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Route of lymphocyte migration in pigs

I. LYMPHOCYTE CIRCULATION IN GUT-ASSOCIATED LYMPHOID TISSUE

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Summary. Evidence is presented which indicates that recirculating lymphocytes originating from the intestine in pigs are returned to the blood circulation at the level of the mesenteric lymph nodes (MLN) and not via efferent intestinal lymph. This was demonstrated by three observations: (1) removal of all MLN resulted in a thirty-fold increase in lymphocyte numbers in efferent lymph of pigs, but not in rats; (2) there are about twenty-five times more lymphocytes in afferent intestinal lymph than efferent intestinal lymph in normal pigs; (3) ^{51}Cr-labelled lymphocytes injected into afferent lymphatics are mostly recovered in the node tissue or efferent lymph of sheep, and very few in the venous drainage, whereas in pigs relatively few labelled cells are recovered in the node or in efferent lymph.

INTRODUCTION

The intestine and its associated lymphoid tissue is a major site for the recirculation of lymphocytes as well as *de novo* generation of lymphoblasts produced in response to enteric antigens. It has been demonstrated in most species that lymphoblasts originating in the gut, together with recirculating small lymphocytes, leave the gut in afferent mesenteric lymphatics, traverse the mesenteric lymph nodes (MLN), exit from the nodes into efferent intestinal lymph and return to blood via the thoracic duct (Yoffey & Courtice, 1970); furthermore, the large and small lymphocytes in thoracic-duct lymph are derived almost exclusively from the intestinal bed (Mann & Higgins, 1950).

The paucity of lymphocytes in efferent intestinal lymph of pigs (Binns & Hall, 1966) has led to speculation regarding the route of migration of lymphocytes from the intestine to blood in this species. Binns & Hall (1966) proposed that recirculating lymphocytes leave porcine lymph nodes via the blood capillaries and this was supported by the demonstration in histological sections of radiolabelled lymphocytes in lymph node blood vessels after their injection into afferent lymphatics (McFarlin & Binns 1973).

Two experiments are reported in this paper which confirm this hypothesis. In the first experiment it is demonstrated that removal of all the MLN results in a dramatic increase in cell numbers in efferent intestinal lymph in pigs, but not in rats. The second experiment compares the fate of radiolabelled lymphocytes injected into efferent lymphatics in pigs and sheep.

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MATERIALS AND METHODS

Experiment 1

Four-month old Large White × Landrace pigs and

adult inbred rats of the hooded strain were used for this experiment. The chains of MLN were removed surgically from animals of both species and 2 months was allowed for lymphatic regeneration and the restoration of normal lymph flow. Efferent intestinal lymphatic duct cannulae were established in six mesenteric lymphadenectomized (MLNx) and five normal pigs and thoracic lymphatic duct cannulae were established in five MLNx and five normal rats. Lymph was allowed to drain continuously from all animals. After a post-operative recovery of 24 hr, lymph was collected daily for 5 days. Each day flow rates were determined, cell counts performed using a Coulter Counter (model FN Coulter Electronic Ltd, Dunstable, England) and, for pig lymphocytes, smears were prepared from washed cells and stained with Giemsa stain for differential cell counts. At the end of the collection period (day 6) afferent intestinal lymphatic ducts were cannulated in four normal and four MLNx pigs and cell counts performed on the lymph collected. The success of lymphadenectomy was established retrospectively by examination of the mesentery after intravenous injection of Evans blue dye.

Experiment 2

Lymphocyte labelling. Porcine lymphocytes were prepared from blood after sedimentation of erythrocytes by addition of 3·0 ml of a 5% w/v solution of dextran (200,000 m.w.) in saline per 10 ml of blood. Lymphocytes were isolated from the supernatant by centrifugation on Ficoll-paque (Pharmacia, Uppsala, Sweden). Ovine lymphocytes were prepared from the buffy coat of centrifuged blood by lysis of erythrocytes by the addition of 0·15 M NH_4Cl.

Lymphocytes were labelled with (^{51}Cr) sodium chromate (Radiochemical Centre, Amersham, England) by incubation at a concentration of 20 μ Ci/10^6 cells/ml of RPMI-1640 (Flow Laboratories, Sydney, Australia) containing 10% foetal calf serum at 37° for 60 min. After labelling, lymphocytes were washed and resuspended in RPMI-1640. They were allowed to stand at room temperature for 1 hr then washed again and resuspended in sterile phosphate-buffered saline. An aliquot was set aside for counting and for viability assay (using trypan blue exclusion). Viability was always better than 95%.

