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The *in-vivo* kinetics of lymphoblast localization in the small intestine

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Summary. We have studied the *in-vivo* kinetics of the accumulation of ^{125}I-UdR labelled mesenteric lymphoblasts in the small intestine of mice. The efficiency with which the labelled cells were extracted from the blood and retained by the intestine was quantified by examination of the accumulations observed over the first 4 hr after cell transfer. The kinetic parameters for the uptake and retention of lymphoblasts determined from these early times were found to provide a good approximation to the entire time course of accumulation observed from 1 hr to 22 hr after cell transfer. For normal mice, approximately 1% of lymphoblasts delivered by the blood stream at any given time gained entry to the small intestine and were retained with an average half-time of 6·5 hr. We also studied the accumulation of lymphoblasts in the small intestine of mice undergoing a self-limited enteric infection with the nematode, *Trichinella spiralis*. There was a greater accumulation of lymphoblasts in the small intestine of these animals. This was the consequence of a prolongation of the half-time for retention of lymphoblasts within the intestine to 15 hr, rather than increased uptake of lymphoblasts from the blood. During a secondary infection with *T. spiralis*, the half-time for retention of lymphoblasts in the intestine was decreased to 3 hr. These studies show that viewing the accumulation of lymphoblasts as the result of a series of first order kinetic processes provides a suitable model for the migration of lymphoblasts to the small intestine.

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INTRODUCTION

The migration of lymphoblasts from the blood to the intestine is an important physiological phenomenon. Lymphoblasts activated in the gastrointestinal lymphoid tissues continuously repopulate the gut mucosa with effector cells reactive to gut derived antigens. These cells travel through abdominal lymphatics and the thoracic duct to return to the gut via the blood stream. The ability of labelled lymphoblasts from thoracic duct (Gowans & Knight, 1964; Hall, Parry & Smith, 1972), mesenteric lymph nodes (Guy-Grand, Griscelli & Vassalli, 1974; Parrott & Ferguson, 1974; McWilliams, Phillips-Quagliata & Lamm, 1975) and intestinal lymph (Hall, Hopkins & Orlans, 1977) to migrate to the lamina propria of the intestine after intravenous transfer has been clearly established, but the mechanisms controlling this migration remain unclear.

In this paper we examine the kinetics of lymphoblast accumulation in the small intestine of mice. Our purpose was to test the applicability of a recently developed kinetic model of the migration behaviour of lymphoid cells (Ottaway & Parrott, 1981a) to observations of the intestinal localization of labelled mesen-

teric lymph-node lymphoblasts under physiological and pathological conditions. The strategy chosen was to examine concurrently the kinetics of lymphoblast localization in the intestine of normal mice and mice in which lymphoblast accumulation in the intestine was perturbed during an experimental enteritis produced by infection with the nematode, *Trichinella spiralis*. The results show that a model in which the uptake and retention of lymphoblasts by the intestine are the independent physiological variables provides a suitable and consistent framework for interpreting lymphoblast localization. The experimentally derived values for the uptake and retention of lymphoblasts by the normal and perturbed intestine, suggest that alterations in the accumulation of lymphoblasts is controlled by changes in their retention rather than by alterations in the recruitment of lymphoblasts from the blood stream.

MATERIALS AND METHODS

Mice

Seven- to nine-week-old female mice of the inbred NIH strain maintained in the Department of Bacteriology and Immunology were used.

T. Spiralis *infections*

Infective larvae were recovered from the muscles of previously infected NIH mice by digestion *in vitro* and enteric infection was established by administration of 400 viable larvae by oral intubation as previously described (Ottaway *et al.*, 1980).

Assessment of cardiac output distribution

The distribution of the cardiac output was measured using ^{86}RbCl as previously described (Ottaway & Parrott, 1979). The proportion of the injected ^{86}RbCl activity found in the small intestine 45 sec after injection was taken as the fraction of the cardiac output received by that organ.

Donor lymphoblast suspensions

Mesenteric lymph-node (MLN) cells from donor animals were prepared at room temperature by teasing of the nodes in RPMI 1640 medium (Gibco-Biocult Ltd.). Cell suspensions were filtered through 2 cm of glass wool to remove debris and the number of viable cells determined by their ability to exclude 0·2% eosin. All cell donors in these experiments were infected with *T. spiralis* 4 days before use. This caused mesenteric node hyperplasia and provided greater numbers of cells for transfer, and has been shown not to affect the intestinal localization of lymphoblasts in recipients (Rose, Parrott & Bruce, 1976a).

