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The role of parasite-induced immunodepression, rank and social environment in the modulation of behaviour and hormone concentration in male laboratory mice (*Mus musculus*)

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Peripheral immune responsiveness in male laboratory mice was reduced by infection with the trichostrongyloid nematode *Heligmosomoides polygyrus*. Responsiveness was also lower among high-ranking (aggressive) males regardless of infection status. Reduced responsiveness in both infected animals and high rankers was associated with elevated serum corticosterone concentration (a potential immunodepressant) and was compounded among high-ranking males by subsequent high aggressiveness. As in previous experiments, only low rankers modulated testosterone secretion in relation to current immunocompetence and corticosterone concentration. The lack of any downregulation of aggression in response to parasite-induced immunodepression contrasted with previous results using antithymocyte serum and may be due to the more localized nature of immunodepression during *H. polygyrus* infection. However, the additional increase in corticosterone concentration resulting from exposure to female odour and destabilized aggressive social relationships did result in downregulation of aggression among high rankers and of testosterone among mice generally, suggesting that modulation rules of thumb are at least partly dependent on the proximate cues associated with immunodepression.

Keywords: immunocompetence; parasite; mice; modulation; aggression; social rank; testosterone; corticosterone

1. INTRODUCTION

The idea that interrelationships between behaviour, immune function and other physiological factors may reflect adaptive life history trade-offs has recently been gaining theoretical and empirical support (e.g. Folstad & Karter 1992; Wedekind & Folstad 1994; Sheldon & Verhulst 1996; Barnard & Hurst 1996; Barnard *et al*. 1996*a*,*b*, 1997*a*). Although interest has mainly focused on sexual selection and the effect on secondary sexual characters of modulating immunodepressive sex steroids to protect immunity (e.g. Folstad & Karter 1992; Owens & Short 1995; Hillgarth & Wingfield 1997), current immunocompetence may act more generally as a constraint on behavioural and physiological decisions where these risk imposing a burden on immune function.

In a series of experiments with male laboratory mice (*Mus musculus*), we have shown that behaviour and serum hormone (testosterone and corticosterone) and immunoglobulin concentrations covary in a rank-dependent fashion that is consistent with the adaptive modulation hypothesis and differences in life history strategy between individuals (Barnard *et al*. 1994, 1996*a*,*b*: see also Klein *et al*. (1997) for evidence from other rodent species). Moreover, the covariation can be manipulated predictably by increasing social stress or depressing immune function experimentally (Barnard *et al*. 1996*b*, 1997*a*,*b*).

However, although these results provide strong support for the adaptive modulation hypothesis, experimental manipulation of immunocompetence has so far been limited to the use of antithymocyte serum (ATS) (to depress thymus-mediated immunity (Doenhoff & Leuchars 1977; Levey & Medawar 1966; Barnard *et al*. 1997*a*,*b*)). While ATS is relatively benign in terms of cytotoxic and other unwelcome side-effects (Levey & Medawar 1966; Lance *et al*. 1973), it is nevertheless an artificial, and fairly extreme, means of compromising immunity. If modulation of behaviour and physiology in relation to immune function is a general feature of decision-making in mice, similar responses should be expected following other means of inducing immunodepression (e.g. direct immune component or pathogen product administration (Friedman *et al*. 1996; Bluthe *et al*. 1997)), particularly those mimicking more natural causes of depressed immunity such as the immunodepressive effects of certain parasitic infections (Playfair 1982; Urban *et al*. 1992).

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Many parasites appear to enhance their survival in the host through a range of immunodepressive strategies (Raybourne *et al*. 1983; Mazingue *et al*. 1983; Liew *et al*. 1987; Cross & Klesius 1989). One such species is the trichostrongyloid nematode *Heligmosomoides polygyrus bakeri*, a natural intestinal parasite of house mice. *H. polygyrus* causes chronic infections (Robinson *et al*. 1989) by downregulating local inflammatory responses in the intestine (Behnke *et al*. 1993), as well as systemic antibody production (Ali & Behnke 1983), probably through interfering with T-cell function (Behnke *et al*. 1993). Although the major impact on host response is locally in the intestine, where mastocytosis is severely depressed (Behnke & Wakelin 1977) and the local mucosal immune response against other intestinal nematodes greatly weakened (Behnke *et al*. 1978), this downregulation is not amenable to intercurrent monitoring. However, systemic downregulation, although weaker, provides a convenient intercurrent yardstick of overall impact. As well as downregulating immune function, low doses of *H. polygyrus* are also known to affect the development of social status (Freeland 1981) and various behavioural attributes (e.g. spatial learning and predator aversion (Kavaliers & Colwell 1995; Kavaliers *et al*. 1998)) in mice. In the present experiment, we repeated Barnard *et al*.'s (1997*a*,*b*) experimental design, but replaced ATS treatment with infection by *H. polygyrus*. As in the ATS experiments, infected and uninfected mice were divided into two treatments on the basis of exposure or otherwise to female odours (and thus potential reproductive opportunity). Following Barnard *et al*. (1997*a*,*b*), we expected infected mice to downregulate testosterone and aggressive behaviour, but maintain or increase time spent sleeping, relative to controls, and for these differences between infected and uninfected mice to disappear when animals were exposed to female odours. Although corticosterone has shown no evidence of modulation in relation to immuncompetence in our previous experiments (see Barnard *et al*. 1996*a*, 1997*a*; Smith *et al*. 1996), glucocorticoids have an inhibitory effect on the secretory function of Th2 cells which characterize the immune response to helminth infections generally (Grencis *et al*. 1991; Finkelman *et al*. 1995; Padgett *et al*. 1995; but see Golding *et al*. 1994) and are known to be effective in protective immunity to *H. polygyrus* (Urban *et al*. 1992). Some reduction in host-induced corticosterone levels might thus be expected among infected animals.

