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The Cellular Transfer of Immunity to *Trichostrongylus colubriformis* in an Isogenic Strain of Guinea-Pig

IV. THE LOCALIZATION OF IMMUNE LYMPHOCYTES IN SMALL INTESTINE IN INFECTED AND NON-INFECTED GUINEA-PIGS

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Summary. Immune and non-immune mesenteric lymph node and Peyer's patch cells were labelled *in vitro* with ^{51}Cr and injected intravenously into infected and non-infected syngeneic guinea-pigs (Heston strain). From the distribution of the radioactive label in the small intestine (the site of infection of *T. colubriformis*) it was concluded that immune lymphocytes: (1) preferentially localize or 'home in' on the infection, (2) come into intimate contact with the parasite in the epithelium, and (3) rapidly undergo 'allergic death' or lysis at the site of infection.

INTRODUCTION

Previous studies have shown that resistance to infection with *Trichostrongylus colubriformis* in the guinea-pig is mediated by lymphoid cells (Wagland and Dineen, 1965; Dineen and Wagland, 1966). In this respect there is a close analogy with transplantation immunity and hypersensitivity reactions of the delayed type. Investigation of the behaviour of immune lymphocytes in this host–parasite system may therefore provide fundamental information on cellular immunity as well as information on the mechanisms of resistance to helminths.

Dineen, Ronai and Wagland (1968) showed that immune allogeneic cells fail to reject the parasite under conditions in which syngeneic cells are functional within 48 hours of injection into infected recipients. They also showed that by comparison with syngeneic transfer only 53 per cent of immune ^{51}Cr-labelled lymph node cells are distributed at the site of infection in allogeneic transfer and that the difference in distribution was statistically significant as early as 6 hours after intravenous injection of the cells. From these observations it was concluded that the failure in function of allogeneic cells might be due to: (a) a rapidly acquired homograft response, (b) 'allogeneic inhibition' as envisaged by Hellström and Möller (1965), or (c) antigenic diversion of immune lymphocytes by the histocompatible antigens of the foreign host; perhaps by a mechanism operating *in vivo* which is similar to the *in vitro* proliferation and de-differentiation of lymphocytes in response to foreign antigens as described by Bain, Vas and Lowenstein (1963).

In this communication results are reported of studies on the distribution of ^{51}Cr-labelled immune and non-immune mesenteric lymph node cells at the site of infection in

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small intestine in infected and non-infected animals. The functional capacity and localization of immune Peyer's patch cells has also been studied. The work has been carried out using only syngeneic recipients to determine the capacity of immune lymphocytes to 'home in' on the target infection without the possible intervention of the factors referred to above.

MATERIALS AND METHODS

Experimental animals

Male and female guinea-pigs 3–4 months of age were used. Both donors of immune lymphocytes and recipients were of the highly inbred Heston strain which are maintained by line mating.

Parasitological techniques

The preparation and administration of infective *T. colubriformis* larvae have been described elsewhere (Wagland and Dineen, 1965; Dineen and Wagland, 1966).

Preparation of lymphocytes for transfer

Guinea-pigs which were donors of immune lymphocytes received a course of four infections each with 1000 larvae given over a 4-week period and were killed 7–10 days after the last dose. The preparation of single cell suspensions from immune and normal mesenteric lymph nodes has been described by Dineen and Wagland (1966). Cells were released from Peyer's patches by careful dissection.

In vitro *labelling of cells with* ^{51}Cr

The method of labelling lymphocytes *in vitro* has been described by Dineen *et al.* (1967). Recipients were injected via the marginal ear vein with 0·5 ml of a suspension containing $20–50 \times 10^6$ ^{51}Cr-labelled cells.

RESULTS

THE LOCALIZATION OF IMMUNE SYNGENEIC LYMPH NODE CELLS IN SMALL INTESTINE OF INFECTED AND NON-INFECTED GUINEA-PIGS

Ten Heston guinea-pigs were each infected with 1000 *T. colubriformis* larvae on day 0 of the experiment. On day 6, the infected animals, and an additional ten non-infected guinea-pigs were each injected intravenously with 0·5 ml of a single cell suspension of immune mesenteric lymph node cells labelled *in vitro* with ^{51}Cr. At 1, 6, 16, 24 and 48 hours after injection, two infected and two non-infected animals were slaughtered, and the small intestines removed in 6 in. segments from duodenum to ileum. Radioactivity in the segments was counted using a Packard Autogamma Spectrometer. This basic experiment was repeated four times. The number of cells injected into both infected and non-infected animals was the same within each experiment but ranged from 20 to 50×10^6 cells between experiments.

