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Distribution of lung-associated lymphocytes from the caudal mediastinal lymph node: effect of antigen

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SUMMARY

Lymphocytes from the efferent lymph of the caudal mediastinal lymph node (CMLN) were labelled *in vitro* with ^{125}I-iododeoxyuridine [^{125}I]UdR and $Na_2{}^{51}CrO_4$. The labelled cells were re-infused i.v. and their distribution in organs/tissues was determined 20–24 hr later. As indicated by tissue ^{125}I-activity, pulmonary lymphoblasts had a marked tendency to relocate in the lung, regional pulmonary lymph nodes and spleen. Localization of efferent CMLN lymphoblasts was greater in antigenically stimulated segments compared to unstimulated segments of the lung. Dual antigen experiments indicated that the increased localization was not specific for the antigen which stimulated production of lymphoblasts used for *in vitro* labelling and reinfusion. Intranodal labelling of blasts by the direct injection of [^{125}I]UdR supported the results obtained from *in vitro* labelling. In these studies, comparisons were made with the localization of lymphocytes obtained from thoracic duct lymph.

INTRODUCTION

There is ample evidence suggesting that the expression of pulmonary immunity is dependent, at least in part, upon the extravasation into lung parenchyma of immunoreactive lymphocytes which have entered the blood from regional pulmonary lymph nodes and/or systemic lymphoid tissues such as the spleen (Bice *et al.*, 1980a; Bice, Harris & Muggenberg, 1980b; Kaltreider *et al.*, 1983).

In this regard, the development of pulmonary immunity resembles the development of intestinal immunity. Although numerous studies have documented the selective relocalization in the gut of intestinal lymphoblasts (Gowans & Knight, 1964; reviewed by Bienenstock & Befus, 1980), there is limited data available describing the distribution of efferent lymph-borne pulmonary immunoblasts.

It has been shown that intrabronchial immunization of the lower lung in sheep stimulates a marked increase in the output of lymphoblasts and specific antibody-forming cells in the efferent lymph of the caudal mediastinal lymph node (CMLN) (Joel, Chanana & Chandra, 1980). When efferent CMLN lymphoblasts are labelled *in vitro* and reinfused i.v. they preferentially reappear in pulmonary lymph and show a marked tendency to extravasate in lung parenchyma (Spencer & Hall, 1984; Joel & Chanana, 1985). Similarly, lymphoblasts obtained from minced intrathoracic lymph nodes of rats have a predilection for localizing in the lung (Spencer, Gyure & Hall, 1983; McDermott & Bienenstock, 1979).

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We have recently demonstrated that certain well-defined subsegments of the sheep lung are drained exclusively by a single lymph node (Chanana & Joel, 1985). The left and right dorsal basal segments drain via the CMLN, the right cranial segment drains via the cranial bronchial lymph node (CrBLN), and the left cranial segment drains via the caudal bronchial lymph node (CdBLN). In the current study, this functional division of the lung was used to define further the influence of antigenic stimulation on the pulmonary distribution of immunoblasts and other lymphocytes emerging in the efferent lymph of the CMLN. The results suggest that immunoblast localization is increased in immunized lung segments; however, it is independent of specific antigen.

MATERIAL AND METHODS

Animals and cannulation procedures

Dorset-cross sheep 6–12 months of age and weighing 25–50 kg were used in this study. The animals were judged healthy and free of pulmonary disease based on routine physical examinations, blood cell counts, and bronchoscopy, including total and differential counts of cells obtained by lavage. During the week prior to surgery the sheep were conditioned to restraining chutes which permitted the animal to stand or lie down as well as limited free movement.

Under general anaesthesia, the efferent lymph duct of the CMLN was cannulated by a modification (Chanana, Chandra & Joel, 1981) of the procedure described by Staub *et al.* (1975). Using the same surgical approach, the thoracic duct (TD) was ligated and cannulated just posterior to the entry of CMLN lymph duct. Aseptic techniques were followed carefully and

Table 1. Radioactivity* in organs/tissues 20–24 hr following the i.v. infusion of labelled† lymphocytes obtained from either the efferent duct of the CMLN or the TD

	^{125}I activity		^{51}Cr activity	
Organ/tissue	CMLN ($n=5$)	TD ($n=4$)	CMLN	TD
Lung	125±44‡	39±26	275±131	208±62
Intestine	67±45‡	290±145	15±9‡	67±42
Spleen	337±253	154±106	1224±488	878±103
CMLN	393±225‡	137±72	1472±860	1661±1089
BLN	423±263‡	109±57	1518±968	1420±866
MLN	134±43‡	401±205	1007±1055	1958±559
PLN	218±228	139±74	1034±933	1365±387

* Radioactivity is expressed as the mean (±SD) c.p.m. per gram tissue and 'normalized' to an injection dose of 10^6 c.p.m. of each isotope.