2. METHODS

(a) *Pre-experimental procedure*

The subjects were two batches of 64 male laboratory mice of the randomly bred CFLP strain (see Barnard *et al*. 1993) purchased from Bantin and Kingman Ltd, Hull, UK, at 42 days of age. Twenty-three female CFLP mice of the same age as the males were purchased from the same suppliers at the same time as the second batch and immediately established in groups of 7–8 animals in large polypropylene cages (48 cm × 28 cm × 13 cm) in a separate room from the males.

The experiment followed Barnard *et al*. (1997*a*,*b*) in being carried out in two parts: a 'no female odour' treatment (batch 1) followed by a 'female odour' treatment (batch 2). This prevented exposure of the former to ambient female odours while maintaining both treatments in the same physical environment (see discussion and references in Barnard *et al*. (1997*b*)). The same parasite stock was used to infect mice in both batches (see below), and any chance batch effects in other variables were taken into account in subsequent analyses (see Barnard *et al*. 1997*b*).

(b) *Pre-isolation/infection period*

Prior to the experiment, animals were maintained, individually marked with black hair dye and blood sampled (figure 1, sample 1 (88 µl from the tail)) exactly as described by Barnard *et al*. (1997*b*). Three days after the pre-experimental blood samples, the males in each batch were re-allocated arbitrarily to 16 groups of four previously unfamiliar individuals (32 groups in total see Barnard *et al*. 1997*b*) and their behaviour recorded over the next 8 d following the comprehensive methods of Hurst *et al*. (1996) and Barnard *et al*. (1997*a*,*b*). A full list of behaviour categories and definitions is given in Table 1 of Barnard *et al*. (1997*a*,*b*).

(c) *Isolation/infection period*

At the end of the pre-infection period, males were weighed again and a second 88 µl blood sample taken from the tail (figure 1, sample 2). Groups were then allocated randomly into either infected or sham-infected control treatments (eight groups per batch each), and mice were separated and housed singly in the same sized cages as pre-infection groups.

On the day after separation, groups in the infection treatment were infected by gavage with 150 L3 larvae of *H. polygyrus* in 0.2 ml of distilled water, while control groups were given 0.2 ml of distilled water only. This dose was chosen because it is well tolerated by mice and is in the low dose range for studies claiming effects of subclinical infections of *H. polygyrus* on behaviour and underlying physiological mechanisms (e.g. Freeland 1981; Kavaliers & Colwell 1995). Moreover, it is within the range causing non-specific systemic depression of antibody (Ali & Behnke 1983) and cell-mediated responses (Ali & Behnke 1984). Mice then remained singly housed for 14 d to allow the worms to develop to the adult immunodepressive stage. On day 13 of separation, mice were weighed for a third time and an 88 µl blood sample taken retro-orbitally (figure 1, sample 3). To confirm that non-specific systemic immunodepression had been induced by infection with *H. polygyrus*, each individual was given an intraperitoneal injection on day 14 of 0.2 ml of a sheep erythrocyte suspension (SRBC) containing 25×10^7 SRBC ml^{-1}, each mouse receiving 5×10^7 SRBC (Barnard *et al*. 1997*a*,*b*; figure 1).

(d) *Post-isolation/infection period*

The day after injection with SRBC, mice were re-established in their pre-isolation/infection groups. 'No odour' groups (batch 1) were re-established on clean sawdust, whereas 'female odour' groups (batch 2) were given soiled sawdust from the cages of two arbitrarily allocated, singly housed females following the procedure of Barnard *et al*. (1997*b*). Behavioural observations were then taken for 8 d exactly as during the pre-isolation/infection period.