Experimental procedure. Four Large White × Landrace pigs at 4 months of age and five adult crossbred wethers were used in this experiment. In each animal the terminal ileal mesenteric lymph node was exposed by laparotomy and the afferent lymphatic vessel cannulated. In sheep there was always a single vein draining the lymph node and this was also cannulated but to maintain blood flow it was necessary to infuse heparin at the rate of 2000 u./hr via a jugular catheter. Cannulation of the venous drainage of porcine nodes proved technically impossible because of the large number and small size of the vessels present. Thus the results for pigs were all obtained from animals in which the efferent lymphatic only was cannulated.

Once cannulae were established, 150 μl of a freshly washed suspension of ^{51}Cr-labelled blood lymphocytes containing 3×10^6 cells was slowly injected, via a 30 gauge needle, into several lymphatic vessels afferent to the cannulated nodes. Lymph from pigs and lymph and blood from sheep were collected over a 48 hr period and at 6-hourly intervals the collections were centrifuged and the cellular component washed and counted in an autogamma scintillation spectrometer (Packard Instruments, Ill., U.S.A.). At the end of the collection period a second laparotomy was performed and the infused node removed for counting.

Calculations. Since it was not possible to cannulate the blood vessels draining the porcine nodes, an estimated value for radioactivity leaving the node via blood in this species was derived from the following calculations.

The proportion (Y) of injected counts which were recovered in venous blood, efferent lymph and the node itself is given by the equation:

$$Y = \frac{N+L+B}{X}$$

where N = radioactivity recovered in node, L = radioactivity recovered in lymph, B = radioactivity recovered in blood and X = radioactivity injected.

For the five sheep studied the mean (±SE) of the Y values was 0·48 ± 0·08. On the assumption that the value for Y, which represents the proportion of injected radioactivity actually reaching the node, would be similar in sheep and pigs, a value for B for pigs was obtained from the above equation by substituting the value for Y obtained in sheep and the measured values for N, L and X obtained in pigs.

RESULTS

Experiment 1

Daily flow rates and cell outputs in the efferent intes-

Table 1. Flow rate and cell output in chronically-drained efferent intestinal lymph from five normal and six mesenteric lymphadenectomized pigs. Values are presented as means ± SE

	Normal pigs		MLNx pigs	
Day	Flow (ml/hr)	Lymphocyte output (cells × 10^{-6}/hr)	Flow (ml/hr)	Lymphocyte output (cells × 10^{-6}/hr)
1	23·72 ± 6·59	6·92 ± 3·49	37·99 ± 3·63	243·48 ± 89·45
2	40·83 ± 11·87	13·40 ± 6·19	34·46 ± 6·68	140·07 ± 44·00
3	39·10 ± 6·35	15·37 ± 6·25	34·79 ± 7·10	174·88 ± 60·78
4	33·02 ± 4·76	11·55 ± 3·05	19·37 ± 4·62	85·55 ± 32·92
5	29·07 ± 4·46	9·65 ± 1·64	28·04 ± 8·19	86·01 ± 40·40

tinal lymph of normal and MLNx pigs are presented in Table 1. Although lymph flow rates in both normal and MLNx pigs were almost identical (approximately 30·0 ml/hr) the lymphocyte output in MLNx pigs was up to thirty-five times greater than in normal pigs ($243·48 \times 10^6$ cells/hr for MLNx compared with $6·92 \times 10^6$ cells/hr for normals on day 1). By the fifth day of chronic drainage the cell output in intestinal lymph from MLNx pigs had fallen to $86·01 \times 10^6$ cells/hr while the cell output in intestinal lymph from normal pigs did not change significantly over the collection period.

Lymphocytes constituted the vast majority of leucocytes in the cell smears from both normal and MLNx pigs. Although there was a high proportion of erythrocytes in smears from normal pigs (mean = 83·9%; range 78·2%–90·5%) this proportion was reduced in MLNx pigs (mean = 40·7%; range 30·7%–48·4%).

At the end of the collection period lymphatic vessels afferent to the MLN were cannulated and the mean lymphocyte counts obtained were $7·59 \pm 4·04 \times 10^6$ cells/ml in normal pigs and $2·73 \pm 0·83 \times 10^6$ cells/ml in MLNx pigs.

To examine whether mesenteric lymphadenectomy itself could cause an increase in lymphocytes leaving the gut, comparisons were made between normal and MLNx rats, a species with conventional lymphocyte recirculation patterns (Gowans & Knight, 1964). The results of these experiments are presented in Table 2. In this species lymphadenectomy did not alter the flow rate of lymph and instead of an increase in cell output, as was observed in MLNx pigs, there was a slight reduction in cell output in MLNx rats.