† Lymphocytes were labelled *in vitro* with both [^{125}I]UdR and Na_2 $^{51}CrO_4$ and reinfused i.v. An average of $2{\cdot}9\ (\pm 1{\cdot}3)\times 10^9$ TD lymphocytes containing $1{\cdot}7\ (\pm 1{\cdot}4)\times 10^6$ c.p.m. ^{125}I and $4{\cdot}1\ (\pm 4{\cdot}8)\times 10^6$ c.p.m. ^{51}Cr were reinfused while an average of $2{\cdot}3\ (\pm 1{\cdot}8)\times 10^9$ efferent CMLN lymphocytes containing $1{\cdot}3\ (\pm 1{\cdot}0)\times 10^6$ c.p.m. ^{125}I and $4{\cdot}7\ (\pm 2{\cdot}4)\times 10^6$ c.p.m. ^{51}Cr were infused.

‡ The values obtained following the infusion of CMLN lymphocytes are significantly different ($P<0{\cdot}05$) from those obtained following the infusion of TD lymphocytes by the Students' *t*-test.

antibiotics were administered routinely for several days post-surgically. Cannulas were composed of a silastic T-tube with a teflon tip. Within 2 cm of the tip, heparin was infused at the rate of 130 U/hr to prevent clotting. An indwelling silastic catheter was placed in the external jugular vein and lymph circulation was maintained in a closed sterile system.

Antigenic stimulation

In order to increase the output of lymph-borne immunoblasts, the CMLN was stimulated by the intrabronchial instillation of antigen into the dorsal basal segment of the right lung. This has been shown to result in an increase in efferent lymphoblasts 4–5 days later (Joel *et al.*, 1980). In experiments designed to study the influence of antigen on the tissue distribution of lymphocytes, a second 'paired' antigen was instilled into the right cranial lung segment which is drained exclusively by the CrBLN. The antigen pairs used were 2×10^9 heat-killed *Escherichia coli* and 2×10^9 heat-killed *Staphylococcus aureus* or *Salmonella minnesota*. Antigens were carefully deposited into the specific segmental bronchi using a fiberoptic bronchoscope as previously described (Joel *et al.*, 1980).

In vitro *labelling of lymphocytes*

Lymph was collected in sterile, siliconized glass bottles kept at 4°. Lymphocytes ($0{\cdot}5$–5×10^9) were concentrated to 10^8 cells/ml in phosphate-buffered saline (PBS) to which 0·5 μCi ^{125}I-iododeoxyuridine ([^{125}I]UdR; New England Nuclear, Boston, MA; specific activity >2,000 Ci mM) and 2–10 μCi $Na_2{}^{51}CrO_4$ (New England Nuclear; specific activity 350–400 mCi/mg) per ml suspension were added. Following 45 min of incubation at 37°, cells were washed once and resuspended in 200 ml of either cell-free autologous lymph or PBS. Aliquots for assessment of cell viability and measurement of radioactivity were removed and the labelled cell suspensions were slowly infused i.v. over a period of 15–20 min.

In vivo *labelling of lymphoblasts*

In an effort to avoid the hazards of *in vitro* labelling, including cell injury and possible lung entrapment, lymphoblasts were labelled *in situ* by the direct intranodal injection of [^{125}I]UdR. Either the CMLN or a mesenteric lymph node (MLN) was exposed and 1 ml PBS containing 20 μCi [^{125}I]UdR was injected via four or five separate punctures using a 30-gauge needle. Injection was done over a period of 15–20 min, during which time 25 mg thymidine in 250 ml saline were infused i.v. to minimize systemic labelling.

