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Suppression of expulsion of *Aspiculuris tetraptera* in hydrocortisone and methotrexate treated mice

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SUMMARY

Hydrocortisone treated male and female mice, given a primary infection with *Aspiculuris tetraptera*, did not reject the worms during the third week of infection. Mice given hydrocortisone during the first week of infection had elevated worm burdens on day 10, suggesting that some worm loss was encountered during the anterior migration in control mice. Furthermore, this temporary period of treatment was sufficient totally to suppress rejection and to allow the parasite to persist until day 28. Methotrexate also significantly delayed rejection, but larval growth was retarded in treated mice. These results, it is suggested, add strength to the hypothesis that the loss of *A. tetraptera* in a primary infection in mice, is an immunological phenomenon.

INTRODUCTION

It has been shown that the mouse pinworm, *A. tetraptera*, establishes and develops equally well in experimentally infected male and female mice (Behnke, 1975). The sex resistance reported by earlier authors (Mathies, 1959; Stahl, 1961) is now known to arise only after the expulsion phase which occurs during the third week of infection. Most female mice reject a high proportion of the infection, but male mice have a weaker response; the sex difference is thus the result of a sexual dimorphism in response to the parasite and not due to differences in susceptibility. There is strong evidence that worm expulsion is the result of an immunological response to infection (Behnke, 1975) rather than a non-immunological event as suggested by Stahl (1966), and that expulsion is followed by a state of resistance to challenge infection.

The present paper describes experiments in which mice were treated with the immunosuppressive drugs hydrocortisone acetate and sodium methotrexate in an attempt to obtain further evidence that the worm loss during the third week of a primary infection is mediated by an immunological reaction.

MATERIAL AND METHODS

Animals. The animals used in these experiments were 4- to 5-week-old, specific pathogen-free, CFLP male and female mice (Carworth Europe Ltd). The main-

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tenance of experimental animals and the procedures used to autopsy mice have been described previously (Behnke, 1974*b*).

Parasite. Mice become infected after the oral intake of the infective eggs of *A. tetraptera* and the parasite, which has a direct life-cycle, becomes sexually mature 24 days after infection (Anya, 1966). The methods used to obtain and embryonate eggs, the preparation of an infective egg suspension and the infection of experimental mice have already been described (Behnke, 1974*b*).

Hydrocortisone. Hydrocortisone acetate (Hydrocortisyl, Roussel, London) was administered subcutaneously (s.c.) in a dose of 1·25 mg. The antibiotic Terramedic (Pfizer Ltd) was given in the drinking water for the duration of the experiment, to reduce secondary bacterial infection in immunosuppressed animals.

Methotrexate. The dose of sodium methotrexate (Lederle) used was 0·125 mg in 0·05 ml of aqueous solution. The drug was injected intramuscularly (i.m.) every two days, alternatively into the left and right hind limbs.

Measurement of worms. Worms for measuring were left in Hanks' saline overnight at 4 °C and were measured by camera lucida on the following day. Not more than ten worms were taken from any one mouse.

Statistical analysis of results. All the results were analysed for significance by the Student's *t*-test (Bishop, 1971) and also by the Wilcoxon test (Sokal & Rohlf, 1969).

RESULTS

Comparison of the effect of hydrocortisone in male and female mice

Mathies (1962) noted that male and female mice treated with cortisone (75 mg/kg body weight) for 11 days post infection had higher worm recoveries than control mice, but that the sex difference found in control mice was not abolished. Autopsy in these experiments was 12 days after infection, and thus the worm burdens could not have reflected the effect of the drug on worm expulsion. In order to distinguish between the effects of hydrocortisone on initial susceptibility and on development of immunity to infection, the following experiment was carried out.

