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Infection with Gastrointestinal Helminths. Effect of Lactation and Maternal Transfer of Immunity

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Abstract. Lactation in sheep and mice was associated with a marked increase in susceptibility to newly acquired infection with *Haemonchus contortus* and *Nematospiroides dubius*, respectively. In sheep this increased susceptibility to infection with *H. contortus* occurred in late pregnancy and during lactation and was seen when the ewes were infected with both a single large dose of infective larvae and a "trickle" infection of 200 and 400 infective larvae of *H. contortus* given weekly for 14 weeks. Nonlactating ewes whose lactation had been terminated were able to reject the parasites which had been acquired in late pregnancy.

A relaxation in the protective immune response was evident in immunized mice infected during lactation with *N. dubius*. However, despite this periparturient relaxation of immunity mice were capable of inducing a marked passive transfer of immunity to their offspring. Thus, neonatal mice born of mothers one or two months after the end of the immunizing schedule were protected against infection with *N. dubius* at 12 or 20 days of age.

Introduction

Lactation has been shown to have a profound influence on a hosts' response to infection with gastrointestinal nematodes. For example, this is manifest in lactating ewes, infected with parasites such as *Haemonchus contortus, Trichostrongylus colubriformis,* and *Ostertagia* species, by an increased establishment of newly acquired infection, an activation of inhibited larvae, an increased fecundity of the parasites present, and an inhibition of the expulsion of the established parasites. Furthermore, when lactation is prevented or prematurely terminated, the ewes regained the capacity to reject newly acquired infection, to expel established parasites, and the fecundity of the parasites, particularly that of *T. colubriformis,* was suppressed (Connan 1968; O'Sullivan and Donald 1970; 1973). Similarly lactating sows have been demonstrated to harbour more *Hyostrongylus rubidus* than their nonlactating counterparts and to show a peri-

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parturient rise in faecal egg counts due to *Oesophagostomum* species and these phenomena terminated abruptly at weaning (Jacobs and Dunn 1968; Connan 1971). In addition, Michel et al. (1979) demonstrated that when cattle were immunized against infection with *Ostertagia ostertagi,* acquired resistance to challenge infection was unaffected by pregnancy but it was largely lost by heifers in early lactation and the proportion of larvae that underwent arrested development in lactating heifers was only marginally greater than the proportion seen in calves, and worms that were not arrested grew more rapidly in lactating heifers than in barren or pregnant heifers.

Experimental infections in laboratory animals have demonstrated also that lactating animals have an increased susceptibility to infection with intestinal nematodes. Thus, during lactation rats were more susceptible to infection with *Nippostrongylus brasiliensis* and the self-cure reaction was inhibited (Connan 1970; Dineen and Kelly 1972). Lactation had a similar effect on infection with *T. colubriformis* in guinea pigs and *Trichuris muris* and *Trichinella spiralis* in mice (O'Sullivan 1974; Selby and Wakelin 1975; Ngwenya 1977).

A colostral transfer of immunity has been demonstrated in a number of parasitic infections. For example, the young of immunized female rats and mice were protected against infection with *N. brasiliensis* and *Nematospiroides dubius,* respectively, through a colostral transfer of immunity (Greenberg 1971, Chiamcumpa et al. 1976). Thus, although the lactating female may herself become more susceptible to infection with gastrointestinal helminths, simultaneously she may be capable of passively transferring immunity to her offspring.

The present study examines the effect of lactation on infection with *H. contortus* in sheep and *N. dubius* in mice. In addition, the ability of these lactating animals simultaneously to passively transfer immunity to their offspring is examined.

Materials and Methods

Parasites

Infective larvae of *N. dubius* were obtained from Dr. J.M. Behnke, University of Nottingham, and the parasite was maintained in NIH mice. Infective third stage larvae were cultured as described by Burren (1980). *H. contortus,* obtained from Dr. R.M. Connan, University of Cambridge, was maintained in lambs.

Animals

Inbred NIH mice were obtained from Hacking and Churchill, Huntingdon. The animals were five weeks old at the start of each experiment.

A flock of Soay sheep is maintained at the Department of Clinical Veterinary Medicine, University of Cambridge. Ewes of mixed ages were used in the experiments. At lambing, lactation was terminated in some ewes by removal of their lambs at birth.

Experimental Design

Five-week-old female NIH mice were immunized as described by Behnke and Wakelin (1977). One hundred infective third stage larvae of *N. dubius* were administered by stomach tube on days 0, 3, 6, 9, and 12 and the mice were treated with pyrantel emboate (150 mg/kg) on days 15, 21, and 28.

