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Lymphocyte Locomotion III. Ultrastructural Studies of the Lymphocyte Traffic over the Postcapillary Venules of Rat Lymph Nodes

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Summary

The direction of lymphocytes with amoeboid movement configuration (AMC) was evaluated by means of electron microscopy of ultrathin sections of post-capillary high-endothelium venules (HE-venules) from rat lymph nodes. Out of 104 lymphocytes, 68 lymphocytes appeared to be on their way towards the lumen of the HE-venule and 36 lymphocytes appeared to be moving away from the venular lumen at the moment of fixation. This difference, which was statistically significant ($p = 0.0024$), is thought to reflect the relative size of the migration stream of lymphocytes at the moment of fixation.

Introduction

The post-capillary high-endothelium venules (HE-venules) of lymph nodes are distinguished by a heavy infiltration of lymphocytes, many with the elongated shape typical of lymphocytes in active locomotion at the moment of fixation. The interpretation of the lymphocyte infiltration in the HE-venules has been a matter of dispute (1); the lymphocytes have been thought to be on their way from the blood of the venular lumen into the lymph node parenchyma (2, 3) or from the lymph node parenchyma towards the lumen of the venule (4, 5, 6, 7).

The polarity of moving lymphocytes with the nucleus at the anterior end and the bulk of cytoplasm at the posterior end appeared to provide us with a possibility to analyse the migration streams of lymphocytes in the HE-venules (8, 9). In a previous study (10) we presented evidence from phase contrast observations on thin sections of rat lymph nodes that the main stream of lymphocytes (70%) was directed from the lymph node parenchyma into the HE-venules, and that the minor lymphocyte stream (30%) was directed out of the HE-venules towards the lymph node parenchyma.

Since the above-mentioned observation has wide implications for the interpretation of lymphocyte kinetics, it seemed desirable to make a complementary study by means of electron microscopy. The cell membrane and the cell organelles are better visible in electron microscopy, thus making the assessment of the direction of lymphocyte locomotion easier. The present ultrastructural study was limited to 4 rats from our previous phase contrast study in 14 rats (10), seeing that the laborious tissue analysis by electron microscopy makes a more extensive investigation unpracticable.

Material and Methods

Lymph node preparations from rats No. 1, 2, 4, 7 of a previous study (10) were subjected to the present ultrastructural study. The caudal parathymical glands were fixed in 4% glutaraldehyde, post-fixed in 1% OsO_4 and embedded in Epon. Ultrathin sections, approximately 0.5 μm thick, were cut with glass knives on an LKB Ultrotome and picked up on 150 mesh grids coated with

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Formvar, post-stained with uranyl acetate in 0.5% water solution and alkaline lead citrate on the grids. The sections were examined in a Zeiss EM 10 at the Zoological Institute of Lund. The microscope was calibrated by means of a grating replica.

All HE-venules encountered by one of us (LR) were first depicted at low magnification and all lymphocytes within the venular wall, in which elongated nuclei suggested locomotion at the moment of fixation were then studied at higher magnification, permitting the observation of cell membrane and cytoplasmic organelles. The probable direction of the individual lymphocytes was then estimated from the microphotos by BN. Lymphocytes were classified as probably moving *towards* or *away from* the venular lumen if they were located:

1. within the basement membrane or in close touch with it
2. inside the membrane within or between the endothelial cells
3. outside the basement membrane in its close vicinity, i.e. approximately with the center of the nucleus within a distance of 10 μm from the external facies of the basement membrane.

The direction of the above-defined lymphocytes was estimated relative to the radius from the lumen (centre) of the HE-venules (Fig. 1).

Statistics. The binominal test with correction for continuity, according to *Siegel* 1956 (11).