(e) *Organ weights, worm burdens and blood assays*

At the end of the post-infection observation period, males were weighed for the final time, killed using chloroform and exsanguinated. The kidneys, adrenal glands, spleen, thymus gland, testes, preputial glands, seminal vesicles, heart and mesenteric lymph nodes (MLN) of each individual were carefully dissected out and weighed. The intestinal worm burden was

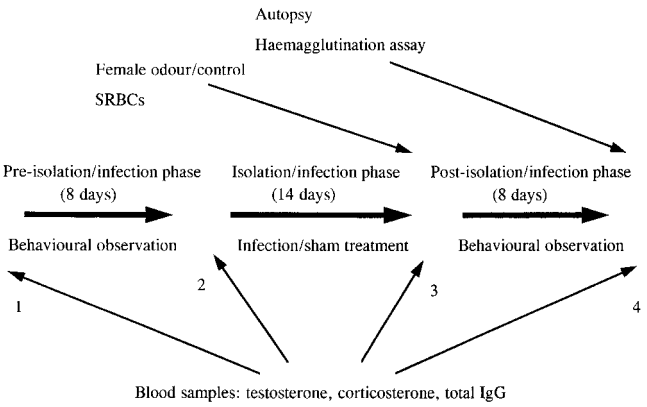

Figure 1. Flow diagram of experimental design (see § 2).

recovered, and worms sexed and counted within 48 h, following the established methods of Jenkins & Behnke (1977). SRBC haemagglutination titres, packed cell volume (PCV) and serum concentrations of testosterone, corticosterone and total IgG (a convenient bystander measure of peripheral immune responsiveness) were assayed using the standard techniques in Barnard *et al.* (1994, 1997*a*,*b*).

(f) *Statistical analyses*

All analyses were done using Statgraphics Plus v. 7 (Manugistics Ltd, Maryland, USA). Parametric analyses were used throughout (data were $\log_{10}$ or square root transformed as necessary and tested for normality using a Kolmogorov–Smirnov one-sample test). Wherever there were *a priori* reasons for expecting trends or differences in a particular direction, probabilities associated with significance tests are indicated as one-tailed.

3. RESULTS

(a) *Social rank*

Following Barnard *et al.* (1993, 1994, 1996*a*,*b*, 1997*a*,*b*), high and low rank categories within groups were defined on the basis of the ratio of attacks initiated and received by each male during the pre-treatment period of grouping, and high-ranking males initiated disproportionately more attacks than low rankers over the pre-treatment period ($t_{52}=4.95$, $p<0.0001$). Five groups, in which the incidence of aggression was very low, were omitted from later analyses because it was not possible to allocate males to rank categories. All other groups comprised either one or two high-ranking males and two or three low-ranking males. All analyses relating to social status were based on high and low rank categories and, where there was more than one individual per category within cages, data were averaged to control for non-independence (Barnard *et al.* 1996*a*,*b*, 1997*a*,*b*).

(b) *Pre-isolation/infection differences*

Three-way analysis of variance (ANOVA) revealed no significant chance pre-treatment biases in any measure of hormone concentration, immunocompetence or body weight with respect to subsequent infection status or odour treatment. Because female odour treatments applied only during the post-isolation/infection phase, results for hormone and IgG concentrations during the period of isolation/infection were analysed with subsequent odour treatments combined.

(c) *Effects of infection, rank and odour treatment on immunocompetence*

(i) *Infection status*

As expected (Ali & Behnke 1983), three-way ANOVA showed that infection with *H. polygyrus* reduced peripheral immune responsiveness as measured by terminal haemagglutination titre ($F_{1,46}=3.87$, one-tailed $p<0.05$, figure 2*a*). Also, as expected with the introduction of foreign antigen and the known IgG1 hypergammaglobulinaemia associated with *H. polygyrus* infection (Chapman *et al.* 1979; Williams & Behnke 1983), total serum IgG concentration increased during the period of isolation/infection (change between sample points 2 and 3 in figure 1) in those animals treated with *H. polygyrus* (two-way ANOVA, $F_{1,49}=93.40$, $p<0.0001$). During the post-isolation/infection phase, and following SRBC challenge, however, further increase in IgG concentration among infected mice was damped compared with the now increased response in control animals (three-way ANOVA, $F_{1,45}=11.98$, $p<0.002$, figure 2*b*). Within infected groups, partial regression analysis revealed a significant negative relationship between worm burden at autopsy and change in IgG concentration over the period of isolation/infection ($t_{20}=-2.23$, $p<0.05$), so that burdens were greater the smaller the IgG response. There was no independent relationship with haemagglutination titre.

Relative (% terminal body weight) MLN ($F_{1,46}=56.66$, $p<0.0001$) and spleen ($F_{1,46}=14.13$, $p<0.001$) weights showed a significant increase among infected animals, but there was no effect of infection on thymus weight.

There was no significant impact of infection on change in body weight or PCV over the period of the experiment, supporting our assumption that our low infection dose did not have deleterious clinical consequences.