Organ/tissue distribution of radioactivity

Twenty to twenty-four hours after the infusion of *in vitro* labelled lymphocytes or 1, 2 and 7 days after intranodal injection of [^{125}I]UdR, sheep were anaesthetized, exsanguinated, and the vascular system 'flushed' with 6–8 litres of saline via carotid–artery, jugular–vein cannulas. Organs were weighed and several representative samples from each organ were taken for analysis. Samples were weighed and radioactivity was determined with a well-type gamma scintillation counter (Searle Analytic Inc., Des Plaines, IL). Activity was expressed as c.p.m./gm tissue, which is referred to as specific activity.

RESULTS

The percentage of injected radioactivity recovered following the infusion of labelled lymphocytes was $20{\cdot}1\pm 10{\cdot}8$ (1 SD) for ^{125}I and $32{\cdot}8\pm 9{\cdot}8$ for ^{51}Cr ($n=9$) with no significant differences in recovery being observed between labelled CMLN lymphocytes and labelled TD lymphocytes. The distribution of radioactivity

Table 2. Radioactivity* in immunized and unimmunized segments of the lung 20–24 hr following the i.v. infusion of labelled †efferent lymphocytes from the caudal mediastinal lymph node

	^{125}I activity		^{51}Cr activity	
Sheep	Immunized segments	Unimmunized segments	Immunized segments	Unimmunized segments
165	134‡	64¶	3608	4328
	141§	77**	3631	4628
106	131	107	2663	2883
	214	108	2821	3294
181	238	238	513	517
	300	248	565	628
159	807	234	940	459
	1257	334	1643	766

* Radioactivity is expressed as c.p.m./gram of lung (mean of two to four samples per lung segment).

† Four to five days after immunization lymphocytes were collected, labelled *in vitro* with [^{125}I]UdR and $Na_2{}^{51}CrO_4$ and reinfused i.v.

‡ Right dorsal basal segment (immunized with the specific antigen).

§ Right cranial segment (immunized with the 'paired' antigen).

¶ Left dorsal basal segment (control).

** Left cranial segment (control).

Table 3. ^{125}I activity* of lymph nodes draining immunized and unimmunized segments of the lung 20–24 hr following the i.v. infusion of labelled †efferent lymphocytes from the caudal mediastinal lymph node

	^{125}I activity				
Sheep	CMLN	CrBLN	CdBLN	MLN	PLN
165	238	395	300	99	131
106	879	974	901	162	292
181	974	794	943	110	198
159	1890	2974	2001	188	661

* Radioactivity is expressed as c.p.m./gram lymph node (mean of two samples per lymph node).

† Four to five days after immunization lymphocytes were collected, labelled *in vitro* with [^{125}I]UdR and $Na_2{}^{51}CrO_4$ and reinfused i.v.

in organs/tissues 20–24 hr following the infusion of labelled cells was independent of the percentage of radioactivity recovered, but was clearly dependent upon the source of lymphocytes used for labelling, particularly for ^{125}I (Table 1). Following the infusion of labelled TD lymphocytes, the ^{125}I-specific activity was considerably higher in the intestine than in lung. In contrast, there was a greater tendency for ^{125}I-labelled efferent CMLN lymphoblasts to relocate in the lung as compared to the intestine. Splenic radioiodine levels were consistently higher following the infusion of CMLN lymphocytes compared to TD lymphocytes, but this difference was not statistically significant. There was also a clear pattern of ^{125}I activity in various lymph nodes. Infusion of labelled TD lymphoblasts resulted in the highest specific activity in the MLN, while infusion of labelled CMLN lymphoblasts resulted in the highest activities in pulmonary lymph nodes. It is of interest to note that although labelled efferent CMLN lymphoblasts were reinfused, relocation of these cells was equally as great in the CrBLN and CdBLN as in the CMLN.

Compared to radioiodine, the tissue distribution of ^{51}Cr was less dependent upon the original source of labelled lymphocytes (Table 1). The only significant difference in specific activity was seen in the intestine, which had very low levels of ^{51}Cr activity in both groups of animals. In all animals ^{51}Cr activity was higher in the lung than in the intestine. As expected, the highest levels of ^{51}Cr were found in lymph nodes and spleen.

