Studies on hemolymph nodes. II. The regional origin of the afferent lymphatics

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INTRODUCTION

The preceding paper (Kazeem, Reid & Scothorne, 1982) provided histological evidence that the renal hemolymph node of rats receives afferent lymphatics, and gave reasons for believing that these provide one route of entry of erythrocytes into the lymph sinuses of the node. In some nodes, erythrocytes adherent to sinus macrophages ('macrophage-erythrocyte rosettes') were found to be localized to one pole of the node. Our interpretation was that erythrocytes entered the node by way of afferent lymphatics and that the polarity of distribution might result if lymph from only one of the possible sources contained erythrocytes. This paper examines in detail the regional drainage territories of the renal hemolymph node.

MATERIALS AND METHODS

Three groups of adult male Swiss albino rats were used, from a closed colony in the Department. Four organs were investigated as possible sites of origin of afferent lymphatics: testis, kidney, diaphragm and adrenal gland. All animals were killed by an overdose of anaesthetic ether before lymphatic injection.

Group 1

In each of 60 rats, the subalbugineal lymphatic plexus in each testis was injected with India ink (Windsor & Newton, diluted 1:4 with distilled water and filtered) by gentle hand pressure, using a syringe and 25 g needle. Ink flowed cephalad in the testicular lymphatic trunk to the regional nodes, encouraged, if necessary, by gentle massage of the testicular lymphatic plexus and collecting trunk.

Group 2

In 26 animals, the regional lymph node of the left kidney was demonstrated by injection of India ink beneath the kidney capsule. In 15 of these animals, the subperitoneal lymphatic plexus of the left hemidiaphragm was also injected, and the collecting trunks traced to the regional nodes.

Group 3

In 33 animals, India ink was injected beneath the capsule of the left adrenal gland, in an attempt to demonstrate the regional lymphatic drainage of the gland.
RESULTS

(1) Regional lymph node of the testis

In all 60 animals of Group 1, the left testicular lymphatic trunk, usually single, but double in about 20% of cases, drained into the ipsilateral renal hemolymph node, either directly or after interruption in one of the lower paraortic group of nodes. The node was blackened by the ink, which could then be traced into its efferent lymphatic, and thence into the closely adjacent cisterna chyli, either directly or after interruption in another small node. Connections of the efferent lymphatic with the adjacent renal and adrenal veins were not found. A significant finding was that, when a lower paraortic node was the first regional node of the testis, it did not show the appearance of a hemolymph node.

On the right side, the lymphatic pattern was similar, except that occasionally the right testicular lymphatic trunk opened directly into the cisterna chyli, without interruption by a lymph node.

(2) Regional nodes of the kidney and diaphragm

Afferents were demonstrated, in all 26 rats of Group 2, from the left kidney to the ipsilateral renal hemolymph node; in about 50% of the animals studied, there were two or even three separate lymphatic channels. In some cases in which there was patchy red coloration of the node, indicating an underlying polarity of distribution of erythrocytes, our impression was that the reddish areas were those which faced the kidney and received lymphatics from it.

Ink-filled lymphatics from the left hemidiaphragm drained to a hemolymph node in only 1 of the 15 animals in which a sub-peritoneal injection had also been made. In the others, the regional node was a small, typical lymph node, situated cranial to the renal node.

(3) Regional nodes of the adrenal gland

The rich vascularity of the adrenal gland made it difficult to inject its lymphatics without inadvertently injecting blood vessels as well. However, in 4 of the 33 animals of Group 3, what were thought to be ink-filled lymphatics were traced from the left adrenal to the left renal hemolymph node. No efferent adrenal lymphatics were demonstrated in the remaining 29 animals.

In some of the animals in which ink was injected inadvertently into veins within the adrenal, retrograde flow occurred from the adrenal vein into small veins draining the renal hemolymph node, which became blackened. In 3 further animals, retrograde flow was encouraged by ligating the left adrenal vein where it entered the left renal vein and then injecting ink deeply into the adrenal gland. Histological sections of the blackened nodes showed ink within postcapillary venules and veins, but none within the lymphatic sinuses.

DISCUSSION

These observations establish that the rat's left renal hemolymph node receives afferent lymphatics consistently from the ipsilateral testis and kidney, possibly from the left adrenal gland and, unusually, from the subperitoneal lymphatics of the left hemidiaphragm. They finally resolve the old controversy about whether or not renal hemolymph nodes (in the rat at least) have afferent lymphatics. They also
show that the efferent lymphatics of the renal hemolymph node usually enter the cisterna chyli and that local lymphaticovenous connections are absent, at least in the particular strain of rat used here.

