Lymphology 9 (1976) 89–96

Lymphocyte Locomotion
I. The Initiation, Velocity, Pattern, and Path of Locomotion In Vitro

L. Rydgren, B. Norberg, C.H. Håkansson*, C. v. Mecklenburg*, N. Söderström

Department of Internal Medicine, University Hospital, Lund, Sweden
* Department of Radiology, University Hospital of Lund, Lund, Sweden

Summary

The locomotive behaviour of human lymphocytes in coverslip preparations of clotted autoplasma was studied at +37°C. Lymphocytes isolated from peripheral blood or from the tonsils did not move prior to membrane activation by means of incubation with phytohemagglutinin (PHA). After PHA stimulation the locomotion of 19 lymphocytes was analysed by time-lapse filming. The locomotion was random, as evidenced by a median locomotive index of 0.64 (Q_1–Q_3 0.40–0.75) and comparatively slow, median velocity 15 μm/min (Q_1–Q_3 12–18). The locomotion of 5 other lymphocytes was studied at high magnification. It is suggested that the characteristic polarity of wandering lymphocytes, indicating the direction of movement, can be utilized in the analysis of the lymphocyte traffic in tissue sections of post-capillary high-endothelium venules.

Introduction

In a previous study (1), we presented evidence that lymphocytes fixed during locomotion retain features indicative of locomotion at the moment of fixation and even indicative of direction of locomotion at the moment of fixation and we suggested that this observation could be utilized in the analysis of short-distance lymphocyte migration.

One region of obvious interest for such an analysis is the post-capillary high-endothelium venules (HE-venules) of the lymph nodes, where the lymphocytes have been thought to recirculate from blood to lymph, or from lymph node to blood, or, maybe, in both directions (for references see e.g. 2). Prior to the analysis of migrating lymphocytes in the endothelium of HE-venules, a closer investigation of the locomotive behaviour of a sample of individual lymphocytes seemed desirable. It was the aim of the present study to define features of lymphocyte locomotion, which are relevant for the analysis of the lymphocyte traffic in tissue sections of lymph nodes.

Material and Methods

Tonsil biopsies.
Fine-needle biopsies from tonsils were performed on 2 healthy medical students with a Franzén instrumentarium as described by *Söderström* 1966 (3). Coverslip preparations sealed with vaseline were made and the preparations were immediately put under the microscope at +37°C for examination and time-lapse microcinematography by means of a Wild Photomicroscope with phase contrast equipment (Table 1).

Tonsilectomies.
Studies were also made on coverslip preparations of imprints from surgically removed tonsils from 4 patients with hyperplasia of the tonsils (Table 1). Immediately after excision, the tonsils were immersed in 0.9% NaCl at room temperature. After washing, the imprints were made from fresh surfaces and treated and examined as previously mentioned.

* This study was supported by the Grant B 76-19X-2294-09A from the Swedish State Board for Medical Research (to N. Söderström and B. Norberg) and grants from the Medical Faculty of Lund.

Table 1 The lymphocyte preparations studied. Several coverslip preparations from each donor were examined. The coverslip preparations from leukocyte-rich plasma contained lymphocytes and granulocytes, but only the granulocytes moved. IF-lymphocytes = lymphocytes isolated by the Isopaque-Ficoll method described by *Böyum* 1968 (4). PHA = phytohaemagglutinin P (Difco) 0.5 µl/ml.