Effect of antigen on the distribution of labelled lymphocytes

In the above studies it was observed that samples of lung taken from the site of intrabronchial immunization (right dorsal basal segment) had consistently higher ^{125}I activity than samples taken from unimmunized lung segments. The following experiment was done in an attempt to answer the question of whether the increase in activity in the immunized lung segment was the result of a non-specific inflammatory reaction or was related to the specific antigen used to stimulate the increase in lymphoblast output in efferent CMLN lymph. As described in the Materials and Methods, the right dorsal basal lung segment was immunized with one of a pair of antigens, and the right cranial

Table 4. Radioactivity* in immunized and unimmunized segments of the lung 20–24 hr following the i.v. infusion of labelled† TD lymphocytes

	^{125}I activity		^{51}Cr activity	
Sheep	Immunized segments	Unimmunized segments	Immunized segments	Unimmunized segments
380	43‡	27¶	687	554
	33§	27**	465	550
397	33	27	120	125
	40	22	140	93
393	20	29	391	992
	23	29	643	809
442††	120	49	1110	638
	100	45	762	574

* Radioactivity is expressed as c.p.m./gram of lung (mean of two to four samples per lung segment).

† Four to five days after immunization lymphocytes were collected, labelled *in vitro* with [^{125}I]UdR and $Na_2{}^{51}CrO_4$ and reinfused i.v.

‡ Right dorsal basal segment.

§ Right cranial segment.

¶ Left dorsal basal segment.

** Left cranial segment.

†† In sheep no. 442, the TD was cannulated in the neck region and therefore lymph contained pulmonary lymphocytes entering the TD via the efferent duct of the CMLN.

Table 5. Radioactivity of lung segments following the labelling of lymphocytes by the injection of [^{125}I]UdR directly into either the CMLN or the MLN (see the Materials and Methods)

			^{125}I activity per gram of lung			
			Immunized Segments		Unimmunized Segments	
Sheep	Lymph node labelled	Time post-labelling	RDB	RC	LDB	LC
166	CMLN	1 day	924	1106	138	183
132	CMLN	1 day	2960	997	433	470
129	CMLN	2 days	1571	1788	222	249
131	CMLN	7 days	247	318	91	96
218	MLN	2 days	40	45	73	43
219	MLN	2 days	43	19	209	34

RDB, right dorsal basal segment.
RC, right cranial segment.
LDB, left dorsal basal segment.
LC, left cranial segment.

segment immunized with the second antigen. Between Days 4 and 5 post-immunization, efferent CMLN lymphocytes were collected, labelled *in vitro*, and reinfused i.v. The animals were killed 20–24 hr later and samples of the right dorsal basal (specific antigen), the right cranial (non-specific antigen), the left dorsal basal (control), and the left cranial (control) lung segments were analysed. The results are shown in Table 2. In all eight paired segments, the specific activity of ^{125}I was higher in the immunized lung segment than in the unimmunized lung segment (in sheep no. 181 the right cranial and left cranial segments had equal activity).

Although the labelled, reinfused immunoblasts were from the CMLN and therefore primarily in response to stimulation by the specific antigen deposited in the right dorsal basal segment, the specific activity of the right dorsal basal segment was not significantly different from the non-specifically stimulated right cranial segment (Table 2).

^{51}Cr activity was not significantly different in immunized and unimmunized segments. In fact, in three out of four animals, unimmunized segments had slightly higher ^{51}Cr than did immunized segments.

As shown in Table 3, the ^{125}I activity of lymph node draining stimulated lung segments (CMLN and CrBLN) was essentially the same as the lymph node draining the unstimulated lung segment (CdBLN). All pulmonary lymph nodes had substantially more radioactivity per gram than either MLN or prescapular/prefemoral lymph nodes (PLN).

To examine whether increased ^{125}I activity in immunized lung segments was dependent upon the source of lymphoblasts, a similar study was done with labelled TD lymphocytes. In three sheep the TD was cannulated posterior to the entrance of the efferent duct of the CMLN. In a fourth sheep, the TD was cannulated in the neck region, and thus lymph contained pulmonary lymphoblasts from the CMLN and the CdBNL as well as intestinal lymphoblasts. The results of this study are shown in Table 4. Of the six paired lung segments from the first three sheep, specific activities were low and there was no evidence that immunized segments had a higher level of ^{125}I activity than control segments. On the other hand, in the fourth sheep where TD lymph also contained pulmonary lymphoblasts, both immunized segments had ^{125}I specific activities greater than the corresponding control segments. There were no consistent differences between immunized and non-immunized lung segments with respect to ^{51}Cr activity.