Cells undergoing DNA synthesis were labelled by incubating MLN suspensions with (^{125}I)-5-iodo-2-deoxyuridine (^{125}I-UdR; Radiochemical Centre, Amersham) as previously described (Ottaway *et al.*, 1980). After labelling, the cells (^{125}I-UdR-MLN) were washed three times with medium and injection doses of 2×10^7 viable cells were given through a lateral tail vein in a 0·4 ml volume. Multiple samples of injection doses were retained to determine the administered radioactivity. The *in-vivo* distribution of labelled lymphoblasts was expressed as a percentage of the injected dose of cells found in the small intestine or per ml of blood.

Treatment of recipients

Uninfected (i.e. naive) mice and mice infected with *T. spiralis* at various times prior to study were examined. Challenge (i.e. secondary) infections were carried out at least 15 days after primary infections by readministration of infective larvae. At intervals after cell transfer (1–24 hr) mice were killed by cervical dislocation and the abdomen immediately opened. The aorta and vena cava were cut and a measured volume of blood collected. The small intestine was removed, gently washed in saline and Peyer's patches were excised prior to counting.

Analysis of experimental data

Results were expressed as the mean ± standard deviation for groups of animals and compared by Student's *t* test.

The time course of accumulation of lymphoblasts in the small intestine was analysed using the observed values for regional blood flow and the lymphoblast localization found over the first 4 hr after cell transfer. When labelled cells are delivered to an organ by the blood stream and the uptake and loss of cells by the tissue are assumed to obey first order kinetics, the time course of labelled cells in the organ can be described by

$$P_i(t) = k_i f_i CO\, e^{-\lambda_i t} \int_0^t P_b(t)\, e^{\lambda_i t} dt + P_i(O)\, e^{-\lambda_i t} \quad \text{(I)}$$

where $P_i(t)$ is the percent of the injected dose of labelled cells in the tissue i at a particular time, k_i is the proportion of cells delivered by the blood that is taken up by the tissue, f_i is the fraction of the cardiac output,

CO, of the animal which goes to the tissue, λ_i is the proportion of the labelled cells in the tissue which is lost per unit time, $P_b(t)$ is the concentration of labelled cells in the blood, and $P_i(O)$ is the percent of the injected dose of cells in the tissue at the start of the time period under consideration (Ottaway & Parrott, 1981a; Ottaway, 1982). The inference of equation I is that the percent of the injected dose of cells found in the tissue at any time is the result of the entry of cells from the blood minus the loss of cells from the tissue integrated over time. To describe the entry and loss of cells as first order kinetic processes, the rate of entry of cells into the tissue has been taken as a constant proportion (k_i) of those delivered by the blood at any time, $f_i CO\ P_b(t)$, and the rate of loss of cells from the tissue has been taken to be a constant proportion (λ_i) of those present in the tissue, i.e. $\lambda_i P_i(t)$.

For a time period when the concentration of labelled cells in the blood is constant, $P_b(t) = B$, the accumulation observed in the small intestine 2 hr and 4 hr after cell transfer (P_2, P_4) can be written as

$$P_2 = \frac{k f CO\ B}{(-lnx)}(1-x) + P_1 x \qquad \text{(II)}$$

$$P_4 = \frac{k f CO\ B}{(-lnx)}(1-x^3) + P_1 x^3 \qquad \text{(III)}$$

where P_1 is the accumulation observed at 1 hr and $ln\ x$ has been substituted for $-\lambda$, so that $x = e^{-\lambda}$ and $x^3 = e^{-3\lambda}$.

Rearrangement of equation II gives

$$k = \frac{(P_2 - P_1 x)(-lnx)}{(1-x) f CO\ B} \qquad \text{(IV)}$$

Replacing k in equation III with this expression gives a quadratic equation in x which has the roots

$$x = \frac{-1 \pm \sqrt{1 - 4\dfrac{(P_2 - P_4)}{(P_2 - P_1)}}}{2} \qquad \text{(V)}$$

from which a value for λ can be obtained from the experimental observations. The uptake coefficient k can then be determined using equation IV.