Table 1. Effect of lactation on infection with *N. dubius*

Group No.	Status of females	No. of animals	Infected with	No. adult *N. dubius* mean ± SE
Experiment I. Parturition occurred one month after the end of the immunizing schedule				
1	Not immunized non-lactating	8	300 larvae	201 ± 9.3
2	Immune non-lactating	8	300 larvae	8 ± 1.3
3	Immune lactating	8	300 larvae	27 ± 2.8
Experiment II. Parturition occurred two months after the end of the immunizing schedule				
4	Immune lactating	4	400 larvae	67 ± 17.9
5	Immune non-lactating	4	400 larvae	0.25 ± 0.25
6	Immune lactating	5	–	0.6 ± 0.4
7	Immune non-lactating	4	–	0.25 ± 0.25

Statistical analysis: Group 1 *vs* Groups 2 and 3; Group 2 *vs* Group 3; Group 4 *vs* Groups 5, 6, and 7 ($P < 0.001$)

Experiments I and II. The effect of lactation on the protective immune response against infection with *N. dubius* was examined. Parturition was timed to occur either one month (Experiment I) or two months (Experiment II) after the end of the immunizing schedule. Lactating mice and groups of control mice of comparable age were infected with 300 or 400 larvae of *N. dubius* given one to five days after parturition. The mice were killed 14 days after infection and the small intestine was removed, opened, and incubated in 0.85% NaCl at 37° C for 30 to 60 minutes. The number of parasites which migrated into the saline or remained attached to the intestinal mucosa was then counted.

Experiments III and IV. Groups of immunized and normal mice were bred in order that parturition occurred either one month (Experiment III) or two months (Experiment IV) after the end of the immunizing schedule. The offspring of these mice were infected with 100 infective larvae of *N. dubius* when 12 or 20 days of age. The mice were killed and the number of adult *N. dubius* in their intestines was counted 25 days after infection.

Experiment V. Eight pregnant and one nonreproductive ewe were removed from pasture in March. Four pregnant ewes and the nonreproductive ewe were infected weekly with 200 infective larvae of *H. contortus* for six weeks followed by 400 larvae a week for eight weeks. Faecal egg counts were performed weekly using the McMaster technique. The ewes lambed $6^1/_2$ to $12^1/_2$ weeks after the commencement of infection. Four uninfected ewes were used to moniter the levels of naturally acquired infection.

Experiment VI. Pregnant and nonreproductive ewes were removed from pasture and treated with albendazole in April. Two pregnant ewes and one nonreproductive ewe were used as uninfected control animals to moniter the levels of naturally acquired infection. Eight pregnant ewes and five non-reproductive ewes were infected with 10,000 larvae of *H. contortus* and the ewes lambed 5 to 28 days after infection. Three infected ewes were deprived of their lambs at parturition and the remaining ewes were each allowed to suckle one lamb. Faecal egg counts were performed weekly.

Results

Effect of Lactation on Infection with N. dubius

Two experiments were carried out and the results are presented in Table 1. A highly significant ($P < 0.001$; Student's t test) level of resistance against infec-

Table 2. Maternal transfer of immunity against infection with *N. dubius*

Immune status of females	Age of neonates at infection (days)	No. of animals	No. adult *N. dubius* mean ± SE	*P* Value
Experiment III. Mice born one month after immunization of mothers				
Immune	12	13	8.8 ± 1.3	–
Normal	12	12	46.4 ± 2.0	< 0.001
Immune	20	11	20.9 ± 1.2	–
Normal	20	13	72.2 ± 1.4	< 0.001
Experiment IV. Mice born two months after immunization of mothers				
Immune	12	12	29.2 ± 1.5	–
Normal	12	14	36.4 ± 1.5	< 0.005
Immune	20	14	55.2 ± 1.8	–
Normal	20	12	70.9 ± 2.6	< 0.001

tion with *N. dubius* was induced in NIH mice following immunization with divided doses of larvae. This protective immunity was suppressed by lactation since a significantly greater number of parasites ($P < 0.001$) was found in the intestines of lactating mice, which had been challenged with 300 or 400 infective larvae one to five days after parturition than was found in comparably treated nonreproductive mice. However, lactating mice still showed considerable resistance to infection when compared with the previously uninfected control mice and this resistance was still apparent two months after the end of immunization.