Counts of radioactivity were also carried out on samples of the cell dose used in each experiment and results were recorded as the percentage of the appropriate cell-dose count obtained for 12 in. of small intestine. The means obtained by pooling results for infected

and non-infected groups within each slaughter period are shown in Fig. 1 and the analysis of variance of the data is given in Table 1.

The analysis shows a highly significant mean square (m.s.) for 'experiments'. This

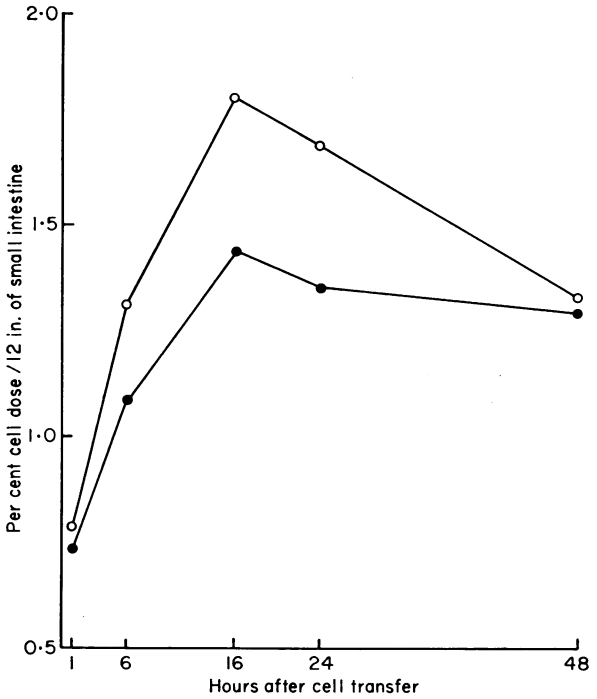

FIG. 1. Distribution of ^{51}Cr-labelled immune cells in infected and non-infected small intestine at 1, 6, 16, 24 and 48 hours after transfer. ○, Infected small intestine; ●, non-infected small intestine.

TABLE 1

ANALYSIS OF VARIANCE ON PERCENTAGE OF CELL DOSE LOCALIZED PER 12 in. OF SMALL INTESTINE AT 1, 6, 16, 24 AND 48 HOURS AFTER INJECTION OF CELLS

Source of variation	Degrees of freedom	Mean square	Significance
Infected *vs.* non-infected	1	0·4575	$0·001 < P < 0·01$
Periods after transfer	4	1·0119	$P < 0·001$
Infection × periods interaction	4	0·0438	
Treatments	9	0·5200	$P < 0·001$
Experiments	3	1·4513	$P < 0·001$
Treatments × experiments interaction	27	0·0545	
Animals within treatments	40	0·0672	
Error	67	0·0621	
Total	79		

main effect is expected as different cell doses were used in each experiment. The non-significant interaction 'treatments × experiments' indicates that the different dosage levels of cells used in the experiments behaved in the same way. It is reasonable, therefore, to

pool the results of all experiments to compare the localization of cells in infected and non-infected animals since each experiment was complete in itself with equal numbers of infected and non-infected animals injected at each dose level and slaughtered at each time interval.

It is apparent from Fig. 1 that more cells are localized in infected small intestine at 6, 16 and 24 hours than in the non-infected tissues. This finding is confirmed by the analysis given in Table 1 which shows a m.s. of 0·4575 for 'infected *vs.* non-infected' which is significant at the 1 per cent level. Fig. 1 also shows that the maximum number of cells localized in infected small intestine occurred at 16 hours after injection of the cells, when the level was 25 per cent above that recorded for non-infected tissues. By 48 hours after injection the amount of radioactivity, and therefore the number of cells localized in infected and non-infected tissues, was the same. Testing for the significance of the difference between means for infected and non-infected groups within sample periods again shows

TABLE 2

SIGNIFICANCE OF THE DIFFERENCE BETWEEN MEANS OF INFECTED AND NON-INFECTED GROUPS WITHIN SLAUGHTER PERIODS

Period after cell transfer (hours)	'*t*' on 67* degrees of freedom	Significance
1	0·48	Not significant
6	1·85	$0·05 < P < 0·10$
16	2·89	$0·001 < P < 0·01$
24	2·49	$0·01 < P < 0·02$
48	0·40	Not significant

* Estimate of error (0·1246) obtained from analysis given in Table 1.

that the differences are highly significant at 16 and 24 hours. The difference obtained at 6 hours approaches significance at the 5 per cent level. The differences obtained at 1 and 48 hours are not significant.

