gradually subsided. This involved cellular proliferation in the splenic white pulp and vascular congestion of the red pulp. Liver weight also increased, though less radically. Moreover, when the mice were challenged with microfilariae *after* the primary infection had disappeared, there was an accelerated removal of the parasites from the circulation, and a marked peripheral eosinophilia.

Grove et al.[25] concluded that the splenic reaction probably represents a response to antigenic stimulation by excretory and secretory products of microfilariae but did not go so far as to suggest that the spleen helped to destroy the microfilariae. Yet it seems quite possible that the spleen helps to suppress the microfilariae in this host-parasite model, because of 1) the existence of a resistance to reinfection, and 2) what is now known about the mechanisms of filarial immunity, discussed in the next section. Microfilariae are also retained predominantly by the lungs in dogs infected with *D. immitis* and probably in humans with lymphatic filariasis, but the spleen appears to be the major organ for trapping microfilariae in some patients with brugian filariasis[49].

Immune mechanisms

Spleen cells, from hamsters that are immune to circulating microfilariae of *D. viteae*, induce amicrofilaremia when transferred to nonimmune, infected individuals, and this is not so if B cells are experimentally depleted[61].

Microfilariae are probably killed in many cases by antibody-dependent cellular cytotoxicity (ADCC)[9,12,49]. For example, spleen and peritoneal cell adherence and cytotoxicity to *Litomosoides carinii* microfilariae in rats requires IgE[41,58]. This antibody has also been implicated in killing of adult *Brugia pahangi* worms in the lymphatics of cats[5]. With other host-parasite experimental models other classes of immunoglobulin are effective[9]. For instance, Tanner and Weiss[60] concluded that IgM promoted macrophage adhesion to *D. vitae* microfilariae in hamsters. The spleen is a major site of IgM synthesis, splenectomy reducing its level in the circulation[42], and is also important to IgG production; as discussed in the next section, its function in the supply of IgE may not be derisory.

The main effector cells appear to be macrophages, eosinophils and neutrophils. For example, the pattern of eosinophilia in the leaf-monkey *Presbytis cristata* following experimental inoculation with infective larvae (*Brugia malayi*) probably reflects the associated presence of microfilariae in the blood, and their destruction in the spleen[10]. This organ is, of course, a central component of the macrophage system, and may also provide a convenient location for granulocytes to interact with microfilariae. The spleen could also influence granulocyte phagocytosis through tuftsin production. It is the site of a critical stage in the production of this tetrapeptide[43], which promotes phagocytosis by granulocytes, and also influences the number of plasma cells in the spleen[22]. Plasma tuftsin concentration is deficient in splenectomised individuals[11].

Toxic substances released by eosinophils appear to be highly active against exsheathed nematode larvae but inactive against larval sheaths, and Butterworth concluded that neutrophils may be more active against ensheathed larvae even though eosinophils appear extensively involved in nematode destruction in vivo[9] – if it is true that the sheaths of microfilariae are derived from the egg capsules[55] then these comments presumably may also apply to the eggs released by oviparous filariae. However, the experiments which found that eosinophils could adhere to, but not damage, sheathed microfilariae may have been using eosinophils that were not functionally activated[9]. Recently, in vitro killing of *B. pahangi* and *B. malayi* (sheathed) microfilariae by eosinophil-granule proteins has been shown[26]. It may therefore be too early to rule out eosinophil cytotoxicity against even sheathed microfilariae (or eggs – filaria species in birds especially are often oviparous rather than ovoviviparous[55]).

There has generally been little success in demonstrating lymphocyte cytotoxicity against helminths. Wakelin[69] commented that the apparent failure of lymphocytes to kill schistosomula, which seem to acquire host MHC antigens on their surface, is remarkable. It has been demonstrated, however, that activated T lymphocytes can kill skin-stage schistosomula in vitro[20]. The method of killing adopted by T lymphocytes, involving distortion or invagination of target cell membranes rather than degranulation of toxic mediators, may not generally be appropriate for helminths[9]. Even so, the release of toxic substances by these cells remains a possibility. Further, it does not preclude delayed-type hypersensitivity responses by T_{dth} lymphocytes.