One hundred mice (50 males and 50 females) were arranged into single sex groups of ten. Twenty mice of each sex were used as controls, half of each group being killed before rejection (day 10) and the remainder after rejection (day 28). Preliminary experiments had shown that continuous treatment with hydrocortisone was not necessary to abolish rejection and therefore 20 male and 20 female mice were given hydrocortisone on days −1, +1, +3, +5 and +7. Half of each group was subsequently killed on day 10 and the remaining half on day 28. Additional groups of ten mice of each sex, treated with hydrocortisone on days +7, +10 and +12 and killed on day 28, were included to ascertain whether the administration of the drug during the second week of infection would still be effective in preventing the onset of spontaneous self cure.

The results of this experiment (Table 1) show that, within the control or cortisone treated groups, there was no difference between the worm burdens in male and female mice on day 10. Hydrocortisone treatment on days −1 to +7 resulted in higher mean worm recoveries on day 10, indicating that there was an early effect of

Table 1. *The effect of hydrocortisone treatment* (1·25 *mg/dose*) *on the development of* Aspiculuris tetraptera *in male and female mice*

Group	Hydrocortisone (days)	Number of worms recovered: Day 10 Mean	Day 10 S.D.	Day 28 Mean	Day 28 S.D.
Male	None	161·9(1)(4)	64·1	67·2(1)(2)(3)	60·2
Female	None	159·1(5)(8)	69·8	27·6(5)(6)(7)	21·8
Male	−1, +1, +3	237·6(4)	63·0	232·4(2)	74·1
Female	+5, +7	208·5(8)	62·7	219·7(6)	62·9
Male	+7, +10,			160·2(3)	71·0
Female	+12			148·0(7)	46·5

Statistical analysis of results:
Groups with the same superscript numbers have the statistical difference shown:

(1) $P < 0{\cdot}01$. (2) $P < 0{\cdot}001$. (3) $P < 0{\cdot}01$.
(4) $P < 0{\cdot}02$. (5) $P < 0{\cdot}001$. (6) $P < 0{\cdot}001$.
(7) $P < 0{\cdot}001$. (8) $P > 0{\cdot}1$.

the drug prior to day 10. Worm rejection did not occur in these groups and consequently the mean worm recoveries on day 28 were similar in these mice to those found on day 10. The group which received hydrocortisone on days +7, +10 and +12 had fewer worms on day 28 than the mice given hydrocortisone on days −1 to +7, but nevertheless there could not have been worm loss after treatment since the mean worm burdens in these mice closely paralleled those in the controls on day 10. This result confirmed that hydrocortisone had an effect early in infection.

The effect of three doses of hydrocortisone administered at different times during infection

The above results indicated that hydrocortisone in the *A. tetraptera* host-parasite system prevented the loss of larvae and that this effect was long lasting, since mice given the last dose of hydrocortisone on day 7 still retained the full worm burden on day 28. In order to obtain further information on immunosuppression induced by short-term drug administration, the second experiment was carried out.

Ten groups of 10–12, 33-day-old female mice were used in this experiment. Three doses of hydrocortisone, a total of 3·75 mg, were given during a period of five days, to each group except the two control groups (groups I and J). The results are shown in Table 2.

The administration of hydrocortisone on days −7, −5 and −3 suppressed worm expulsion and this group (group A) had significantly more worms than the control group (group J) on day 28 ($P < 0{\cdot}001$). Although most worms were found in groups C, D and E, the result for the latter two groups is particularly interesting, since here treatment was not initiated until after infection (day +1, group D; day +5, group E) and both groups had more worms ($P < 0{\cdot}02$, $P < 0{\cdot}05$, respectively) than group I ($104{\cdot}4 \pm 50{\cdot}2$ larvae; day 10 control group) and group F (hydrocortisone treatment days +9, +11 and +13). The higher worm burdens

Table 2. *The effect of three doses of hydrocortisone administered at different times during a primary infection of* Aspiculuris tetraptera

