

21 Interactions among immune, endocrine, and behavioural response to infection

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1 Introductory remarks

Macroparasite infection is common among small mammals. As such, extensive coevolution between parasite and host species has occurred. In this chapter, we consider several aspects of the physiology of host-parasite relationships. In the first section, we discuss the generalities of host immune defences against macroparasites and the myriad tactics that parasites employ to avoid or minimize these responses. In the second section, we describe known effects of macroparasites on host behaviour and how hosts behave to eliminate or control macroparasite infections. In the final section, we consider the role of hormones in modulating behavioural and immune responses to parasites, highlighting the seasonal interplay between changes in host immunophysiology and macroparasite abundance and diversity that has been described in some species. Throughout the chapter, we discuss phenomena from both ultimate and proximate perspectives. However, we focus primarily on the regulatory processes that determine how micromammals avoid or resist macroparasite infections in natural contexts.

2 The vertebrate immune system

The vertebrate immune system is a complex network of cells, tissues, and soluble substances that either prevents macroparasite infections or eliminates or controls parasitic agents once infection occurs. Although much has been learned about host-parasite immune interactions in model species of domestic rodents (e.g., rats, mice, and guinea pigs), it is not apparent how relevant these studies are for understanding how free-living small mammals defend themselves against macroparasite attacks. The scant evi-

dence available indicates that wild animals rarely control and/or eliminate infection as readily as domesticated species. Further, wild animals rarely exhibit sterilizing immunity; i.e., they generally attempt to control but not eliminate most macroparasite infections (Wikel 2002). Below, we discuss how domestic and wild small mammals defend themselves immunologically against the various macroparasites discussed elsewhere in this volume. Instead of discussing the minutiae of each macroparasite-host interaction however, we highlight generalities among these interactions, as the immunological activities involved in each host-parasite relationship vary slightly.

Macroparasites preceded the phylogenetic development of the vertebrate immune system by millions of years, so it is to be expected that the immune defences of small mammals have been shaped over time to control these infections (Barriga 1999). The vertebrate immune system can be coarsely divided into two components: innate and adaptive (Janeway et al. 1999). The major distinction between these two lines of defence is that the innate system non-specifically destroys non-self substances whereas the adaptive system generates immunological memory of encounters with non-self substances and effects targeted attacks against these substances upon secondary interactions. The innate immune system includes cells such as macrophages, basophils, granulocytes, and other soluble compounds such as complement, acute phase proteins, and a host of enzymes and lytic molecules, all of which are often managed by the adaptive immune system. The adaptive immune system is comprised of two groups of lymphocytes, one of which is derived predominantly from bone marrow (B-cells). These cells either produce antibodies (immunoglobulins: IgG, IgM, IgE, IgA etc.) that control infection directly or more often target parasites or parasite-derived molecules for destruction (through a process called opsonization). The other group of lymphocytes (T-cells) originates in the thymus. These cells can also be divided into classes. T helper cells are responsible for managing immune responses, particularly the activity and movement of innate system effector cells. T killer cells, or cytotoxic T lymphocytes, attack and destroy self cells infected by intracellular pathogens. A third group of T cells, T regulatory cells, has been proposed, but their function and identity remains in debate. The actions of all of these cells are regulated by cytokines and chemokines. Although cytokines and chemokines are diverse in structure and function, they predominantly orchestrate one of two immune defence strategies. These strategies are referred to as Th1 versus Th2 responses, with Th representing the predominant T helper cell type involved in the immune response. Generally, Th1 responses induce inflammatory and/or cytotoxic cell activity and are best at controlling intracellular infections, such as those caused by viruses. Th2 responses on

the other hand drive humoral, or B-cell mediated immune responses, and hence are better at controlling extra-cellular parasites. Specific cytokine mediators of each response are variable and depend on the parasite and location within the body where infection occurs (Mahida 2003). However, some general patterns occur during responses to particular parasite types.

3 Immune responses to macroparasites

Macroparasites differ from bacteria, viruses, and protists in terms of how the vertebrate immune system must deal with them to prevent or control infection. First, macroparasites are generally larger and hence contain many more antigens to which immune responses can be generated. Intestinal helminthes in particular have been estimated to possess 7 to 20 thousand protein encoding genes, giving their hosts ample targets for immune attack (Pearce and Tarleton 2002). From the host's perspective, this characteristic can make macroparasite control difficult because parasites change antigenicity over the period of host colonization (i.e. – across developmental stages). In other words, hosts must constantly generate and mount immune responses against “moving targets” (Grzych et al. 1991). Second, parasites in most cases have an interest in promoting the survival of their host. Although macroparasites must subvert some immune defences in order to reproduce successfully, their tendency to replicate outside hosts generally precludes high virulence. The optimal strategy for a chronically infective macroparasite therefore is to suppress components of the immune system that prevent parasite persistence and reproduction but do not compromise the host to the point that it is killed by another infectious agent (Pearce and Tarleton 2002).

Several traits of macroparasites allow them to be successful in these efforts. First, they can often migrate to other areas of the body and hence avoid some immune responses generated against them. Second, they typically progress through multiple developmental stages within hosts, so immune defences generated against them may lose effectiveness over time. Finally, some parasites possess traits that allow them to avoid immune detection altogether. Cysts of *Echinococcus granulosus*, for instance, incorporate host complement regulatory factors into their outer membranes, which allow them to avoid innate-mediated immune attacks (Finkelson 1995).

From the perspective of hosts, these traits make macroparasites better candidates for control rather than eradication. Indeed, in the wild, it is rare to find animals that engage sterilizing levels of immune activity (i.e., com-

plete clearance) of intestinal parasites (Viney 2002). The more common pattern is maintenance of some low level of infection. By maintaining such a modest infection, hosts keep intact resistance they generate against primary infections, which some have suggested may provide them with increased defence against secondary infections (Viney 2002). This concomitant immunity, as it is commonly referred, effectively represents the hosts use of the parasite itself as a first line of defence against further infection.

A second and less obvious benefit of parasitism is the avoidance of autoimmune diseases that animals may experience late in life if they are infected (with low levels of parasites) early in life. Non-obese diabetic (NOD) mice, for example, show signs of diabetes soon after they reach adulthood. If they are experimentally infected early in life, onset of diabetes in adulthood never occurs. These early-life infections, which are often associated with elevated proinflammatory cytokine levels, are believed to oppose autoimmune cytokine production and induce greater T-regulatory cell activity in adulthood (Thomas et al. 2004).