Immune responses to the microfilariae L_1 stage have been studied more intensively than other stages, but immunity against infective larvae, and the inhibition of microfilariae production by the adult female worms

have also been demonstrated[12]. The transfer of splenic T lymphocytes from mice that have been immunised with attenuated infective larvae, provides considerable protection to naive mice[30].

Thus, although the mammalian spleen's activities against filaria are far from understood, there is a variety of evidence for an important splenic role in controlling at least some species. In addition to filtering and destroying microfilaria, the spleen could influence events outside it through antibody production, T_h cell help and any T_{dth}-mediation, as well as tuftsin production.

Gastrointestinal nematodes

With internal stages

The relationship between the immune system and the spontaneous expulsion of adult worms has been intensively studied with the rat nematode *Nippostrongylus brasiliensis*, although it is not fully understood. Worm expulsion appears to be associated with 1) release, from intestinal mucosal mast cells, of amines which damage worms directly or indirectly by inducing intestinal inflammation (including mucus secretion by intestinal globlet cells and possible antibody damage) (see also Wakelin[71]), and 2) accumulation of eosinophils in the intestinal mucosa which degranulate when appropriately stimulated by antibody[9,69]. IgE antibody appears to be intimately involved in both these activities; but intestinal nematodes can also be expelled by mice that are deficient in IgE production[64,69]. T lymphocytes are clearly involved because mice depleted of T helper cells do not, in some experimental models at least, expel adult *N. brasiliensis* worms, increase IgE production or exhibit mast cell hyperplasia[64]. (Although IgE is a prominent antibody in helminth immunity, it has recently been revealed that IgA can be highly effective in mediating the killing of metazoan parasites, namely schistosomes, by human eosinophils[18]).

In what ways could the spleen be involved? In mice inoculated orally with eggs of *Ascaris suum* (normally a nematode of swine) or subcutaneously with infective larvae of *N. brasiliensis*, cells of the spleen as well as those of mesenteric lymph nodes demonstrate a preferential increase in the expression of IgE[35,64]. The life cycle of *A. suum* cannot be completed in mice, and migration within the body is limited to the lung stage, but Urban et al.[64] argued that the increased expression of IgE in infected mice is consistent with IgE-related responses to this infection in the pig, such as cutaneous anaphylaxis and degranulation of intestinal mucosal mast cells.

This association between IgE and the spleen might seem surprising given that Mitchell[42], for example, concluded that IgE (and IgA) production is essentially extrasplenic in mammals studied to date. Work by Katona et al.[35] placed a different perspective on the synthesis of IgE by the spleen. While 40% and 35%, respectively, of mesenteric lymph node and spleen cells (or, more specifically, over 95% of mesenteric lymph node, and about 60% of splenic, B lymphocytes) acquire surface IgE, following subcutaneous inoculation of mice with infective larvae of *N. brasiliensis*, most of it is probably cytophillic rather than intrinsic[35]. The percentage of cells containing much intracytoplasmic IgE (indicative of IgE synthesis) peaks at about 0.6% in both organs[35]. However, according to Katona et al. [35], this is not an insignificant amount. Both intracytoplasmic and surface IgE are essentially undetectable in uninfected mice. Thus, although the percentage of B cells acquiring intrinsic surface IgE is small, these cells are responsible for the synthesis of large quantities of IgE[35].

This contribution, together with the extensive cytophillic acquisition of IgE by splenic B cells points to some splenic involvement in controlling intestinal nematodes with tissue stages, and should also be seen in the context of the general proliferation of cells. Between 6 and 16 days after infection there is, respectively, a quadrupling and doubling in mesenteric lymph node and spleen cell numbers, and in both organs a three-fold increase in the percentage of large cells[35]. By the 10th day of infection adult worms are cleared from the intestine. Another pointer to splenic production of IgE during infections by this parasite is that the high IgE response shown by certain strains of rat coincides with an increase in the IgE secreting cells in the spleen that is not matched by low responders; moreover, when the IgE response is artificially stimulated the increase in serum levels of this antibody is paralleled by a substantial rise in splenic IgE secreting cells in low and intermediate as well as high responders[15].

