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Lymphocyte Migration through the Walls of the Post-capillary Venules

B. Norberg, L. Rydgren

Department of Internal Medicine, University Hospital of Lund, S-221 85 Lund, Sweden

Summary

The results of morphological analyses of the direction of the lymphocyte traffic through the walls of the HE-venules are conflicting. The aim of this paper is to provide a short analytical review of the evidence available for the bi-directional hypothesis and for the uni-directional hypothesis.

The generally accepted present-day view is that lymphocytes recirculate from blood to efferent and central lymph through the walls of the postcapillary high-endothelium venules (HE-venules) of the lymph nodes (see e.g. 1,2). The aim of the present paper is to analyse the direction of polarized lymphocytes in the walls of the HE-venules at the moment of fixation.

The migrating lymphocyte has an elongated shape (3, 4, 5), and is distinguished by an anterior thin granula-free lamellipodium, an anterior or middle position of the nucleus, and the bulk of granulated cytoplasm in the tail (cf. Fig. 1). The characteristic behaviour of the migrating lymphocyte can be observed directly in vital preparations or visualized by time-lapse filming or by microphotography (4, 5).

Lymphocytes fixed during migration retain the elongated shape of wandering cells (4, 6, 7, 8). This polarity of wandering lymphocytes offers an opportunity to study the migration of lymphocytes in the endothelium of the HE-venules (4, 5, 6, 7, 8), provided that the direction of the lymphocytes at the moment of fixation reflects the size of the migration streams of lymphocytes, i.e. no valve mechanism or chemotactic process prevents the wandering lymphocytes from entering the venous blood stream or the lymph node parenchyma (Fig. 1).

Three studies on the direction of polarized lymphocytes in the endothelium of HE-venules have hitherto been performed (Tab. 1). When thin sections of rat lymph nodes were studied by phase contrast microscopy, 82 elongated lymphocytes appeared to be directed towards the lumen of the HE-venule and 36 elongated lymphocytes appeared to be directed towards the lymph node parenchyma (7). This difference was highly significant ($p < 0.001$). An electron microscope study of rat lymph nodes yielded essentially the same result (8); 68 elongated lymphocytes appeared to be directed

Tab. 1 Open analyses of the direction of elongated lymphocytes in the walls of post-capillary HE-venules. EM: electron microscopy. PCM: phase contrast microscopy. BF: assessment of direction blind-fold.

Source	Animal	Method	Lc: s directed towards		p ≤	BF
			Blood	Node		
Rydgren et al. Lympho. 9:96, 1976	Rat	PCM	82	36	0.001	–
Rydgren et al. Lympho. 9:150, 1975	Rat	EM	68	36	0.002	–
Anderson & Anderson Immunol. 31:731, 1976	Rat	EM	10	127	0.001	–

