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The Suppression of Rejection of *Nippostrongylus brasiliensis* in Lactating Rats: the Nature of the Immunological Defect

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Summary. Lactating female rats infected with 3000 third-stage larvae of *Nippostrongylus brasiliensis* showed significant increases in worm fecundity and total worm burdens when compared with infected nulliparous controls. Statistically significant differences were recorded for each of the three periods of infection, although these differences were of greatest magnitude during Period 3 (16–30 days of infection).

Immune mesenteric lymph node cells (100×10^6), obtained from nulliparous female donors on Day 15 of a primary infection, were transferred syngeneically to lactating female recipients. The transferred cells invariably caused suppression of worm fecundity, reduction in the number of eggs per uterus in gravid female worms and rejection of a substantial proportion of worms by Day 10 of a challenge infection in the lactating recipients. The results of this study showed that immune cells were functional in lactating female recipients and that transfer of immune cells repaired the deficit in the rejection mechanism.

Mesenteric lymph node cells (100×10^6), obtained from lactating female donors on Day 15 of a primary infection, were transferred syngeneically to nulliparous female recipients. The transferred cells caused suppression of worm fecundity, reduction in the number of eggs per uterus in gravid female worms and rejection of the majority of parasites by Day 10 of a challenge infection in the nulliparous recipients. Clearly, potentially immune lymphoid cells were present in the mesenteric nodes of lactating females at the time that the rejection mechanism was severely impaired.

Mesenteric lymph node cells obtained from infected lactating donors were substantially less effective in lactating recipients than in nulliparous recipients. These cells caused the expulsion of 51 per cent of worms by Day 10 in lactating recipients, whereas they caused expulsion of 99 per cent of worms in nulliparous recipients.

These observations suggest that the inductive processes of the immune response occur normally, but that differentiation of induced cells to effector cells is impaired in lactating animals.

INTRODUCTION

The regular seasonal occurrence, in sheep, of an increase in faecal nematode egg output ('spring rise' phenomenon) was first described in lactating ewes by Zawadowsky and Zvjaguintzer (1933) in Russia and by Taylor (1935) in England. This phenomenon has

since been reported by Crofton (1954; 1958) and Connan (1967a; 1968) in England; Morgan, Parnell and Rayski (1950; 1951) in Scotland; Seghetti and Marsh (1945) in America; Naerland (1952) in Norway; Gibbs (1964; 1967) in Canada; Brunsdon (1964; 1970) in New Zealand and by Dunsmore (1965), Arundel (1967) and O'Sullivan and Donald (1970) in Australia. As the rise in faecal nematode egg counts in lactating ewes occurred in both spring and autumn, Crofton (1958) suggested that the phenomenon was associated with parturition and lactation rather than the season of the year. Consequently he proposed that the rise in faecal egg output in parturient and lactating females be called a 'post-parturient' rise.

The association of lactation with increased nematode egg output has been confirmed by Brunsdon (1964), Dunsmore (1965) and Connan (1968). In addition O'Sullivan and Donald (1970) observed that ewes which aborted or ewes which had their lambs removed soon after birth, did not show an increase in faecal egg output in the post-parturient period. Similarly Connan (1967b) and Jacobs (1967) reported a post-parturient rise in faecal egg output in lactating sows, but not in sows which aborted or had their litters removed soon after birth. Evidence of an increase in susceptibility to nematode infections during lactation has also been reported in pony mares (Poynter, 1954), rabbits (Dunsmore, 1966), guinea-pigs (Connan, 1968, 1970) and rats (Connan, 1970). O'Sullivan and Donald (1970) have further shown that, if lactation is prevented or prematurely terminated, the ewe is capable of rejecting both newly acquired larvae and established mature infections and that in the case of at least one species of nematode (*Trichostrongylus colubriformis*) the remaining adult female nematodes may have their fecundity severely restricted.

The factors responsible for the 'post-parturient' rise are not understood. Dunsmore (1965) cited numerous examples in which there is an association between alterations in the host's hormonal status and the reproductive activity of an associated commensal organism. Since the major difference between lactating and non-lactating females is in their circulating hormone levels, it is reasonable to assume that lactogenic hormones play an important role in the 'post-parturient' rise.

