Immunology, 1972, **22**, 199.

The Cellular Transfer of Immunity to *Nippostrongylus brasiliensis* in Inbred Rats (Lewis Strain)

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(*Received* 16*th March* 1971)

Summary. Mesenteric lymph node cells obtained from highly inbred donor rats (Lewis strain), resistant to *Nippostrongylus brasiliensis* infection, were syngeneically transferred by intravenous injection into previously uninfected recipients. The adoptively immunized recipients were then challenged with either 1500 or 3000 third stage *N. brasiliensis* larvae on the day of cell transfer. The degree of resistance transferred was assessed by monitoring daily faecal egg output, differential worm burdens on days 6 and 10 of infection and the number of eggs per uterus in gravid worms.

The syngeneic transfer of 100×10^6 immune mesenteric lymph node cells invariably resulted in suppression of egg production, a two- to four-fold reduction in the number of eggs per uterus in gravid females and rejection of at least 75 per cent of adult worms by days 6 and 10 of infection.

It was also noted that mesenteric lymph node cells obtained from donors on day 15 of a primary infection were more effective than those obtained from donors immunized by multiple infections.

Immune cells transferred from donors on day 4 of infection were equally effective with those transferred on day 0. However, immune cells transferred on or after day 10 of infection had little or no effect and this shows that the parasite is less susceptible to an attack mounted by the transferred cells during the later stages of infection.

INTRODUCTION

It was first shown by Africa (1931) and Schwartz, Alicata and Lucker (1931) that rats developed an actively acquired immunity to the nematode *Nippostrongylus brasiliensis* (Yokogawa, 1920). This immunity was manifested by decreased numbers of eggs passed in the faeces, inhibition of ovulation and egg release in adult female worms, and terminally by rejection of the worms.

Various workers have investigated the mechanisms of immunity involved in *N. brasiliensis* infections in rats by attempting to passively transfer immunity with serum (Graham, 1934; Porter, 1935; Sarles and Taliaferro, 1936; Chandler, 1937; Sarles, 1939; Thorson, 1953, 1954; Mulligan, Urquhart, Jennings and Nielson, 1965; Ogilvie and Jones, 1968; Jarrett, Urquhart and Douthwaite, 1969; Jones, Edwards and Ogilvie, 1970). In most

of the work cited above, protective immunity conferred by passive transfer of serum was extremely variable, large volumes of serum were required to produce an effect, and the level of protection transferred was seldom comparable to that produced by active infection.

Unsuccessful attempts to passively transfer immunity with serum against other helminth infections include those of Larsh, Goulson and Weatherly (1964a,b) against *Trichinella spiralis* in mice; Wagland and Dineen (1965), Dineen and Wagland (1966) and Rothwell (1969) against *Trichostrongylus colubriformis* in guinea-pigs, Panter (1969) against *Nematospiroides dubius* in the mouse and Dhar and Singh (1970) against *Oesophagostomum columbianum* in goats.

In contrast to the controversy that exists regarding the ability of passively transferred serum to protect recipients against helminth infection, there is an increasing number of host–parasite relationships in which resistance to challenge infection has been transferred with immune lymphocytes. Larsh *et al.* (1964a,b) investigated delayed hypersensitivity reactions in mice infected with *T. spiralis* and transferred immunity with peritoneal exudate cells from immune animals. They concluded that the mechanism of expulsion of adult *T. spiralis* is mediated by a specific delayed hypersensitivity reaction.

The cellular transfer of immunity has been reported in other host–parasite systems, including *T. colubriformis* infections in guinea-pigs (Wagland and Dineen, 1965; Dineen and Wagland, 1966; Rothwell, 1969), *Fasciola hepatica* infections in mice (Lang, 1967; Lang, Larsh, Weatherly and Goulson, 1967) and *Hymenolepis nana* infections in mice (Friedberg, Neas, Friedberg and Faulkner, 1967a,b; Okamato, 1968, 1970).

The first attempt to demonstrate cellular transfer of immunity in rats infected with *N. brasiliensis* was reported by Hunter and Leigh (1961). However, these workers were unable to demonstrate any degree of immunity in recipients given spleen and lymph node cells from highly immunized donors. Ogilvie and Jones (1968) transferred resistance passively with immune serum and adoptively with immune cells, but with both methods results were variable and the degree of protection achieved was much less than that produced by active immunization. Recently Kassai and Szepes (1970) also attempted to assess the role of cell mediated immunity in resistance to *N. brasiliensis*. However, they failed to transfer immunity with either spleen or lymph node cells and concluded that resistance to challenge infections involves one or more factors which cannot be transferred by sensitized lymphoid cells.