Similar processes involving mucosal mast cells, eosinophils, IgE and/or T cell mediation are evident against other species of nematode and other life stages – such as the L_3 infective larvae (e.g. refs 23, 37, 53, 79). Lymphocytes collected from the thoracic duct of rats infected with *Trichinella spiralis* induce eosinophilia and increased numbers of mucosal mast cells in the intestine when adoptively transferred to normal, uninfected recipients[72]; this operation can also confer protection against challenge infection[72]. The recipients of the helper lymphocytes that yield intestinal eosinophilia also reveal rising numbers of B cells, in the spleen and mesenteric lymph nodes, secreting antibody against adult *T. spiralis*; it is the same subset of helper T cells that produces protection against the adult nematodes[72].

In *Toxocara canis*-infected mice, numbers of haemopoietic stem cells in peripheral blood and spleen increase in parallel with peripheral blood eosinophilia, these stem cells apparently migrating from bone marrow in response to increased demand for eosinophils[31]. Spleen cells produce interleukin-3 and interleukin-5 and these in turn can induce eosinophilia in vivo[31]. Inter-

leukin-3 is also known as mast cell growth factor, and intestinal mastocytosis is elicited by systemic elevation of this interleukin after infection with tissue-migrating larvae[31].

Despite these findings, the interaction between these components of the immune system in eliciting protection is by no means transparent. One controversy is the role of eosinophils as effector cells against gastrointestinal nematodes in vivo since artificial depletion of these white blood cells does not enhance worm survival[21]; further, resistant strains of mice have higher counts of these cells in the peripheral circulation and in bone marrow but not in intestinal tissue during *T. spiralis* infections[39]. On the other hand, it has also been suggested that mast cells of the intestinal mucosa are not implicated in the expulsion of these parasites during primary infection[72].

Increasingly the immune system is being revealed to be extraordinarily versatile as well as intricate. Interpretations are complicated because, for instance: 1) any given agent may be capable of diverse activities (perhaps the eosinophil adopts a more regulatory influence in the nematode context); and 2) in the absence of a particular functional effector, other agents may take over its normal role. As a manifestation, different arms of the defence mechanism come into play in different host species, as well as in different ways in the same host-parasite combinations depending on circumstances. One illustration is that in gerbils (*Meriones unguiculatus*), infection by *Strongyloides venezuelensis* produces a gradual increase in mast cell number in the jejunal mucosa, while *N. brasiliensis* worms are expelled in association with goblet cell hyperplasia[33].

'Solely' intestinal

Finally, it is known that certain T cells regulate the protective immunity, IgE levels and worm fecundity in mice inoculated orally with the infective larvae of *Heligmosomoides polygyrus* (*Nematospiroides dubius*)[65]. Ali and Behnke[3] found that this parasite species, which resides in the lumen and mucosal wall of the small intestine, but does not migrate in the blood circulation even during its larval phase, induces splenic enlargement; the involvement of the spleen is secondary to that of the mesenteric lymph nodes but the spleen's support appears to be prominent when the amount of antigen emanating from the intestine is high and the lymph nodes are unable to process all of it themselves (see also Parker and Inchley[46]). This may be especially pertinent to birds since they generally lack lymph nodes, and many of their gut nematodes enter the mucosa (see John[34]), suggesting that corresponding avian studies would be intriguing.

Conclusions

There is some experimental evidence for splenic involvement in immunity against each of the three categories of nematode: filarial, intestinal with internal stages, and 'solely' intestinal. However the precise nature of the spleen's activities – in particular exactly how much these contribute to successful resistance to, or complete expulsion of, the parasites – remains to be elucidated. There has also been little study of the immunological role of the spleen in freeliving wild animals encountering natural infections. Here, at least, comparative studies[34] can complement histological and immunological experimental findings, following Claude Bernard's[7] dictum that observation and experiment advance together.

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