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Lymphocyte Locomotion
II. The lymphocyte Traffic over the Post-Capillary Venules Analysed by Phase Contrast Microscopy of Thin Sections of Rat Lymph Nodes

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Summary

Thin sections of lymph nodes from 14 rats were examined by phase contrast microscopy as regards direction of lymphocytes with amoeboid movement configuration (AMC) relative to the basement membrane of post-capillary high-endothelium venules (HE-venules). Out of 118 lymphocytes with AMC, 82 appeared to be on their way into the venule from the lymph node parenchyma. This observation suggests that the lymphocyte traffic over the HE-venules is bi-directional, with the main migratory stream of lymphocytes from the lymph node parenchyma into the post-capillary venules.

Introduction

It was proposed by *Gowans* and co-workers (1, 2) that some lymphocytes recirculate from blood to lymph nodes through the high endothelium of the post-capillary venules (HE-venules) of the lymph nodes. This hypothesis was challenged by *Sainte-Marie* and co-workers (3, 4, 5), who provided evidence for afferent lymphatic entry of lymphocytes into the lymph nodes of rat (6) and dog (5) and, in addition, obtained more lymphocytes from the lymph node vein than from the lymph node artery in rats (3). It is also conceivable that there may be two migration streams of lymphocytes over the endothelium of the post-capillary venules of lymph nodes, as pointed out by *Yoffey and Courtice* 1970 (7).

Hitherto, methods have been lacking to assess the directional flow of lymphocytes over the post-capillary venules. The distinct polarity of wandering lymphocytes appears, however, to provide a tool for the mentioned analysis (8, 9). The aim of the present study is to assess the direction of lymphocytes with *amoeboid movement configuration* (AMC) in thin sections of HE-venules

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Table 1 The direction of locomotion at the moment of fixation of lymphocytes in the endothelium of post-capillary venules (HE-venules) of lymph nodes from 14 rats. Thin sections examined by phase contrast microscopy. TB = toluidine blue. MGP = methyl-green pyronine. SEC = sheep erythrocytes

Rat	Treatment	Stain	Embedding	Examined HE-venules	Lymphocytes In	Out
1	Non-treated control	TB	Epon	33	32	11
2	"	"	"	7	2	0
3	"	"	"	19	15	7
4	1,9 ml 0,2% SEC i.p. 6 h before killing	"	"	2	1	0
5	"	"	"	11	8	7
6	"	"	"	10	1	2
7	As 4, but 16 h before killing	"	"	25	17	4
8	Non-treated control	"	"	1	0	0
9	0,2 ml typhus vaccine i.p. 1, 4, 22 and 29 days before killing	MGP	Paraplast	7	1	3
10	"	"	Epon	3	1	0
11	1 ml ferritin in tail 3, 14, 21 and 28 days before killing	"	"	4	2	0
12	"	"	"	2	1	0
13	"	"	"	2	1	0
14	"	"	"	2	0	2
14				125	82	36

from rat lymph nodes. It is assumed that the numbers of lymphocytes caught during movement *into* the HE-venules and *out of* the HE-venules, respectively, will reflect the size of the migration stream(s).

Material and Methods

The basic material is summarized in Table 1. Lymph node preparations were drawn from the material of the laboratory and consisted of lymph nodes from 14 rats with or without preceding antigenic stimulation. In rats 1–7 the caudal parathymic glands were examined, in rats 8–14 abdominal and mesenterial glands were examined. The lymph nodes were fixed in 4% glutaraldehyde, post-fixed in 1% OsO_4 and embedded in Epon or Paraplast (rat no. 9). Thin sections, appr. 1 μm, were cut with an LKB Ultrothome, stained and examined in a Zeiss Photomicroscope with phase contrast equipment at 400x magnification. All encountered HE-venules were photographed and the microphotographs were later examined and the direction of lymphocytes with amoeboid movement configuration was assessed relative to the basement membrane of the post-capillary venules.

Chemicals and solutions. Glutaraldehyde, Epon and Paraplast were obtained from Grave AB, Solna Sweden, toluidine blue from Merck, Darmstadt, BRD, methyl green from Fluka AG, Buchs Switzerland, and pyronin from Chroma Gesellschaft, Stuttgart-Untertürkheim, BRD. The other chemicals used were of analytical grade. Double distilled water was used for the preparation of solutions.