Donor	Preparation	Moving lymphocytes
Male 13 yrs	Tonsilectomy, immediate microscopy	0
? ?	" "	0
Female 15 yrs	" "	0
Male 49 yrs	" "	0
Female 25 yrs	Tonsil, fine-needle aspiration biopsy	0
Male 25 yrs	" "	0
Nine healthy donors, 20–30 yrs	Leukocyte-rich plasma immediate microscopy	0
Female 25 yrs	IF-lymphocytes from leukocyte-rich plasma, immediate microscopy	0
Male 23 yrs	" "	0
Female 25 yrs	IF-lymphocytes from leukocyte-rich plasma, incubated with PHA	11
Male 23 yrs	" "	2
Female 20 yrs	" "	6

Isolation of mononucleated leukocytes from peripheral blood.
Venous blood, five 10 ml glass tubes with heparin to a final concentration of 16 IU/ml, was obtained from the antecubital fossa of 3 healthy donors by means of Vacutainer®. To each tube was added 1 ml of a solution containing 0.15 M l-histidine hydrochloride and 5% w/v Dextran T 250. The content of each tube was then mixed with an equal volume of 2% w/v Dextran T 500 in 0.9% NaCl, and allowed to sedimentate in long plastic tubes at an angle of 45° for 30 min. at +37°C. Lymphocyte preparations were obtained from the leukocyte-rich supernatant by separation on an Isopaque-Ficoll column, as described by *Böyum* 1968 (4), washed 3 times with 0.9% NaCl and resuspended in 0.5 ml autologous plasma. Differential counts of smears stained according to May-Grünwald-Giemsa showed that the isolated cell suspension contained 3–4% granulocytes and 80–96% small lymphocytes. The other cells encountered were large lymphocytes and monocytes. In order to induce locomotion, the lymphocyte suspension was incubated with phytohaemagglutinin P (Difco) in a concentration of 0.5 µl PHA-P/ml cell suspension, at +37°C. Observations on moving cells were performed after 6, 24, 48 or 72 hours (Table 2). A droplet of cell suspension was clotted with thrombine in a coverslip preparation and individual lymphocytes were followed by 30 frames/min. in a Wild Photomicroscope with phase contrast and time-lapse equipment, basic magnification x40. The film was projected on a paper and the trajectories of 19 individual lymphocytes were depicted (Fig. 2).

Analysis of lymphocyte locomotion at a higher basic magnification, x400, was performed by means of a Zeiss Photomicroscope equipped with phase contrast, flash illumination and heated slide. The temperature of the heated slide was adjusted to +38°C to compensate for the heat loss due to the open system. The depth of the visual field was approximately 0.4 µm. The optical section of the cell was adjusted to the plane of the anterior lamellipodium. For time-lapse analysis of non-purified leukocyte preparations the leukocyte-rich supernatant from 9 healthy donors was centrifuged and a droplet of the sediment was clotted between slide and coverslip.

Chemicals and solutions.
Dextran T 250, Dextran T 500 and the Ficoll solution were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden), chromatographically homogenous l-histidine hydrochloride from British Drug House Chemicals Ltd. (London), and bovine thrombine from Parke-Davis (Detroit,

Table 2 The moving lymphocytes studied by means of time-lapse filming. PHA = phytohaemagglutinin P (Difco) 0.5 µl/ml Q_1–Q_3 = interquartile range.

Donor	Cell	Observation time (secs)	Length of path (um)	Velocity (um/min)	Incubation with PHA (hrs)
1	1	450	112.5	15.0	48
	2	310	130.0	25.2	48
	3	430	92.5	12.8	48
	4	140	27.5	11.8	48
	5	380	130.0	20.5	48
	6	510	140.0	16.5	48
	7	460	140.0	18.3	72
	8	460	120.0	12.0	72
	9	190	45.0	14.2	72
	10	180	30.0	10.0	72
	11	190	35.0	11.0	72
2	12	240	45.0	11.2	24
	13	90	27.5	18.3	48
3	14	140	30.0	12.9	6
	15	220	67.5	18.4	6
	16	120	30.0	15.0	6
	17	80	20.0	15.0	6
	18	160	20.0	7.5	6
	19	190	20.0	7.5	6
Median		190		15.0	
Q_1–Q_3		140–430		11.8–18.3	
Extreme values		80–510		7.5–25.2	

Mich.). The dextran solutions were kept at -18°C, the other solutions at +4°C until just prior to each experiment.
The Isopaque solution was obtained from Nyegaard & Co. (Oslo, Norway).