(ii) *Rank*

High rankers had significantly lower haemagglutination titres than low rankers (three-way ANOVA, $F_{1,46}=10.54$, $p<0.005$, figure 3) and there was a pronounced, but just non-significant ($F_{1,46}=3.72$, $p=0.059$), interaction between rank and infection status, with infected low rankers showing a much reduced titre compared with uninfected controls (mean ± s.e. titre (1/dilution) in infected groups $=52.61\pm8.00$, $n=12$; in uninfected groups $=88.89\pm9.36$, $n=15$) but high rankers showing similarly low titres in both (mean ± s.e. in infected groups $=40.08\pm5.69$, $n=13$; in uninfected groups $=40.53\pm11.53$, $n=15$). There were no significant effects of rank and no interaction for changes in IgG concentration during either the isolation/infection or post-isolation/infection phases, and no difference between rank categories in the regression relationship between worm burden and IgG (see above). Rank showed no effects (main or interaction) on worm burden, or weight of MLN, spleen or thymus.

(iii) *Odour treatment*

There were no significant biases in IgG measures with respect to post-isolation/infection odour treatment during the isolation/infection period, and no significant effect of odour treatment on post-isolation/infection measures of

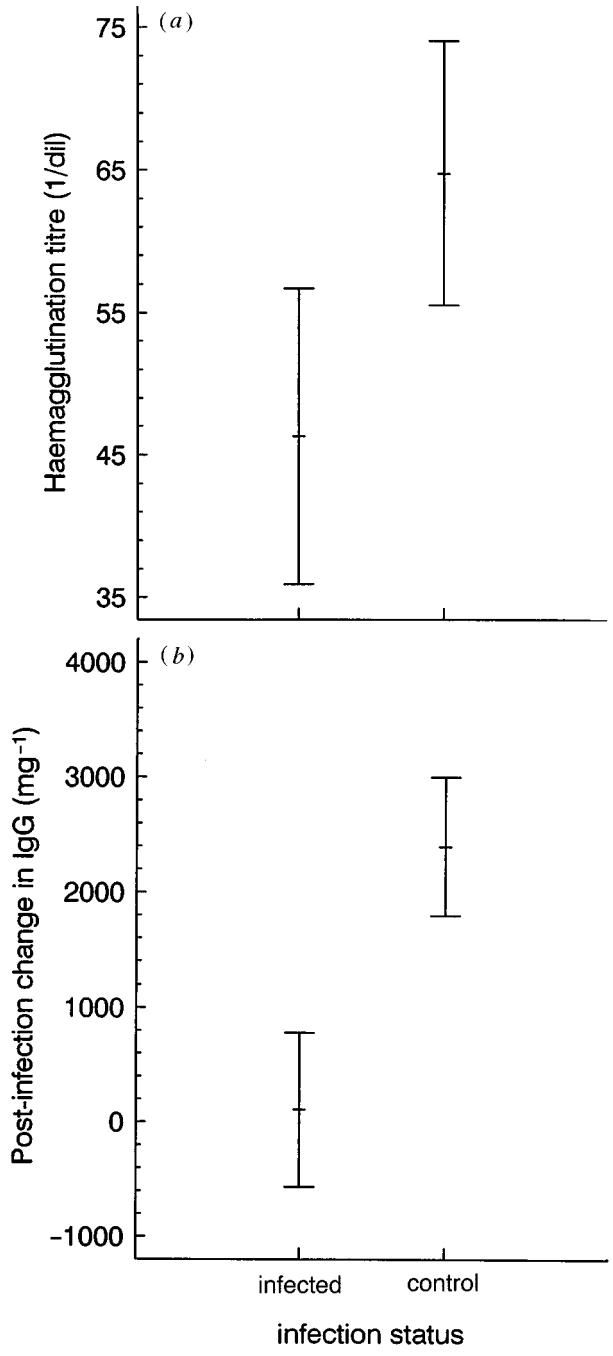

Figure 2. Effect of infection status on (*a*) haemagglutination titre and (*b*) post-isolation/infection phase change in total IgG concentration (means ± least squares deviations (l.s.d.) from ANOVA). See text.

IgG or on haemagglutination titre. However, terminal worm burden among infected animals was significantly lower following exposure to female odour (two-way ANOVA by odour treatment and rank, $F_{1,19}=45.30$, $p<0.0001$), with no interaction between odour treatment and rank. There were no effects of odour on MLN, spleen or thymus weight, but, in keeping with the reduced worm burden, there was a significant interaction between infection status and odour treatment on spleen weight ($F_{1,46}=5.64$, $p<0.05$), with spleen weight being reduced among infected animals following the presence of female odour.