Group (days of hydrocortisone treatment)	No. of mice	No. of worms recovered at 28 days		
		Mean	±	S.D.
A (−7, −5, −3)	8	129·6		37·6
B (−5, −3, −1)	12	123·8		55·2
C (−3, −1, +1)	10	146·4		90·2
D (+1, +3, +5)	12	155·4		39·1
E (+5, +7, +9)	12	145·9		28·9
F (+9, +11, +13)	11	109·1		41·5
G (+13, +16, +17)	10	86·1		21·5
H (+17, +19, +21)	10	38·7		42·8
J (Control)	10	22·6		38·1

found in hydrocortisone treated mice are not, therefore, dependent on administration of the drug before infection and do not reflect an elevated susceptibility to infection, but rather follow the drug's action on an early loss phase (*i.e.* after day +5 but before day +9). Group G and particularly group H had fewer worms than group I, but still more than group J, indicating that partial suppression of worm loss could still be achieved by commencing the drug treatment as late as on day 13 (group G) and 17 (group H). The worms recovered from group H and those from the control group (J) were stunted, small and immature and consequently many of the females were without eggs, whereas worms from groups A–F were larger and contained numerous eggs.

Suppression of worm loss by methotrexate

This experiment was designed to study the effect of methotrexate on the rejection of *A. tetraptera* from mice. Nineteen control and eleven experimental female mice were infected with 750 eggs of *A. tetraptera* and the experimental group was given methotrexate every second day commencing one day before infection. Eight control mice were killed on day 11 to assess the worm burden before rejection and then both remaining groups were killed on day 21. The results (Table 3) clearly show that methotrexate significantly delayed rejection ($P < 0{\cdot}001$). Whereas worm expulsion occurred in the usual way in control mice ($P < 0{\cdot}001$), methotrexate-treated mice harboured on day 21 worm burdens no different from those of control

Table 3. *The effect of methotrexate on the development and survival of* Aspiculuris tetraptera *in female mice*

Group	No. of mice	Mean worm recovery	± S.D.	No. of larvae measured	Mean length (mm)	± S.D.
Control mice killed day 10	8	136·9(1)(3)	64·1	—	Not measured	
Control mice killed day 21	11	7·5(1)(2)	13·9	17	2·28(4)	0·47
Methotrexate treated mice killed day 21	11	140·4(2)(3)	110·2	29	1·67(4)	0·48

Statistical analysis of results:
Groups with the same superscript numbers have the statistical difference shown:
(1) $P < 0{\cdot}001$. (2) $P < 0{\cdot}001$. (3) P = N.S. (4) $P < 0{\cdot}001$.

mice on day 10 ($P < 0{\cdot}6$). In addition, methotrexate inhibited the growth of larval *A. tetraptera* and the worms recovered from this group were significantly smaller than those of control mice ($P < 0{\cdot}001$).

DISCUSSION

The results reported in this paper show that the loss of *A. tetraptera* during a primary infection in mice could be suppressed by treatment with hydrocortisone and methotrexate. The administration of hydrocortisone before infection resulted in higher worm recoveries on day 10, suggesting either that treated mice were more susceptible to infection or that control mice underwent a partial minor loss phase during the first week of infection. Subsequently, it was shown that the latter explanation was valid and it was established that a partial reduction in worm numbers occurred after day 5, coinciding with the anterior emigration by the larvae in the colon (Behnke, 1974*b*).

Expulsion proper occurred after day 10, but administration of hydrocortisone as late as on day 13 still partially inhibited this process. By day 17, however, most worms were lost and treatment with hydrocortisone no longer prevented stunting and damage to the worms.