Observations

The present assessment of direction of lymphocyte locomotion at the moment of fixation in ultrathin sections of rat lymph nodes was based on criteria presented in our previous studies on lymphocyte locomotion in vital preparations (8, 9). Lymphocytes move in vitro by equatorial and longitudinal contractions, co-ordinated in contraction cycles. The contraction cycle starts with the herniation of a small thin granula-free pseudopodium (lamellipodium) in the anterior end. The nucleus is squeezed into the lamellipodium by contractions in the membrane-associated cytoplasm layer. The forward movement of the nucleus within the elongated cell body is followed by forward movement of cytoplasmic granula (mitochondria, endoplasmatic reticulum, centrioles). The tail shortens, and a new contraction cycle is marked by the development of a new lamellipodium.

It follows from the above-mentioned observations that the lamellipodium, when present, always marks the direction which the lymphocyte is about to take, that the end of the elongated nucleus which is closest to the pole points out the direction of lymphocyte locomotion at the moment of fixation, that the bulk of granulated cytoplasm is located in the posterior part of the cell.

In the present study the probable direction of all lymphocytes with the elongated shape typical of locomotion (AMC) and a location in the venular wall as already described were recorded. The direction was defined as *towards* or *away from* the venular lumen relative to the radius of the venule (Fig. 1). We

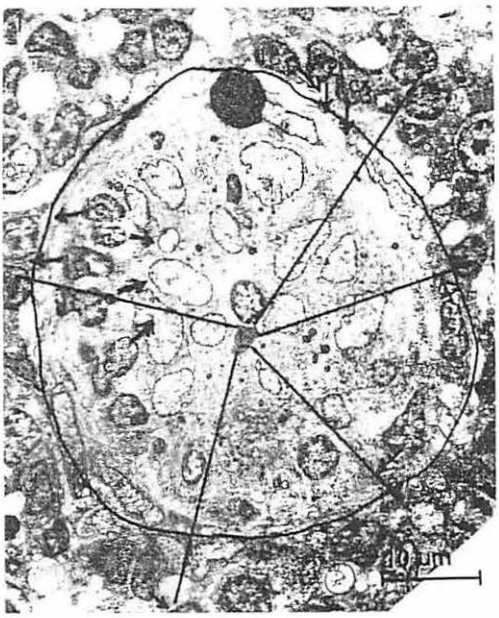

Fig. 1 The estimation of the direction of elongated lymphocytes, presumably fixed during locomotion, in ultrathin sections of lymph nodes. The radius was drawn from the lumen (centre), L, of the post-capillary high-endothelium venule (HE-venule). The direction of elongated lymphocytes was assessed relative to the mentioned radius.

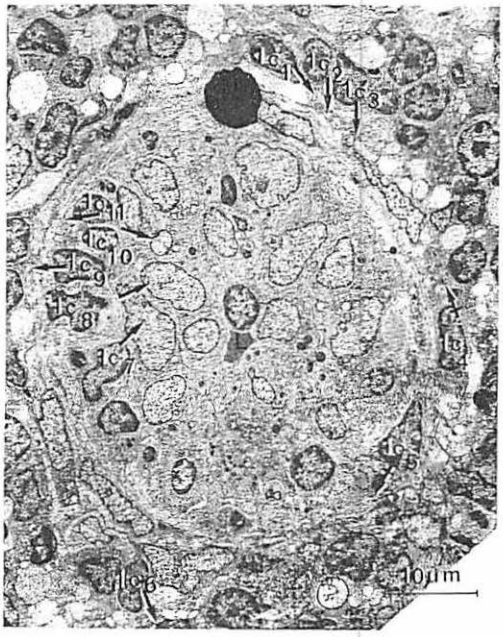

Fig. 2 Overview picture of a HE-venule from rat No. 4. In the endothelium of the venule or in the vicinity of the periendothelial sheath of the venule, there are seen 11 lymphocytes ($1c_{1-11}$) with the elongated shape suggestive of locomotion at the moment of fixation. L: lumen of the HE-venule. Arrows indicate presumed direction of locomotion at the moment of fixation. Barred arrow: the direction of locomotion cannot be determined relative to the periendothelial sheath or relative to the lumen of the HE-venule. Further comments, see "Observations". Magnification x 1.250.