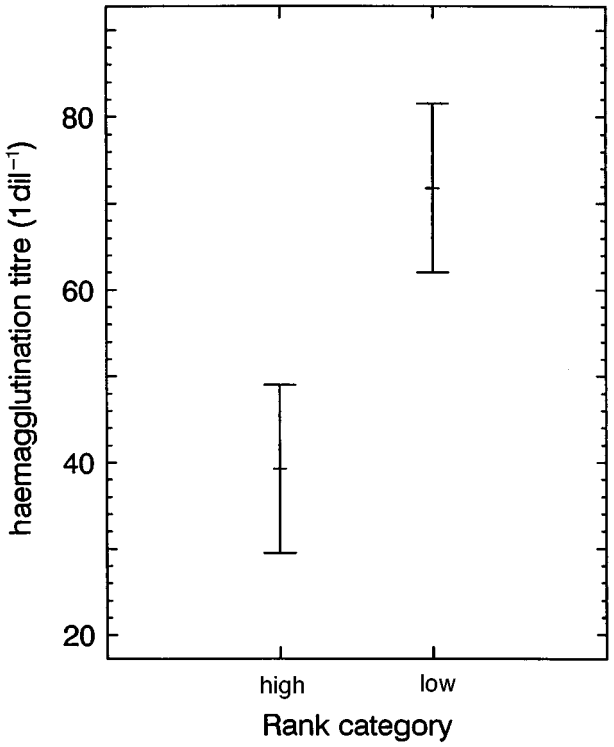

Figure 3. Effect of rank category on haemagglutination titre (means ±1.s.d. from ANOVA). See text.

(d) *Immunocompetence and hormone modulation*

(i) *Isolation/infection phase*

Two-way ANOVA of changes in hormone concentrations over the period of isolation and infection showed no significant main effects of infection status or rank for testosterone. However, the interaction between infection status and rank was significant at the 10% level, with high rankers tending to reduce testosterone relative to low rankers in infected groups (from 10.11 ±2.34 to 7.21 ±3.50 (mean±s.e.) ng ml^{-1} in high rankers but 6.64±1.8 to 9.10±1.59 ng ml^{-1} in low rankers) but to increase it in control groups (7.36±2.27 to 12.69±3.58 ng ml^{-1} in high rankers compared with 6.53±1.04 to 4.47±1.26 ng ml^{-1} in low rankers) ($F_{1,40}=3.03$, $p=0.089$). As before (Barnard *et al.* 1994, 1996*a*,*b*), partial regression analysis revealed a rank difference in the change in testosterone relative to measures of immunocompetence, testosterone over the period of infection showing a significant increase with IgG among low rankers ($t_{24}=2.53$, $p<0.05$), but not among high rankers ($t_{22}=-0.39$, n.s.).

In contrast, two-way ANOVA of change in serum corticosterone concentration over the period of infection showed strongly significant differences with both infection status ($F_{1,45}=7.25$, $p<0.01$) and rank ($F_{1,45}=6.21$, $p<0.02$). Corticosterone levels declined sharply over the period in uninfected groups (from 118.22±18.50 to 55.63±4.12 ng ml^{-1} versus 88.38±6.96 to 77.82± 19.70 ng ml^{-1} in infected groups) and showed a sharper decline among low rankers (from 124.52±19.65 to 56.45±3.96 ng ml^{-1} compared with 84.22±6.18 to 74.42±17.64 among high rankers). However, the decline among low rankers was reduced in infected groups while infection resulted in an increase among high rankers (interaction in figure 4). Again as previously (Barnard *et*

al. 1994, 1996*a*,*b*; Smith *et al*. 1996), corticosterone showed no significant change relative to immunocompetence measures. Partial regression analysis showed that worm burden increased significantly with increasing corticosterone concentration ($t_{20}=2.89$, $p<0.01$), but there was no effect of change in testosterone and no difference in either relationship between rank categories.

(ii) *Post-isolation/infection phase*

Taking odour treatment into account, post-isolation/infection phase testosterone showed a precipitate drop (from 8.15 ± 1.62 to 2.10 ± 0.55 ng ml^{-1}), rather than the expected increase, in mice exposed to female odour (three-way ANOVA, $F_{1,41}=11.01$, $p<0.01$), but with no independent effects of infection status or rank and no interactions. Corticosterone concentration, however, increased, with the increase being greater among infected individuals (77.83 ± 19.70 to 440.80 ± 38.69 ng ml^{-1} compared with 55.63 ± 4.12 to 308.85 ± 33.77 ng ml^{-1} in uninfected controls; $F_{1,44}=9.90$, $p<0.005$) and, as expected (Smith *et al*. 1996), those exposed to female odours (79.59 ± 16.48 to 409.10 ± 39.83 ng ml^{-1} compared with 50.39 ± 4.90 to 329.85 ± 33.77 ng ml^{-1} in those not exposed to odours; $F_{1,44}=3.93$, one-tailed $p<0.05$). The greater increase in corticosterone among mice exposed to female odour was reflected in significantly greater relative adrenal weights in these individuals ($F_{1,46}=4.78$, $p<0.05$).

Partial regression analyses by rank category once again showed no significant association between change in testosterone levels and immunocompetence measures among high rankers, but a significant positive association with haemagglutination titre ($t_{25}=3.48$, $p<0.01$) among low rankers. Testosterone among low rankers also decreased with increasing corticosterone concentration ($t_{25}=-2.65$, $p<0.02$). Change in corticosterone showed no significant relationships with measures of immunocompetence and no significant relationships emerged between changes in hormone or IgG concentrations and eventual worm burden.