Host hormones may act on the host parasite relationship in two ways (a) by a direct effect on the parasite or (b) by an indirect effect on the parasite mediated via the tissues of the host. A direct effect of lactogenic hormones on the parasite has been suggested by Gibbs (1967), Ford (1967) and Salisbury and Arundel (1970) on the basis of an increase in faecal nematode egg count in non-pregnant ewes, following treatment with diethyl stilboestrol. An indirect effect of lactogenic hormones on the parasite via the host's immune system has been postulated by Dunsmore (1966), Connan (1968) and O'Sullivan and Donald (1970). These workers suggest that the 'post-parturient' rise is due to a temporary, non-specific suppression of immunological response due to variations in the levels of hormones associated with lactation. There is little direct evidence to support either hypothesis, but as pointed out by O'Sullivan and Donald (1970) the differences in behaviour of parasite populations in lactating and non-lactating animals are qualitatively the same as the differences reported between susceptible and resistant non-pregnant animals. A hypothesis proposing a direct effect of lactogenic hormones on the parasite is less acceptable than one which implicates both host hormones and host immune response.

Connan (1970) showed that 'self-cure' was inhibited or at least markedly depressed in lactating female rats infected with the nematode *Nippostrongylus brasiliensis* (Yokogawa, 1920). In view of the recent report by Kelly and Dineen (1972) that resistance to

N. brasiliensis can be transferred in the rat with immune lymphocytes, this cell-transfer system was used in the present studies to analyse the immunological deficit in lactating females.

MATERIALS AND METHODS

Animals

Rats used were inbred females of the Lewis strain (Kelly and Dineen, 1971). Nulliparous females, of about 200–250 g weight, were mated over a 2-day period to ensure that litters were born with a minimal time spread. Approximately 20 days after mating, pregnant females were removed to individual cages, and supplied with a small quantity of nesting material (wood shavings), which was replaced at 4-day intervals throughout the experiments to minimize the possibility of reinfection. Only parous females which actively suckled their litters for at least 21 days were used in the experiments to be described. Dry, parous females were obtained by weaning litters within 24 hours of birth.

Preparation and administration of infective larvae

Third stage infective larvae of *N. brasiliensis* were obtained from faeces-charcoal cultures (Leigh, 1956), washed in normal saline and suspended in an appropriate volume for counting by a dilution technique. The final volume of the larval suspension was adjusted so that the required number of larvae for each dose was contained in 0·5 ml of the suspension. Rats were infected by subcutaneous inoculation in the lumbar-dorsal area.

Estimation of worm eggs/g of faeces and worm burdens

Estimates of daily faecal eggs/g on fresh 2 g faecal samples, were obtained using a modification of the McMaster slide counting technique of Gordon and Whitlock (1939). Total worm counts were performed on bulked intestinal digests and contents.

Preparation and transfer of lymphoid cells

Mesenteric lymph node cells were prepared for transfer by intravenous injection as previously described by Kelly and Dineen (1971).

RESULTS

EFFECT OF LACTATION ON DAILY FAECAL OUTPUT

Previous workers have shown that the daily output of faeces is increased in lactating females (Murray, 1941 and Pike, Suder and Ross, 1954). Consequently egg production, as measured by eggs/g of faeces, should be corrected to allow for faecal output. An experiment was therefore carried out to determine the daily output of faeces in seven lactating Lewis-strain rats and seven nulliparous females of the same strain, weight and age, which were infected with 3000 third-stage larvae on the day of parturition. The results are given in Table 1.

The results given in Table 1 show that the daily faecal output in infected lactating rats is increased four- to five-fold by comparison with nulliparous animals. In subsequent studies the daily faecal egg output was obtained by multiplying the estimated eggs/g of faeces by the appropriate daily faecal output shown in Table 1.