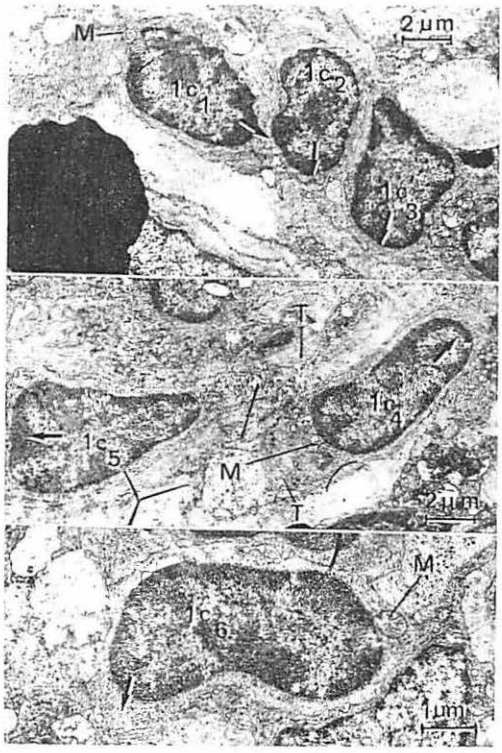

Fig. 3 Higher magnification of lymphocytes $1c_{1-6}$ from Fig. 2.
3A Lymphocyte $1c_1$ was classified as an immigrant towards the HE-venule due to the direction of the long axis of the nucleus and the presence of several mitochondria (M) in the pole distal to the HE-venule. The bulk of cytoplasm was thought to mark the posterior pole of $1c_2$, which thus was classified as an immigrant according to the direction of the long axis of the nucleus. The blunt end of the nucleus of $1c_3$ was thought to mark the direction of locomotion at the moment of fixation. Magnification x 3.650.
3B The hand-mirror shape of $1c_5$ is evident. The mitochondria (M) of the anuclear pole (T = tail) of $1c_4$ and $1c_5$ are now visible. Magnification x 6.800.
3C Lymphocyte $1c_6$ is symmetrical. The mitochondrium (M) suggests the posterior pole of this cell. Magnification x 14.800.

are fully aware of the exacting and apparently arbitrary character of this estimation and find it necessary to exemplify our considerations related to concrete directional problems as visualized in Figures 2–4.

The lymphocyte $1c_1$ was classified as immigrant towards the HE-venule due to the diection of the long axis of the nucleus relative to the radius from the lumen of the HE-venule and the presence of several mitochondria in the distal pole (Figs. 1, 2, 3A). Lymphocyte $1c_2$ was classified as immigrant due to the direction of the long axis of the nucleus and the bulk of cytoplasm indicating the posterior pole (Figs. 2, 3A). The blunt end of the pear-shaped nucleus of $1c_3$ was thought to mark the direction of locomotion at the moment of fixation (Figs. 2, 3A).

The direction of $1c_{4,5}$, perpendicular to the radius from the venular lumen, was indicated by the bulk of granulated cytoplasm in the anuclear pole, which contained several mitochondria. Both nucleus and cytoplasm of $1c_5$ had the handmirror shape typical of moving lymphocytes (Figs. 2, 3B).

Table 1 The lymph node material analysed by electron microscopy in the present study (cf. Figs. 1-4). Lymphocytes with amoeboid movement configuration (AMC) apparently fixed during movement *out of* a post-capillary HE-venule (emigrants) towards the lymph node parenchyma and lymphocytes apparently fixed during movement *into* a post-capillary HE-venule (immigrants) from the lymph node parenchyma. Examined preparations: number of lymph node preparations with sections containing HE-venules and elongated lymphocytes, the direction of which could be assessed. Rat number refers to a previous phase contrast study (10).