Finally, individual hosts vary in their ability to control parasite infections depending on the time of year or current life stage through which they are progressing. For example, after experimental infection with *Nippostrongylus brasiliensis* pregnant female rats harboured more adult nematodes in their intestines and more eggs in their colons than age-matched virgin rats (Houdijk et al. 2003). Although it is not apparent what specific immune mediators produced this outcome, other work suggests that multiple aspects of immune activity vary with physiological state and time of year in small mammals (Nelson 2004).

3.1 Endoparasites

Helminths represent the most prevalent macroparasite group. The most common infectious genera for small mammals include *Ascaris*, *Trichuris*, *Strongyloides*, and *Trichinella*. Endoparasitic macroparasites can enter the host three ways: directly through the skin, via food or water consumption, or through the bite of blood-feeding insects. Unlike bacterial and viral infections, the number of macroparasites that hosts harbour reflects the number of times they came into contact with parasites; rarely do endoparasites replicate within hosts (Scott and Grencis 2002). Once parasites enter the host's body, the host's basic strategy is to make the local environment intolerable. Depending on the parasite, this strategy results either in expulsion (*Nippostrongylus*, *Trichinella*, and *Strongyloides*) or chronic mild infection (*Trichuris* and *Heligosomoides*). Regardless of the outcome, similar strategies of defence are enacted. Indeed, although hosts are gener-

ally able to recognize intestinal parasites, they show little ability to distinguish among different types (at least upon primary infection) to orchestrate more coordinated immunological attacks (Finkelman et al. 1997).

Four generalizations can be made about the immune processes engaged to combat intestinal parasites: (1) CD4⁺ T-cells are critical for protection, (2) IL-12 and IFN γ can counteract protective immunity generated by CD4⁺ cells and the effector mechanisms they induce, (3) IL-4 is either imperative for protection, important for limiting severity of infection, or pivotal in inducing redundant forms of protection, and (4) other cytokines increase in circulation during infection, but do not directly affect parasites (Finkelman et al. 1997). In terms of the specifics of immune control, Th1 mediated immune responses predominantly control initial infection whereas Th2 responses provide defence against chronic infection (Tarleton et al. 2000). Th1 responses are characterized by abundant IL-2 and IFN γ production, which leads to high cytolytic activity and increased complement activity. Such activities are effective at eliminating infiltrating larval stages of parasites. However, if a Th1 bias persists for long within a host, that host can become more susceptible to a persistent infection. High IL-12 production, which occurs late during Th1 responses, can increase helminth susceptibility (Else and Finkelman 1998).

Th1 biased responses activate many innate effector cells. Mast cells in particular are important for nematode resistance, and their activity is T-cell-dependent (Else and Finkelman 1998). Eosinophils are also important for resolving helminth infections (Butterworth 1984), but their efficacy depends on the developmental stage and parasite identity. Generally, eosinophils prevent larval establishment (Meeusen and Balic 2000). However, no studies have demonstrated that these cells affect adult parasite stages *in vivo*. Further, eosinophilia is only effective against some endoparasites; *Taenia taeniformis*, *Trichuris muris*, and *N. brasiliensis* resistance is positively correlated with capacity to generate eosinophilia in mice, but other parasites show no such relationships. Also, elimination of eosinophils during *T. spiralis* infections delays expulsion of parasites, but the same treatment has no effect for *N. brasiliensis* infections (Meeusen and Balic 2000). Eosinophilia is mediated by IL-5 secretion by T-cells or NK-cells, but B-cells serve important roles in these responses as antibodies must label parasites for eosinophils to have an effect (Hunter and Sher 2002).

Although larval stages of endoparasites are susceptible to innate effector cells, adult stages are better controlled by antibody-mediated defences, especially once they have established (Hunter and Sher 2002). Th2 cytokines, such as IL-4, IL-5, IL-10, and IL-13 are produced in abundance in response to adult parasite infections, and they are effective at mast cell activation, inducing eosinophilia, and suppression of Th1 responses (Mahida

2003). In some cases, Th2 cytokines can induce other parasite defences that are not immunological in nature. For instance, some Th2 cytokines alter gut mucosa structure and composition, including the intestinal cell phenotype composition and smooth muscle structure (Mahida 2003). Although Th1 versus Th2 biases affects host success against controlling infections, the effector mechanisms that are induced by these cytokine cascades are not well understood. For instance, production of IL-4 and IL-13 is related to control of *Heligonomoides* infection in mice, but not *Trichuris* or *Nippostrongylus* (Scott and Grencis 2002).

Until recently, these mechanisms were thought to be the primary pathways through which endoparasitic infections were fought. Recently, two other cell types, CD5+ B1-cells and $\gamma\delta$ T cells, were discovered to have anti-helminth properties. CD5+ B1-cells combat helminths by producing low affinity poly-reactive antibodies that recognize a variety of polysaccharides. These B cells respond to IL-10, a Th2 cytokine, and are present in large numbers in the body cavity. CD5+ B1-cells proliferate and produce IL-10 in response to *Schistosoma mansoni* eggs in mice (Vellupillai and Harn 1994). $\gamma\delta$ T cells express a diverse array of receptors, and are abundant in epithelial and mucosal tissue. In rats, *N. brasiliensis* infection induced proliferation of these cells and production of IL-4 (Rosat et al. 1995) initiating further Th2 mediated immune activity.

In common with these cell types, other soluble compounds have roles in parasite defence that were only recently recognized. One particularly promising line of research involves mannan-binding protein, which is produced by hosts and is capable of detecting mannose residue on parasite surfaces (Hunter and Sher 2002). Upon contacting mannose, these proteins initiate lectin-mediated complement activity, which allows hosts to eliminate parasites before they become established in tissue. Additional comparable mechanisms of detection may also occur in mammals. Some have even predicted that immune cells may possess receptors for particular components of parasite tissue much like Toll-like receptors are receptive to components of bacteria (Medzhitov et al. 1997).