Statistics. The binominal test with correction for continuity, according to *Siegel* 1956 (10).

Observations

The post-capillary HE-venules are distinguished by their high endothelium, an often split basement membrane and an accumulation of lymphocytes to the venule endothelium. A large pro-

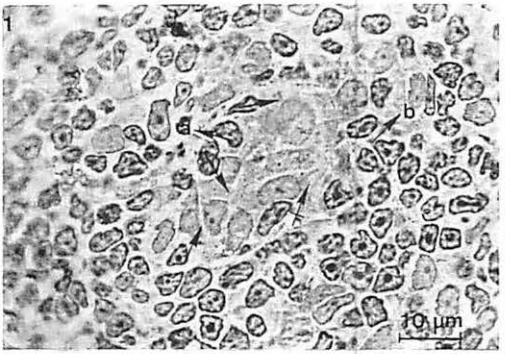

Fig. 1 A post-capillary high-endothelium venule with infiltrating lymphocytes. Arrows indicate presumed direction of locomotion of elongated lymphocytes at the moment of fixation. The polarized position of the nucleus and the elongated cell shape with granulated cytoplasm at the anuclear cell pole suggests that lymphocytes (a) and (b) were leaving the HE-venule at the moment of fixation. Barred arrow indicates that the direction of locomotion could not be classified as out of or into the HE-venule. From rat no. 1. Basic magnification x200.

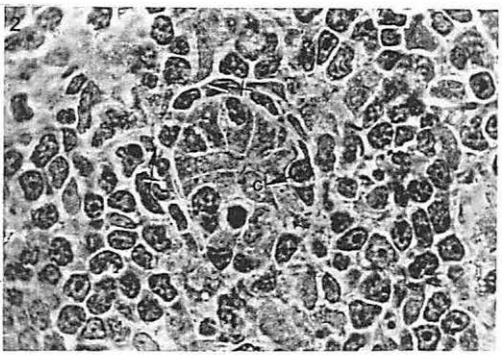

Fig. 2 HE-venule from rat no. 1 with infiltrating lymphocytes. Lymphocyte (c) has the classical hand-mirror shape of a moving lymphocyte. It appears to be bending towards the lumen of the venule after sliding along the basement membrane. Basic magnification x200.

portion of the lymphocytes within the venular endothelium have the elongated shape typical of cells fixed during locomotion (Figs. 1–5).

During examination of thin sections of rat lymph nodes, we found lymphocytes with *amoeboid movement configuration* (AMC), some without any reasonable doubt caught on their way into the venule (Figs. 2c, 3d, 5e), others apparently caught on their way out of the vein into the lymph node parenchyma (Figs. 1a, 1b). The direction of many lymphocytes with AMC could not be determined due to oblique sectioning or due to sliding of the lymphocytes along the periendothelial sheath (Figs. 1–5).

In order to get more quantitative information about the flow of lymphocytes into and out of the post-capillary venules, we examined thin sections of lymph nodes from 14 rats, photographed the HE-venules encountered and determined the direction of elongated lymphocytes within the level of the section relative to the basement membrane of the HE-venules by means of the morphological criteria of the direction of lymphocyte locomotion described in two previous studies (8, 9). It was assumed that a random orientation of moving lymphocytes would produce approximately equal numbers of apparent in-movers and apparent out-movers ($P = Q = 1/2$). It was further assumed that the elongated lymphocytes caught in the examined thin sections represented a random sample from the HE-venules of rat lymph nodes.

The basement membrane was chosen as the reference point in the present study: only lymphocytes close to this structure were classified as directed out of or into the HE-venule, i.e. the lymphocytes with the distant pole maximally one lymphocyte length (approximately 10 μm) from the basement membrane. The direction of locomotion could be assessed in 118 lymphocytes. The quotient between in-movers and out-movers was approximately 2:1; 82 lymphocytes with AMC were classified as in-movers and 36 as out-movers. This difference was highly significant ($p < 0.001$).