THE LOCALIZATION OF NON-IMMUNE SYNGENEIC LYMPH NODE CELLS IN SMALL INTESTINE OF INFECTED AND NON-INFECTED GUINEA-PIGS

The design of this experiment was similar to that used in the previous section. Thus, twelve Heston guinea-pigs were each infected with 1000 *T. colubriformis* larvae on day 0 of the experiment. On day 6 the infected animals and an additional twelve non-infected guinea-pigs were each injected intravenously with 31×10^6 non-immune mesenteric lymph node cells which were labelled *in vitro* with ^{51}Cr. At 1, 18 and 48 hours after injection of the cells, four infected and four non-infected animals were killed and the small intestines removed in 6-in. segments from duodenum to ileum. Radioactivity in the segments was counted in the Gamma Spectrometer. Counts of radioactivity were carried out on samples of the cell dose used in the experiment and results were again recorded as the percentage of cell dose per 12 in. of small intestine. The results obtained for infected animals were $0·335 \pm 0·022$, $0·555 \pm 0·054$ and $0·530 \pm 0·056$ per cent at 1, 18 and 48 hours, respectively. The corresponding results for non-infected guinea-pigs were $0·26 \pm 0·054$, $0·567 \pm 0·039$ and $0·549 \pm 0·010$ per cent. Clearly there is no significant difference in the localization of non-immune cells in the intestines of infected and non-infected animals.

THE FUNCTIONAL ACTIVITY OF IMMUNE PEYER'S PATCH CELLS

Before consideration was given to the localization of immune Peyer's patch cells in the small intestine of infected and non-infected animals it was necessary to show that these cells are active against the parasite.

Twelve Heston guinea-pigs were each infected with 5000 *T. colubriformis* larvae on day 0 of the experiment. Six animals were then each injected intravenously with 60×10^6 immune Peyer's patch cells on day 6. The remaining six guinea-pigs did not receive cells but were used as controls on infectivity and development of the challenge infections.

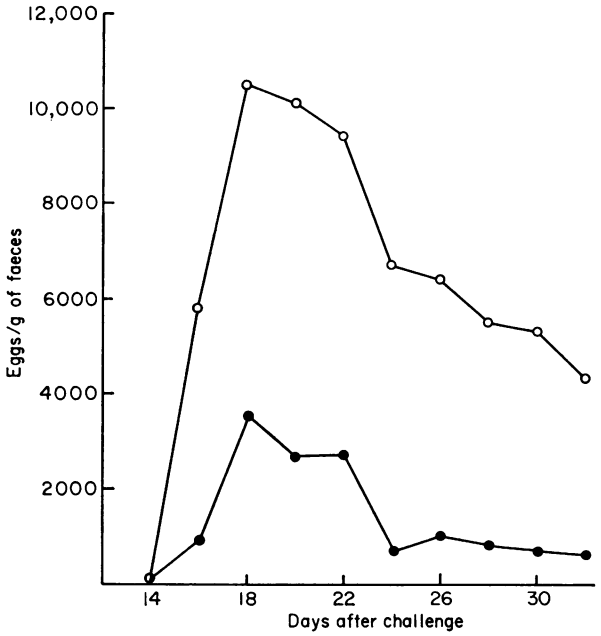

FIG. 2. The effect of adoptive immunization with Peyer's patch cells on challenge with 5000 *T. colubriformis* larvae. ●, Adoptively immunized; ○, control group.

Faecal worm egg counts were carried out at frequent intervals on both groups during patency. Group mean egg counts are shown in Fig. 2.

Fig. 2 shows that the degree of infection was greatly reduced by the intravenous injection of immune Peyer's patch cells.

THE DISTRIBUTION OF IMMUNE PEYER'S PATCH CELLS BETWEEN PEYER'S PATCHES AND THE TISSUES OF THE SMALL INTESTINE OTHER THAN PEYER'S PATCHES FOLLOWING TRANSFER TO INFECTED AND NON-INFECTED RECIPIENTS

During the course of the preceding studies it was noted that radioactivity was increased in some segments of small intestine by comparison with adjacent segments, and that this increased activity was correlated with the presence of Peyer's patches. It was relevant, therefore, to determine whether immune lymphocytes were preferentially attracted to Peyer's patches or to tissues of the small intestine other than Peyer's patches in infected animals.