In view of our finding that the renal hemolymph node receives at least 2 afferent lymphatics – and some receive 4 or even 5 – an explanation of the negative findings of some previous studies may be called for. The most likely reason is that it is undoubtedly easier to find slender, thin-walled afferent lymphatics when they are seen under the dissecting microscope, in continuity and distended with ink, than when they are sought, collapsed and empty, in sections. In his careful search, Turner (1969) relied exclusively on serial sections; it may also be that cleaning of adventitious tissue from the surface of the node prior to processing tore away the delicate afferent vessels. Nopajaroonsri, Luk & Simon (1974) found ‘a few’ afferent lymphatics but did not determine their regional origin, other than by injecting ink into the adjacent retroperitoneal connective tissue. Andreasen & Gottlieb (1946) accepted Job’s (1915) findings, by injection, that the left renal hemolymph node received lymph from the lumbar lymphatic trunk and drained, in a proportion of cases, into the left renal vein. Tilney (1971), using an injection technique, like ourselves, found afferent lymphatics from testis, kidney and adrenal to a node which he allocated to the upper paraaortic group and called ‘renal’. Although, somewhat surprisingly, he did not recognize its hemolymph character, we have little doubt that it was the same node as that studied by ourselves and by the others cited in this paper.

Taken in conjunction with the histological observations of the previous paper, these results throw some light on the source of the erythrocytes found in abundance in the lymphatic sinuses of hemolymph nodes. Reflux from the renal vein into efferent lymphatics of the renal node was already thought unlikely as a possible route. It is ruled out by our failure to find local lymphaticovenous communications.

If erythrocytes do reach the hemolymph node from an external source, through afferent lymphatics, then it is important to determine the organ from which they come. Of the four possibilities examined – testis, diaphragm, kidney and adrenal gland – the testis may be confidently excluded, since in those animals in which the lower paraaortic nodes primarily interrupted the testicular lymphatics, they were not of the hemolymph variety.

It is well known that diaphragmatic lymphatics provide a major route for uptake of particulate material from the peritoneal cavity (see e.g. Casley-Smith, 1964) and we anticipated that the renal hemolymph node might derive its erythrocytes principally from that source. Our findings do not however support this idea: in 14 out of 15 animals, lymphatics from the left hemidiaphragm drained into an ordinary lymph node.

We had little success in injecting the lymphatics of the adrenal gland and cannot therefore exclude it as a possible source. It is not known if adrenal lymph contains many erythrocytes, but it is worth recalling the rich vascularity of the gland (see e.g. Coupland, 1975), the demonstration by Bloodworth & Powers (1968) of extravasated erythrocytes in the capsular interstitium, and Merklin’s (1966) finding of the communication between capsular lymphatics and lymphatics in the adventitia of the central vein of the adrenal. However, Selye & Schenker (1939) found that unilateral adrenalectomy did not alter the hemolymph character of the node.

Of the four possibilities studied, the kidney remains as the most likely source of erythrocytes. We found in some cases that renal lymphatics drained selectively into reddish areas of renal hemolymph nodes, but this evidence is no more than circum-
stantial. Nephrectomy in the rat leads to disappearance of erythrocytes from the renal hemolymph node of rats (Selye & Schenker, 1939). Their interpretation of this effect was not, however, that this deprived the node of its lymph-borne source of erythrocytes but that "the blood destroying function of the node is dependent upon the presence of the kidney". Andreasen & Gottlieb (1946) also found that, after nephrectomy, the renal node assumed the appearance of an ordinary lymph node: they thought that this was because concomitant ligation of the renal pedicle prevented reflux of blood into efferent lymphatics from the renal vein.

If erythrocytes do reach the renal hemolymph node via afferent lymphatics draining the kidney, then renal lymph must contain many more erythrocytes than peripheral lymph from most other parts of the body. Whether this is the case is not known for the rat, but McIntosh & Morris (1971) have shown that the renal lymph of sheep, aged more than 1 year, contains abundant erythrocytes. Indeed, they suggested that this might explain the hemolymph character of the renal node in that species.

**SUMMARY**

The left renal hemolymph node of the male Swiss albino rat receives lymph consistently from the ipsilateral testis and kidney. It may occasionally receive lymph from the left adrenal gland, although this is technically difficult to prove conclusively. It does not usually receive lymph from the peritoneum of the left hemidiaphragm.

Lymph from the kidney, reaching the node in its afferent lymphatics, is suggested as the most likely source of the erythrocytes in the nodal lymphatic sinuses, the salient characteristic of hemolymph nodes.

**REFERENCES**