Because of the conflicting results obtained in the studies cited above, the present investigation was undertaken to re-examine the role of immune lymphoid cells in resistance to *N. brasiliensis* infection in the rat.

MATERIALS AND METHODS

Animals

The rats used were usually of the highly inbred Lewis strain which were maintained by line mating. Rats of about 200 g were used in the experiments. In one experiment, involving allogeneic transfer of cells, DA strain rats were used in addition to Lewis rats. These animals were also maintained by line mating and were about 200 g when used in the experiment.

Preparation and administration of infective larvae

Third stage infective larvae of *N. brasiliensis* were obtained from faeces–charcoal

cultures, using a modification of the technique of Leigh (1956). Larvae were filtered through surgical gauze, washed thoroughly 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 either 0·5 or 1·0 ml of the suspension. Rats were infected by subcutaneous inoculation in the abdominal region.

Active immunization of cell donors

Donors for cell transfer were immunized by one of two schedules.

Schedule 1. Infected with *ca.* 3000 third stage larvae, then a month later with *ca.* 6000 third stage larvae and again followed 1 month later with *ca.* 6000 third stage larvae. Mesenteric lymph nodes were removed from the donors 7 days after the third infection.

Schedule 2. Infected once with *ca.* 3000 third stage larvae 15 days before mesenteric lymph nodes were removed.

Preparation and transfer of lymph node cells

Mesenteric lymph nodes were removed from donors, using sterile technique, and placed in chilled Hanks' solution (pH 7·3).

The intact nodes were dissected free of fat and excess connective tissue and diced with fine scissors. The diced fragments were vigorously drawn up and down into a wide-mouthed pipette and then allowed to settle. The supernatant was then pipetted off and held in an iced flask. This procedure was repeated twice. The cell suspension was then filtered through sterile gauze and centrifuged for 6 minutes at 200 ***g*** in a refrigerator. The supernatant was removed and cells were resuspended in fresh Hanks's solution and again centrifuged. Following removal of the supernatant, washed cells were resuspended in fresh, chilled Hanks's solution and the number of cells per ml of the suspension was estimated using a haemocytometer. The volume of the single-cell suspension was then adjusted so that 1 ml contained 100×10^6 mesenteric lymph node cells. Cell viability was assessed by the capacity of cells to exclude eosin or 2 per cent trypan blue. Cell suspensions were transferred to recipients within 2 hours of killing of donors by intravenous injection into the lateral tail veins.

Estimation of faecal worm-egg counts and worm burdens

Worm-eggs per gram of faeces were estimated using a modification of the McMaster slide counting technique (Gordon and Whitlock, 1939).

Total worm counts were made on bulked intestinal digests and contents. The digestion technique was carried out using a pepsin–hydrochloric acid solution. Incubation proceeded for 8–12 hours at 39°. When digestion was complete, concentrated formalin (36 per cent) was added so that the final concentration of formalin in the digest was 5 per cent. The stomach and distal large intestine were not routinely included in the digests as Haley (1962) found very few, or no parasites in these locations.

Formalized digests were concentrated by removal of the supernatants and the sediment, containing worms, was spread in marked Petri dishes. Prior to counting, the sediment was stained with Lugol's iodine for 5 minutes and then decolourized with sodium thiosulphate.

RESULTS

Effect of the syngeneic transfer of immune cells on relative worm-egg production

Two series of experiments were carried out. Inbred Lewis rats weighing about 200 g

were used in these experiments. In the first series of five experiments the relative worm-egg production (egg production) was estimated by integration of the daily count of eggs per gram of faeces by numerical quadrature for the period 6–10 days of infection (period 1) and for the period subsequent to day 11 (period 2). The rats of both control and cell-recipient groups were each infected with 3000 third stage larvae and the cell-recipients were each injected intravenously with 100×10^6 mesenteric lymph node cells as a single-cell suspension on the day of infection. In this series of experiments the cells were obtained from Lewis donors which were immunized by prior multiple infection with *N. brasiliensis* (schedule 1). These results are shown in Table 1.