Rat	Examined preparations	Lymphocytes with AMC	
		Emigrants	Immigrants
1	4	25	37
2	5	6	11
4	1	2	8
7	5	3	12
Sum	14	36	68

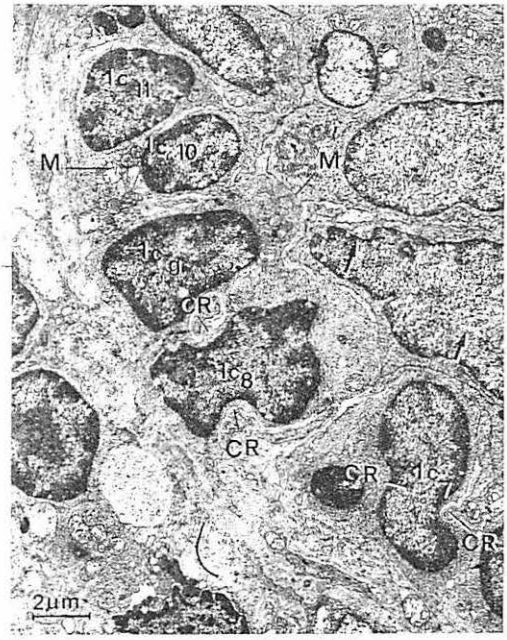

Fig. 4 Higher magnification of lymphocytes $1c_{7\text{-}11}$ allows better identification of mitochondira (M) and cell membranes. CR: contraction ring, a typical feature of wandering lymphocytes. Further comments, see "Observations". Magnification x 7.100.

Lymphocyte $1c_6$ had a symmetrical shape of both nucleus and cytoplasm. The mitochondrium was thought to mark the posterior pole, and the $1c_6$ was accordingly classified as an emigrant (Figs. 2, 3C). Lymphocytes $1c_{7,8}$ were classified as immigrants towards the lumen of the HE-venule due to the hand-mirror shape of nucleus and cytoplasm (Figs. 2, 4). The bulk of granulated cytoplasm with numerous mitochondria marked the posterior pole of $1c_{9,10,11}$ (Figs. 2, 4).

In the total material of the present study, the direction of 104 lymphocytes was determined relative to the radius from the lumen (centre) of the HE-venules (Table 1). The lymphocytes which appeared to be directed *towards* the venular lumen at the moment of fixation were approximately twice as many as the lymphocytes which appeared to be directed *away from* the venular lumen, 68:36 (Table 1). This difference was significant ($p = 0.0024$).

Discussion

In the present ultrastructural study, 68 lymphocytes with amoeboid movement configuration (AMC) appeared to be directed towards the lumen of the HE-venules of rat lymph nodes. Only 36 lymphocytes with AMC appeared to be directed from the venular lumen towards the lymph node parenchyma. The ratio between "immigrants" and "emigrants", approximately 2:1, was in agreement with a previous analysis by means of phase contrast microscopy (10), despite slight differences in the assessment of direction of locomotion with the basement membrane (10) and the radius from the venular lumen as respective points of reference.

The present study is meant as a contribution to the discussion on the direction of lymphocyte traffic between lymph node parenchyma and blood through the walls of the HE-venules. The results support the impression gained by us during several years of attention to these curious vessels, that the passage of lymphocytes through the basement membrane is bi-directional and that a considerably fraction of the migrants are leaving the parenchyma. The idea of bi-directional lymphocyte traffic over the HE-venules was proposed by *Yoffey* and *Courtice* 1970 (1) and

supported by the observations of *Sainte-Marie* et al. 1975 (6) on the migration of labelled lymphocytes in dog lympho nodes.

It should be remembered, however, that the main stream of lymphocytes might well be imagined to change direction in different functional conditions. From a morphological point of view the HE-venules look like relative obstacles to the lymphocyte traffic, causing an accumulation of cells. For obvious reasons this accumulation is easier to detect inside the basement membrane than in the perivenular lymphocyte cuff. The capillaries of the lymph node parenchyma may represent a less conspicuous but more important site for lymphocyte migration (cf. 12), which is still poorly investigated.

In summary, the present studies support the general conclusions of *Sainte-Marie* and co-workers (4, 5, 6, 7), based on other methods and observations, that there is reason to question the current concept of lymphocyte re-circulation from blood to lymph nodes mainly via the HE-venules. The phenomenon of lymphocyte lodging in the endothelium of HE-venules may reflect other lymphocyte functions, e.g. recording of antigeneic information in the tissue fluid which returns to the blood stream of the HE-venules.

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