TABLE 1

DAILY OUTPUT OF FAECES IN LACTATING AND NULLIPAROUS LEWIS RATS

Days after parturition and infection	Mean ± S.D. (g)	
	Lactating females	Nulliparous females
1	16·6 ± 2·1	3·1 ± 0·8
3	15·3 ± 3·6	3·3 ± 0·5
4	15·2 ± 3·2	3·5 ± 0·5
6	16·7 ± 3·3	3·4 ± 0·5
8	17·2 ± 3·3	3·5 ± 0·6
9	18·6 ± 4·2	3·5 ± 0·6
10	20·0 ± 3·0	3·2 ± 0·6
12	20·0 ± 3·6	3·9 ± 0·7
14	20·6 ± 3·5	4·2 ± 0·7
16	22·0 ± 4·3	4·0 ± 0·8
18	22·0 ± 4·7	3·8 ± 0·8
20	20·5 ± 3·5	3·4 ± 0·8
22	17·5 ± 4·9	3·5 ± 0·8
24	14·3 ± 4·9	3·4 ± 0·8
26	14·4 ± 6·0	3·4 ± 0·6
28	11·4 ± 3·8	3·7 ± 0·6
30	9·6 ± 2·4	3·7 ± 0·7

EFFECT OF LACTATION ON WORM FECUNDITY

The daily output of eggs was estimated from eggs/g of faeces and faecal output in groups of lactating females, dry parous females and nulliparous females infected with 3000 third-stage larvae. Eight animals were included in each group. From these data the total egg production (fecundity) during each period of infection was approximated by numerical quadrature. Fecundities were calculated separately for the three distinct phases of infection i.e. Period 1 (plateau phase 1, Jarrett, Jarrett and Urquhart, 1968)—6–10 days of infection; Period 2 (logarithmic phase 2)—11–16 days of infection; and Period 3 (the residual worm burden phase)—the period of egg production following Day 16. The results of these experiments are summarized in Table 2. As the fecundities estimated for the three periods are not independent, the data obtained for the periods were analysed separately. These analyses are given in Table 3.

The results given in Table 2 show that fecundity during periods 1 and 2 were marginally greater in lactating females than in either dry parous or nulliparous females. During Period 3 the fecundity in lactating females (751,382) was much greater than in dry parous and nulliparous females (4,387 and 2,779, respectively) and this difference was significant at the 0·1 per cent level (m.s. 3,691,187,787 for 'group 3 *vs* groups 1+2,' Period 3 (see Table 3).

EFFECT OF LACTATION ON WORM COUNT

Worm counts were carried out in groups of nulliparous and lactating female rats of the same age and weight which were infected with 3000 third-stage larvae. Groups of animals were killed for differential worm counts on Days 10, 22 and 28 of infection. The results are given in Table 4. As larval counts were invariably insignificant these are not included in Table 4.

TABLE 2

EFFECT OF LACTATION ON PARASITE FECUNDITY

Groups	No. of animals	Period		
		1 (Days 6–10)	2 (Days 10–16)	3 (Days 16–30)
1. Nulliparous females	8	482,583	451,960	2,779
2. Dry parous females	8	500,483	447,251	4,387
3. Lactating females	8	516,805	638,559	751,382

TABLE 3

ANALYSES OF VARIANCE OF PARASITE FECUNDITIES* IN NULLIPAROUS (GROUP 1) DRY PAROUS (GROUP 2) AND LACTATING FEMALES (GROUP 3)

Source of variance	D.F.	S.S.	M.S.	
Period 1				
3 *vs* 1+2	1	16,394,056	16,394,056	$0{\cdot}01 < P < 0{\cdot}05$
1 *vs* 2	1	32,581	32,581	N.S.
Groups	2	16,426,637	8,213,319	N.S.
Error	21	72,394,184	3,447,342	
Total	23	88,820,821		
Period 2				
3 *vs* 1+2	1	10,074,169	10,074,169	N.S.
1 *vs* 2	1	196	196	N.S.
Groups	2	10,074,365	5,037,183	N.S.
Error	21	98,416,371	4,686,494	
Total	23	108,490,736		
Period 3				
3 *vs* 1+2	1	3,691,187,787	3,691,187,787	$P < 0{\cdot}001$
1 *vs* 2	1	140,944,384	140,944,384	N.S.
Groups	2	3,832,132,171	1,916,066,086	
Error	21	1,293,944,335	61,616,397	
Total	23	5,126,076,506		

* Fecundities transformed to $\log_{10} \times 10^4$.

