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Lymphocyte Locomotion

Morphological Criteria of the Direction of Lymphocyte Movement

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Summary

Moving lymphocytes leave the round configuration of the resting cell and demonstrate cyclic elongations and shortenings. In fixed preparations it is, thus, possible to identify lymphocytes killed during active locomotion. The direction of lymphocyte movement can often be inferred from the following features: the hyaline pseudopod, the granulated tail, the position and the configuration of the nucleus.

Introduction

Lymphocytes, many of them at least, are motile cells; studies of the destination of tagged cells reflect the *long distance* migrations of lymphocytes carried by circulating blood and lymph. Within the tissues they move actively, evidently aiming at local targets which are of fundamental interest, though difficult to define.

In histologic and ultrastructural studies, lymphocytes are now and then caught during their passage through blood and lymph vessels. If the direction of their movement could be determined, details of current hypotheses on lymphocyte kinetics could be tested. This possibility has, however, been denied by several authors (1, 2, 3, 4).

In contrast to the authors mentioned, we believe that some features of moving lymphocytes could indicate their direction; this idea is based on some observations of our own (5, 6) and of previous authors (7, 8, 9).

The scope of the present work was to study living lymphocytes, try to define the features that reveal the direction of movement and seek these features in dead lymphocytes of sections and smears.

Material and Methods

Vital lymphocytes were studied in cell cultures and in coverslip preparations of blood and bone marrow.. The cell cultures of human adenoid tissue and pleural exsudate were grown in Rose chambers and the emperipolesis of lymphocytes was recorded by means of microcinematography at +37°C. Blood and bone marrow preparations from healthy volonteers or from patients without known blood disease were obtained in two ways:

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1. A droplet of bone marrow or capillary blood was allowed to coagulate between slide and coverslip, the preparation was sealed with vaseline and observed in time-lapse microcinematography at +37°C with a basic magnification of 80 x or 160 x.

2. The erythrocytes of heparinized blood (10 IU/ml) were allowed to sedimentate, the leukocyte-rich supernatant was removed, the leukocytes were spun down at 175 g for 10 min., the sediment was resuspended in 0.5 ml of plasma, a droplet of this suspension was spread between slide and coverslip, coagulated with thrombin and then treated as previously described.

The cell in Fig. 1 a, b was studied with a Zeiss Photomicroscope using phase contrast equipment at a basic magnification of 400 x, at room temperature (+24°C). The lymphocyte movements apparently retained their general character at this temperature.

For histologic and ultrastructural studies, thin slices of lymph nodes were immersed in 2% glutaraldehyde and processed as specified in the legends of Figs. 1 c, 2.

Results

The main features of lymphocyte movement have been described by previous authors (7, 8, 9). Anybody working with living lymphocytes cultured *in vitro* soon becomes familiar with the curious morphology of the moving lymphocyte, labelled the "hand-mirror" shape, with the handle at the rear (Fig. 1). The cell body moves *in toto*, headed by the nucleus, which appears to be squeezed in one direction by contractions in the superficial layer of the cytoplasm, sometimes discernible as distinct contraction rings (CR, Fig. 1 a) in a plane perpendicular to the direction of movement. Nuclear sap is thereby often pressed forward to form a more or less clear bulging bleb (Fig. 1 b). A small hyaline pseudopod is often seen ahead of the nucleus but the main part of the cytoplasm is usually found at the rear (the "handle").

During direct observation, the wandering lymphocytes often changed their direction of movement. Such a change always started with the formation of a small expanding pseudopod in the new direction. Then the nucleus advanced through the contraction ring towards the stem of the cell. Since the contraction ring regularly was formed at the base of the pseudopod, this ring acquired a position just anterior to the angle of the bent cell body (Fig. 1 a). The lymphocytes were not observed to move "backwards", i.e. in the direction of the hand-mirror handle. The position of the nucleus was always in the middle or anterior part of the cell body.

In thin sections, the direction of dead lymphocytes could be assessed only when the cells were sectioned approximately in the plane of movement. In Fig. 1 c a lymphocyte is caught during its passage through the basement membrane of a postcapillary venule. The direction of this lymphocyte into the venule is evidenced by the hand-mirror form, the streamer configuration of the chromatine, the nuclear sap and the thin brim of intraluminal cytoplasm around that part of the nucleus.