(e) *Effects of treatment on behaviour*

ANOVA revealed no significant biases with respect to subsequent infection or odour treatment in any behaviour category showing treatment effects after infection. During the post-isolation/infection phase, however, the highly significant difference between rank categories in the amount of aggression initiated remained ($F_{1,45}=13.50$, $p<0.001$) and there was a strong significant interaction between rank and odour treatment ($F_{1,45}=19.28$, $p<0.001$) with high rankers showing less aggression and low rankers more when female odours were present (figure 5). As expected following a period of isolation (Cairns *et al*. 1985, Hurst *et al*. 1994), low rankers showed a greater tendency to challenge high rankers in post-isolation/infection groups, with the number of attacks received by high rankers increasing significantly over pre-isolation/infection phase levels ($F_{1,45}=5.60$, $p<0.05$). Again, there was a suggestive interaction between rank and odour treatment ($F_{1,45}=3.41$, $p=0.073$), high rankers showing a greater increase in attacks received, and low rankers a marked reduction, when female odours were present. There were no significant main or interaction effects of infection status on aggression initiated or received during the post-isolation/infection phase.

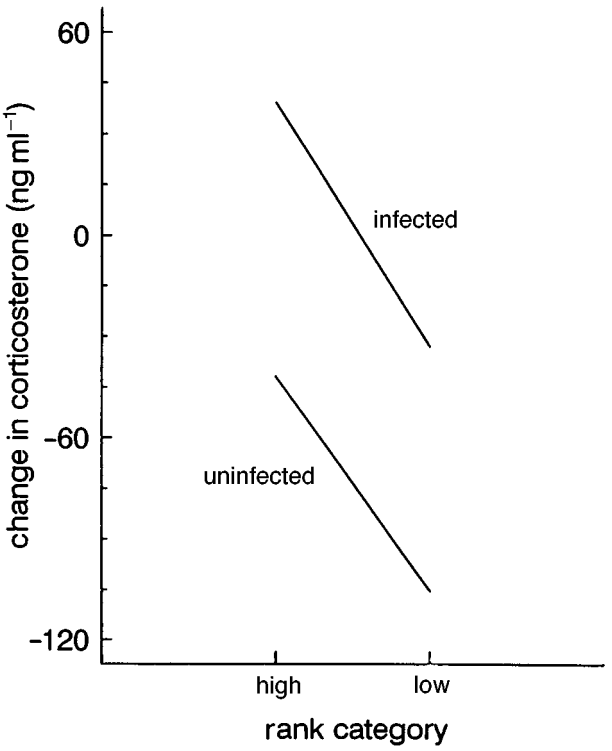

Figure 4. Effects of rank category and infection status on change in corticosterone concentration during the isolation/infection phase (interaction plot from ANOVA, error bars omitted for clarity). See text.

The only other behaviour showing a significant effect of experimental treatments was mounting, the frequency of mounts initiated during the post-isolation/infection phase increasing when female odours were presented ($F_{1,46}=13.50$, $p<0.001$; see also Barnard *et al*. 1997*b*). There were no effects of infection status and no interaction with either infection status or rank.

(f) *Behaviour, hormone concentrations and immune function*

Partial regression analysis was used to investigate the effect of post-isolation/infection phase behaviour on hormone concentrations. Rank categories were analysed separately with infection status and odour treatment included as dummy variables and post-isolation/infection phase body weight and testosterone concentration as additional independent variables. Analyses revealed a significant positive effect of aggression initiated on change in corticosterone concentration during the post-isolation/infection phase among high rankers ($t_{23}=2.19$, $p<0.05$), but not among low rankers. There were no significant effects of aggression received or any other behaviours included simultaneously as independent variables. There were no significant effects of any behaviour category on post-isolation/infection phase measures of testosterone concentration. However, inclusion of the change in corticosterone concentration over the post-isolation/infection period showed a significant negative correlation with the change in testosterone in both rank categories when female odour was present ($t_{10}=-2.32$, one-tailed $p<0.05$ for high rankers: $t_{12}=-2.38$, one-tailed $p<0.01$ for low