TABLE 4

WORM COUNTS IN LACTATING AND NULLIPAROUS FEMALES

Group	No. of animals	Day of infection	Adult worm count		
			Males	Females	Total
Nulliparous females	6	10	510	520	1030
Lactating females	6	10	731	756	1487
Nulliparous females	5	22	4·4	0·4	4·8
Lactating females	5	22	472	423	895
Nulliparous females	6	28	5·6	4·1	9·7
Lactating females	6	28	305	324	629

The mean total worm count in lactating females on Day 10 was 1,487 and in nulliparous females this was 1,030. The difference between these means was highly significant ($t = 3{\cdot}61$ on 10 d.f.: $P<0{\cdot}01$). While most worms were rejected in nulliparous females by Days 22 and 28 of infection, substantial numbers remained in lactating females at these times (means of 895 and 629 respectively).

THE TRANSFER OF CELLS FROM IMMUNE NULLIPAROUS DONORS TO LACTATING RECIPIENTS

The experiments described above show that rejection of the parasite is markedly suppressed in lactating females. This finding suggests an immunological deficiency in lactating animals and this deficiency might be repaired by the injection of syngeneic mesenteric lymph node cells obtained from immunized donors. Consequently the following experiment was carried out.

Eighteen nulliparous Lewis rats and eighteen lactating Lewis rats were infected with 3000 third-stage larvae. On the day of infection, nine of the nulliparous and nine of the lactating females were each injected intravenously with 100×10^6 cells. These cells were obtained from the mesenteric nodes of a group of Lewis strain donors on Day 15 of a primary infection with 3000 third-stage larvae. Both cell-recipient and non-recipient groups were killed on Day 10 of infection for differential worm counts, and worm fecundities were estimated during Period 1 (Days 6–10). Group mean worm counts and fecundities are given in Table 5 and the appropriate analyses in Table 6.

The analysis of the worm counts shows that the immune-cells were effective (see m.s. 13,883,076 Groups 1+2 *vs* Groups 3+4, $P<0{\cdot}001$; Table 6) and that they were as effective in lactating female recipients as they were in nulliparous female recipients (m.s. 12,534 Groups 3 *vs* 4, n.s.). In addition the difference between means of the control groups (Groups 1 and 2) confirms that lactating females are more susceptible than nulliparous females (m.s. 680,166, Groups 1 *vs* 2, $P<0{\cdot}001$). The same conclusions may be drawn from the group mean worm fecundities shown in Table 5 and the analysis of these data is included in Table 6.

THE TRANSFER OF CELLS FROM INFECTED LACTATING DONORS TO NULLIPAROUS RECIPIENTS

Four lactating Lewis donors and four nulliparous donors of the same strain and age were each infected with 3000 third-stage larvae on the day of parturition of the lactating females. On Day 15 of infection single cell suspensions were prepared from the pooled mesenteric lymph nodes of the lactating and nulliparous donors. Syngeneic recipients were injected intravenously with either 100×10^6 cells obtained from the lactating donors, or the non-lactating nulliparous donors. Eight animals were included in each recipient group and these with eight non-recipient controls were each dosed with 3000 third-stage larvae on the day of cell transfer. The three groups of animals were killed on Day 10 of infection for differential worm counts and worm fecundities were estimated for Period 1 (Days 6–10) as described above. In addition the number of eggs per uterus was counted in gravid females obtained from the non-recipient control animals and from the recipients of cells from lactating donors as described previously (Kelly and Dineen, 1971). These results are summarized in Table 7.

Comparison of the worm count in non-recipient controls (group mean of 569) with the count in lactating-cell recipients (group mean of 65) shows that highly effective immune

lymph node cells can be obtained from lactating donors although these cells are not quite as effective as cells obtained from non-lactating donors (group mean of 0).