In electron micrographs, the streamer configuration in the posterior part of the nucleus and the nuclear sap in the anterior part of the nucleus are often the most prominent signs of the direction of movement (Fig. 2).

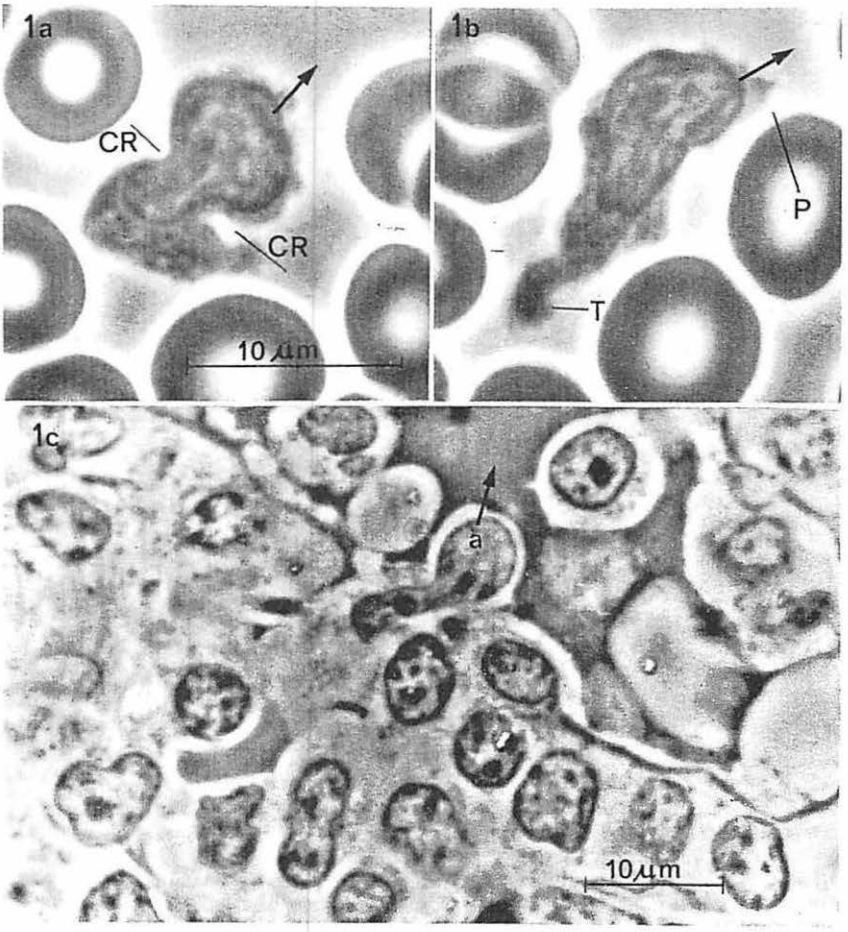

Fig. 1. Phase contrast observations on a vital lymphocyte (Fig. 1 a, b). Capillary blood was allowed to coagulate between slide and coverslip. The preparation was sealed with vaseline and the cell was observed for 80 min at room temperature (+24 °C), during several contraction cycles.
In Fig. 1 a (21 min) the nucleus has passed through the contraction ring (CR) into the anterior part of the cytoplasm. The posterior part of the nucleus is still compressed by the contraction ring, which gives the nucleus a pear-shaped configuration with the blunt end pointing in the direction of locomotion (arrow).
In Fig. 1 b (37 min) the same lymphocyte is seen with a granula-free anterior pseudopod (p) and granulated cytoplasm at the tail part (T). The blunt end of the pear-shaped nucleus indicates the direction of movement (arrow)
The vital lymphocyte of Fig. 1 a, b should be compared with the cells of the thin section depicted in Fig. 1 c (human lymph node, biopsy specimen, glutaraldehyde/osmium tetroxide/May-Grünwald-Giemsa, phase contrast microscopy). A lymphocyte is passing through the basement membrane into the lumen of a venule, as evidenced by the position and configuration of the nucleus.