The expected accumulation for the entire time course of the experiments was calculated with equation I by starting with the lymphoblast accumulation observed at 1 hr, using the derived values for the kinetic parameters, and the observed concentration of lymphoblasts in the blood throughout the experiment. The standard deviations of the derived kinetic parameters were calculated using the multivariate error analysis methods described by Clifford (1973).

RESULTS

Preliminary experiments

Initial experiments were carried out to examine the localization of lymphoblasts and regional blood flow in the small intestine during challenge infection with *T. spiralis*. We have previously shown that there is a relationship between lymphoblast accumulation and the delivery of blood-borne lymphoblasts within the small intestine (Ottaway & Parrott, 1980) and that induced alterations in blood flow can be associated with corresponding changes in the localization of lymphoblasts (Ottaway & Parrott, 1981b). Primary enteric infection with *T. spiralis* lasts for approximately 12 days in NIH strain mice before expulsion of the intestinal worm burden occurs (Manson-Smith *et al.*, 1979; Ottaway *et al.*, 1980). In mice undergoing primary *T. spiralis* infection, the greatest change in the 24-hr localization of mesenteric lymphoblasts occurs within 2 days of initiation of the infection (Rose, Parrott & Bruce, 1976b; Ottaway *et al.*, 1980).

When previously infected mice were challenged 15 days after the initial infection, expulsion of parasites from the gut was rapid and very few worms were recoverable from the small intestine by 2 days after the challenge. In contrast to the effect of primary infection, no significant alteration in the 24-hr accumulation of mesenteric lymphoblasts was observed in the small intestine of animals at different stages of the challenge (Table 1). This was so in spite of the

Table 1. The 24-hr localization of ^{125}I-UdR-MLN in the small intestine of mice undergoing secondary infection with *T. spiralis**

Stage of infection	% Injected dose in small intestine
Naive	4·1 ± 0·2
1° (15 days)	3·8 ± 0·4
2° + 12 hr	4·2 ± 0·9
2° + 24 hr	3·9 ± 0·6
2° + 48 hr	3·5 ± 0·6
2° + 96 hr	3·9 ± 0·2

* The results are the means ± SD for four animals per group.

Secondary infection was carried out on the 15th day of a previous infection at the times shown before cell transfer.

observation that a greater proportion of the cardiac output went to the small intestine of challenged animals (Table 2).

Table 2. Distribution of cardiac output (%*CO*) to the small intestine in infected and challenged mice*

Stage of infection	% *CO* to small intestine
Naive	17·2±1·5
1° (2 days)	17·4±2·0
1° (15 days)	17·2±2·4
2°+12 hr	18·6±3·1
2°+24 hr	19·6±4·2
2°+36 hr	21·3±4·5
2°+48 hr	22·6±2·7†

* Results are the means±SD for five to six animals per group. Challenge was carried out on the 15th day of a previous infection at the times shown before the assessment of ^{86}RbCl distribution.
† $P<0·01$ significantly different from that value in animals subjected to a 15-day primary infection only.

Kinetics of lymphoblast localization

To exploit this difference in the migration of lymphoblasts during the primary and secondary infection, we compared the time course of lymphoblast accumulation in naive mice, mice undergoing a primary infection initiated 2 days before cell transfer and mice that were given a challenge with *T. spiralis* 1 day before cell transfer on the 15th day of a previous infection (Table 3). More lymphoblasts were found in the small intestine of mice undergoing primary infection compared with naive mice throughout the time course. In contrast, in mice with the challenge infection, the proportion of the injected lymphoblasts in the small intestine was similar to that in naive animals for the first 4 hr, but with a more rapid fall thereafter. By 22 hr, there was no difference between the localization observed in the naive and challenge infected mice, but the animals undergoing the primary infection showed marked enhancement of lymphoblast accumulation in the small intestine.