In vivo labelling of pulmonary lymphoblasts

In an attempt to study immunoblast tissue distribution more physiolocially, pulmonary lymphoblasts were labelled *in situ* by the injection of [^{125}I]UdR directly into the CMLN. Four sheep were immunized with paired antigens 4 days prior to intranodal labelling. Two sheep were killed on Day 1, one sheep on Day 2, and one sheep on Day 7 post-labelling. As shown in Table 5, at all time periods, the specific activity of immunized lung segments was consistently higher than unimmunized segments. Although the specific activity of the right dorsal basal segment (antigen specific) was higher than the right cranial segment (non-specific paired antigen) in sheep no. 132, this difference was not evident in the other three animals. The specific activity of bronchial lymph nodes was, in general, more than twice that of either MLN or PLN (data not shown) indicating that labelled lymphoblasts from the CMLN had a much greater tendency to relocate in other lung-associated lymph nodes.

In two sheep the MLN was injected with [^{125}I]UdR 4 days after paired intrabronchial antigen immunization. Both animals were killed 2 days after labelling. Although the overall levels of radioactivity in these two animals were comparatively low, there was no evidence of increased specific activity in immunized lung segments compared to non-immunized segments. As would be expected, the highest levels of radioactivity in these animals were found in the intestine and MLN.

DISCUSSION

In studies utilizing *in vitro* labelling techniques, it was assumed that tissue ^{125}I activity was associated with migrant lymphoblasts (immunoblasts) which had incorporated [^{125}I]UdR during DNA synthesis, while ^{51}Cr activity was associated with a

broader population of lymphocytes including small, non-dividing lymphocytes.

It has been shown in both rats (Gowans & Knight, 1964) and sheep (Hall, Hopkins & Orlans, 1977) that lymphoblasts emerging in intestine efferent lymph are destined to extravasate primarily in the small intestine. More recently, Spencer & Hall (1984) reported that lymphoblasts in pulmonary lymph efferent to the CMLN have a marked tendency to relocate in the lung and spleen with only a small fraction relocating in the intestine. Our results are basically in agreement with these findings, although we found a somewhat greater tendency for pulmonary immunoblasts to relocate in the intestine than was reported by Spencer & Hall (1984). There was also a clear difference between pulmonary and TD lymphoblasts with regard to their relocation in lymph nodes. When efferent CMLN lymphoblasts were labelled and reinfused, [^{125}I] activity was significantly higher in lung-associated lymph nodes than in either MLN or PLN. Although the lymphoblasts used for labelling originated primarily from the antigenically stimulated CMLN, they relocated to an equal degree in other non-stimulated bronchial lymph nodes. When TD lymphoblasts were reinfused, specific activity of the MLN was about three times higher than either lung-associated or PLN. ^{51}Cr activity per gram tissue was, as expected, highest in lymph nodes and spleen. Following the infusion of ^{51}Cr-labelled efferent CMLN lymphocytes, radiochromium levels were consistently higher in pulmonary lymph nodes than in either the MLN or PLN. Similarly, ^{51}Cr-labelled TD lymphocytes tended to relocate to a greater degree in the MLN than in the CMLN or PLN.

This differential accumulation of radioactivity in lymph nodes could result from either an increased rate of entry or the selective retention of labelled lymphocytes in the lymph node. Retention of labelled lymphocytes in a lymph node would result in a decreased output of labelled cells in efferent lymph, which is not consistent with published results. Spencer & Hall (1984) found that when lymphocytes in efferent lymph of the CMLN were labelled with ^{51}Cr or rhodamine and reinfused i.v. they reappeared in efferent CMLN lymph to a relatively greater degree than in efferent intestinal lymph. The reverse was also demonstrated. It was observed further that efferent CMLN lymphocytes recirculated with equal facility through the CMLN and the prefemoral lymph node. We reported recently similar findings for ^{125}I-labelled pulmonary lymphoblasts (Joel & Chanana, 1985). Efferent CMLN lymphoblasts produced in response to intrabronchial antigen, labelled and reinfused i.v., reappeared with a higher specific activity in efferent CMLN lymph than in TD lymph. The reverse was observed with labelled TD lymphoblasts. In our studies the differences with respect to ^{51}Cr-labelled lymphocytes were not as evident as those reported by Spencer & Hall (1984). This may be due to the fact that TD lymph, as opposed to intestinal lymph, contains a large fraction of recirculating lymphocytes from peripheral lymph nodes.

