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Route of lymphocyte migration in pigs

II. MIGRATION TO THE INTESTINAL LAMINA PROPRIA OF ANTIGEN-SPECIFIC CELLS GENERATED IN RESPONSE TO INTESTINAL IMMUNIZATION IN THE PIG

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Summary. Intestinal lymph-duct cannulae were established in normal and mesenteric lymphadenectomized (MLNx) pigs for the 6-day duration of a local intestinal immune response to a protein antigen (ovalbumin). The daily output of anti-ovalbumin-containing cells (AOCC) in intestinal lymph and the numbers of AOCC in the intestinal lamina propria at the end of the experiment were recorded. Very few AOCC were recovered in the intestinal lymph of normal pigs whereas in MLNx pigs large numbers were recovered reaching a peak output on day 4. However, there were significantly more AOCC detected in the jejunal lamina propria of normal pigs than MLNx pigs despite continuous drainage of intestinal lymph throughout the response.

The absence of AOCC from efferent intestinal lymph of normal pigs, the failure of chronic intestinal-lymph drainage to abrogate the AOCC response in the intestine of these pigs and the reversal of these findings in MLNx pigs indicate that, in contrast to other species, lymphoblasts are diverted from porcine intestinal lymph, and probably enter the blood circulation at the level of the mesenteric lymph node (MLN).

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INTRODUCTION

In rats and sheep, IgA-specific antibody-containing cells in the intestine have been shown to be generated in Peyer's patches and to migrate through mesenteric lymph nodes (MLN) to enter thoracic-duct lymph then blood and eventually become resident as plasma cells in the intestinal lamina propria (Husband, Monié & Gowans, 1977; Husband, Beh & Lascelles, 1979).

Lymphocyte migration patterns in the pig are radically different to that in most other species. Binns & Hall (1966) drew attention to the paucity of lymphocytes in pig efferent lymph despite normal numbers in blood and suggested that in this species there is either a failure of lymphocyte recirculation, or that recirculating lymphocytes exit from lymph nodes via blood rather than efferent lymph. Bennell & Husband (1981a) obtained evidence to support the latter hypothesis, at least with respect to mesenteric lymph nodes (MLN), by showing that mesenteric lymphadenectomy in pigs resulted in a thirty-fold increase in cell numbers in efferent intestinal lymph and by observing the fate of ^{51}Cr-labelled lymphocytes injected into afferent lymphatics.

The aims of the experiments reported here were to use an intestinal immunization protocol to generate antibody-containing cells of IgA specificity and to study their migration pathway from gut-associated lymphoid tissue to the intestinal lamina propria. In

particular it was of interest to determine whether antigen-specific lymphoblasts reaching the MLN in afferent lymph were diverted from efferent lymph at the level of the MLN, as we have described for the majority of lymphocytes in porcine intestinal lymph (Bennell & Husband, 1981a). This has been achieved by observing the numbers of anti-ovalbumin-containing cells (AOCC) in efferent intestinal lymph in normal and mesenteric lymphadenectomized (MLNx) pigs following local immunization with ovalbumin and the effect of chronic intestinal duct drainage on AOCC numbers in the intestinal lamina propria in normal and MLNx pigs.

MATERIALS AND METHODS

Animals

Eleven Large White x Landrace pigs were used. They were housed under intensive conditions but after cannulation of the intestinal lymph duct they were kept in metabolism cages. The pigs were offered a commercial ration and water *ad libitum*.

Antigen and immunization

Crystalline ovalbumin was prepared according to the method of Campbell, Garvey, Cremer & Sussdorf (1964). For intraperitioneal (i.p.) immunizations ovalbumin (10 mg/ml) was emulsified with an equal volume of Freund's complete adjuvant (FCA) and administered as a 3·0-ml dose. Repeated intra-duodenal infusions were performed by daily injections of 120 ml of a 1 mg/ml solution of ovalbumin containing 50 mg/ml DEAE-dextran (Pharmacia, Uppsala, Sweden) for 6 days into a surgically-inserted duodenal cannula.

Experimental procedure

Laparatomies were performed on eight pigs at 2 months of age (Groups 1 and 3), the chains of mesenteric lymph nodes removed, then 2 months allowed for lymphatic regeneration and the restoration of normal flow before experiments were commenced. After this time all pigs were immunized according to a protocol developed previously which gave an optimal response in terms of IgA-specific antibody-containing cells in the intestine (Bennell & Husband, 1981b). Briefly, pigs were immunized i.p. and 14 days later laparotomies were performed, duodenal cannulae inserted and for pigs in Group 1 and 2 the intestinal lymph duct was cannulated using the technique described by Bennell & Watson (1979). Antigen plus DEAE-dextran was repeatedly infused into the duodenum of all pigs over a period of 6 days during which time intestinal lymph from pigs in Groups 1 and 2 was allowed to drain continuously. The treatment schedules are summarized in Table 1.