Corticosteroid drugs are known to enhance the survival of helminth parasites to maturity (Wakelin, 1970) and to prolong the infection after maturity has been attained (Ogilvie, 1965). These effects are thought to be achieved by immunosuppression, since similar results can be obtained by treatment with anti-lymphocyte serum, irradiation, neonatal thymectomy and thoracic duct drainage (Ogilvie & Jones, 1967; Dineen & Adams, 1971; Hopkins, Subramanian & Stallard, 1972; DiNetta, Katz & Campbell, 1972), but it is not altogether clear how this effect is achieved since immunity to helminths is thought to be *T*-cell dependent (Ogilvie & Jones, 1971, 1973) but the *T*-lymphocyte is known to be relatively cortisone resistant (Cohen, Fischbach & Claman, 1970; Cohen & Claman, 1971). Cohen (1971) suggested that the suppression by cortisone of the G.V.H. response is not achieved by its direct effect on *T*-cells but rather on the non-specific cellular

elements involved in the host response. It has lately become apparent that the non-specific component of the processes involved in worm expulsion is of vital importance to the success of spontaneous self-cure (Dineen & Kelly, 1973; Dineen, Kelly, Goodrich & Smith, 1974; Smith, Goodrich, Kelly & Dineen, 1974) and it is therefore possible that cortisone acts on this component. Recent results by North (1972) have suggested that a short lived *T*-cell population is also cortisone sensitive and it is possible that this cell type is involved.

The enhancement by hydrocortisone of the worm recoveries on day 10 is particularly interesting. It cannot be stated at present whether this effect was achieved by immunosuppression, although immune-mediated worm expulsion can follow as early as day 7 after infection in other systems (*e.g. Nippostrongylus brasiliensis* in the rat, Ogilvie & Jones, 1971) and there is sufficient host–parasite contact in *A. tetraptera* in the mouse during the first week to generate such a response. There is evidence that the early worm loss is greater in the abnormal host (Behnke, 1974*b*) where it can also be suppressed by hydrocortisone (Behnke, 1974*a*), suggesting that perhaps a non-specific inflammatory response may be involved in both cases.

The long-term survival of worms in mice given only a short period of hydrocortisone treatment in the first week of infection is not easy to explain. In other host-parasite systems involving the mouse, *e.g. Trichinella spiralis* (Coker, 1955), *N. brasiliensis* (Ogilvie, 1965) and *Hymenolepis diminuta* (Hopkins *et al.* 1972), the termination of cortisone treatment is followed a week later by a resumption of the normal events leading to a spontaneous self-cure response, the exceptions to this rule being *Taenia taenaeformis* (Olivier, 1962) and *Trichuris muris* (Wakelin, 1970). Wakelin & Selby (1974) recently showed that the long-term survival of *T. muris* in cortisone treated mice was not unlike a state of drug-induced immunological tolerance (Many & Swartz, 1970) and it is possible that, in part at least, a similar mechanism may be operating in *A. tetraptera* in the mouse. The antigenic stimulus is thought to be recognized by the host in the first week of infection, during the crypt-phase (Behnke, 1975) and concurrent treatment with hydrocortisone might therefore be expected to bring about a tolerant state (Wakelin & Selby, 1974).

Mice treated with the cytotoxic antimetabolite methotrexate were unable to reject *A. tetraptera*. Furthermore, the worms grew more slowly in methotrexate treated animals, an observation previously made by Wilson (1971) for larval *Dictyocaulus viviparous* in guinea-pigs. In contrast Hopkins *et al.* (1972) found that methotrexate did not affect the growth or the maturation of *Hymenolepis diminuta* in mice, but the parasite grew better than in controls, presumably because the worms were protected from the host immune response. Methotrexate delayed the rejection of *D. viviparus* by guinea-pigs (Wilson, 1971) and Chinese Hamsters given methotrexate developed 50 times as many *T. spiralis* as did control animals (Ritterson, 1968). These results are pertinent since methotrexate is known to have a powerful effect on antibody synthesis (Friedman, Buckler & Baron, 1961) and to depress cell-mediated immunity (Berenbaum, 1963; Friedman, 1964).

It has been shown, therefore, that hydrocortisone and methotrexate suppressed

the rejection of *A. tetraptera* from mice and this, it is suggested, is consistent with the hypothesis that the rejection is immune-mediated since, although the two agents affect different components of the host response and have other more general effects, both are known to be potent suppressors of cell-mediated immunity.

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