Although not all of the mechanisms that small mammals use to prevent or control intestinal parasite infection have been identified, their immune defences are effective against the parasites that infect them. In rats, *Strongyloides ratti* become shorter and less fecund over the course of an infection. If the same individual parasites are transplanted into a naïve host rat, then parasites re-grow to their original size and produce eggs at higher rates, although these changes last only until their new hosts generate immune responses once again (Schad et al. 1997). In other words, changes in parasite fitness are directly related to the activity of the hosts' immune system, even in captive-housed animals. Indeed, shrinkage and decreased fe-

cundity of parasites in the case above was caused by a plug being generated over the oral cavity of worms; this plug, which is partly composed of host immunoglobulins, effectively starves the worm. If hosts are treated with immunosuppressive glucocorticoids, these effects are eliminated (Wilkes et al. 2004).

3.2 Ectoparasites

Ectoparasitic macroparasites take one of two general forms: (1) individuals attach and feed on blood or other bodily fluids of the host over an extended period (e.g. – ticks and lice), or (2) they attack and feed rapidly (e.g. – biting flies and most fleas). Because these two groups use different strategies to obtain resources from hosts, the tactics they use to subvert host immune defence and the immune defences that hosts engage to combat them vary. Much like endoparasitic helminths, ectoparasites that use an attachment strategy tend to down-regulate host immune defences to a level that promotes feeding but not host mortality. Rapid feeding ectoparasites also depress some aspects of the hosts' immune system to obtain a blood meal. Even though they encounter components of the host immune system for brief periods of time, they expose themselves to pre-programmed immune cells and more importantly cells primed by exposure to arthropod-specific antigens from previous ectoparasitic attacks.

Hosts use a variety of immune defences to combat ectoparasites. These mechanisms include up-regulation of antigen-presenting cell and T and B cell activity, antibody mediated complement activity, and mast cell and granulocyte activity. Ectoparasites do not passively avoid these barriers however. Almost all ectoparasites possess anti-hemostatic compounds in their saliva. In some cases, such as the ixodid ticks, parasites maintain substances in their saliva (e.g. kininase), which decreases host grooming activity by destroying bradykinin, a mediator of skin irritation (Wikel 2002).

3.2.1 Ticks

Ticks are one of the best studied groups of ectoparasites. As in other ectoparasites, salivary products produced by these arthropods change over the course of engorgement, making immune defence difficult for the host because of changes in the antigen environment. Indeed, ticks may use this salivary antigen variability as a defence mechanism itself. High production of several different proteins forces the host to make antibodies that are often ineffective for parasite control. In other words, by forcing the host to generate abundant ineffective antibodies, generation of sufficient protec-

tive antibodies is prevented (Barriga 1999). Besides antibody responses, hosts can engage many other immune defences against macroparasites, which can lead to impaired feeding, reduced engorgement weight, a diminished number and decreased viability of ova, and even parasite death. Further, once hosts have developed immunity to ticks, this immunity can be transferred to naïve animals either by infusing immune serum or lymphocytes (Wikel and Allen 1976). Avoidance or resistance of tick infestation is important to hosts, as tick parasitization can have strong negative consequences. Guinea pigs experimentally infected with *Dermacentor Andersoni*, for instance, were less able to produce antibodies to another novel antigenic substance compared to uninfected control animals (Wikel 1982). Although this effect disappeared once ticks stopped feeding, these data indicate that tick infections may suppress overall immune activity in hosts, which could lead to infections by more virulent disease agents.

To successfully feed on hosts, ticks must overcome the host's immune responses and other defences such as blood coagulation agents, platelet aggregation, and pain and/or irritation responses. Ticks are not passive in their efforts to avoid these defences. Their saliva can affect the host immune system in many ways including inhibition of complement activity, depression of pro-inflammatory cytokine production, T lymphocyte proliferation, Th1 cytokine secretion, antibody production, and natural killer (NK) cell function (Wikel 1999; Trinchieri 1995). One of the main targets of tick-induced immunosuppression is T lymphocyte activity, as salivary gland extracts reduce T- but not B-cell lymphocyte proliferation *in vitro* (Wikel 1999). Interestingly, tick saliva often contains prostaglandins (particularly PGE₂). Currently, no data exist to indicate that this substance has immunomodulatory effects in these contexts, but prostaglandins are generally well-known modulators of vertebrate immune activity (Wikel 1999). Surprisingly, little is known about the immune activity that takes place at the site of tick bites. Some evidence indicates that Langerhans cells, which serve as the major antigen presenting cells in skin, are less able to recognize parasite antigen post-bite (Brossard and Wikel 2004).

3.2.2 Fleas

Fleas are another common parasite of small mammals. Most fleas are solenophagous, meaning that they feed on blood from small blood vessels of hosts, but there is extensive variability among species in terms of how long they remain on hosts and how they obtain blood meals (Jones 1996). Only one genus (*Tunga* sp.) remains attached to its host for an extended period, although many remain on the skin for weeks or longer. Flea bites incite multiple immune activities in hosts including mast cell and basophil infil-

tration and IgE production. Generally, in the skin, a stereotyped progression of immune activity occurs. Upon the first infection, inflammatory immune activity is induced at the site of the bite, but outward signs are not obvious. After a second bite, a delayed hypersensitivity response is induced (T cell-mediated inflammation) characterized by infiltration of mononuclear leukocytes within 24 hours. With a third bite, both immediate and delayed hypersensitivity reactions are induced, and eosinophils infiltrate as rapidly as 20 minutes after the bite. A fourth bite generates only immediate hypersensitivity, but a fifth bite induces no skin reactivity (Larivee et al. 1964). As in ticks, substances in flea saliva change over the course of the feeding process, with different substances being released during blood vessel probing versus feeding.

3.2.3 Anoplura lice

Lice (*Anoplura* sp.) resemble ticks in terms of the strategies they use to exploit and evade their hosts. They evade grooming activity by hiding among hairs, and their elongate, flattened body form allows them to avoid easy removal. Overall, they feed rapidly and rarely remain attached to hosts for long (Jones 1996). In mice, resistance to *Anoplura* is correlated with increased numbers of multiple immune cell types at site of bite (Nelson et al. 1972). Sometimes, hosts control lice infection by modifying the structure and immunological access to their skin. In mice, lice infection increased epidermal thickness over the four weeks of infestation. During this period, neutrophils, eosinophils, and lymphocytes increased in number in tissue, followed by degranulation and subsequent tissue destruction at the site of infection over this period (Nelson et al. 1972).