Laparotomies were performed on day 7 and intestinal biopsies obtained by excision, using diathermy, of a 1-cm diameter piece of tissue from the wall of the intestine in the mid-jejunum region. This was fixed and processed for fluorescent histology (Sainte-Marie, 1962) and the intestine was repaired with absorbable suture. Before closure of the abdominal incision, the success of lymphadenectomy was established retrospectively by examination of the mesentery of MLNx pigs after intravenous injection of Evans blue dye.

Table 1. Treatment schedules

Group	No. of pigs	*Day 0	Day 7	
1 (MLNx)	6	Intestinal lymph duct and duodenum cannulated	R.i.d. + lymph drainage	Biopsy
2 (normal)	5	Intestinal lymph duct and duodenum cannulated	R.i.d.+ lymph drainage	Biopsy
3 (MLNx)	2	Duodenum cannulated	R.i.d.	Biopsy

MLNx = mesenteric lymphadenectomized.
R.i.d. = repeated intraduodenal infusions of antigen
* All pigs were primed i.p. 14 days previously.

Lymph collection

Lymph collections from pigs in Group 1 and 2 were not made until 2 days after cannulation to allow animals to recover from the effects of surgery. After this period daily lymph samples were collected over a 2 hr period from four pigs in each group. Flow rates were recorded and leucocyte concentrations were determined using a Coulter Counter, (model FN, Coulter Electronic Ltd, Dunstable, England). Smears were prepared from washed intestinal lymph cells and were fixed in 96% ethanol for 10 min, at 4°.

Enumeration of anti-ovalbumin-containing cells (AOCC)

AOCC were enumerated in smears using fluorescein-conjugated anti-ovalbumin in the indirect labelling technique described by Beh (1977). AOCC and their immunoglobulin class specificity were detected in tissue sections using the double fluorochrome labelling and enumeration technique described previously (Husband, 1978). Fluorescein-conjugated monospecific anti-porcine immunoglobulin reagents were prepared as described by Bennell & Watson (1979).

Statistics

The significance of differences between means was tested using Student's *t* test.

RESULTS

The output of AOCC in the intestinal lymph of MLNx and normal pigs during the local immunization period are presented in Table 2. There were very few AOCC recovered in the lymph of normal pigs (Group 2) and on days 2 and 4 no AOCC were detectable. The output of AOCC in intestinal lymph of MLNx pigs (Group 1) was much greater than normal pigs and reached a peak on day 4 when the mean recovery was $98{\cdot}74 \times 10^6$ AOCC/hr.

Table 3. Density and immunoglobulin class specificity of AOCC in the jejunum of pigs given 6 days of repeated intraduodenal antigen infusion starting 14 days after i.p. priming. Group 1 (MLNx) and Group 2 (normal) had chronically draining intestinal lymph-duct fistulae. Group 3 (MLNx) were not cannulated. Values are presented as means ±SE.

Group	No. of pigs	AOCC/cm	IgA (%)	IgG (%)
1	6	46·98±20·98	30·96± 5·30	40·67± 4·81
2	5	202·17±41·22	40·95±15·07	39·76±13·10
3	2	301·34±99·07	37·08±16·25	55·00± 5·00

The density and immunoglobulin class specificity of AOCC in the jejunum of MLNx and normal pigs following chronic drainage of the intestinal lymph duct are presented in Table 3. Significantly more AOCC were detected in the jejunal lamina propria of normal pigs than MLNx pigs ($P < 0{\cdot}05$) although the distribution of immunoglobulin class specificity of AOCC was similar in both groups of animals.

Thus, chronic drainage of intestinal lymph failed to affect the intestinal response in normal pigs but there was a depletion of AOCC in the gut of MLNx pigs. This result indicates that the route of migration of AOCC to the gut is not via intestinal lymph, unless MLN are removed.

To ensure that the depletion of AOCC in the gut of MLNx pigs was due to chronic lymphatic drainage and not an effect of mesenteric lymphadenectomy *per se*, a further two MLNx pigs were similarly immunized

Table 2. Output of AOCC in intestinal lymph of Group 1 (MLNx) and Group 2 (normal) pigs during the period of repeated intraduodenal infusions. All pigs had been primed i.p. 14 days previously. Lymph was allowed to drain throughout the infusion period

Time after commencement of infusion (days)	AOCC output (cells × 10^{-6}/hr)							
	Group 1 (MLNx) (Pig no.)				Group 2 (normal) (Pig no.)			
	37	39	19	20	14	21	22	59
2	0·00	0·00	84·63	30·64	0·00	0·00	0·00	0·00
3	2·63	14·48	30·60	43·03	0·00	0·00	0·00	2·14
4	80·61	201·93	—	13·68	0·00	0·00	0·00	—
5	43·20	93·53	—	28·93	0·00	0·90	0·00	—
6	12·02	80·53	—	10·03	0·00	5·93	1·53	—

but not cannulated (Group 3). The response in these animals was comparable with that in Group 2 and to the response recorded previously in intact pigs immunized with this protocol (Bennell & Husband, 1981b), which indicates that the depletion in MLNx pigs (Group 1) was a result of chronic drainage and not mesenteric lymphadenectomy *per se*.