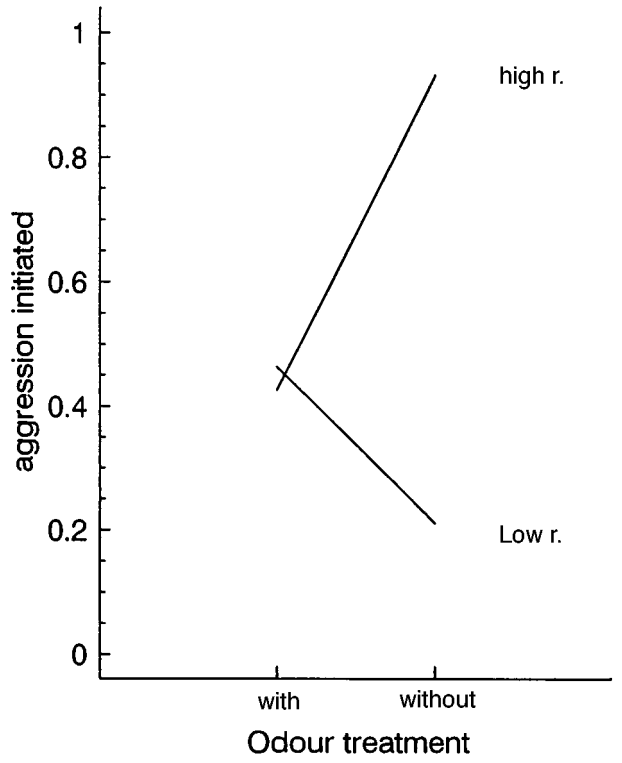

Figure 5. Effects of odour treatment and rank category on the ($\log_{10}$+1) number of aggressive acts initiated during the post-isolation/infection phase (interaction plot from ANOVA, error bars omitted for clarity). See text.

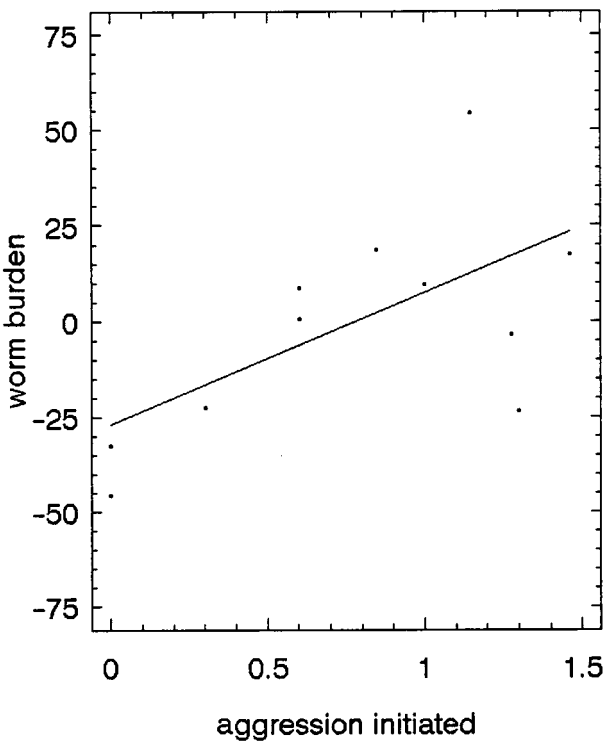

Figure 6. Component plot from partial regression analysis of the relationship between the ($\log_{10}$+1) number of aggressive acts initiated by high-ranking males in the post-isolation/infection phase and terminal worm burden. Regression equation: $y = 126.60 + 34.50(\text{agg})$, where agg = ($\log_{10}$+1) no. aggressive acts initiated; no other independent variables entered the equation. See text.

rankers), but only among low rankers ($t_{11} = -3.69$, one-tailed $p < 0.001$) when odour was not present. Analyses of the effects of behaviour on haemagglutination titre and (in infected animals only) worm burden showed a significant reduction in haemagglutination response with increased aggression received ($t_{25} = -2.35$, one-tailed $p < 0.01$) and an increase in worm burden with increased aggression initiated ($t_{10} = 2.26$, one-tailed $p < 0.05$, figure 6) but, again, only among high rankers. No significant effects of any other behaviour on measures of immune response or worm burden emerged in either rank category.

4. DISCUSSION

The results showed the expected reduction in immune responsiveness among animals infected with *H. polygyrus*. However, associations between infection status and changes in behaviour were less direct than those resulting from ATS-induced immunodepression (Barnard *et al.* 1997*a*) and appeared to be mediated by rank-related changes in corticosterone levels. As found by Smith *et al.* (1996), changes in corticosterone also appeared to underlie behavioural responses to the substrate odours of females. However, as Barnard *et al.* (1997*a*) have stressed, these apparent effects of hormones do not necessarily reflect direct causal relationships at the level of measurement (in this case, total serum concentrations), but instead may correlate with associated underlying metabolic pathways and mechanisms (e.g. the activity of precursors, metabolites and the modulation of receptors and binding globulins) (see, for example, Kotani *et al.* 1974; Grossman & Roselle 1986; Folstad & Karter 1992; Roberts *et al.* 1996).