Table 3. Effect of lactation on infection with *H. contortus* (continued on page 185)

Week of Experiment			0	1	2	3	4	5	6	7
No. larvae administered			200	200	200	200	200	200	400	400
Group No.	Status of ewe	Ewe No.	Egg counts per gramme of faeces							
1	Lactating infected	1	0	0	0	50	0	50	0	150
		2	0	0	50	1,100	1,250	3,350	2,750	5,300
		3	0	0	200	850	900	2,800	3,500	4,000
		4	0	0	50	300	550	750	850	2,300[a]
2	Non-reproductive infected	5	0	0	0	0	0	50	50	0
3	Lactating not infected	11	0	0	0	0	0	50	0	0[a]
		12	50	50	50	100	0	0	0	0[a]
		13	0	0	0	0	0	0	0[a]	0
		14	0	0	50	0	0	0	100	0

[a] Date of lambing [b] Treated with thiabendazole – = Not done

That the increased number of parasites found in the intestines of lactating mice was associated with an increased susceptibility to newly acquired infection and was not associated with reactivation of inhibited parasites arising from the immunizing infections is evident from the results detailed in Table 1 in that the worm burdens were low and comparable in both immune lactating and immune nonlactating animals not challenged with larvae.

Maternal Transfer of Immunity Against Infection with N. dubius

Mice born of immunized females or comparable normal females, one month (Experiment III) or two months (Experiment IV) after the end of the immunizing schedule, were infected with 100 larvae of *N. dubius* when 12 or 20 days of age. The results are presented in Table 2. Neonatal mice born of immunized females one month after the end of the immunizing schedule were markedly resistant to infection when compared with those born of normal females. A highly significant ($P < 0.001$) degree of resistance was apparent when the neonatal mice were infected at both 12 and 20 days of age. Neonatal mice born two months after the end of the immunizing schedule were still significantly protected against infection when 12 or 20 days of age. However, the maternal transfer of immunity appeared to have waned slightly two months after the end of the immunizing schedule since the level of infection seen in these neonates was greater than the level of infection seen in neonates born one month after the end of the immunizing schedule.

Table 3 (continued from page 184)

8	9	10	11	12	13	14	15	16	17	18	19
400	400	400	400	400	400	–	–	–	–	–	–
0	150	400	150	950[a]	1,600	2,250	2,300	2,200	1,600	2,500	1,950[b]
3,050[a]	2,750	4,950	1,950	3,850	1,300[b]	–	–	–	–	–	–
1,850	1,700[a]	3,950	2,300	2,100	4,400[b]	–	–	–	–	–	–
850	1,200	1,950	1,350	1,100	4,000[b]	–	–	–	–	–	–
0	50	50	50	50	100[b]	–	–	–	–	–	–
0	0	50	0	0	50[b]	–	–	–	–	–	–
0	0	0	0	0	100[b]	–	–	–	–	–	–
0	0	0	50	0	0[b]	–	–	–	–	–	–
0	0	0	0	50	0[b]	–	–[a]	–	–	–	–

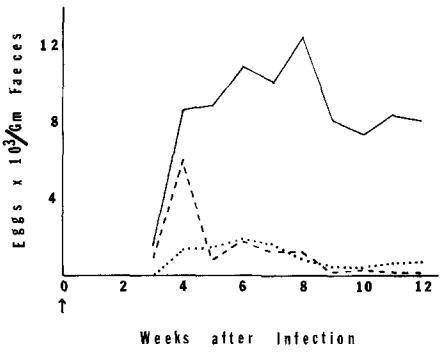

Fig. 1. Effect of lactation on infection with *H. contortus*. Group 1: — Lactating ewes; Group 2: – – – Ewes in which lactation was terminated at parturition; Group 3: · · · Nonreproductive ewes; ↑ Infection with 10,000 larvae of *H. contortus*

Effect of Lactation on Infection with H. contortus

The results of the two experiments are depicted in Table 3 and Fig. 1. When ewes were infected at weekly intervals with a "trickle" infection of 200 and 400 larvae of *H. contortus* such ewes showed an increase in faecal egg counts in late pregnancy and during lactation (Group 1; Table 3). The faecal egg counts of the ewes would suggest that this susceptibility to infection had increased as early as three to nine weeks prior to parturition. The nonreproductive ewe (Ewe 5) remained resistant to infection throughout the experiment. Those ewes which were lactating but which were not infected with *H. contortus* (Group 3) showed the presence of a low level of infection with gastrointestinal trichostrongyles but no post-parturient rise in faecal egg counts was evident in these ewes. This demonstrated that the increase in faecal egg counts in the ewes was due to increased susceptibility of the lactating ewes to newly acquired infection with *H. contortus* and was not due to activation of any inhibited larvae that may have been present.