As egg production estimated for the two periods are not independent the data obtained were analysed separately. Egg production was logarithmically transformed ($\log_{10}$) for statistical analysis. The appropriate 'analysis of variance' based on a total of 47 degrees of freedom (d.f.) carried out on the transformed period 1 data showed no significant difference between mean egg production of worms in controls and immune-cell recipients. A similar analysis carried out on the period 2 data showed highly significant mean squares

TABLE 1

EFFECT OF IMMUNE MESENTERIC LYMPH NODE CELLS ON EGG PRODUCTION (SERIES 1)

Experiment	No. of animals	Group mean egg* production		Significance of the difference between means of immune-cell recipients and controls for period 2
		Period 1	Period 2	
1. Infection controls	5	78,005	137,035	$t = 2{\cdot}61$ on 8 d.f.; $0{\cdot}02 < P < 0{\cdot}05$
Immune-cell recipients	5	84,590	78,615	
2. Infection controls	6	214,840	56,045	$t = 5{\cdot}51$ on 10 d.f.; $P < 0{\cdot}01$
Immune-cell recipients	6	207,923	5,254	
3. Infection controls	3	176,523	167,157	$t = 2{\cdot}03$ on 4 d.f.; $0{\cdot}1 < P < 0{\cdot}02$
Immune-cell recipients	3	137,490	30,348	
4. Infection controls	5	167,210	188,698	$t = 13{\cdot}90$ on 8 d.f.; $P < 0{\cdot}01$
Immune-cell recipients	5	35,204	9	
5. Infection controls	6	149,632	104,346	$t = 2{\cdot}93$ on 8 d.f.; $0{\cdot}01 < P < 0{\cdot}1$
Immune-cell recipients	4	129,948	22,260	

* See 'Materials and Methods' for estimation of egg production.

(m.s.) for 'between experiments', 'controls *vs* immune-cell recipients' and for 'interaction' between these main effects. Consequently egg production of worms in 'immune-cell recipients' and controls were compared within experiments by simple *t*-tests and these results are included in Table 1. Analysis of the results given in Table 1 show that in the first series of experiments, transfer of mesenteric lymph node cells from syngeneic donors immunized by multiple infection, had a statistically significant effect on egg production in recipients during period 2. No effect was detected during period 1.

In the second series of six experiments egg production was calculated for the first period alone as these animals were killed for worm counts on day 10. In these experiments recipients were injected intravenously with 100×10^6 cells obtained from the mesenteric nodes of donor Lewis rats 15 days after a primary infection with 3000 third stage larvae (schedule 2). Analysis of variance showed a significant interaction between experiments and treatment (controls *vs* recipients). Consequently egg productions of worms in 'immune-cell recipients' and 'controls' were again compared within experiments. These results are shown in Table 2. In addition to the usual 'infection control', groups of animals (normal-

cell recipients) were included in experiments 5 and 6 which were intravenously injected with 100×10^6 mesenteric lymph node cells obtained from uninfected animals. The analysis of these experiments is shown in Table 3.

The analyses summarized in Tables 2 and 3 show that mesenteric lymph node cells

TABLE 2

EFFECT OF IMMUNE MESENTERIC LYMPH NODE CELLS ON EGG PRODUCTION (SERIES 2)

Experiment	No. of animals	Group mean egg production; period 1	Significance of the difference between means of immune-cell recipients and controls
1. Infection controls	4	131,292	$t = 3{\cdot}56$ on 6 d.f.; $P < 0{\cdot}01$
Immune-cell recipients	4	26,386	
2. Infection controls	4	125,621	$t = 1{\cdot}64$ on 6 d.f.; $0{\cdot}1 < P < 0{\cdot}2$
Immune-cell recipients	4	68,572	
3. Infection controls	4	128,058	$t = 3{\cdot}11$ on 6 d.f.; $0{\cdot}02 < P < 0{\cdot}05$
Immune-cell recipients	4	59,947	
4. Infection controls	7	175,104	$t = 3{\cdot}29$ on 12 d.f.; $P < 0{\cdot}01$
Immune-cell recipients	7	93,156	
5. Infection controls	6	141,099	(See Table 3 for analyses)
Normal-cell recipients	6	127,038	
Immune-cell recipients	6	45,035	
6. Infection controls	6	127,904	(See Table 3 for analyses)
Normal-cell recipients	6	124,159	
Immune-cell recipients	6	86,471	