Similarly it is apparent that cells obtained from infected lactating donors reduced worm fecundity in recipients and that these cells were not as effective as cells obtained from

TABLE 5

THE CELLULAR TRANSFER OF RESISTANCE TO LACTATING RECIPIENTS WITH CELLS FROM IMMUNE NULLIPAROUS DONORS

Group	No. of animals	Adult worm count			Fecundity Period 1 (Days 6–10)
		Males	Females	Total	
1. Nulliparous females (non-recipients)	9	570	558	1128	402,387
2. Lactating females (non-recipients)	9	749	768	1517	861,831
3. Nulliparous females (recipients)	9	48	59	107	171,726
4. Lactating females (recipients)	9	25	29	54	232,591

TABLE 6

ANALYSIS OF WORM COUNTS AND FECUNDITY IN LACTATING RECIPIENTS

Source of variance	D.F.	S.S.	M.S.	
1. Worm counts				
Nulliparous *vs* lactating females (group 1 *vs* group 2)	1	680,166	680,166	$P< 0{\cdot}001$
Nulliparous *vs* lactating recipients (group 3 *vs* group 4)	1	12,534	12,534	N.S.
Non-recipients *vs* recipients (groups 1+2 *vs* groups 3+4)	1	13,883,076	13,883,076	$P< 0{\cdot}001$
Groups	3	14,575,776	4,858,592	
Error	32	1,082,225	33,820	
Total	35	15,658,001		
2. Fecundity* Period 1				
Nulliparous *vs* lactating females (group 1 *vs* group 2)	1	40,464,007	40,464,007	$0{\cdot}001< P< 0{\cdot}01$
Nulliparous *vs* lactating recipients (group 3 *vs* group 4)	1	9,439,513	9,439,513	N.S.
Non-recipients *vs* recipients (groups 1+2 *vs* groups 3+4)	1	249,403,156	249,403,156	$P< 0{\cdot}001$
Group	3	299,306,676	99,768,892	
Error	32	158,542,710	4,940,460	
Total	35	457,849,386		

* Fecundity transformed to $\log_{10} \times 10^4$.

infected non-lactating donors. The significances of the differences between group means were determined by Duncan's (1955) new multiple-range test. This test showed that the difference between means of fecundity in controls (non-recipients) and recipients of cells from non-lactating donors (the difference between groups 1 and 2) was significant at the 1 per cent level, and these differences for controls and recipients of cells from lactating

TABLE 7

THE EFFECT OF MESENTERIC LYMPH NODE CELLS OBTAINED FROM INFECTED LACTATING DONORS IN NULLIPAROUS RECIPIENTS

Group	No. of animals	Adult worm count			Fecundity Period 1 (Days 6–10)	Eggs per uterus
		Males ± S.D.	Females ± S.D.	Total		
1. Infection control (non-recipients)	8	280 ± 140	289 ± 159	569	833,944	28 ± 4*
2. Recipients of cells from non-lactating donors	8	0	0	0	312,442	
3. Recipients of cells from lactating donors	8	28 ± 43	28 ± 25	65	483,626	11 ± 4*

* Significance of a difference between means, $t = 8{\cdot}5$ on 14 d.f.: $P < 0{\cdot}01$

donors (groups 1 and 3) and for recipients of cells from non-lactating and lactating donors (groups 2 and 3) were significant at the 5 per cent level.

In addition to the reduction in worm count and fecundity which was observed in cell recipients, the results summarized in Table 7 also show that egg production per female, as measured by eggs per uterus, was suppressed in gravid females obtained from recipients of cells from lactating donors (11 ± 4) compared with females obtained from non-recipient controls (28 ± 4).