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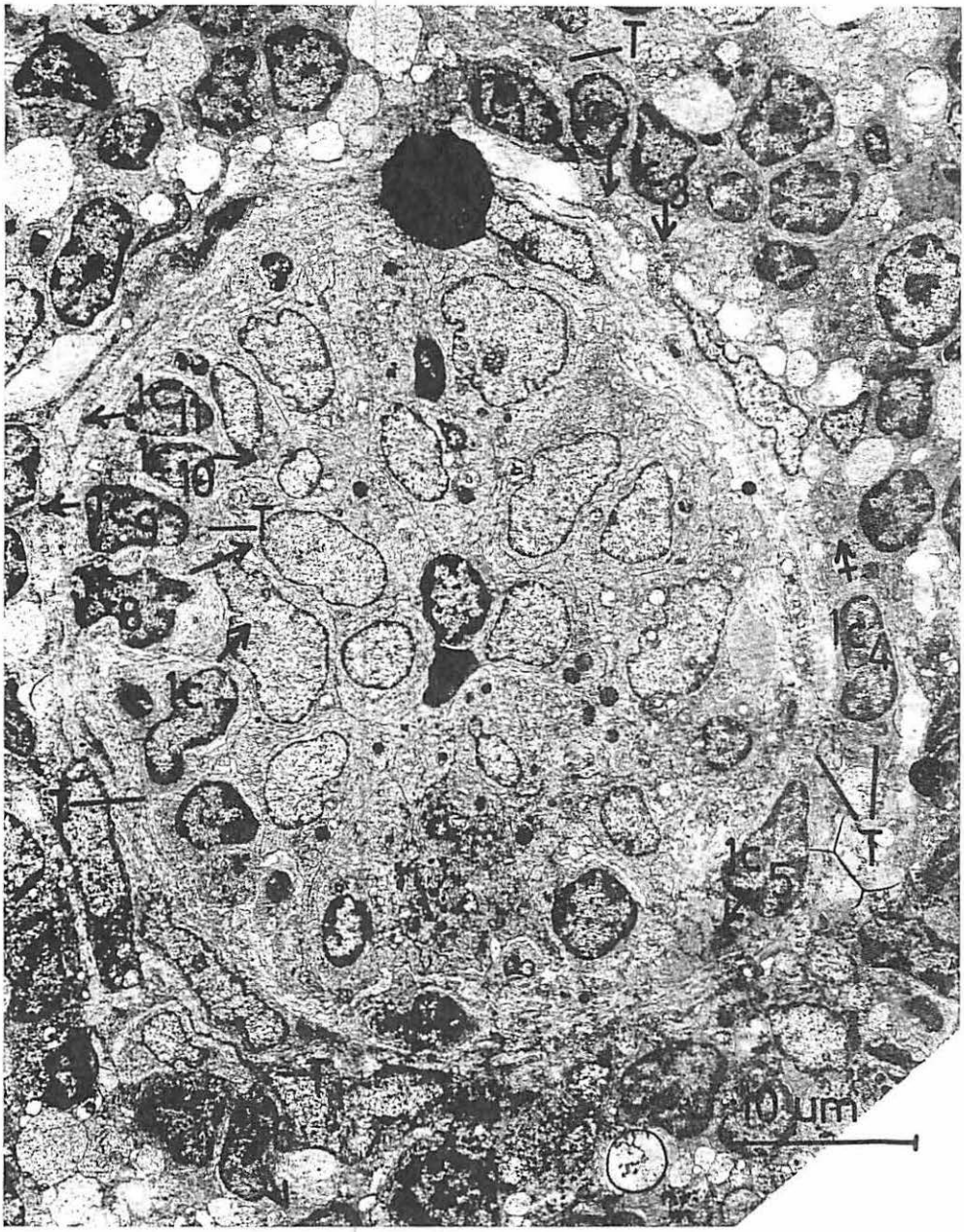

Fig. 1 Overview picture of a HE-venule from a rat lymph node with several lymphocytes infiltrating the endothelium. Lymphocytes $1c_{1-11}$ have the elongated shape suggestive of locomotion at the moment of fixation. Arrows indicate presumed direction of locomotion at the moment of fixation. Barred arrow: the direction of locomotion cannot be determined relative to the lumen(L) of the HE-venule. T: tail of the elongated lymphocyte. Magnification x 2,500.

towards the lumen of the HE-venules, and 36 elongated lymphocytes appeared to be directed towards the lymph node parenchyma ($p = 0.002$). In contrast, *Anderson & Anderson* (9) found that 10 elongated lymphocytes appeared to be lumen-directed and 127 elonga-

ted lymphocytes appeared to be parenchyma-directed ($p < 0.001$).

It is evident that the results available from direction analyses of lymphocytes fixed in the walls of HE-venules are conflicting. This conflict is not new. The HE-venules were discovered in 1898 by *Thomé* (10). The accumulation of migrating small lymphocytes in the walls of the HE-venules was noted by *Schumacher* in 1899 (11). Like *Schumacher,* subsequent investigators interpreted this picture as a migration of lymphocytes produced in the node into the blood stream (12, 13, 14). *Gowans* and co-workers proposed in 1964 that the HE-venules were the main site of lymphocyte recirculation from blood to lymph node and efferent lymph (15, 16). This hypothesis was questioned by Sainte-Marie and co-workers in a series of studies (17, 18, 19, 20, 21).

The "extranodal" arguments for lymphocyte recirculation over the walls of the HE-venules appear to be based on the recovery of labelled lymphocytes from the thoracic duct after infusion into venous blood, beginning with the study of *Gowans & Knight* in 1964 (15).

The "extranodal" arguments against the lymphocyte recirculation through the walls of the HE-venules appear to be based on the observation, that the venous blood from the lymph node contains more lymphocytes than arterial blood (11, 17, 18, 19).

The routes of lymphocyte recirculation could be regarded as a series of compartments with many parallel couplings (cf. 1). The information available on the circulation time of lymphocytes in an isolated compartments is often scanty. It is evident from this compartment concept, that the lymphocyte recirculation is a complex process, most variables of which are not controlled in an isolated experiment.

It should be emphasized, that morphological analyses of the direction of elongated lymphocytes within the walls of the HE-venules could at best be expected to provide information about the relative sizes of the migration streams of lymphocytes, not of the size of lymphocyte turnover between the blood and the lymph node; the area of the walls of the HE-venules and the average penetration time of the lymphocytes are not known.