Experiment 2

The radioactivity recovered over 48 hr in the MLN tissue, the efferent lymph and venous blood in sheep and in the node tissue and efferent lymph in pigs is shown in Table 3.

Table 2. Flow rate, cell outputs and proportion of large lymphocytes in chronically-drained thoracic duct lymph from five normal and five mesenteric lymphadenectomized rats. Values are presented as means ± SE

	Normal rats			MLNx rats		
Day	Flow (ml/hr)	Lymphocyte output (cells × 10^{-7}/hr)	Large lymphocytes (%)†	Flow (ml/hr)	Lymphocyte output (cells × 10^{-7}/hr)	Large lymphocytes (%)†
*1	3·38 ± 0·68	3·07 ± 1·09	11·14 ± 1·51	3·62 ± 0·55	2·34 ± 1·30	14·87 ± 1·35
2	2·36 ± 0·45	1·51 ± 0·39	18·33 ± 2·58	3·50 ± 1·04	1·67 ± 0·12	16·40 ± 0·64
3	1·70 ± 0·20	1·04 ± 0·25	22·76 ± 1·61	2·10 ± 0·34	0·88 ± 0·12	20·94 ± 1·68
4	1·88 ± 0·12	0·74 ± 0·04	27·27 ± 2·14	2·74 ± 0·87	0·50 ± 0·05	24·21 ± 1·55
5	1·58 ± 0·19	0·45 ± 0·09	28·30 ± 2·28	1·94 ± 0·29	0·47 ± 0·13	27·48 ± 1·41

* Means from four rats on Day 1.

† Determined as a percentage of total lymphocytes by cell counts with Coulter Counter (Model FN, Coulter Electronics Ltd, Dunstable, England) with size threshold setting raised to 55% of size distribution.

Table 3. Radioactivity (c.p.m.) in dose of ^{51}Cr-labelled lymphocytes injected into afferent lymphatics and in ileal mesenteric node tissue, efferent lymph and venous blood recovered during 48 hr period after injection in five sheep and four pigs

	Dose injected	Node	Lymph	Blood
Sheep				
1	18,921	8779	602	31
2	19,613	8773	4155	0
3	27,150	7517	739	0
4	80,121	20,488	1100	0
5	42,775	25,167	2672	0
Pigs				
1	18,282	379	453	—*
2	9606	48	105	—
3	65,741	8410	806	—
4	43,617	9549	47	—

* Blood vessels not cannulated in pigs.

The recovery of ^{51}Cr-labelled lymphocytes in these compartments, expressed as a percentage of total counts recovered in each over 48 hr, is shown in Table 4. Whereas low counts were recovered in the efferent lymph of porcine nodes, a greater proportion of injected counts appeared in sheep efferent lymph. On the other hand, negligible counts were recovered in the venous drainage of sheep nodes but, using the equation, it was estimated that in pigs most counts would have left the node in venous blood (Table 4). It is of interest to note that there was a much greater retention of counts within the node in sheep than in pigs.

Table 4. Radioactivity recovered in ileal mesenteric lymph nodes, efferent lymph and venous blood, as a percentage of total counts recovered, after afferent infusion of ^{51}Cr-labelled lymphocytes. Values presented are means ± SE from five sheep and four pigs

	Node (%)	Lymph (%)	Blood (%)
Sheep	87·50 ± 4·98	12·43 ± 4·99	0·07 ± 0·07
Pigs*	19·49 ± 10·47	3·07 ± 0·71	77·95 ± 9·73

* Estimated values according to the equation, based on Y value of 0·48.

DISCUSSION

To explain the extraordinarily low numbers of lymphocytes present in pig efferent lymph it has been hypothesized that pig lymphocytes recirculate from lymph nodes directly into blood (Binns & Hall, 1966) but little data to support this contention is available (Binns, 1973). The data we present here are consistent with this hypothesis, at least with respect to the recirculation of cells from the gut.

The results in Table 1 indicate that mesenteric lymphadenectomy resulted in a dramatic increase in the number of lymphocytes in efferent intestinal lymph. Indeed the cell output recorded in MLNx pigs was similar to data obtained in intestinal lymph of adult sheep (Beh, Husband & Lascelles, 1979) an animal of comparable size with the pigs used in these experiments. Since removal of the MLN results in elevated cell output in porcine efferent lymph without any change in the flow rate, it is reasonable to assume that the MLN normally direct lymphocytes into blood, rather than efferent lymph, in this species.