The Isopaque and Ficoll solutions were mixed according to *Böyum* 1968 (4) and were kept sterile in the dark at +4°C until just prior to each experiment.

Bacto-Phytohaemagglutinin P was provided by Difco Laboratories (Detroit, Mich.).

The other chemicals used were of analytical grade. Double distilled water was used for the preparation of solutions. The glassware was washed with bichromate-H_2SO_4 and rinsed in double distilled water. The 50 ml plastic tubes were soaked in 50 ml Pyroneg overnight, rinsed in distilled water, 95% ethanol and double distilled water. The other plastic material was soaked in ethanol for 30 min. and rinsed in double distilled water.

Statistical methods.
The Mann-Whitney U test according to *Siegel* 1956 (5).

Observations

It is obvious from Table 1 that lymphocytes with spontaneous motility or the cell shape suggestive of motility – *amoeboid movement configuration* (AMC) – were extremely rare in fresh coverslip preparations from excised tonsils, fine-needle aspiration biopsies from the tonsils, leukocyte-rich autoplasma clots and Isopaque-Ficoll-isolated lymphocytes without previous membrane stimulation. In preparations from leukocyte-rich plasma the locomotive behaviour of the lymphocytes thus contrasted with that of the polymorphonuclear leukocytes (PMNs), which showed an intense and recurrent motility in fresh coverslip preparations of the present type (Tables 1, 3).

Table 3 The locomotive index (straight distance/travelled distance) of wandering leukocytes. IF-cells = cells separated by the Isopaque-Ficoll method. Non-purified leukocytes were obtained from the sediment of leukocyte-rich plasma. Q_1–Q_3 = interquartile range.

Cell type	Donors	Cells	Locomotive index Median	Q_1–Q_3
IF-lymphocytes without obvious attractant	3	19	0.64	0.40–0.75
Non-purified PMNs without obvious attractant	9	129	0.63	0.50–0.75
Non-purified PMNs during accidental chemotaxis	1	21	0.89	0.76–0.98

When the lymphocyte membranes were activated by means of PHA stimulation for 6–72 hrs some of the lymphocytes moved. The velocity of 19 lymphocytes analysed by means of the time-lapse equipment was 15 μm/min (median value) with an interquartile range of 12–18 μm per min (Table 2). The path of the lymphocyte locomotion was tortuous, as evidenced by microphotographic mapping (Fig. 1A) and by the trajectories (Fig. 2).

The lymphocyte locomotion appeared to be random, i.e. no accumulation of cells around an attractant or goal could be observed or otherwise defined. The locomotive indices – the quotient straight distance/actually travelled distance of the IF-lymphocytes – were low (Table 3), on the same level as the locomotive indices of 129 non-purified PMNs without obvious attractant ($p < 0.05$) and lower than the locomotive indices of 21 non-purified PMNs during accidental chemotaxis ($p < 0.001$). The behaviour of the PMNs was analysed in detail in another study (6). The present observations thus suggest that the lymphocyte locomotion was random under the prevailing conditions.

It should be emphasized that the direction of the lymphocyte was always marked by the protrusion of a granula-free anterior lamellipodium at the beginning of a new contraction cycle (Fig. 1 B-P). Then the nucleus advanced into the lamellipodium. The granulated tail contracted, a pseudopodium protruded, and a new contraction cycle started. Sometimes a lamellipodium was withdrawn and a new lamellipodium developed in another direction (Fig. 1 F-G, I-L). In the absence of one definite lamellipodium, the tail, or better, the long axis of the nucleus, marked the direction of lymphocyte locomotion at the moment of observation.

To sum up, the present observations confirmed previous observations on moving lymphocytes regarding the anterior position of the nucleus, which is preceded by a granula-free, short, thin, anterior lamellipodium and followed by a long, granulated tail containing the main part of the cytoplasm.