DISCUSSION

The intestine, together with 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 MLN, exit from the nodes into efferent intestinal lymph and return to the blood circulation via the thoracic duct (Yoffey & Courtice, 1970). Thus MLN differ from peripheral lymph nodes in that they receive large numbers of migrating cells via afferent lymph whereas most cells passing through peripheral lymph nodes have originated from blood. The importance of the lymphatic route in transmitting enterically-stimulated lymphoblasts from the gut-associated lymphoid tissues to the gut lamina propria has been demonstrated in rats (Pierce & Gowans, 1975) and sheep (Husband *et al*, 1979) by showing that diversion of intestinal lymph by chronic lymphatic drainage before intestinal immunization with a specified antigen prevents the appearance in the intestine of antibody-containing cells of corresponding specificity.

The paucity of lymphocytes in efferent lymph of pigs (Binns & Hall, 1966) and the detection in lymph node blood vessels of radiolabelled lymphocytes after their injection into afferent lymphatics (McFarlin & Binns, 1973) led to speculation that recirculating lymphocytes leave porcine lymph nodes via the blood capillaries. Support for this hypothesis, at least with regard to MLN, has been provided by the observation that there were many more lymphocytes in lymph afferent to MLN than in efferent intestinal lymph in pigs and in the observation that mesenteric lymphadenectomy resulted in a thirty-fold increase in lymphocyte numbers in porcine efferent intestinal lymph (Bennell & Husband 1981a). These findings indicate that in normal pigs the majority of lymphocytes are diverted from intestinal lymph at the level of the MLN. The recovery of only small numbers of ^{51}Cr-labelled lymphocytes in MLN or efferent lymph after their injection into afferent lymph also supports this hypothesis (Bennell & Husband, 1981a).

In the light of these findings, the present study was undertaken to determine the route of migration of IgA-specific antibody-containing cells generated in the gut-associated lymphoid tissues in response to a specific antigen. Pigs were immunized using a procedure which has previously been shown to generate antibody-containing cells predominantly of IgA specificity in the intestine (Bennell & Husband, 1981b). The results in Table 2 indicate that in normal pigs the MLN divert the majority of AOCC from the efferent intestinal lymph since it was only when the nodes were removed that significant numbers of AOCC were recovered in lymph. This supports the suggestion that, in normal pigs, lymphocytes leave MLN via blood.

Further support for this hypothesis was obtained by recording AOCC numbers in the intestine of chronically drained normal and MLNx pigs (Table 3). The failure of chronic drainage of efferent intestinal lymph to abrogate the intestinal response in normal pigs is in contrast to findings in rats (Pierce & Gowans, 1975) and sheep (Husband *et al.*, 1979) and indicates that the AOCC must be entering the circulation distal to the point of cannulation. Presumably this occurs within the MLN because the gut AOCC response is depleted by chronic drainage if MLN are removed.

The finding that chronic drainage did not completely abrogate the response in the intestine of MLNx pigs could be accounted for by the possibility that some AOCC precursors migrated to the gut via a route other than intestinal lymph, or they could have arisen as a result of compensatory proliferation of the few cells which arise in the gut following an i.p. priming dose of antigen (Bennell & Husband 1981b). This latter phenomenon was demonstrated with respect to IgA cells in the gut of rats when the supply of IgA cell precursors was interrupted by thoracic duct drainage (Mayrhofer & Fisher, 1979).

The results presented here support the hypothesis that lymphocytes migrate from afferent lymph directly into blood in porcine MLN and that this occurs not only for recirculating small lymphocytes (Bennell & Husband, 1981a) but also for *de novo* generated lymphoblasts, the precursors of IgA plasma cells in the gut. In the light of the very low numbers of lymphocytes present in porcine peripheral lymph (Binns & Hall, 1966) it is likely that this route of lymphocyte migration also occurs in peripheral lymph nodes in pigs.

This unusual route of lymphocyte migration may have some bearing on the way in which pigs respond to intestinal antigen, and it should also be taken into consideration wherever the pig is used as an experimental animal for medical research because of the differences between the porcine and human immune systems.

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