The fact that lymphocytes constituted the vast majority of leucocytes in smears from both normal and MLNx pigs indicates that myeloid or monocytic cells did not contribute disproportionately to the increase in cell output in MLNx pigs. The predominance of lymphocytes and relative depletion of erythrocytes in smears from MLNx pigs also discounts the possibility that lymph/blood anastomoses distal to the point of efferent duct cannulation accounted for the elevated cell count in MLNx pigs. The abundance of erythrocytes in efferent intestinal lymph of normal pigs is consistent with other reports in this species (Binns & Hall, 1966).

In rats, a species with conventional lymphocyte recirculation patterns (Gowans & Knight, 1964), mesenteric lymphadenectomy resulted in a slight reduction in cell output in thoracic duct lymph rather than an increase as occurred in pigs. Presumably the fall in cell output in MLNx rats was due to the absence from lymph of cells which normally recirculate from blood to lymph through the MLN. It is well documented that in chronically drained rats a fall in lymphocyte output occurs as a result of a depletion of recirculating lymphocytes (Mann & Higgins, 1950). This is reflected in the fall in lymphocyte output for both normal and MLNx rats over the 5-day collection period (Table 2). Thus the dramatic fall in cell output in lymph from MLNx pigs during the course of the experiment (Table 1) indicates that the bulk of the cells

in efferent lymph of MLNx pigs are indeed recirculating lymphocytes.

If pig lymphocytes leave intestinal lymph at the level of the MLN, the corollary to this is that there should be high numbers of lymphocytes in afferent intestinal lymph, that is, lymph obtained by cannulation, at a point distal to the MLN, of lymphatics draining the gut. The number of cells observed in afferent lymph was about twenty-five times greater than the corresponding values in normal efferent lymph and is comparable to the lymphocyte concentration per millilitre in efferent lymph of MLNx pigs on the first day of collection (Table 1) before depletion of recirculating lymphocytes occurred. The fact that there were fewer lymphocytes per millilitre in afferent lymph of MLNx pigs than in afferent lymph of normal pigs is probably a result of the lymphocyte depletion resulting from prior chronic efferent-lymphatic drainage in MLNx pigs (since afferents were cannulated on day 6).

The results obtained in Experiment 2 demonstrate by a more direct method that the majority of lymphocytes reaching porcine MLN are not recovered in the node tissue or in efferent lymph and it is presumed that they leave the nodes via blood. The lower proportion of counts retained in the node itself in pigs is probably explained by its loose cortical structure (McFarlin & Binns, 1973) allowing a more rapid transit of cells than in sheep nodes.

Since it was not possible to obtain direct measurements of radioactivity leaving the porcine nodes via blood, this value was derived by subtraction of the activity in the node and that recovered in lymph from the activity estimated to have reached the node. This estimate was calculated from the equation and its validity depends on three assumptions: (1) that the proportion of labelled cells in the injected dose which fail to reach the node is similar in both pigs and sheep; (2) that lymphocytes which do reach the node must all be recovered in either the node tissue, the efferent lymph or venous blood; and (3) that the rate of loss of label is similar for both porcine and ovine lymphocytes. There appears to be no reason why the first two assumptions should not be valid. Indeed, even if only half the proportion of injected cells reached the node in pigs as in sheep (that is if $Y=0{\cdot}24$ in pigs), the estimated recovery in blood-draining porcine nodes would still represent 56% of counts recovered compared to 5% in lymph. The validity of the third assumption has been confirmed by the unpublished observations of the authors that there is no greater loss of label from porcine lymphocytes than ovine lymphocytes cultured *in vitro*.

Thus, the combined evidence from the two experiments presented here indicates that the paucity of lymphocytes in efferent lymph of pigs, at least with respect to intestinal lymph, is the result of migration of lymphocytes into blood within the MLN and does not occur through a failure of cells to recirculate. We suggest that this route of lymphocyte recirculation is made possible by the peculiar architecture of the pig lymph node where germinal centres are located centrally and medullary tissue arranged around the periphery of the nodes (McFarlin & Binns, 1973). In this connection we have observed up to five major veins draining single mesenteric nodes in pigs, these exit at various points over the surface of the node. The vascular drainage from lymph nodes of sheep and most other species is characteristically represented by a single vein located at the hilus near the efferent lymphatic vessel. This might indicate a role in cell collection for veins draining porcine nodes.

The significance of those lymphocytes which are present in efferent intestinal lymph of normal pigs remains unexplained but probably represents imperfect partitioning of cells between blood and lymph within the nodes.

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