Discussion

The locomotive behaviour of 24 lymphocytes from 3 healthy donors is reported. Under the prevailing conditions, coverslip preparations of clotted autoplasma, +37°C, it was never difficult to tell the direction of moving lymphocytes at any moment – the position of the nucleus in the anterior end of the cell, the small granula-free lamellipodium in the front end and the bulk of granulated cytoplasm in the tail marked the direction of locomotion. Our observations on lymphocyte locomotion are in close agreement with the observations reported by *Lewis and Webster* 1921 (7), *McCutcheon* 1924 (8), *Lewis* 1931 (9), and *DeBruyn* 1945 (10).

In a previous study one occasional lymphocyte from a fresh coverslip preparation of peripheral blood was caught in locomotion (1). The present material shown in Table 1, which represents hundreds of thousands of examined cells, indicates that locomotion is extremely rare in human

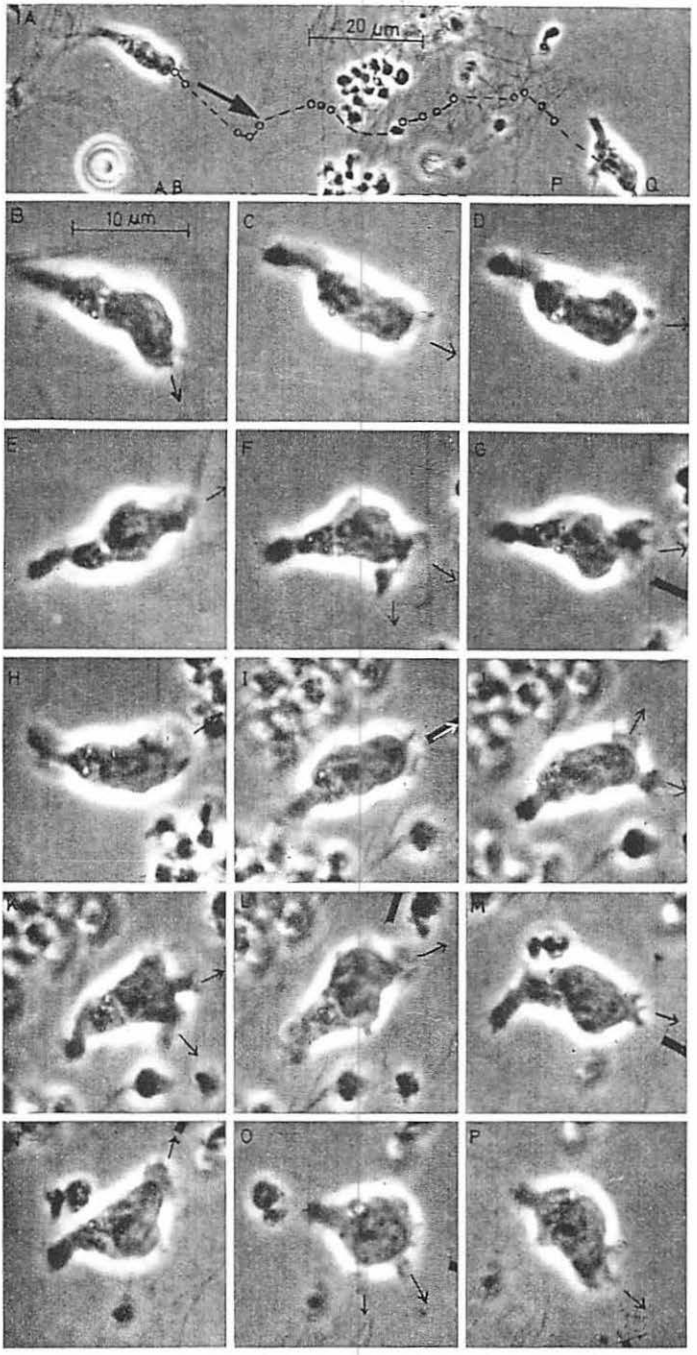

Fig. 1 A. A Ficoll-separated lymphocyte from peripheral blood which had been incubated with PHA-P for 6 hours. It was wandering in an autologous plasma clot in a coverslip preparation at 37°C. Basic magnification 400x. The first and the last picture in the series are put together to show the path. Circles mark the point of the anterior pseudopod of the cell in the positions B-P. The observation time can be approximated from the known velocity of lymphocytes, under the prevailing conditions (Table 2), to 6–7 minutes.
B-P The same lymphocyte in the different positions marked in A. Arrows indicate the direction of movement suggested by the anterior pseudopod. Further comments, see „Results".