3.2.4 Mosquitoes and flies

Although more often recognized as vectors for viral and bacterial parasites, biting flies themselves induce changes in the immune systems of their hosts. For instance, bites of the sand fly, *Simulium vittatum*, induces hosts to make IgM, IgE, and IgG reactive saliva antigens (Cross et al. 1993). Such immune responses serve a protective purpose; female sand flies (*Phlebotomus argentipes*) were less able to obtain blood meal from repeatedly bitten versus unbiten hamster hosts (Ghosh and Mukhopadhyay 1998). In common with other ectoparasites, fly attacks are chemically aggressive. Extracts from salivary glands of multiple species can affect the immune system by changing antigen presentation capacity and decreasing T and B cell proliferation (Titus 1998). Some of these processes are beneficial to the infectious agents that flies carry in saliva, as evidenced by increased

transmission of disease causing agents when saliva components are inoculated into animals in addition to infectious agents (Titus and Ribeiro 1990). However, the main purpose of these salivary compounds seems to be host immunosuppression. In rats, salivary gland extract of female but not male *Aedes* mosquitoes limit TNF α release from mast cells (Bissonette et al. 1993). Because males do not feed on blood in this mosquito species, they probably do not need, and thus do not produce, immunosuppressive salivary substances.

4 Behavioural responses to infection

Animal models of infection and parasitism have been studied for years. However, until recently, the role of behaviour in host defence and pathogen transmission was largely ignored. In this section we consider host behaviour from two broad perspectives: (1) behaviours mediated by the parasite and (2) behaviours mediated by the host. First we consider the phenomenon of parasite modulation of host behaviour because of mounting evidence that parasites can (and do) alter host behaviour to facilitate their own fitness (Klein 2003). In addition, we consider some of the underlying neuroendocrinological and immunological mechanisms by which this occurs. Second, some host-mediated behaviours that have evolved in order to prevent or control parasitic infections will be described. Behavioural responses can be complementary to immune function in the avoidance and regulation of macroparasites (Hart 1997). Hart (1990) described two conditions for which a behavioural pattern has a parasite defence function: (1) the parasite in question must have a detrimental effect on host fitness and (2) the behaviour must have the effect of removing, avoiding, or otherwise controlling the parasite. Specifically, behavioural mechanisms that are complementary to immune function in preventing or managing parasites, such as grooming behaviour and sickness responses, are presented. In addition, the social recognition and avoidance of parasitized conspecifics are considered. Finally, we discuss the role of major histocompatibility complex in resistance to parasites and the phenomenon of disassortive mating that occurs in some rodent populations.

4.1 Parasite modulation of host behaviour

The study of the modulation of host behaviour by parasites has received enormous interest in recent years (Moore 2002). Parasitic modulation of behaviour can occur through several different pathways including: (1) di-

rectly cellular infection, (2) immunologically mediated changes in the nervous system, or (3) alteration of the chemical messengers modulating behaviour (Klein 2003). The type of behavioural alterations that parasites induce is often dependent on the life cycle of the parasite in question. Although the specific responses vary among parasite-host responses, some generalities have emerged. For instance, parasites with direct life cycles are more likely to alter host behaviour to increase contact between the infected organism and vulnerable conspecifics. On the other hand, parasites with intermediate hosts often act to increase the probability of predation in order to facilitate transmission into the definitive host. Most of the work on parasitic alterations in behaviour as focused on microparasites (Klein 2003). However, some evidence exists for macroparasitic infections altering behaviour.

Parasites alter host neurochemistry as a proximate mechanism to alter behaviour. Parasitization and parasite-associated cues are able to induce analgesia in some rodent species. Analgesia, a reduction in pain thresholds, is part of a larger suite of defensive responses associated with real or potential danger. For instance, parasitization with *S. mansoni* and *N. brasiliensis*, but not *H. polygyrus*, induces a state of analgesia. Although some of these responses may be mediated by the host brain, *S. mansoni* produces and releases proopiomelanocortin (POMC), a peptide precursor to opiate molecules, as well as other opiate-like peptides (Duvaux-Miret et al. 1992). For parasites with intermediate hosts, natural selection would presumably favour organisms that could facilitate the intermediate host's depredation. The best known example of this phenomenon is that ants infected with the trematode "brain worm" (*Dicrocoelium dendriticum*) are more likely to ascend blades of grass, which increases the possibility that they will be consumed by sheep. Several lines of evidence suggest that this sort of manipulation may be occurring in mammals. As some other stressors, exposure to predator odours induces analgesia as well as number of other physiological alterations. Remarkably, animals infected with *H. polygyrus* and *Taenia crassiceps* fail to exhibit the normal analgesic responses to predatory stress (Kavaliers and Colwell 1995b; Gourbal et al. 2001). Future research should focus on identifying parasite induced susceptibility to predation and the proximate mechanisms underlying them.

Other behavioural responses are mediated via alterations in peripheral tissues. For example, a tape worm (*T. crassiceps*) induces a behavioural and physiological feminization in its male hosts. The disruption in normal neuroendocrine signaling interferes with the expression of reproductive behaviour. However, tapeworms (e.g., *T. crassiceps* and *T. taeniaformis*) inhibit mating behaviour in parasitized animals by adjusting testosterone signaling rather than acting on brain circuits that mediate mating. Reduced

mating behaviour in these animals can be restored with exogenous testosterone (Morales et al. 1996). Other androgen dependent behaviours including aggression are also reduced (Gourbal et al. 2002).

4.2 Behavioural avoidance of parasitization

Simple motor behaviours can be powerful defences against parasites. Lab rats may spend up to 1/3 of their waking time grooming (Bolles 1960). Also, biting and blood-sucking flies can be repelled with twitches, tail and ear flipping. Key largo wood rats (*Neotoma floridana*) exposed to mosquitoes demonstrate ~3-fold increase in the number of fly-repelling behaviours per hour (Edman and Kale II 1971). Mice infected with malaria fail to exhibit normal anti-mosquito behaviours resulting in increased feeding success for the mosquito vector (Day and Edman 1983) and potentially greater transmissibility for the malarial parasite.

4.2.1 Recognition of parasitized individuals

Physical contact represents a major mechanism by which parasites can be transmitted from infected to uninfected individuals (Kavaliers et al. 2005a). Thus, hosts have evolved a number of mechanisms to minimize their exposure to parasitized individuals. In rodents, the olfactory system is paramount among sensory systems. Chemical signals can provide key information about a conspecific (e.g., sex, reproductive condition, social status, etc.). As such, the accessory olfactory or vomeronasal systems are critical in detecting and avoiding parasitized conspecifics (Kavaliers et al. 2005b). Avoidance of parasitized conspecifics confers a fitness advantage to individuals in two distinct ways: (1) avoidance of close social contact with parasitized conspecifics can prevent transmission of the parasite, and (2) because resistance to many types of parasites is genetic, females select for males that are apparently resistant to parasites (for discussion see next section).