In Experiment VI, ewes were infected with a single dose of 10,000 infective larvae of *H. contortus* just prior to parturition and there was a marked and rapid increase in faecal egg counts in the reproductive ewes (Groups 1 and 2; Fig. 1) compared with the faecal egg counts evident in the nonreproductive ewes (Group 3). In addition, in those ewes in which lactation was prevented by removal of their lambs at birth (Group 2), but which were given a single dose of 10,000 larvae, after an initial increase there was a rapid fall in faecal egg counts to levels comparable to those seen in the nonreproductive animals. Three ewes, two lactating and one nonreproductive, were not infected with *H. contortus* and the faecal egg counts remained unchanged throughout the experiment.

Discussion

Lactation in sheep was associated with a marked increase in susceptibility to newly acquired infection with *H. contortus*. This increase in susceptibility to infection occurred when the ewes were infected with either a single large dose

of 10,000 infective larvae or a "trickle" infection of 200 or 400 larvae given weekly for 14 weeks. The ewes were able to reject the infection acquired in late pregnancy if their lactation was terminated. Faecal egg counts fell rapidly within two weeks of the termination of lactation to levels comparable to those in nonreproductive ewes. The putative defect in these lactating sheep remains unclear. However, cell mediated immune mechanisms may be important since the defect in lactating rats could be repaired by syngeneic cells from immune nonreproductive rats (Dineen and Kelly 1972) and, in sheep, in vitro lymphocyte responsiveness to antigens of *H. contortus* and to the mitogens phytohaemagglutinin and pokeweed mitogen is markedly reduced in late pregnancy and early lactation (Chen and Soulsby 1976; Burrells et al. 1978).

Lactation in mice was associated with suppression of the protective immune response against infection with *N. dubius*. Thus, NIH mice immunized with multiple oral doses of larvae of *N. dubius* developed a marked protective immune response against infection confirming the results of Behnke and Wakelin (1977). However, lactation suppressed this immune response since greater numbers of parasites were recovered from immune lactating mice when compared with immune nonreproductive mice. This effect of lactation on the protective immune response was similar to that reported previously for other species of parasites, *N. brasiliensis* in rats (Connan 1970; Dineen and Kelly 1972), *T. colubriformis* in guinea pigs (O'Sullivan 1974), and *T. muris* and *T. spiralis* in mice (Selby and Wakelin 1975; Ngwenya 1977). However, lactation did not completely suppress the protective immune response and lactating mice still showed considerable resistance against infection when compared with unimmunized animals. Connan (1972) and Selby and Wakelin (1975) had demonstrated also that lactating rats and mice showed considerable resistance to reinfection with *N. brasiliensis* and *T. muris*, respectively, when compared with previously uninfected animals.

Immunity against infection with *N. dubius* is manifested primarily by destruction of larvae in intestinal granulomata (Bartlett and Ball 1974; Jones and Rubin 1974) but this process appears not to be rapid since, although there was a gradual reduction in the numbers of larvae capable of resuming their development presumably due to their destruction in intestinal granulomata, a proportion of larvae was still capable of resuming development in corticosteroid treated mice for at least 22 days after challenge infection (Behnke and Parish 1979). In lactating sheep the increase in the numbers of parasites in the intestines is associated with both an increased susceptibility to newly acquired infection and an activation of previously acquired infective larvae. Therefore it was possible that the increased numbers of *N. dubius* in the intestines of lactating mice resulted not from the newly acquired challenge infection but from larvae which might have become arrested late in the course of the immunizing infections. However, lactating mice which had been immunized but which had not received a challenge infection showed no increase in the number of parasites in their intestines. Thus, the relaxation in protective immunity against infection with *N. dubius* in lactating mice was manifested, in this experiment, by an increased susceptibility to newly acquired infection.

Despite the relaxation in their protective immune response during lactation,

lactating mice were nevertheless still capable of transferring immunity against infection with *N. dubius* to their offspring. Neonatal mice born of immunized mothers were protected against infection when infected at 12 or 20 days of age. The length of this period of protection suggests that immunity was transferred *via* the colostrum. A colostral transfer of immunity is in agreement with the findings of Greenberg (1971) and Chiamcumpa et al. (1976) who demonstrated that immunity against infection with *N. brasiliensis* and *N. dubius* in rats and mice, respectively, was transferred *via* the colostrum.

The mice used to examine peri-parturient relaxation of immunity and those mice used to examine maternal transfer of immunity were immunized in an identical manner in order that the results of the two groups of experiments could be compared. Thus, mice simultaneously demonstrated a relaxation in their own protective immune response during lactation and a marked passive transfer of immunity to their offspring. The immunological mechanisms associated with protection against intestinal infection in an immunized adult animal and with maternal transfer of immunity may be similar, and the suppression of protective immunity in the lactating female may be associated with the transfer of committed cells or immunoglobulins to the mammary gland to result in the passive transfer of immunity to the neonate.

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