From a morphological point of view, the walls of the HE-venules are thicker than the walls of blood capillaries (cf. 22, 23, 24), and would seem more like a lymphocyte trap than a lymphocyte expressway to the efferent lymph (cf. Fig. 1). In this context, it may be pertinent to remember that mitotic inhibition by anti-tubulins like colchicine was originally thought to be a mitotic stimulation, since metaphase arrest produced an increased number of mitoses in the preparations (25).

The available analyses of the direction of elongated lymphocytes within the walls of the HE-venules suggest that the migration stream of lymphocytes is bi-directional, as proposed by *Yoffey & Courtice* in 1970 (26). It is conceivable that the relative amounts of blood-directed lymphoytes and node-directed lymphocytes may reflect species variations, regional node variations, functional variations and, to some degree, the strength of the bias of the assessing scientist. It is thus desirable to have more analyses of lymphocyte direction within HE-venules from different nodes and with blindfold assessment of lymphocyte direction, i.e. the assessor is not allowed to know the position of the venule lumen, the endothelium and the lymph node parenchyma.

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B. Norberg, L. Rydgren, Department of Internal Medicine, University Hospital of Lund, S–221 85 Lund, Sweden

Discussion

Haljamäe: I think it was a very nice picture of moving lymphocytes. Do you have any idea of how they are moving in the tissues? Do you think they pass in between the meshwork of the fibrillous structures in a random type of movement or do they also have some kind of enzymatic activity with which they can affect the composition of the ground substance to make it easy for them to move through the interstitious spaces.

Norberg: The lymphocytes are able to move between cells, within tissues and, as shown in the movie, within fibrin clots. The external and internal variables which initiate and control lymphocyte locomotion are, however, almost unknown (5).

Ford: I cannot share your assumption that electromicroscopy can give any clue to which direction lymphocytes are moving. I think moving in vitro pictures and moving at the electromicroscopic appearance one simply cannot correlate them.

Norberg: I agree, that the lymphocyte gait may be modified in tissues, as compared with the lymphocyte gait within fibrin clots and within fibroblasts, which can be observed directly.

Engeset: Yoffey was, at least years ago, of the opinion that newly formed lymphocytes left the node mainly by the blood vessels. At that time we performed some experiments in rats to see if we

could prove this. We cannulated the vein of the lumbar node and a small vein of the abdominal wall and compared the lymphocyte number and morphology in blood samples from these two vessels for two hours. There was no significant difference. May I also mention experiments where others have induced a primary respons on one leg, ligated the efferent lymphatic of the regional node on that side and after some days injected the same antigen on the other leg. The regional node on this second leg also gave a primary immune response and not a secondary response. I think that two experiments indicate that few lymphocytes leave the node via the blood vessels. If node-directed lymphocytes meet some type of hinder between the endothelial cells, caused for instance by desmosomes, they will probably temporarely change direction and may appear as if they were blood-directed. I wonder if such mechanism might explain why so many appear blood directed by electronmicroscopy?

Norberg: The hypothesis that the relative numbers of blood-directed and node-directed lymphocytes reflect the sizes of the lymphocyte migration streams is based on the assumption that there is no one-way valve or chemotactic process which influences the drop-out of cells into the blood stream or into the lymph node. Previous authors reported that the number of lymphocytes which leave the lymph node by the venous blood stream exceeds the number of lymphocytes which enter the lymph node by the arterial blood stream (11, 17, 18, 19). How do these observations agree with the hypothesis of lymphocyte recirculation from blood to node through the walls of the HE-venules?

Ford: It is needless to say you cannot measure the flux of cells in a static section. You can only look on the concentration of cells.

Norberg: I agree, that a drop-off of marginated lymphocytes during preparation could explain the excess recovery of lymphocytes from the venous blood of the lymph nodes.

It seems to me, that in the absence of a one-way valve, a one-way trap or a chemotactic process, the proportion of blood-directed and node-directed lymphocytes will reflect the relative sizes of the migration streams. If the migration streams are of approximately the same size, the proportion of blood-directed lymphocytes and node-directed lymphocytes will reflect the sizes of the migration streams closely, since the errors involved tend to balance each other. If one of the migration streams is minute, the errors involved will favour the bi-directional hypothesis.

From a morphological point of view, it is more easy to imagine that the large-scale flux of lymphocytes in the lymph nodes is localized to the walls of the capillaries, which provide a wider and thinner surface than the walls of the HE-venules.