Rodents can often discriminate between parasitized and unparasitized conspecifics (Kavaliers and Colwell 1995a). The nematode, *Heligmosomoides polygyrus*, has been used extensively to study the effects of parasitic infection on social behaviours (Ehman and Scott 2002). This gastrointestinal nematode is shed in the feces and then after a short period is infective of other mice. Importantly, infected animals do not display classical sickness behaviours (see next section), so the number of interactions in which they engage with uninfected conspecifics does not change post-infection. Unparasitized individuals can discriminate between parasitized

and unparasitized conspecifics however, and not surprisingly, they prefer to mate with unparasitized animals (Ehman and Scott 2002). In addition, mice also prefer the urine of uninfected animals relative to parasitized ones despite the lack of parasite components contained in the urine (Kavaliers et al. 2004). The phenomenon of female avoidance of parasitized males also occurs in rats infected with the nematode *Hymenolepis diminuta* (Willis and Poulin 2000) and meadow voles (*Microtus pennsylvanicus*) harboring *T. spiralis* (Klein et al. 1999). The presence of ectoparasites also evokes avoidance responses. Female mice could discriminate between uninfected males and males infected with the louse, *P. serrata* (Kavaliers et al. 2003).

Although much of the interest in detection of parasitized conspecifics has focused on female choice, there is evidence that males prefer uninfected conspecifics and benefit from this choice. Female house mice harbouring *T. crassiceps*, *Echinostoma revolutum*, and *Echinostoma caproni* had smaller litter sizes than uninfected mice (Moore 2002), so males may do well to choose uninfected females, particularly if they exhibit a tendency towards monogamy and/or extended parental care. Indeed, male mice avoid females infected with *T. spiralis*, *T. crassiceps*, and *H. polygyrus* (Edwards and Barnard 1987; Kavaliers et al. 1998; Gourbal and Gabrion 2004). Male mice also demonstrate aversive responses to the odours of other infected males (Kavaliers et al. 2004), indicating that these behavioural tendencies may be driven by factors other than mate choice.

4.2.2 MHC diversity

Another area wherein olfactory cues may be important in regulating social behaviour is in relationship to the major histocompatibility complex (MHC). The MHC genes are among the most variable loci in the vertebrate genome (Penn and Potts 1998). MHC genes encode two types of large glycoproteins (class I and II molecules) involved in presenting peptide antigens to T cells and hence initiating some immune responses (Falk et al. 1991). MHC class I molecules present antigens from infected or cancerous cells. MHC II molecules present antigens from extracellular pathogens and parasites and are expressed on phagocytes and antigen presenting cells (Janeway et al. 1999).

The relationship between macroparasites and MHC is an area that has received much interest for two reasons: (1) MHC diversity is associated with greater resistance to parasitism (possibly due to greater T cell diversity (Dyall et al. 2000). For instance, specific MHC alleles were negatively associated with nematode burdens in wild yellow-necked mice (*Apodemus flavicollis*; (Meyer-Lucht and Sommer 2005); (2) Parasites appear at least in part to drive MHC variation to greater diversity. For instance, MHC di-

versity was positively correlated with prevalence of macroparasites in blind mole rats (*Spalax ehrenbergi*, Nevo and Beiles 1992).

Laboratory mice prefer to mate with individuals with dissimilar MHC alleles in the laboratory (Yamazaki et al. 1976; Egid and Brown 1989) and in semi-natural enclosures (Potts et al. 1991). The major hypotheses underlying the preference for this disassortive mating are increased pathogen/parasite resistance for offspring and avoidance of inbreeding (Brown and Eklund 1994). Importantly, mice can distinguish between genetically identical conspecifics that differ only in MHC haplotype (Yamazaki et al. 1979). The exact mechanism by which MHC proteins alter olfactory cues is not known, although this appears to be volatile components in the urine that may be components of MHC glycoprotein itself, or specific peptides that bind MHC molecules (Singer et al. 1997).

4.2.3 Sickness behaviour

Many behavioural responses to parasites are evoked by the parasite in an effort to enhance its own fitness. However, sickness responses are primarily mediated by the host. Sick or infected animals display a coordinated suite of physiological and behavioural responses (Exton 1997) collectively termed the acute phase response (Baumann and Gauldie 1994). The behavioural sequelae of bacterial infections are particularly salient and include lethargy, anorexia, adipsia, anhedonia, and reduced social interactions (Hart 1988). Additionally, the reproductive neuroendocrine axis is inhibited at multiple physiological levels (Rivier and Vale 1990; Avitsur and Yirmiya 1999). These responses collectively termed “sickness behaviour”, along with the induction of fever, are thought to be part of a coordinated, adaptive effort to aid in recovery from infection (Hart 1988; Kent et al. 1992). Indeed, interference with components of the sickness response can negatively impact recovery from infection. Force-feeding of mice infected with *Listeria monocytogenes* (e.g. – preventing anorexia) resulted in a nearly 100% increase in mortality over mice that were allowed to eat *ad libitum* (Murray and Murray 1979). The primary mediators of the sickness response are the proinflammatory cytokines, IL-1 β , IL-6, and TNF α (Kent et al. 1992; Dantzer 2001). Glucocorticoid secretion is potently activated by cytokines that feed back to inhibit cytokine gene expression (Turnbull and Rivier 1995; Goujon et al. 1997).

So far, sickness behaviours have not been shown to occur in response to macroparasitic infection. One component of sickness behaviour, anorexia, in response to parasitic infection is fairly common. The best studied example involves rats infected with the nematode, *N. brasiliensis*. These animals display a biphasic pattern of anorexia followed by a hyperphagic pe-

riod that occurs once the parasite has been cleared. The anorectic period is associated with the increased expression of mRNA for neuropeptide Y, which is a potent stimulator of food intake (Horbury et al. 1995). Additionally, the proinflammatory cytokine IL-6 is released and corticosterone concentrations are elevated during the early period of infection with *T. spiralis* (Roberts et al. 1999). Suppression of cytokine signaling with the synthetic glucocorticoid betamethasone reduced the anorectic affects of *T. spiralis*, although the authors attributed this effect to alterations in intestinal rather than neural inflammation (Faro et al. 2000).