TABLE 3

ANALYSIS OF VARIANCE OF EGG PRODUCTION* OBTAINED IN EXPERIMENTS 5 AND 6, SERIES 2

Source of variance	Degrees of freedom	Sum of squares	Mean square	
EXPERIMENT 5				
I-C R† *vs* N-CR‡+Infection control	1	90,849,492	90,849,492	$P < 0{\cdot}001$
N-CR *vs* Infection control	1	363,661	363,661	N.S.
Groups	2	91,213,153	45,606,577	
Error	15	23,558,009	1,570,534	
Total	17	114,771,162		
EXPERIMENT 6				
I-CR *vs* N-CR+Infection control	1	21,379,835	21,379,835	$0{\cdot}01 < P < 0{\cdot}05$
N-CR *vs* Infection control	1	6,422,571	6,422,571	N.S.
Groups	2	27,802,406	13,901,203	
Error	15	61,303,084	4,086,872	
Total	17	89,105,490		

* Egg production transformed to $\log_{10} \times 10^4$.
† I-CR = immune-cell recipients.
‡ N-CR = normal-cell recipients.

obtained from donors on day 15 of a primary infection with *N. brasiliensis* significantly reduced egg production in recipients during period 1. Mesenteric lymph node cells obtained from normal uninfected donors showed no effect.

EFFECT ON RELATIVE WORM-EGG PRODUCTION OF IMMUNE CELLS TRANSFERRED TO SYNGENEIC RECIPIENTS ON DAYS 4, 10 AND 14 OF INFECTION

Twenty Lewis strain rats (*ca.* 200 g) were randomly allotted to four groups of five animals and each was infected with 3000 third stage larvae. Group 1 (infection control) were given no further treatment. Group 2 animals were injected intravenously with 100×10^6 mesenteric lymph node cells obtained from Lewis donors which were immunized by multiple infection. The immune cells were transferred to the group 2 recipients on day 4 of infection. The animals in group 3 each received 100×10^6 immune cells on day 10 of infection and group 4 were injected with 100×10^6 immune cells on day 14 of infection. Faecal egg counts were carried out daily and egg production estimated for periods 1 and 2. Group mean egg production is shown in Table 4.

Analysis of variance on 19 d.f. of the period 1 data showed no significant difference between mean egg production of worms in cell recipients and controls. A significant difference ($P < 0{\cdot}01$) was obtained for the comparison of the 4-day recipients with infection controls in period 2 by Dunnett's (1955) method. However, egg production in the 10- and 14-day recipients was not significantly different from infection controls (see Table 4).

TABLE 4

EFFECT ON EGG PRODUCTION OF IMMUNE MESENTERIC LYMPH NODE CELLS INJECTED ON DAYS 4, 10 AND 14 OF INFECTION

Group	No. of animals	Group mean egg production		Significance of a difference between period 2 means*
		Period 1	Period 2	
1. Infection controls	5	208,192	177,328	
2. Day 4 recipients	5	193,630	64,200	$P < 0{\cdot}01$
3. Day 10 recipients	5	179,200	121,340	N.S.
4. Day 14 recipients	5	259,940	171,029	N.S.

* Significance of the difference between means of recipient groups and infection-control group estimated by Dunnett's (1955) method following $\log_{10}$ transformation of egg production.

EFFECT OF THE SYNGENEIC TRANSFER OF IMMUNE CELLS ON WORM BURDEN

In the second series of six experiments (see Results, Part 1) worm counts in cell recipients were compared with infection controls on day 10 of infection. Group mean egg productions obtained in these experiments are given in Table 2. Mean worm counts for control infections and immune-cell recipients are given in Table 5, experiments 1–6. The significance of the difference between means of the two groups of animals within experiments 1–4 was calculated by the *t*-test and the values of these are also included in Table 5. The appropriate analysis of variance for experiments 5 and 6 was carried out separately as groups of recipients which were injected with normal mesenteric lymph node cells obtained from uninfected donors were also included as additional controls in these experiments. These analyses are given in Table 6. In addition to the six experiments described above a seventh experiment was carried out using a challenge infection of 1500 third stage larvae. The results of this experiment are also included in Table 5.

The results and analyses given in Tables 5 and 6 show that immune mesenteric lymph node cells caused rejection of a substantial proportion of worms of a challenge infection

in syngeneic recipients by day 10. Lymph node cells obtained from normal uninfected donors did not cause the rejection of worms (see m.s. for N-CR *vs* infection controls, experiments 5 and 6, Table 6).