The relative contribution of the delivery of lymphoblasts by the blood, the extraction of the cells from the blood stream and their retention by the small

Table 3. Kinetics of lymphoblast localization in uninfected and infected small intestine*

Tissue	Recipients	Time after injection of cells: 1 hr	2 hr	3 hr	4 hr	6 hr	10 hr	22 hr
Small intestine (% injected dose)	Naive	8·12 (0·85)	9·08 (0·71)	10·41 (0·60)	10·72 (1·26)	11·74 (0·86)	9·24 (0·29)	5·53 (0·84)
	Primary	10·00† (0·31)	11·20† (0·91)	11·71 (0·83)	13·44‡ (0·90)	14·59† (1·77)	14·28§ (0·28)	10·47‡ (1·24)
	Secondary	8·12 (1·31)	8·88 (0·89)	9·38 (0·70)	9·97 (0·72)	8·46† (0·74)	6·42† (0·71)	4·86 (0·64)
Blood (% injected dose/ml)	Naive	2·07 (0·32)	1·94 (0·52)	2·24 (0·51)	1·78 (0·45)	1·07 (0·57)	0·47 (0·09)	0·31 (0·25)
	Primary	2·28 (0·37)	2·16 (0·29)	1·97 (0·27)	1·75 (0·30)	0·80 (0·21)	0·46 (0·07)	0·20 (0·05)
	Secondary	2·30 (0·28)	2·08 (0·26)	2·26 (0·09)	1·62 (0·72)	0·75 (0·20)	0·36 (0·07)	0·23 (0·06)

* Results are the means (±SD) for four mice per group at each time. Infections were carried out either 2 days before cell transfer for the primary infection, or 1 day before cell transfer, on the 15th day of a previous infection, for the secondary.
† $P<0·05$ significantly different from that value in the naive group of animals.
‡ $P<0·01$.
§ $P<0·001$.

intestine, to the accumulation of lymphoblasts was assessed by examining the kinetics of the accumulation over the first 4 hr of the experiment. During that time, there was no significant change in the concentration of lymphoblast label available in the blood of the uninfected or infected animals (Table 3). Although the proportion of injected cells which was present in the blood decreased between 4 hr and 22 hr in each of the groups studied, there was no significant difference in the concentration of blood-borne label present in the infected or naive groups at any of the times examined after the cell transfer (Table 3). For the analysis of the 1-, 2- and 4-hr observations of lymphoblast localization in the intestine (see 'Materials and Methods'), the concentration of lymphoblasts available in the blood was taken as the mean value observed for all the animals throughout the 4-hr period (2·05% injected dose/ml) and the cardiac output of the mice was taken as 7·5 ml/min (Ottaway & Parrott, 1981a). The fraction of the cardiac output delivered to the small intestine was taken from Table 2 as 0·172 for naive animals, 0·174 for the primary infection and 0·196 for the challenged animals.

The results of the analysis (Table 4) gave values for the uptake of lymphoblasts by the small intestine of both the primary and challenge infections which were the same as that for naive animals. There was a large difference, however, in the values obtained for the loss or retention of labelled lymphoblasts by the intestine in the three groups of animals.

Expected and observed lymphoblast localization

To assess the stability of this kinetic interpretation of the observations, the time course for lymphoblast accumulation that would be expected from the derived values for entry and retention of the cells was calculated (Fig. 1). For the small intestine of naive animals and those undergoing primary infection a good approximation to the entire time course of lymphoblast localization resulted when the values derived for the kinetic parameters were taken to apply throughout the experiment (Fig. 1). For the challenged small intestine, the calculated time course was in good agreement with the experimental data up to 10 hr, but underestimated the observed localization at 22 hr.

Table 4. Kinetic parameters obtained from the analysis of ^{125}I-UdR-MLN localization in the small intestine*

Status of infection	k	$t_{\frac{1}{2}}$(hr)	λ(hr^{-1})
Naive	0·012±0·002	6·5±0·9	0·106±0·014
Primary	0·011±0·001	15·1±5·6	0·046±0·017
Secondary	0·015±0·005	3·1±1·8	0·226±0·129

* Results are the means±SD (see analysis methods): k = the proportion of delivered cells which enter the tissue; λ = the proportion of labelled cells which leave the intestine per hour; $t_{\frac{1}{2}}$ = the half-time for the lymphoblasts in the intestine calculated from $t_{\frac{1}{2}} = \ln 2/\lambda$.

DISCUSSION

The purpose of this study was to examine the applicability of a simple model of the kinetics of lymphoid cell migration to observations of lymphoblast localization within the intestine over the first 24 hr after cell transfer. A means for successfully interpreting the *in-vivo* migration of cells could offer a way of resolving and evaluating the contribution of different mechanisms to the migration of cells under normal and experimentally manipulated conditions.