Although specific examples of host-mediated sickness behaviours can be found in the macroparasite literature, they are not as common as in bacterial infections. Classical sickness behaviours may not be adaptive responses to chronic parasitic infections. Many animals face some parasite burden and as such the generalized behavioural depression associated with sickness responses would compete with other critical behaviours (Kavaliers et al. 2000). In addition, there is mounting evidence that sickness responses are plastic and can be modulated by the proximate social or physical environment (Aubert 1999; Bilbo et al. 2002a; Weil et al. 2006). Therefore, some chronic parasitization might not induce the sickness responses indicative of other types of infections. It would be interesting to determine if some parasites are interfering with the signal transduction pathways associated with sickness behaviours in order to enhance their own fitness.

5 Neuroendocrine regulation of immune function

Most individuals in a population are infested with small numbers of macroparasites whereas a minority of individuals within the same population harbours large numbers of macroparasites (Wilson et al. 2002). What accounts for this variation within populations? Variation in macroparasite infection may be partly due to the demographic composition of a population. For instance, prepubertal and aged individuals are generally more susceptible to macroparasites than adults (Klein 2004; Gryseels 1994). Thus the distribution of age classes within a population may affect the diversity and intensity of infection within a population. Part of this variation in infection status of the population may be reflective of differences in endocrine regulation among age classes. In a two-year study, male field voles (*M. agrestis*) displayed higher number of fleas than female cohorts (Smith et al. 2005). Flea numbers were maximal in autumn, likely reflecting the increased social densities (Smith et al. 2005). Importantly, this population

of voles displayed a convex age-prevalence pattern implicating the development of immunity for flea-borne protozoan (*Trypanosoma microti*) (Smith et al, 2005).

Host genetic differences in macroparasite susceptibility may also exist (Wilson et al. 2002). In part, such differences may reflect differences in hormone-modulated immune function. Individuals that perceive stressors tend to compromise immune function as compared to individuals that do not perceive stressors in their environment (Ader et al. 1995; Padgett and Glaser 2003). Glucocorticoids are released during stress responses and chronic exposure to these adrenal steroid hormones suppresses immune function (Ader et al. 1995; Padgett and Glaser 2003). Other possible proximate sources of variation in macroparasite prevalence include genetic differences among parasites, variation in host behaviour, and the time of year (Wilson et al. 2002).

5.1 Sex steroid hormones

One proximate mechanism underlying variation in macroparasite infection is differential endocrine modulation of behaviour and physiology, especially immune function. For example, male mammals and birds tend to be more infected than female conspecifics, which may be due to steroid-mediated differences in immune function (e.g., Grossman 1985; Klein 2000a, b). Generally, androgens compromise immune function whereas estrogens promote immune function (Klein 2000a, b). Other proximate explanations include the observation that the sex difference in body size favours male infection by macroparasites simply because they are easier to locate than the smaller females (Klein 2004).

The evolutionary pressures and trade-offs underlying sex differences in parasite load and immune activity have been previously described (e.g., Zuk 1990; Folstad and Karter 1992; Norris and Evans 2000); briefly, the original Hamilton-Zuk hypotheses stated that elaborate secondary sex characteristics were preferred by females because they provided evidence for the presence of genes that conferred resistance to parasites (Hamilton and Zuk 1982). Later Folstad and Karter (1992) expanded on this concept by proposing that the high androgen concentrations necessary for the expression of many sexually selected traits could suppress immune function. Thus, parasitized males would have to suppress their androgen secretions in order to enhance their defences and inversely males that can survive potential infection despite high testosterone concentration would be selected for.

Males are more susceptible to most parasitic infections than females. In a recent review of the sex differences in parasite literature of the 58 parasite species listed, 49 had male-biased infection patterns including the macroparasite genera nematodes, trematodes, and arthropods (Klein 2004). In already cited study, males of *M. agrestis* were heavier parasitized by fleas than females (Smith et al. 2005). The study of the proximate actions of androgens on the immune system is an active area of research. Androgen receptors are present on many immune cells (Abraham and Buga 1976). Much of the sexually dimorphic immune responses may be accounted for by a Th2 dominated response in males that is mediated in part by androgens. Testosterone also slows cutaneous wound healing, and inhibits delayed-type hypersensitivity responses (Mendenhall et al. 1990; Ashcroft and Mills 2002)

In contrast to testosterone, estradiol, the primary estrogen secreted by females, generally enhances immune function (Grossman 1985; Klein 2000a, b). Consistent with the finding that estrogen receptors are on lymphoid tissue, estrogen may have a direct action on lymphocytes. T-cell function can be modified *in vitro* by estrogen treatment (Paavonen et al. 1981). For example, *in vitro* B-cell activity is enhanced after the addition of estrogen, presumably due to estrogen inhibition of T-cell-mediated suppression (Paavonen et al. 1981). Similarly, macrophages increase lysosomal enzyme activity and phagocytosis in response to the addition of estrogen in tissue culture (Stimson 1983).

5.1.1 Glucocorticoids

The hypothalamic-pituitary adrenal (HPA) axis regulates the production of glucocorticoids from the adrenal glands. Glucocorticoids are steroid hormones that are important regulators of several physiological systems including carbohydrate metabolism, neuronal function, and immune function. Further, glucocorticoids have been conceptualized as “stress” hormones because they are elevated in responses to stressors.

Glucocorticoid hormones (corticosterone in most rodents and cortisol in primates) are intimately involved in regulating the immune system. The relationship between glucocorticoids and immune activity has been studied in two parallel but separate contexts. In general, immunological activation is associated with potent activation of the HPA axis. Proinflammatory cytokines are capable of activating the HPA axis at all three anatomical levels (Turnbull and Rivier 1995). Inversely, glucocorticoids feed back to inhibit the expression of proinflammatory cytokines.

Because of their effects on cytokines, as well as other cellular signaling pathways, glucocorticoids tend to suppress inflammation and because of their induction by inflammatory stimuli they have been conceptualized as “brakes” on the immune system having evolved to prevent runaway inflammation. Indeed, clinically, synthetic corticosteroids are used to treat inflammatory conditions.