THE TRANSFER OF CELLS FROM INFECTED LACTATING DONORS TO LACTATING RECIPIENTS

Lactating Lewis females to be used as cell donors were infected with 3000 third-stage larvae of *N. brasiliensis* on the day of parturition. All rats were infected by subcutaneous inoculation in the lumbar-dorsal area. Only parous females which actively suckled their litters for at least 15 days after infection were used as donors of mesenteric lymph node cells. Fifteen days after infection, lactating cell donors were killed and single cell suspensions were prepared from their mesenteric lymph nodes. The syngeneic recipients, both lactating and nulliparous females, were then injected intravenously with 100×10^6 mesenteric lymph node cells.

The lactating and nulliparous recipient groups, as well as a non-recipient, nulliparous control group were each infected with 3000 *N. brasiliensis* third-stage larvae on the day of cell transfer. Only those lactating cell recipients which actively suckled their litters for a period of 10 days after cell transfer and infection were used in the experiment.

All groups were killed on Day 10 of the challenge infection for total worm counts and the fecundity of infection was estimated during the first 6 to 10 days of infection. Group mean fecundities and total worm counts for the three groups are shown in Table 8.

For statistical analysis fecundities were transformed logarithmically and the significances of the differences between group means estimated by Duncan's new multiple-range test. This analysis showed that the difference between means of lactating cell recipients and infection controls (211,513 and 575,739 respectively) was significant at the 5 per cent level and the differences between means of nulliparous recipients and infection controls (11,474 and 575,739 respectively) and lactating and nulliparous recipients (211,513 and 11,474 respectively) were both significant at the 1 per cent level. A similar analysis was

TABLE 8

COMPARISON OF THE EFFECT OF MESENTERIC LYMPH NODE CELLS OBTAINED FROM INFECTED LACTATING DONORS IN LACTATING AND NULLIPAROUS RECIPIENTS

Group	No. of animals	Mean fecundity (6–10 days of infection)	Worm counts (day 10)		
			Males	Females	Total ± S.E.
1. Infection controls	18	575,739	414	514	928 ± 169
2. Lactating recipients	10	211,513	213	238	451 ± 76
3. Nulliparous recipients	11	11,474	3	5	8 ± 8

carried out on the total worm counts. Again the difference between means of these counts for lactating cell recipients (451 ± 76) and infection controls (928 ± 169) was significant (at the 5 per cent level). The differences between means of nulliparous recipients (8 ± 8) and infection controls was significant at the 1 per cent level and the difference between mean worm counts in lactating and nulliparous cell recipients was significant at the 5 per cent level.

DISCUSSION

As the post-parturient rise in faecal nematode egg output is associated with lactation (Brunsdon, 1964; Dunsmore, 1965; and O'Sullivan and Donald, 1970) lactogenic hormones have been implicated in the phenomenon. Conceptually host hormones may act either by a direct effect or by an indirect effect on the parasite. The indirect effect of hormones may be mediated through the immunological response of the host as advocated by Dunsmore (1966), Connan (1968), O'Sullivan and Donald (1970) and others. It was the purpose of the present study to investigate this possibility using the *N. brasiliensis* inbred rat system.

The results described in the present communication confirm Connan's (1970) preliminary study that the lactating rat is more susceptible to infection with *N. brasiliensis* than nulliparous controls. Increased worm fecundity was observed in lactating females infected with 3000 third stage larvae and this was most evident during the third period of infection (Days 16–30) (see Table 2). This result showed that survival of the parasite was prolonged in lactating females. In the first experiment worm fecundity in lactating females was greater during the first two periods than in either nulliparous or dry parous females although the magnitude of the differences was small (see Table 2). In a subsequent experiment the difference between mean worm fecundity in nulliparous females (402,387) and lactating females (861,831) during Period 1 (see Table 5) was marked. As the daily output of worm eggs during this period was constant it is reasonable to assume that this is due to the establishment of more adult worms in lactating females than in nulliparous females.

Worm counts carried out on groups of nulliparous and lactating females on Days 10, 22 and 28 of infection showed that more adult worms were recovered from lactating females (mean of 1,487) than from nulliparous females (1,030) on Day 10 and the difference became greater during the later periods of infection (see Table 4). These worm counts are in accord with the worm fecundities and support the conclusion that there is increased

establishment and prolonged survival of worms in lactating, compared with nulliparous females.