Discussion

Gowans and co-workers (1,2) concluded from experiments with labelled lymphocytes that the

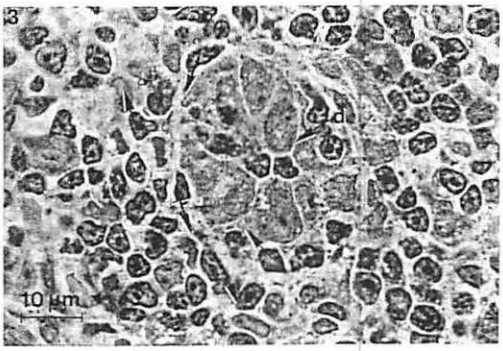

Fig. 3 HE-venule from rat no. 1 with infiltrating lymphocytes. Another lymphocyte (d) with the hand-mirror shape typical of moving lymphocytes. It appears to be turning from the basement membrane towards the lumen of the venule. Basic magnification x200.

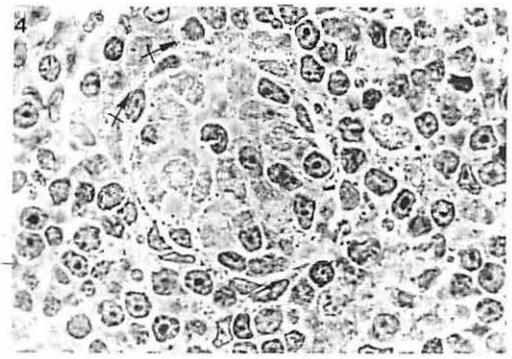

Fig. 4 HE-venule from rat no. 1 with lymphocytes sliding along the basement membrane. Basic magnification x200.

flow of lymphocytes over the post-capillary venules was likely to be unidirectional, from the lumen of the venule into the lymph node parenchyma. The technically complicated evidence for this point war, however, in our opinion mainly inferential.

Saint-Marie and co-workers (3) concluded from cell counts in the lymph node vein and in arterial blood that the main migration stream of lymphocytes over the post-capillary venules was from the lymph node parenchyma into the blood stream of the venule. Although the simple experimental design of *Sainte-Marie* et al. (3) seems to make their results unquestionable as regards the net flow of lymphocytes, no unequivocal evidence was provided regarding the place und process of lymphocyte immigration from the lymph node parenchyma into the venous blood stream. In recent studies, *Sainte-Marie* and co-workers have provided evidence that a considerable number of labelled lymphocytes transfused into the blood stream of dogs (5) or injected into the mediastinum of the rat (6) arrive at the lymph nodes via the afferent lymphatics and the marginal sinuses.

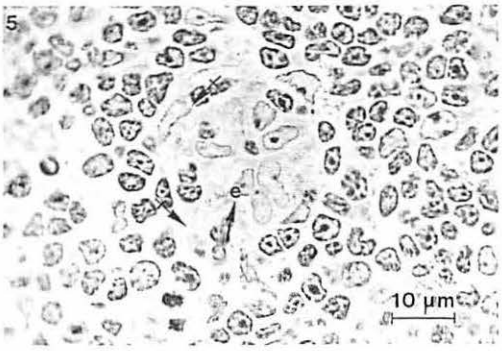

Fig. 5 HE-venule from rat no. 3. Lymphocyte (e) appears to be penetrating into the venule. Basic magnification x200.

The important observation in the present study is that the lymphocyte traffic over the HE-venules appears to be bi-directional; lymphocytes with AMC are found to be directed into the venule as well as out of the venule. The net flow of lymphocytes is, however, from the lymph node parenchyma into the HE-venules, provided that the numbers of observed lymphocytes with AMC reflect the migration streams. It is reasonable to assume that a net input of cells into the HE-venules from the lymph node parenchyma will produce a spill-over of lymphocytes into the venous blood stream. This conclusion is in agreement with the finding of *Sainte-Marie* et al. (3) that lymphocytes are more numerous in the blood from the lymph node vein than in arterial blood.

The present observations support the hypothesis of *Yoffey and Courtice* (7) that there are at least two migration streams of lymphocytes in the endothelium of the post-capillary HE-venules. The quantitative dominance of the cell stream from the lymph node parenchyma into the HE-venules may have a bearing on the interpretation of data on lymphocyte circulation, recirculation and kinetics.

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