Twelve Heston guinea-pigs were each infected with 5000 *T. colubriformis* larvae so that on the day of cell transfer, four had been infected for 3 days, four for 6 days and four for 8 days. On the appropriate day each animal received 14×10^6 immune Peyer's patch cells intravenously. An additional control group of six non-infected guinea-pigs were each injected with 14×10^6 immune Peyer's patch cells at the same time. All animals were

TABLE 3

DISTRIBUTION OF IMMUNE PEYER'S PATCH CELLS BETWEEN PEYER'S PATCHES AND OTHER TISSUES OF THE SMALL INTESTINE IN NON-INFECTED ANIMALS AND ANIMALS INFECTED FOR 3, 6 AND 8 DAYS

	No. of cells localized per 1×10^6 injected cells			
	Non-infected group	Groups infected for		
		3 days	6 days	8 days
Tissues other than Peyer's patches	25,900	33,100	30,800	34,800
Peyer's patches	11,700	12,500	10,200	8,200
Total	37,600	45,600	41,000	43,000

TABLE 4

ANALYSIS OF VARIANCE OF THE DISTRIBUTION OF IMMUNE PEYER'S PATCH CELLS BETWEEN PEYER'S PATCHES AND OTHER TISSUES OF THE SMALL INTESTINE IN NON-INFECTED ANIMALS AND ANIMALS INFECTED FOR 3, 6 AND 8 DAYS

Source of variation	Degrees of freedom	Mean square	Significance
(1) *Tissues of the small intestine other than Peyer's patches*			
Infected *vs.* non-infected	1	19,834*	$0{\cdot}001 < P < 0{\cdot}01$
Between infections (3, 6 and 8 days)	2	1,676	N.S.
Treatments	3	7,728	$0{\cdot}001 < P < 0{\cdot}01$
Remainder (error)	14	1,191	
Total	17		
(2) *Peyer's patches*			
Infected *vs.* non-infected	1	850	N.S.
Between infections (3, 6 and 8 days)	2	1,843	$0{\cdot}10 < P < 0{\cdot}20$
Treatments	3	1,512	$0{\cdot}10 < P < 0{\cdot}20$
Remainder (error)	14	744	
Total	17		

N.S., Not significant.

* Estimates from the original data $\times 10^{-2}$.

slaughtered 20 hours after injection and 3-in. segments of small intestine from duodenum to ileum were removed for counts of radioactivity. In addition, the presence or absence of Peyer's patches in each segment was observed macroscopically and recorded. The γ-counts of samples of the cell dose were also determined.

To determine the distribution of cells between Peyer's patches and in other tissues of the small intestine the mean count for segments of small intestine in which patches were not observed was first calculated. This count was then subtracted from the count for each

segment in which a Peyer's patch was observed to obtain an estimate of the count for the Peyer's patch in that segment. To obtain the total count for other tissues the mean count for Peyer's patch negative segments was multiplied by the number of segments (eighteen to twenty) in the total length of small intestine. A total γ-count for all Peyer's patches observed in each small intestine was obtained by summation (six to eight Peyer's patches were usually observed in each small intestine). The counts for Peyer's patches and other tissues of the small intestine were related to the radioactivity of the cell dose, and results were recorded as the number of cells localized in the tissues per million cells injected. Means for the infected and non-infected groups of animals are given in Table 3 and the appropriate analysis of the results is shown in Table 4.

It is apparent from the results shown in Table 3 that the increased localization of immune Peyer's patch cells in small intestine is due to their distribution in other tissues. This finding is confirmed by the analysis given in Table 4 which shows m.s. (19,834) for 'infected *vs.* non-infected' which is significant at the 1 per cent level. The corresponding m.s. (850) for Peyer's patches in infected and non-infected animals is not significant. It is interesting to note, however, that the m.s. (1843) for counts in Peyer's patches 'between infections' (3, 6 and 8 days) approaches significance at the 10 per cent level. While this level of significance can in no way be considered conclusive, it is suggestive that the age of infection may have some effect upon Peyer's patches and this is also indicated by the group means given in Table 3 which show a progressive reduction in cell count, i.e. 12,500 for 3-day, 10,200 for 6-day and 8200 for 8-day infections.

DISCUSSION

The results presented in this communication show that ^{51}Cr-labelled immune lymphoid cells preferentially accumulate in infected small intestine by comparison with the small intestine of non-infected animals. Preferential accumulation in infected small intestine was not observed when non-immune cells were used. It is reasonable to assume, therefore, that either immune lymphoid cells 'home in' on the target, in this case the helminth *T. colubriformis*, or that these cells reach the site of infection by random processes but are then retained so that they do not return to the circulation as rapidly as non-immune cells. It is likely that the magnitude of this phenomenon is much greater than the results presented suggest. It is unlikely that cells obtained from lymphoid tissues of immune donors are exclusively immune or functionally competent cells. The preferential localization of immune cells must, therefore, be masked to some extent by the non-immune and non-functional cells in preparations from 'immune' nodes.