TABLE 5

EFFECT OF THE SYNGENEIC TRANSFER OF IMMUNE CELLS ON WORM BURDENS ON DAYS 6 AND 10 OF INFECTION

Experiment	No. of animals	Challenge infection no. of larvae	Group mean adult worm count			Significance of a difference between means of immune-cell recipients and infection controls
			Males	Females	Total	
DAY 10 OF INFECTION						
1. Infection controls	4	3000	507	520	1027	$t = 11{\cdot}50$ on 6 d.f.; $P<0{\cdot}01$
Immune-cell recipients	4	3000	8	11	19	
2. Infection controls	4	3000	580	578	1158	$t = 6{\cdot}25$ on 6 d.f.; $P<0{\cdot}01$
Immune-cell recipients	4	3000	88	107	195	
3. Infection controls	4	3000	717	699	1416	$t = 7{\cdot}03$ on 6 d.f.; $P<0{\cdot}01$
Immune-cell recipients	4	3000	113	150	263	
4. Infection controls	7	3000	921	1097	2018	$t = 5{\cdot}99$ on 12 d.f.; $P<0{\cdot}01$
Immune-cell recipients	7	3000	424	478	902	
5. Infection controls	6	3000	739	737	1476	See Table 6
Immune-cell recipients	6	3000	117	98	215	
Normal-cell recipients	6	3000	736	737	1472	
6. Infection controls	6	3000	634	639	1273	See Table 6
Immune-cell recipients	6	3000	196	176	372	
Normal-cell recipients	6	3000	627	624	1251	
7. Infection controls	8	1500	280	290	570	
Immune-cell recipients	8	1500	0	0	0	
DAY 6 OF INFECTION						
Infection controls	8	3000	457	572	1029	$t = 3{\cdot}70$ on 13 d.f.; $P<0{\cdot}01$
Immune-cell recipients	7	3000	86	142	228	

TABLE 6

ANALYSIS OF VARIANCE OF TOTAL WORM COUNTS OBTAINED IN EXPERIMENTS 5 AND 6; SERIES 2

Source of variance	Degrees of freedom	Sum of squares	Mean square	
EXPERIMENT 5				
I-C R* *vs* N-CR† + Infection control	1	6,342,842	6,342,842	$P<0{\cdot}001$
N-CR *vs* Infection control	1	37	37	N.S.
Groups	2	6,342,879	3,171,440	
Error	15	324,001	21,600	
Total	17	6,666,880		
EXPERIMENT 6				
I-CR *vs* N-CR + Infection control	1	3,164,841	3,164,841	$P<0{\cdot}001$
N-CR *vs* Infection control	1	1,587	1,587	N.S.
Groups	2	3,166,428	1,583,214	
Error	15	476,476	31,765	
Total	17	3,642,904		

* I-CR = Immune-cell recipients.
† N-CR = Normal-cell recipients.

An experiment was also carried out to determine whether the worms were rejected by transferred immune cells before day 10 of infection. Accordingly fifteen Lewis strain rats

(*ca.* 200 g) were each infected with 3000 third stage larvae. Seven animals (cell recipients) were each injected intravenously with 100×10^6 mesenteric lymph node cells obtained from Lewis donors on day 15 of a primary infection. The remaining eight animals were used as infection controls. All animals were killed on day 6 of infection for differential worm counts. These results are included in Table 5. A substantial proportion (78 per cent) of worms of the challenge infection were rejected by the immune cells by day 6 of infection.

EFFECT OF THE SYNGENEIC TRANSFER OF IMMUNE CELLS ON THE NUMBER OF EGGS IN THE UTERI OF WORMS

In the second series of six experiments (see 'Results', Part 1), the number of eggs per female worm was estimated in a random sample of usually fifty females, obtained from each rat killed on day 10 of infection. The mean number of eggs per uterus was calculated by dividing the number of eggs counted by the number of females. These mean values of eggs per uterus for infections in each animal were then averaged for controls and immune-cell recipients and this data is given in Table 7. In experiment 1 (Table 5), the average