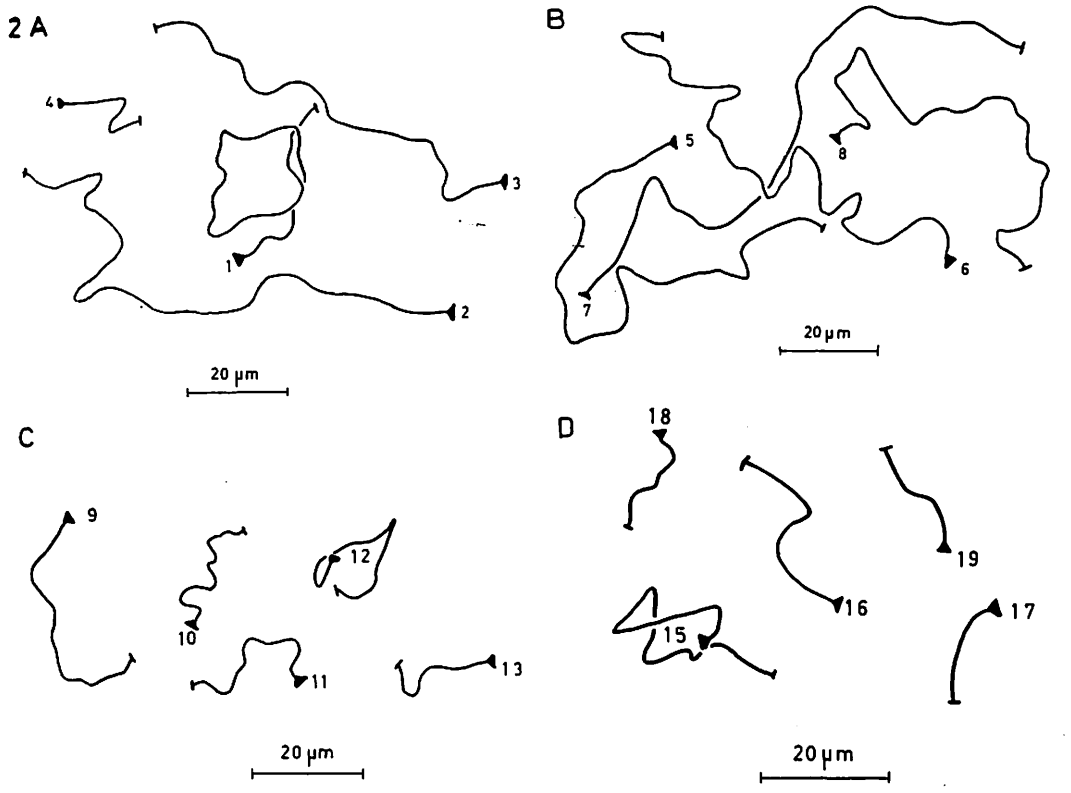

Fig. 2 A-D Trajectories of the 19 wandering lymphocytes (Table 2). The locomotive indices suggested that the locomotion of these lymphocytes was random (Table 3). ▶ starting point ▮ end point.

lymphocytes prepared directly from leukocyte-rich plasma and mounted in a fibrin coagulum between slide and coverslip. This observation applies also to human lymphocytes from tonsil preparations.