In a previous study Kelly and Dineen (1971) showed that immune cells can be obtained from the mesenteric lymph nodes of rats 15 days after infection with *N. brasiliensis*. Intravenous injection of these cells into non-immune syngeneic recipients caused accelerated rejection of a challenge infection. We infer, with others studying the rejection of *N. brasiliensis* in the rat, that the rejection mechanism is basically immunological. As a corollary, it is reasonable to assume that the failure of rejection in lactating females is due to a deficiency in the mechanism of rejection. There is an accumulating weight of evidence that the rejection mechanism is di-phasic (Urquhart, Mulligan, Eadie and Jennings, 1965; Ogilvie and Hockley, 1968; Keller, 1970a, b). One phase is immunologically specific and the other probably involves myeloid cells and the production and release of amines. It is not necessary to discuss the roles and interaction of these two components of the rejection mechanism here, but it is relevant to note that hormones could affect and suppress either, or both, of these components. Thus failure of rejection in the lactating female could involve suppression of either or both components. With this deduction in mind, it was highly pertinent to investigate whether immune lymphoid cells transferred syngeneically in lactating females were functional. The results summarized in Table 5 clearly show that immune cells are functional in lactating recipients and transfer of immune cells repaired the deficiency in the rejection mechanism. Therefore we infer that there is no deficiency in the myeloid-amine component of the rejection mechanism.

As immune lymphoid cells were functional in lactating recipients it was particularly interesting to determine whether immune cells could be obtained from lactating females during the period that the rejection mechanism is seriously impaired. Consequently mesenteric lymph node cells were transferred syngeneically from lactating donors on Day 15 of infection to normal recipients which were then challenged with 3000 third-stage larvae. The results of the worm counts of recipients are summarized in Table 7 and these show that the cells from infected lactating donors caused a 90 per cent reduction in worm count when compared with the worm count in the non-recipient control group. Clearly potentially functional immune lymphoid cells were present, at least in the mesenteric nodes of lactating females, at the time that the rejection mechanism was seriously impaired.

The worm counts given in Table 8 show that lymphoid cells obtained from infected lactating donors are substantially less effective in lactating recipients than in nulliparous recipients. Thus the cells from lactating donors caused the expulsion of 51 per cent $\left(\frac{(928-451)}{928} \times 100\right)$ of worms by Day 10 of infection in lactating recipients, whereas these cells caused the expulsion of 99 per cent $\left(\frac{(928-8)}{928} \times 100\right)$ of worms in nulliparous recipients. This conclusion is supported by the results of the faecal egg counts. It is relevant to note that the worm counts in lactating animals on Days 22 and 28 of infection (895 and 629 respectively) were approximately 50 per cent of the worm count in similar animals on Day 10 (1487) (see Table 4). Therefore the reduced immunological reactivity of cells from lactating donors, provides an adequate explanation of the failure of immunological control during lactation.

The results of the present study show that (a) potentially reactive cells are present in

lactating donors. (b) effector cells can react in lactating recipients, but (c) the action of potentially reactive cells from lactating donors is substantially inhibited in lactating recipients.

These observations suggest that the inductive processes of the immune response occur normally in lactating rats, but that differentiation of induced cells to effector cells is substantially inhibited. The results of the present study may be interpreted if it is assumed that differentiation of induced cells from infected lactating donors is inhibited when they are transferred to lactating recipients, but that the cells complete differentiation to effector cells in nulliparous non-lactating recipients. However cells obtained from infected nulliparous donors may have completed differentiation to effector cells before transfer to lactating recipients. It is possible that lactogenic hormones either directly or indirectly, exert an inhibitory effect on differentiation of lymphoid cells.

O'Sullivan and Donald (1970) reported that faecal nematode egg counts fell to low levels in ewes that ceased to lactate within 10 days of weaning. They suggested that if lactation is terminated as a result of weaning, immunological control is rapidly re-established. This finding is also compatible with the view that inductive processes occur normally in lactating animals and that when lactation is terminated, differentiation to effector cells is completed with a rapid acquisition of immunological responsiveness.

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