TABLE 7

EFFECT OF THE SYNGENEIC TRANSFER OF IMMUNE CELLS ON UTERINE EGG COUNT ON DAYS 6 AND 10 OF A CHALLENGE INFECTION

Experiment	No. of eggs per uterus		Significance of difference between means of immune cell recipients and controls
	Infection control	Immune cell recipients	
DAY 10 OF INFECTION			
1	23·0	10·7	$t = 6{\cdot}50$ on 6 d.f.; $P < 0{\cdot}01$
2	21·5	9·6	$t = 9{\cdot}65$ on 6 d.f.; $P < 0{\cdot}01$
3	22·3	8·5	$t = 12{\cdot}61$ on 6 d.f.; $P < 0{\cdot}01$
4	34·1	12·8	$t = 12{\cdot}20$ on 12 d.f.; $P < 0{\cdot}01$
5	25·3	11·0	$t = 8{\cdot}62$ on 10 d.f.; $P < 0{\cdot}01$
6	25·2	9·7	$t = 13{\cdot}22$ on 10 d.f.; $P < 0{\cdot}01$
DAY 6 OF INFECTION			
	28·3	7·0	$t = 13{\cdot}22$ on 10 d.f.; $P < 0{\cdot}01$

number of female worms obtained from each immune-cell recipient was eleven. In this case the average number of eggs per uterus was estimated from the counts obtained in the total of forty-four worms in the immune-cell recipient group.

The results presented in Table 7 show a significant reduction in the number of eggs per uterus per female worm in immune-cell recipients. The number of eggs per uterus was also estimated in gravid females obtained from the infection control and immune-cell recipient animals killed on day 6 of infection. These results, which are also included in Table 7, show that the number of eggs per uterus was significantly reduced in gravid worms in immune-cell recipients (7·0) compared with infection controls (28·3).

DISCUSSION

The results of the present studies show that mesenteric lymph node cells obtained from actively immunized donors invariably caused suppression of relative egg production of

worms (egg production), reduction in the number of eggs per uterus in gravid females and rejection of at least a substantial proportion of parasites by days 6 and 10 of infection. The magnitude and the reproducibility of the effects of syngeneically transferred immune lymphoid cells on the parasite were greater than those recorded by Ogilvie and Jones (1968) for passive serum immunization in their rat–*N. brasiliensis* system. In addition, the results of the present studies contrast sharply with those of Ogilvie and Jones (1968) who reported only limited success, and Kassai and Szepes (1970) who were unsuccessful when they attempted to adoptively immunize in this host–parasite system.

In the first series of five experiments on the effect of immune-cell transfer on egg production, there was generally little or no effect on egg production during period 1 (6–10 days of infection), although some reduction was observed in experiment 4 (Table 1). This period is the plateau phase described by Jarrett, Jarrett and Urquhart (1968). However, over all five experiments during period 2 (the period subsequent to day 10) the mean for 'infection controls' was 130,656 whereas it was 27,297 in 'immune-cell recipients'. Period 2 combines log phase 2 and the threshold phase described by Jarrett *et al.* (1968). The actively acquired immune response of the host usually becomes effective during this second period so that the effect observed in 'immune-cell recipients' was due to the effect of adoptive immunization superimposed upon actively acquired immunity. However, it is reasonable to assess the effect of 'immune-cells' on egg production during this second period by comparison of cell recipients with controls, as it is expected that actively acquired immunity would also be operational in the control groups.

In the second series of six experiments, egg production was estimated in control and recipient animals during period 1 (Table 2). The mean egg production in 'infection controls' over all experiments was 138,180 and this was 63,261 for 'immune-cell' recipients. From these means and from the data recorded in Table 2 it can be seen that immune mesenteric lymph node cells can affect egg production during the 6–10 day period of infection and before the actively acquired response of the recipient host becomes effective.

It is particularly interesting to note that in the first series of experiments there was little or no effect of immune cells on egg production during period 1 while egg production was markedly reduced during this same period in cell recipients of the second series of experiments. Throughout the course of our studies we noted great variation in the magnitude of effects between experiments and for this reason statistical analysis was usually confined to group comparisons within experiments. However, despite the considerable variation in magnitude of effects between experiments egg production was always reduced in cell recipients.