After membrane stimulation by incubation with PHA, some lymphocytes displayed active locomotion (Table 2). This observation is in agreement with the findings of previous authors that membrane stimulation by PHA (11), anti-immunoglobulin (12), macrophages and isologous lymphocytes (13) induced active locomotion or at least the hand-mirror shape associated with active locomotion in lymphocytes. Membrane receptors and cytoplasmic enzymes are reported to be confined to the posterior part of the lymphocytes (11, 14). During interaction with fixed complementary receptors, the lymphocyte translocation is reported to be inhibited by adherence for varying periods of time (13, 15, 16, 17); during tail adhesion little or no translocation is performed but the cell may retain the hand-mirror shape.

The hypothesis of membrane stimulation as a trigger of lymphocyte locomotion has interesting corollaries. The lack of active locomotion in fresh preparations of lymphocytes from peripheral blood and fresh tonsil preparations can then be interpreted as an absence of membrane stimulation of these lymphocytes. The finding of numerous lymphocytes with amoeboid movement configuration in sections of lymphoid thyroiditis (18), in the HE-venules of lymph node sections from man and rat (1, 19) and in bone marrow smears from healthy donors (20) can in analogy be interpreted as a presence of membrane stimulation of these lymphocytes.

Leukocyte locomotion is thought to be due to a complex interaction between the suspension fluid of the cells with signal substances (cf. 21), the substrate of cell locomotion (15, 17), the cell membrane (21, 22) and the contractile machinery of the cell. The contractile machinery of the leukocytes is thought to be localized in close association to the cell membrane and composed of actin-like and myosin-like proteins (23, 24).

The difference in locomotive behaviour of PMNs and lymphocytes are obvious from the present study. PMNs display an intense and recurrent motility immediately after isolation from peripheral blood, the lymphocytes of the same preparations do not. This discrepancy is in agreement with previous observations that PMN chemotaxis is membrane-mediated (21, 22) and can be induced by several attractants (6, 21, 22), while the attractant of directional lymphocyte locomotion in unknown (25). The present observations are consistent with the idea that the observed differences in initiation and direction of locomotion between PMNs and lymphocytes reflect differences in basic conditions of membrane activation.

Despite the rapidly varying morphology of moving lymphocytes, the basic "hand-mirror" shape was remarkably constant (Fig. 1). It seems reasonable to conclude that the distinct polarity of the moving lymphocytes provides a solid basis for the analysis of the lymphocyte traffic between HE-venules and lymph node parenchyma.

References

1 *Norberg, B., L. Rydgren, N. Söderström:* Lymphocyte locomotion. Morphological criteria of the direction of lymphocyte movement. Lymphology 6 (1973) 187–191

2 *Yoffey, J.M., F.C. Courtice:* Lymphatics, lymph and the lymphomyeloid complex, p. 525. Academic Press, London 1970

3 *Söderström, N.:* Fine-needle aspiration biopsy used as a direct adjunct in clinical diagnostic work, p. 81. Almqvist and Wiksell, Stockholm 1966

4 *Böyum, A.:* Isolation of mononuclear cells and granulocytes from human blood. Scand. J. clin. lab. Invest. Suppl. 97 (1968) 77–89

5 *Siegel, S.:* Nonparametric statistics for the behavioral sciences, p. 116. McGraw-Hill, London 1956

6 *Rydgren, L., G. Simmingsköld, U. Bandmann, B. Norberg:* The role of cytoplasmic microtubules in polymorphonuclear leukocyte chemotaxis. Evidence for the release hypothesis by means of time-lapse analysis of PMN movement relative to dot-like attractants. Exp. Cell Res. 99 (1976) 207–220

7 *Lewis, W.H., L.T. Webster:* Migration of lymphocytes in plasma cultures of human lymph nodes. J. exp. Med. 33 (1921) 261–269

8 *McCutcheon, M.:* Studies on the locomotion of leukocytes. III. The rate of locomotion of human lymphocytes in vitro. Amer. J. Physiol. 69 (1924) 279–282

9 *Lewis, W.H.:* The locomotion of lymphocytes. Bull. Johns Hopk. Hosp. 49 (1931) 29–36

10 *De Bruyn, P.P.H.:* The motion of the migrating cells in tissue cultures of lymph nodes. Anat. Rec. 93 (1945) 295–315