Following intrabronchial deposition of antigen, specific immunoreactive lymphocytes can be demonstrated in lung parenchyma and lavage cell suspensions (Bice *et al.*, 1980b; Kaltreider *et al.*, 1983; Bice & Schnizlein, 1980); however, the source of these cells remains to be fully clarified. It has been shown in dogs that the antigenic stimulation of a single lung lobe results in a significantly higher number of specific antibody-forming cells in the immunized lobe as compared to control lobes and that the likely source of these cells was the blood (Bice *et al.*, 1980a). We have shown that the deposition of antigen in the right dorsal basal segment of the sheep lung results in a marked increase in the output of lymphoblasts and specific antibody-forming cells in the efferent lymph of the CMLN (Joel *et al.*, 1980). When efferent CMLN lymphoblasts were labelled and reinfused i.v., these cells had a tendency to relocate in lung parenchyma, pulmonary lymph nodes and spleen. Further, it was consistently found that the immunized dorsal basal lung segment contained a greater amount of radioiodine per gram tissue than unimmunized lung segments.

We utilized the natural 'compartmentalization' of the sheep lung to address the question of whether the apparent increased lymphoblast extravasation into the immunized segment was antigen specific. In these studies the efferent duct of the CMLN was cannulated and one of a pair of antigens deposited in the right dorsal basal lung segment. In this situation, the increased output of lymphoblasts, which were labelled and reinfused, was in response to this 'specific' antigenic stimulation. (Unpublished results from our laboratory indicate that antigenic stimulation of the right cranial segment alone does not result in a measurable increase in the output of lymphoblasts from the CMLN.) The right cranial segment, which is drained by the CrBNL, was immunized with a second paired antigen. Sections of the left dorsal basal segment and the left cranial segment, along with the unstimulated CdBLN, were used for comparison. In seven of the eight paired observations, samples of the immunized segments had higher ^{125}I-specific activities than unimmunized segments. There was, however, no evidence that efferent CMLN lymphoblasts, relocated in the dorsal basal lung segment to any greater degree than in the right cranial lung segment, stimulated with a paired, non-cross-reacting antigen.

The increase in ^{125}I-associated lymphoblast activity in immunized lung segments does not appear to have been due simply to an increase in lymphocyte extravasation in general, since a corresponding increase in ^{51}Cr activity, which would reflect entry by a broader population of lymphocytes, was not observed. Also, this was not a response characteristic of all lymphoblasts. Increased ^{125}I activity in immunized lung segments was not observed following the infusion of labelled TD lymphoblasts, except in the one instance where the TD lymph also contained pulmonary blasts due to cannulation of the TD in the neck region.

These findings suggest that lymphoblasts produced in the CMLN have a surface molecule which is recognized by a complimentary receptor on pulmonary endothelial cells and that this lymphoblast–endothelial interaction increases non-specifically in response to immunization. An alternative explanation is that lymphoblasts entered the lung randomly, with subsequent retention in the immunized segments. In this latter situation one would expected an increase in ^{125}I activity in lymph nodes draining unimmunized lung segments which did not retain randomly extravasated lymphoblasts. As shown in Table 3 there was no consistent pattern of radioactivity in the various pulmonary lymph nodes. Specific activity of the unstimulated CdBLN was essentially the same as the lymph nodes draining immunized lung segments.

These results are consistent with those of Bice *et al.* (1982), who reported that immunization with two non-cross-reacting antigens in separate lung lobes of the dog resulted in equal numbers of specific antibody-forming cells to each antigen in

both lobes. They suggested that this was the result of non-specific recruitment of antibody-forming cells which entered the blood from regional pulmonary lymph nodes. The data of Hall *et al.* (1977) indicate that the homing of intestinal immunoblasts to different segments of the gut in sheep also is not 'antigen-driven'.

The *in vivo* labelling experiments are somewhat difficult to interpret because of the failure to avoid totally systemic labelling from [^{125}I]UdR entering the circulation during the injection procedure. Nevertheless the high levels of radioactivity found in the lung, particularly in immunized lung segments, and in bronchial lymph nodes strongly suggest a selective relocation in these tissues of labelled lymphoblasts originating in the CMLN. The distribution of radioactivity was markedly different after labelling of the MLN, although the systemic component should have been roughly the same in both experiments. The results from this study basically support the findings obtained with *in vitro* labelling and argue against non-specific trapping of labelled cells as a major contributing factor to the accumulation of radioactivity in the lung.

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