The variation in the magnitude of effects may be due to the different batches of infective larvae and immune cells used in the experiments. However, we believe that the striking difference in the effect of immune cells on egg production during period 1 in the two series of experiments was due to the different schedules used to immunize cell donors. In the first series of experiments the donors were immunized by multiple infection and in the second series immune cells were prepared from mesenteric nodes of donors killed only 15 days after a primary infection (see 'Materials and Methods' for schedules). The cells obtained after single-infection immunization appear to be more effective than those obtained after multiple-infection immunization. Studies are under way to confirm this observation as it may have important implications for the understanding of the role of the immune response in control of parasite populations in natural host–parasite relationships.

In experiments 5 and 6, series 2, additional groups of normal-cell recipients were in-

cluded to show that mesenteric lymph node cells obtained from normal uninfected donors had no effect on egg production (see m.s., for N-CR *vs* infection control, experiments 5 and 6, Table 3). In addition to this control, cells were transferred allogeneically from immune Lewis donors to DA strain recipients and vice versa. Immune cells were not effective in either allogeneic system. The failure to transfer resistance allogeneically served as a useful control, as it precluded the possibility that either antigenic material or preformed antibodies contained within the lymph nodes of the immune donors, acted as mediators of the immune response. The results of this study, which are not reported in detail in this communication, emphasize that cell transfer is only effective in a syngeneic system as reported above, and this finding is consistent with the results obtained by Dineen, Wagland and Ronai (1968) in the *Trichostrongylus colubriformis*–guinea-pig system. It is likely, therefore, that survival of the transferred cells is necessary for the transfer of immunity.

Dineen and Wagland (1966) showed that immune mesenteric lymph node cells affected *T. colubriformis* in the guinea-pig when injected before day 10 of infection though not later than this time. They concluded that adult worms were not susceptible to an immune response mounted by the transferred cells. In the present studies immune mesenteric lymph node cells were injected on days 4, 10 and 14 of infection in recipients. Mean egg production for periods 1 and 2 of 4, 10 and 14 day recipients are compared with 'infection controls' in Table 4. In this study the 'immune-cells' were obtained from multiple-infection donors and again little or no effect on egg production was noted during period 1. However, the period 2 egg production showed a significant effect of immune-cells injected on day 4, whereas egg production in the day 10 and 14 recipients was not significantly different from controls. Again it is evident that the parasite is less susceptible to an attack mounted by the transferred cells during the later stages of infection.

The effect of immune mesenteric lymph node cells on worm burdens was determined in the second series of experiments in which the animals were killed for worm counts on day 10 of challenge infection. The effect of immune cells on worm burden was even more striking than the effect on egg production (see Table 5). The mean number of worms counted in immune-cell recipients over all seven experiments was 281 and for 'infection controls' this was 1277. Again, as was the case with egg production, normal cells were not effective (see m.s. N-CR *vs* infection control, experiments 5 and 6, Table 6). Although counts of adult worms alone are recorded in Table 5, larval forms were also differentiated during worm counting but the numbers present were so small that they are not recorded.

In a subsequent experiment cell-recipient and control animals were killed on day 6 of infection for worm counts and for uterine worm-egg counts to determine whether the transferred cells affected the parasite at this early stage of infection. The results of this study showed that the immune cells caused rejection of 78 per cent of the adult worm burden when compared with controls (mean worm count in immune-cell recipients was 228 and this count in controls was 1029, see Table 5).

An attempt was made to calculate the number of eggs produced per gravid female by estimating the total egg output on the day the animals were killed for worm counts (days 6 and 10). Because of the time required for passage of faecal material through the intestines and because worms were being rapidly rejected during the 6–10 day period, more females would have contributed to faecal egg output than those recorded in the subsequent worm counts. As an alternative, an estimate was made of the number of eggs per uterus per female. These results are shown in Table 7. There was a highly significant two- to four-fold reduction in eggs per uterus of females from immune-cell recipients compared with

'infection controls'. We assume that this demonstrates a suppressive effect of immune-cells on egg production per female although it is recognized that we are making a deduction concerning a dynamic function from a static morphological observation.

It is interesting to note that the immune response initiated or mounted by the transferred cells was already effective during the early stage of patency of the parasite (day 6 of infection). Currently studies are under way to determine whether transferred cells cause rejection or arrested development of the parasite during larval stages and whether the cells are effective only at the gut level of the host or whether the parasite is also affected during tissue migratory phases.

ACKNOWLEDGMENTS

We wish to thank Peter Maxwell and John Pearce for their excellent technical assistance. One of us (J.D.K.) was the holder of the George Aitken Pastoral Research Scholarship while this work was undertaken.

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