11 *Biberfeld, P.:* Uropod formation in phytohaemagglutinin (PHA) stimulated lymphocytes. Exp. Cell Res. 66 (1971) 433–445

12 *Unanue, E.R., K.A. Ault, M.J. Karnovsky:* Ligand-induced movement of lymphocyte membrane macromolecules. IV. Stimulation of cell movement by anti-Ig and lack of relationship to capping. J. exp. Med. 139 (1974) 295–312

13 *McFarland, W., D.H. Heilman, J.F. Moorhead:* Functional anatomy of the lymphocyte in immunological reactions in vitro. J. exp. Med. 124 (1966) 851 to 858

14 *Smith, C.W., J.C. Hollers:* The pattern of binding of fluoresceinlabelled concanavalin A to the motile lymphocyte. J. reticuloendothelial Soc. 8 (1970) 458–464

15 *Smith, C.W., A.S. Goldman:* Interaction of lymphocytes and macrophages from human colostrum: Characteristics of the interacting lymphocyte. J. reticuloendothelial Soc. 8 (1970) 91–104

16 *Rutishauser, U., I. Yahana, G.M. Edelman:* Morphology, motility, and surface behaviour of lymphocytes bound to nylon fibers: Proc. Nat. Acad. Sci. USA 71 (1974) 1149–1153

17 *Unanue, E.R., M.J. Karnovsky:* Ligand-induced movement of lymphocyte membrane macromolecules. V. Capping, cell movement, and microtubular function in normal and lectin-treated lymphocytes. J. exp. Med. 140 (1974) 1207–1220

18 *Söderström, N., A. Björklund:* Organization of the invading lymphoid tissue in human lymphoid thyroiditis. Scand. J. Immunol. 3 (1974) 295–301

19 *Rydgren, L., B. Norberg, C. v. Mecklenburg, C.H. Håkansson, N. Söderström:* Lymphocyte locomotion. III. Ultrastructural studies of the lymphocyte traffic over the post-capillary venules of rat lymph nodes. Lymphology, submitted for publication

20 *Norberg, B., L. Rydgren, M. Stenstam:* Amoeboid movement configuration. A cell configuration observed in tumour cells from 3 cases of bone marrow neoplasia. Scand. J. Haematol. 13 (1974) 294–304

21 *Gallin, J.L., J.R. Durocher, A.P. Kaplan:* Interaction of leukocyte chemotactic factors with the cell surface. I. Chemotactic factor-induced changes in human granulocyte surface charge. J. Clin. Invest. 55 (1975) 967–974
22 *Keller, H.V., M. Bessis:* Migration and chemotaxis of anucleate cytoplasmic leukocyte fragments. Nature (London) 258 (1975) 723–724
23 *Norberg, B.:* Amoeboid movements and cytoplasmic fragmentation of glycerinated leukocytes induced by ATP. Exp. Cell Res. 59 (1970) 11–21
24 *Senda, N., H. Tamura, N. Shibata, J. Yoshitaka, K. Kondo, K. Tanaka:* The mechanism of the movement of leukocytes. Exp. Cell Res. 91 (1975) 393–407
25 *Ward, P.A., C.D. Offen, J.R. Montgomery:* Chemoattractants of leukocytes, with special reference to lymphocytes. Fed. Proc. 30 (1971) 1721–1724

Dr. L. Rydgren, Department of Internal Medicine, University Hospital, S-221 85 Lund/Sweden

Lymphology 9 (1976) 96–100

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

L. Rydgren, B. Norberg, C. v. Mecklenburg*, C.-H. Håkansson*, N. Söderström

Department of Internal Medicine, University Hospital, Lund, Sweden
*Department of Radiology, University Hospital, Lund, Sweden

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

* This study was supported by the Grant B 76-19X-2294-09A from the Swedish State Board for Medical Research (to N. Söderström and B. Norberg) and grants from the Medical Faculty of Lund.