The other major area in which glucocorticoids and the immune system have been studied is in response to stressors. Both psychological and physiological stressors are potent activators of the HPA axis. A relatively new field (psychoneuroimmunology) has developed to study the complex interactions between psychological variables and the immune system (for a review see McEwen et al. 1997; Kiecolt-Glaser et al. 2002). Generally, acute stressors and the associated rise in glucocorticoids enhance several aspects of immune function via alterations in immune cell distribution. In contrast, chronic stressors (or chronically high glucocorticoid exposure) tend to suppress immune function (Dhabhar and McEwen 1997). Glucocorticoid suppression of inflammation can also be characterized as a biasing towards a Th1 mediated immune response (Padgett et al. 1995) and thus shifting away from the Th2 response involved defences against many parasites.

The relationship between glucocorticoids and parasites is likely to be complex. For instance, elevated glucocorticoids may be associated with reduced resistance to infection. However, parasitization and the associated immune responses are likely to induce glucocorticoid production. In addition, the effects of glucocorticoids on the host-parasite system can be mediated in at least two ways: (1) increased glucocorticoid concentrations can be associated with immunosuppression and thus enhanced parasite survival, reproduction, or both, or (2) glucocorticoids may be important for limiting parasite-induced inflammation and tissue damage.

The direct evidence for glucocorticoid interactions with macroparasites is somewhat limited. In general, synthetic glucocorticoids, which often have high affinity for glucocorticoid receptors, tend to increase the susceptibility to parasites. For instance, rats infected with *S. ratti* and treated with betamethasone had more surviving worms and increased parasite fecundity as compared to uninfected rats (Wilkes et al. 2004). Two populations of bank voles *Clethrionomys glareolus* that differed in helminth burdens showed a positive correlation between circulating corticosterone concentrations and parasite numbers (Barnard et al. 2002; Barnard et al. 2003).

5. 2 Seasonal influences

The most salient (and best studied) seasonal fluctuation observed among small mammals is seasonal breeding. Small mammals tend to breed in the spring and summer when conditions are most conducive to offspring survival (Prendergast et al. 2002). Although several proximate factors, such as inanition and extreme ambient temperatures, can inhibit breeding, the most reliable cue used by animals to phase breeding to the appropriate season is photoperiod (day length). Photoperiodic information is transduced from the eyes to an endocrine signal in the pineal gland. Pineal melatonin is secreted only at night; as night lengths increase during short days, the duration of melatonin secretion increases. Prolonged exposure to short day lengths (<12.5 h/light/day), or extended duration of nightly melatonin secretion, induces gonadal regression in many species of small mammals. In addition to extended duration of melatonin secretion, other hormones are influenced by short days; short days reduced circulating concentrations of prolactin, gonadotropin-releasing hormone (GnRH), gonadotrophins, and sex steroid hormones (Prendergast et al. 2002). Gonadal regression in response to short days leads to diminished gonadal steroidogenesis and spermatogenesis, which results in infertility and cessation of androgen-dependent behaviours.

In response to reduced testosterone, individual males suppress territorial and reproductive behaviours during winter and tend to form group aggregations to improve retention of heat and humidity in communal burrows comprised of mixed species of rodents (Madison et al. 1984). Lab studies have indicated a strong relationship among androgens, aggressive behaviours, and macroparasite infection. For example, dominant male mice display elevated testosterone concentrations and are both more aggressive and more likely to be infected with macroparasites *Babesia microti* and *H. polygyrus* than low ranking individuals (Barnard et al. 1994; Barnard et al. 1998). Also, higher helminth loads were observed in bank voles (*C. glareolus*) in northeast Poland that had heavier adrenal glands, testes, and seminal vesicles (Barnard et al. 2002; Barnard et al. 2003). Thus, the seasonal pattern of macroparasite infection may represent a complicated pattern associated with reduced testosterone concentrations during winter, which would seem to reduce macroparasite infections via improved immune function, as well as a seasonal change in social structure, from largely solitary to group-huddling, which improves the opportunities for macroparasite infection.

Several studies have indicated seasonal changes in macroparasites or in the prevalence and severity of macroparasitic infections (reviewed in Nelson et al. 2002; Klein 2004). As noted, the causes underlying seasonality in

macroparasites can range from seasonal changes in climate to seasonal changes in the physiology or behaviour of intermediate or host species. Small mammals, especially rodents and bats, are often the intermediate hosts for many macroparasites that have medical implications for humans. Because many of these mammalian species display robust seasonal fluctuations in breeding, territorial, and other social grouping behaviours, the prevalence of macroparasites varies across the year (Read 1990)

For example, maras (*Dolichotis patagonum*), a hystricomorph rodent species from Argentina, display a unique social organization comprising either monogamous pairs or communal nests (Porteous and Pankhurst 1998). In these animals, intensity and prevalence of Strongyloidea egg counts was highest among the communal family groups as compared to adult pairs of maras living in a zoological park in the UK. These results support the idea that seasonal changes in social group size can contribute variation in macroparasite infection.

In addition to seasonal changes in social organization, the energetic bottleneck during winter results from increased thermoregulatory demands when food availability is scarce; this makes winter a particularly difficult time to breed and survive. Immune function often varies on a seasonal basis; it is generally decreased during the winter in the wild, but is enhanced in the laboratory during short-day conditions when all other factors are held constant (Nelson and Demas 1996; Nelson 2004). Because (1) immune function is compromised by the chronic stressors of winter and (2) winter stressors are seasonally predictable, we have previously proposed that individuals use photoperiodic information to anticipate winter and accordingly redistribute energy among competing reproductive and survival functions (Nelson et al. 2002; Nelson 2004). Obviously, mounting an immune response requires resources that could otherwise be allocated to other biological functions (Sheldon and Verhulst 1996). Thus, it is reasonable to consider immune function in terms of energetic trade-offs. Individuals may partition resources among the immune system and other biological processes, such as reproduction, growth, or thermogenesis. Consequently, animals may maintain the highest level of immune function that is energetically possible given the constraints of processes essential for survival, growth, reproduction, thermogenesis, foraging, and other activities (Festa-Bianchet 1989; Deerenberg et al. 1997; Nelson et al. 2002). The observations that immune function fluctuates seasonally and is compromised during stressful times are consistent with this idea (Zuk 1990; John 1994).