The values obtained here for the entry and retention of lymphoblasts in the small intestine were derived from the observations over the first 4 hr after cell transfer. Nevertheless, these estimates appear applicable to the entire experiment. For naive small intestine and for the small intestine of animals undergoing primary *T. spiralis* infection, the values obtained for the kinetic parameters k and λ provide a description of the whole time course which is consistent with the experimental observations (Fig. 1). In the small intestine of animals undergoing challenge with *T. spiralis*, a consistent interpretation of the observed accumulation is also obtained, except at 22 hr when the observed localization was greater than that predicted by the parameters obtained from the early data (Fig. 1). This discrepancy may reflect the increasing regional blood flow delivered to the challenged small intestine during the time of the experiment (Table 2) which was not accounted for in the calculations. Alternatively, there may be selective retention of a subpopulation of the injected lymphoblasts by the challenged gut.

The principal criteria by which the suitability of a model for an experimental system can be judged are its logical plausibility, its consistency with the experimental data and its ability to account rationally for perturbations of the system (Hertz, 1900). The kinetic model used here is based upon physiological considerations of the distribution of blood flow throughout the

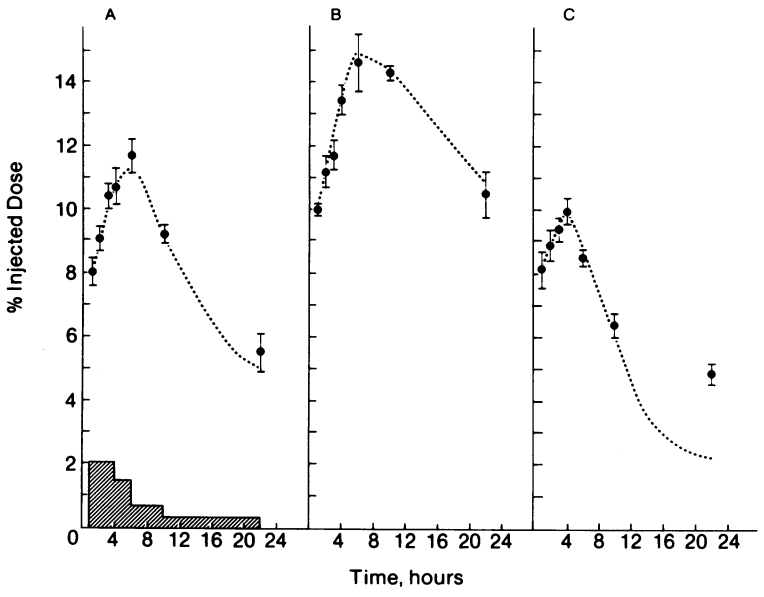

Figure 1. Time course of lymphoblast localization in the small intestine, ($\bar{x} \pm$SEM). (A) Naive mice. (B) Primary infection initiated 2 days before cell transfer. (C) Secondary infection 1 day before cell transfer on the 15th day of a previous infection. The expected accumulation (- - - -) from 1 hr to 22 hr was calculated from equation 1 using the values for k and λ obtained from the analysis. The concentration of lymphoblasts (*hatched area*) available in the blood during the experiment was approximated by the mean value for all animals for each interval.

body and the assumption, based on experimental observations, that the entry of cells into tissues and the retention of cells by a tissue are governed by simple first order kinetic processes (Ottaway & Parrott, 1981a). We have previously found that the use of the model provides a consistent interpretation of the localization of a variety of lymphoid-cell populations in both lymphoid and non-lymphoid tissues of the mouse (Ottaway & Parrott, 1981a; Ottaway, 1982). Those experiments were limited to short time periods after cell transfer during which the changes in cell accumulation were essentially monotonic in character. The present results show that the kinetic parameters derived for the localization of lymphoblasts in the small intestine provide a consistent interpretation of experimental data over prolonged periods and for observations which display distinct maxima. Thus, these experiments provide a rigorous test of the consistency of the model.

The values for the uptake of transferred lymphoblasts into the small intestine are about an order of magnitude lower than that which has been found for the entry of lymphoid cells into mesenteric node and Peyer's patch lymphoid tissue (Ottaway & Parrott, 1981a; Ottaway, 1982). Contrary to our expectation (Ottaway *et al.*, 1980), there was no difference in the uptake of lymphoblasts into the small intestine of naive, infected or challenged animals (Table 4). The retention of lymphoblasts in the bowel, however, was markedly altered in the infected animals. The normal turnover time of lymphoblasts in the intestine (6·5 hr) increased to approximately 15 hr in the primary infection, but was decreased to about 3 hr in the challenge. Alteration in the way in which arriving lymphoblasts are retained by the mucosa appears to be a major control mechanism in the perturbations induced by *T. spiralis*.