Overall, high rankers showed reduced immune responsiveness compared with low rankers, with the main effects of infection with *H. polygyrus* appearing to be due to a reduction in responsiveness among low rankers. The difference between ranks may be explicable in terms of the interrelationship between aggression, corticosterone concentration, immunocompetence and resistance to *H. polygyrus* among high rankers in which post-infection aggressive behaviour correlated positively with corticosterone concentration and negatively with haemagglutination titre and resistance to *H. polygyrus* (measured as terminal worm burden). There was no evidence that infected animals downregulated corticosterone concentration. Instead, the post-infection phase relationships compounded a tendency for high rankers and infected mice generally to maintain corticosterone concentration during the period of isolation and infection, when concentrations dropped among low rankers and uninfected controls. The difference between ranks is consistent with a previously reported link between elevated corticosterone levels and reduced resistance among high-ranking males (Smith *et al.* 1996). The maintenance of corticosterone levels among infected animals, however, could reflect a confounding effect of parasite-induced glucocorticoid secretion (Hillgarth & Wingfield 1997) as a manipulative strategy to reduce the efficacy of the host's immune response (see also Behnke *et al.* 1992). Although glucocorticoids have a stimulatory effect on the initial cell proliferation phase of T lymphocytes, and thus

some elevation might have been expected on this account (e.g. Maier *et al*. 1994; Padgett *et al*. 1995), the change in corticosterone concentration during the infection phase was the best hormone-measure predictor of eventual worm burden. The negative relationship between the two is more in keeping with the later impact of glucocorticoids on the secretion of Th2 cytokines (Padgett *et al*. 1995; Rook & Zumia 1997) and thus depression of the Th2 arm of the immune response. This is consistent with effects of glucocorticoid drugs in prolonging intestinal nematode infections (Wakelin & Selby 1974), increasing the susceptibility of rodent hosts to *H. polygyrus* (Quinnell *et al*. 1991) and depressing the expression of acquired resistance to *H. polygyrus* (Behnke & Parish 1979).

In contrast to corticosterone, testosterone once again showed rank-related modulation, with correlations between hormone concentration and measures of immunocompetence being evident only among low rankers. However, unlike the situation with *Babesia* infections (Barnard *et al*. 1994, 1996*a*,*b*), there was no testosterone-dependent increase in parasite burden among high rankers in the present experiment, perhaps because resistance to the two parasites relies on a different emphasis on the Th1 and Th2 arms of the acquired immune response. Androgens tend to modulate Th1 responses, which are broadly associated with combating intracellular parasites such as *Babesia* (but see Allen & Maizels 1997).

These interrelationships suggest that, as before (e.g. Barnard *et al*. 1996*a*,*b*, 1997*a*,*b*), aggression was costly in terms of reduced immunocompetence, but, unlike the experiments in which immunocompetence was reduced by administering ATS (Barnard *et al*. 1997*a*), mice immunocompromised by *H. polygyrus* did not downregulate aggressive behaviour. This may reflect a difference in the modes of immunodepression by *H. polygyrus* and ATS treatment, the major impact of *H. polygyrus* on host immune response being locally in the intestine, with a weaker reduction in peripheral responsiveness compared with ATS treatment (cf. haemagglutination titres in figure 2*a* and Barnard *et al*. (1997*a*,*b*)). Such localized immunodepression may be less likely to trigger compensatory responses than systemic downregulation (see below).

The main effect of introducing female odour on the behaviour of males was to increase the amount of aggression initiated by low-ranking individuals against high rankers, which showed a marked reduction in aggressiveness as a result. The resulting destabilization of competitive social relationship (see Smith *et al*. (1996) for a fuller discussion) caused a general elevation of corticosterone levels and adrenal weights, to which the reduction in aggression by high rankers, and the general downregulation of testosterone concentration, may have been compensatory responses (Barnard *et al*. 1996*a*). Interestingly, partial regression by rank and odour treatment revealed that testosterone was downregulated in relation to corticosterone concentration rather than aggression and that downregulation occurred in high rankers only when female odours were present. We have shown previously (Barnard *et al*. 1996*a*) that testosterone concentration in male CFLPs of both ranks drops sharply in situations of increased social conflict. Increased aggression in groups may also account for the discrepancy with some other studies, including our own, that have shown an increase in testosterone in male mice exposed to the odours of females (Macrides *et al*. 1975; Batty 1978; Smith *et al*. 1996), but in single rather than grouped animals.

Taken together, the results support our earlier conclusions (Barnard *et al*. 1994, 1996*a*,*b*) that high- and low-ranking males differ in their tendency to modulate potential immunodepressants (in this case testosterone) and trade-off future susceptibility to infection. However, the results also suggest that the difference may vary with the proximate cues relating to immunodepression. Thus, socially salient cues such as increased rate of being attacked, which are associated with elevated levels of immunodepressive corticosterone, reduced peripheral immune responsiveness and short-term changes in reproductive potential, may have acted as a stronger selection pressure for modulatory rules of thumb than helminth infections, which tend to be chronic and ubiquitous, with a more localized and perhaps manipulative impact on host immune function. The study therefore points to the potential importance of life history considerations and proximate rules of thumb in decisions relating to the conservation of immune function.

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