“Stress” is a notoriously ethereal concept that has been used to describe any factor that increases glucocorticoid secretion including injury, pain, infection, overcrowding, harsh ambient temperature, food deprivation, noise,

restraint, and aversive social interactions (Nelson et al. 2002). The ecological literature illustrates that environmental factors perceived as stressors, such as low food availability, low ambient temperatures, overcrowding, lack of shelter or increased predator pressure can be seasonal. Oftentimes, seasonal changes in environment correlate with seasonal fluctuations in immune function among individuals and seasonal changes in population-wide disease and death rates (Lochmiller et al. 1994). Thus, winter survival, at least among non-human animals, is hypothesized to require a positive balance between short-day enhanced immune function and glucocorticoid-induced immunosuppression (Nelson and Demas 1996). The balance between short-day enhanced immune function (i.e., to the point where autoimmune disease becomes a danger) and stressor-induced immunosuppression (i.e., to the point where opportunistic macroparasites overwhelm the host) must be met for animals to survive and become reproductively successful (Raberg et al. 1998). Indeed, the stressor of reduced food availability during winter may contribute to macroparasite infections. In some cases, starved hosts are preferred to well-nourished hosts (Krasnov et al. 2005). For example, egg production of fleas (*Xenopsylla ramesis*) was significantly increased when parasitizing underfed as compared to control gerbils (*Meriones crassus*). Although inanition of hosts affected survival of flea eggs and larvae on these rodents, survival of pupae was unaffected. These results suggest nutritional state in combination with the energetic costs of host resistance can affect parasites (Krasnov et al. 2005).

Photoperiod affects the immune system of many rodent species. Short days increase the number of circulating blood leukocytes, lymphocytes, T cells and NK cells, as well as spontaneous blastogenesis in whole blood and isolated lymphocytes and the cytolytic capacity of natural killer cells (Yellon et al. 1999; Bilbo et al. 2002b). Moreover, short days suppress phagocytosis and oxidative burst activities of granulocytes and monocytes (Yellon et al. 1999). Short days also enhance lymphocyte proliferation in species ranging from mice to primates (Mann et al. 2000; Nelson et al. 2002; Nelson 2004). There are species differences in photoperiodic influences on immune function. In addition, certain specific components of immune function might be more costly to maintain, although methods for precise measurements are generally not available. Finally, the types of immune responses, such as enhanced primary defences in the skin, lymph nodes and gastrointestinal tract, could vary because the types of infectious risks vary seasonally. However, the general pattern is that short day lengths are usually associated with enhanced immune function.

Melatonin transduces photoperiodic information and also influences immune function both directly and indirectly (reviewed in Nelson et al.

2002). For example, melatonin receptors have been localized on lymphocytes, and *in vitro* melatonin treatment enhances splenocyte proliferation (Pozo et al. 1997). Enhancement of several components of immune function in mice is mediated directly through type 2 melatonin receptors on lymphocytes (Drazen and Nelson 2001). Melatonin also stimulates production of endogenous opioids directly from T cells, which might mediate the immunoenhancing effects of melatonin; melatonin also modulates the effects of stressors on immune function during the winter (Moore and Siopes 2003; Nelson 2004). Finally, melatonin increases survival of mice infected with *Schistosoma mansoni* (El-Sokkary et al. 2002).

Short days inhibit prolactin in all small mammals examined (Goldman and Nelson 1993), and prolactin influences immune function. For example, hypophysectomy suppresses hematopoiesis and immune cell proliferation; these effects are reversed after administration of prolactin (Berczi et al. 1991). Bromocriptine, a drug that inhibits prolactin release, suppresses antibody formation and cell-mediated immune activity; this immunosuppression is reversed with prolactin (Nagy et al. 1983). Finally, prolactin stimulates several immune parameters in untreated animals (Nagy et al. 1983). There is also evidence that prolactin may be produced locally in immune cells to regulate immune parameters. For example, antibodies directed against prolactin inhibit lymphocyte proliferation *in vitro* (Hartmann et al. 1989) suggesting that immune-derived prolactin may enhance lymphocyte proliferation.

Estrogens tend to enhance immune function while androgens tend to suppress it. Therefore, if photoperiodic changes in immune function are due to fluctuations in sex steroid hormones, then female rodents housed in short days, that have low estrogen concentrations, should reduce immune function compared to long-day animals. Alternatively, if the effects of short days or melatonin on immune function are independent of changes in gonadal steroid hormones, then immune enhancement should be observed among both males and females housed in short days regardless of circulating concentrations of gonadal steroids. Most studies support the latter hypothesis. Specifically, both male and female deer mice (*Peromyscus maniculatus*) housed in short days display enhanced lymphocyte proliferation compared to long-day housed animals, regardless of gonadal status (Demas and Nelson, 1998). Gonadectomized male and female animals that lack circulating testosterone and estradiol, respectively, display similar enhancement of immune function compared to intact animals; exogenous hormone replacement does not change these results (Demas and Nelson 1998). Furthermore, the photoperiodic effects on immune function in this species do not appear to be due to changes in glucocorticoids because corticosterone concentrations do not differ between short- and long-day deer

mice (Demas and Nelson 1998). Thus, it appears that some of the immunoenhancing effects of short days are probably caused by direct effects of melatonin.

6 Future directions

A few host-macroparasite systems have been extensively investigated, but to date the general principles governing interactions between macroparasites and micromammals have not been identified. In particular, most of the work on host immune responses to parasites has been conducted using *in vitro* models in the laboratory. Studies on immune responses to macroparasites in wild mammals would greatly extend our understanding of these interactions. Such field experiments, although methodologically difficult at present, would provide the ecological validity that exclusively lab-based studies do not.

One particularly promising line of research involves the interplay between macroparasites and host behaviour. Indeed, it remains unclear whether and in what contexts parasites induce sickness behaviour. More specifically, we know little about how animals parse the competing processes of growth, feeding, reproduction and social behaviours when sickness behaviours makes these activities partially incompatible. Have some hosts simply evolved to forgo systemic inflammation typically associated with sickness behaviour? Perhaps some parasites actively suppress the chemical signals that orchestrate these responses. If so, do hosts become more susceptible to chronic infection? From a human health perspective, neuroendocrine-immune interactions have become an important research topic. However, this work can only provide us with direction through which we can begin to understand how wild animals use their immune defences to combat macroparasites. Overall, the physiological interactivity between micromammals and their macroparasites is fascinating, but there is still much to learn.

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