The alterations in retention of the transferred lymphoblasts presumably reflect changes in the interaction of lymphoblasts with other elements of the mucosa. It has previously been shown that the localization of lymphoblasts from subcutaneous lymph nodes responding to contact sensitizers is also enhanced in the small intestine during primary *T. spiralis* infection (Manson-Smith *et al.*, 1979; Rose *et al.*, 1976b). Increased retention of any available lymphoblast populations may reflect alterations in the activity of macrophages or dendritic cells within the

mucosa disrupted by the enteritis. On the other hand, the decreased retention of lymphoblasts during the early time course in the challenged intestine may result from the release of cell products of mucosal mast cells. Pronounced mast-cell hyperplasia develops in the intestine of *T. spiralis*-infected NIH strain mice by day 8 and persists through to day 15 of the primary infection (Brown *et al.*, 1981), and these cells may discharge their cytoplasmic granules in the face of challenge with the nematode at the times used in our experiments.

The precise means by which cellular mechanisms or cell products may influence the retention of lymphoblasts are not yet known, but could arise through regulation of the exit of cells from the intestine or changes in the death of lymphoblasts *in situ* after arrival in the mucosa. Cells can leave the intestine to enter either the lumen or the lymphatics. Lymphoid cells have been demonstrated in the lumen of the gut in *Giardia muris* infections (Owen, Nemanic & Stearns, 1979) and *T. spiralis* infections in mice (Bruce & Parrott, unpublished observations), but the significance of this route of exit to the economy of lymphoblasts in the bowel has so far been difficult to quantify. Although some lymphoblasts may recirculate via the lymph after entering the intestine (Howard, 1972; Smith, Martin & Ford, 1980), the majority of them probably do not do so. Lymphoblasts are, however, quite fragile cells (Parrott & Wilkinson, 1981) and their life-span after entry to a tissue may offer a limiting factor through which the effector-cell population represented in the intestine can be regulated. Mechanisms for modulating the retention of admitted lymphoblasts combined with antigen driven proliferation of some of the retained cells (Husband & Gowans, 1978) may allow the effector-cell population of the mucosa to be adjusted rapidly in response to pathological processes in the gut.

This study shows that the process of lymphoblast migration to an organ such as the small intestine is amenable to characterization by a simple kinetic model of the accumulation of migrating cells. The model as used is a phenomenological one. Specific assumptions regarding the nature of the affinity of lymphoblasts for other cells or the mechanistic details of migration have been deliberately avoided. The kinetic model provides a good accounting of the phenomenon of lymphoblast migration, however, and quantitatively resolves the contribution of delivery, entry and retention processes to that migration. The potential contribution of specific signals, triggers, targets or receptors to these processes may be amenable to *in-vivo* experimental evaluation, through the elaboration of secondary models defining the dependence of the kinetic parameters on specified cell–cell or cell–ligand interactions.

For example, mesenteric lymphoblasts accumulate in the small intestine more readily than lymphoblasts generated in other tissues such as subcutaneous lymph nodes. This specificity is likely the result of selective mechanisms controlling the interaction of these cell populations with signals within the mucosa after their arrival, as we have found that there is no significant difference in the efficiency with which different lymphoblast or other lymphoid-cell populations are admitted to the small intestine from the blood (Ottaway & Parrott, 1981a; Ottaway, 1982). Mesenteric lymphoblasts, however, are much more likely to be retained within the mucosa than are lymphoblasts from subcutaneous nodes or Peyer's patches (Ottaway, 1982). Kinetic studies aimed at quantifying the effect of experimental manipulations on the retention of lymphoblasts within the intestine could help to elucidate the significance of putative receptors on different cell populations.

We conclude that time-course experiments are a useful adjunct to cell-migration studies. The requirement for being able to carry out analysis of such experiments is to have observations of cell accumulation in a tissue during a time period when the concentration of labelled cells in the blood is constant, along with a concomitant estimate of the blood flow to the tissue under study.

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