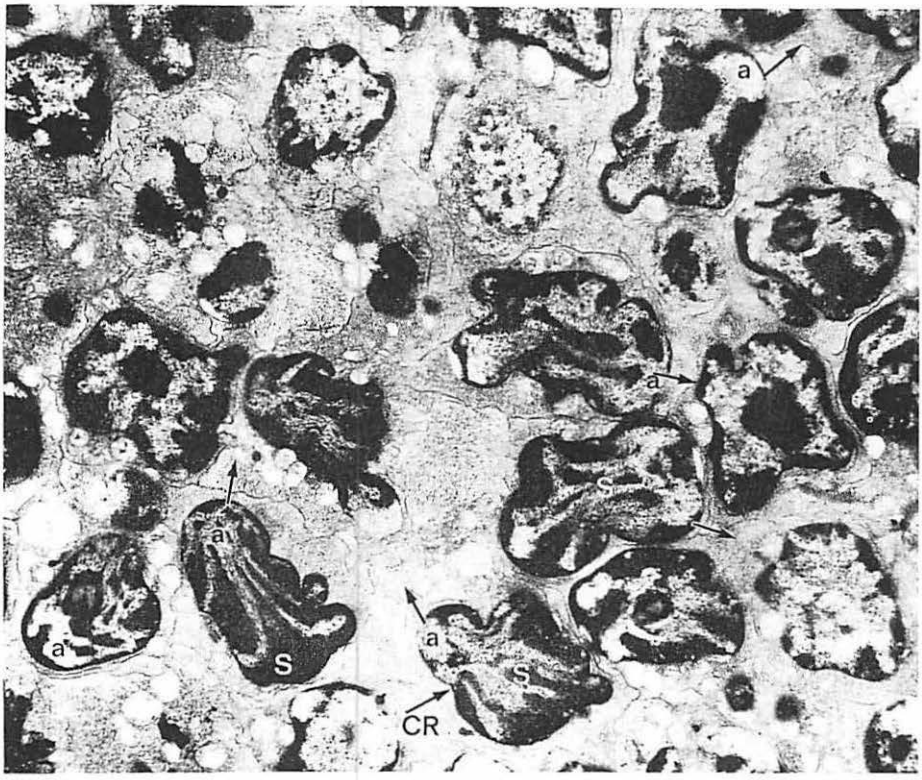

Fig. 2. Rat spleen, detail from the marginal zone of a Malpighian body. Nearly all lymphocytes present pictures of active movement. Thick arrows mark the direction of the movement where this can be assessed.
a. The nuclear "bulging bleb" marking the direction of the movement.
s. Chromatine "streamers" marking the rear portion of the nucleus.
CR contraction ring, more or less visible also in other nuclei.
Glutaraldehyde/osmium tetroxide/lead acetate, EM micrograph, 3600x.

Discussion

We suggest in this communication that the morphology in the dead lymphocytes in a section may allow the conclusion that many lymphocytes were killed in active motion and that even the direction of this movement may be assessed, at least if this direction was approximately in the plane of the section. This idea has previously been proposed by *Schoefl* (10) but has not been worked out in detail.

Sometimes moving cells retain their configuration of movement even in air-dried smears of needle aspirates. We have recently observed such a case, classified as acute myeloblastic leukemia; approximately 40% of the malignant cells had the configuration of active movement in bone marrow smears. Time-lapse microcinematography on coverslip preparations verified that the motile-looking cells actually moved, at a maximum velocity of 18-24 μm/min, but with a gait much like lymphocyte movement.

From observations in living lymphocytes we consider that the granule-free anterior pseudopod and the elongated tail part of the granulated cytoplasm at the rear are the most reliable land-marks of the direction of movement (Fig. 1 a, b). The position of the nucleus may also suggest the direction of movement; it us usually located in the middle or anterior part of the cell. The streamer configuration often marks the posterior end of the nucleus, the nuclear sap the anterior end. From the features mentioned, it is often possible to infer the direction of lymphocyte movement with a reasonable degree of reliability.

Contraction rings are perpendicular to the direction of movement (11) but do not themselves indicate the stem or the rear of the cell. The formation and position of streamer configuration of the chromatine seems to be due to the deformation of the nucleus by the contraction ring during the passive transport of the nucleus within the cell.

We have become interested in directional signs during a study of the direction of lymphocytes moving through the basement membrane of postcapillary venules in lymph nodes or through the walls of the marginal sinus in the spleen. We feel justified in drawing attention to this type of still picture of the movement of lymphocytes at the moment of death as a way of mapping out the short distance migration of lymphocytes through lymphatic tissue, a very important sector in the natural history of migrating lymphocytes which has hitherto escaped analysis.

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