Comparison of the localization of immune mesenteric lymph node cells in infected and non-infected small intestine (see Fig. 1) showed that: (1) a difference approaching significance at the 5 per cent level between group means occurred as early as 6 hours after cell transfer; (2) the maximum difference between means occurred about 16–24 hours; and (3) there was no significant difference by 48 hours after cell transfer (see Table 2). The return of the level of immune cells in infected small intestine to the level recorded for immune cells in non-infected small intestine at about 24 hours after the occurrence of the maximum difference indicates that the competent effector cells are rapidly eliminated on contact with the infection. This suggests that the cells are rapidly lysed or undergo 'allergic death' on intimate contact presumably with the antigens of the parasite. If this interpretation is correct then the label released on cell lysis can not be taken up by other

cells in the tissues and must be rapidly removed from the site of infection. McCall, Sutherland, Eisentraut and Lanz (1955) showed that ^{51}Cr released from mechanically disrupted labelled leucocytes would not label either red cells or other leucocytes, while Ronai (1967) showed that lysis of ^{51}Cr-labelled mouse peritoneal leucocytes was followed by rapid removal of label from the site of lysis.

Gowans and McGregor (1965) discussed the mechanism of destruction of skin homografts by adoptively transferred immune lymphocytes. They suggest that the active cells migrate into the grafts and exert some cytocidal effect, but they also pointed out that attempts to demonstrate significant numbers of transferred cells in the homografts by autoradiographic techniques have failed. Prendergast (1964) and more recently Hall (1967) concluded that at the time of rejection, cells originating in local nodes do reach skin homografts in significant numbers but that they are not specifically attracted to the site of the homograft. The failure to observe an increase of structurally intact lymphocytes in the graft beyond that expected as a result of purely random distribution has been explained by the view that either a 'homing' phenomenon does not occur or that the specifically attracted cells are rapidly lysed following contact with antigen. Our results are consistent with the latter view.

In the studies presented in the current series of papers we are concerned with the behaviour of lymphocytes in relationship to their function as prime instigators and mediators of resistance to infection with the parasite. As it was desirable to investigate the behaviour of Peyer's patch cells it was necessary to demonstrate the capacity of these cells to cause rejection of the parasite. The results shown in Fig. 2 demonstrate this point.

During the course of experiments on the localization of mesenteric node cells in small intestine it became apparent that the cell count was increased in segments of tissue which contained Peyer's patches. This finding is to be expected, since Mitchison (1955) has previously drawn attention to the 'homing instinct' of lymphocytes for lymphoid organs. Although, in addition, infection may cause hyperplasia in local primary lymphoid nodules these phenomena in no way invalidate the conclusions drawn above since interpretation rested upon the localization of non-immune cells as well as immune cells in both infected and non-infected tissues. There was no increase in the localization of non-immune lymph node cells in infected small intestine by comparison with non-infected tissues.

Because the cell count increased in segments containing Peyer's patches it was of interest to compare the distribution of immune lymphoid cells between Peyer's patches and the tissues of the small intestine other than Peyer's patches in both infected and non-infected animals. In this study immune Peyer's patch cells labelled with ^{51}Cr were used. The group means given in Table 3 show that in infected animals more cells per million injected are localized in other tissues of the small intestine when compared with non-infected animals. This finding is confirmed by the analysis shown in Table 4. Contrary to expectation this difference was not detected in Peyer's patches. Indeed there is some indication from the results that as the infection aged, the number of cells localized in Peyer's patches may decrease.

As the localization of immune lymphoid cells is increased in the small intestine of infected animals, and these cells are distributed in tissues other than Peyer's patches it is likely that the immune cells come into intimate contact with the infection. This conclusion gains considerable support from the observation that when the mucosal epithelium of infected small intestine was treated with dilute hydrochloric acid, the radioactivity count was decreased by 60 per cent and this was correlated with the removal of about 50 per cent

of the epithelium in stained sections of the treated tissues. The results given in Table 3 show that about 30 per cent of radioactivity in small intestine is localized in large Peyer's patches. Therefore, the above finding shows that the remaining activity is almost wholly distributed in epithelial layers.

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