Lymphocyte Locomotion and Attachment on Two-dimensional Surfaces and in Three-dimensional Matrices

WENDY S. HASTON, JAMES M. SHIELDS, and PETER C. WILKINSON
Department of Bacteriology and Immunology, University of Glasgow, Glasgow G11 6NT, Scotland

ABSTRACT   The adhesion and locomotion of mouse peripheral lymph node lymphocytes on 2-D protein-coated substrata and in 3-D matrices were compared. Lymphocytes did not adhere to, or migrate on, 2-D substrata such as serum- or fibronectin-coated glass. They did attach to and migrate in hydrated 3-D collagen lattices. When the collagen was dehydrated to form a 2-D surface, lymphocyte attachment to it was reduced. We propose that lymphocytes, which are poorly adhesive, are able to attach to and migrate in 3-D matrices by a nonadhesive mechanism such as the extension and expansion of pseudopodia through gaps in the matrix, which could provide purchase for movement in the absence of discrete intermolecular adhesions. This was supported by studies using serum-coated micropore filters, since lymphocytes attached to and migrated into filters with pore sizes large enough (3 or 8 μm) to allow pseudopod penetration but did not attach to filters made of an identical material (cellulose esters) but of narrow pore size (0.22 or 0.45 μm). Cinematographic studies of lymphocyte locomotion in collagen gels were also consistent with the above hypothesis, since lymphocytes showed a more variable morphology than is typically seen on plane surfaces, with formation of many small pseudopodia expanded to give a marked constriction between the cell and the pseudopod. These extensions often remained fixed with respect to the environment as the lymphocyte moved away from or past them. This suggests that the pseudopodia were inserted into gaps in the gel matrix and acted as anchorage points for locomotion.

Though lymphocytes are motile, they adhere and move poorly on protein-coated glass or plastic under conditions in which fibroblasts, macrophages, and neutrophils adhere and move well. In consequence, generalizations about cell locomotion obtained from studies of the latter cells do not fit the more puzzling locomotor behavior of lymphocytes, a knowledge of which would help us to understand how lymphocytes recirculate, cooperate with each other and with other cell types, and infiltrate and migrate through extravascular sites.

Cells moving on 2-D surfaces must make close enough contact with the substratum to provide traction for locomotion. In fibroblasts, it has been postulated that locomotion takes place because the cells form areas of contact named focal adhesions (1) where there is cross-bridging of the space between cell and substratum with an intermediate protein, fibronectin (27), which allows traction to be generated. Focal adhesions are not seen in faster-moving cells such as neutrophil leukocytes, but these cells do form rapidly shifting areas of close contact while moving on protein-coated substrata (3).

In early studies of lymphocyte movement, the polarized morphology was described, with (from front to back) a leading hyaline veil, a constriction ring, the cell body with the nucleus well forward, and the tail or uropod (8, 15, 16). This morphology was described as amoeboid, and comparisons with the locomotor behavior of amoebae were made. These lymphocytes were usually studied within the 3-D matrix of a fibrin clot (8, 18) but sometimes apparently on flat surfaces (5). Later work in which lymphocyte locomotion was studied in relation to other, larger cell types growing in culture, for example fibroblasts (12, 19) or lymph node reticular cells (14), showed that, whereas lymphocytes on the bare substratum hardly moved at all, lymphocytes that penetrated between the cultured cells and the substratum were highly motile.

The requirement for close adhesion may be much less critical for cells moving in 3-D environments, as described by earlier workers, than for cells moving on plane surfaces. It is the major aim of this paper to explore this hypothesis. We compare the adhesion and locomotion of lymphocytes on 2-D substrata and

in 3-D matrices. These experiments lead us to suggest that lymphocytes may gain sufficient traction for locomotion within a 3-D framework by a pushing and pulling mechanism without making and breaking close, adhesive contacts.

We show that lymphocytes attach in larger numbers to 3-D collagen matrices than to 2-D collagen films. From this, it does not follow that lymphocytes are more adhesive to 3-D than to 2-D collagen, and, throughout this article, we use the word "attachment" to be distinguished from the word "adhesion," which implies particular molecular interactions between two surfaces.

MATERIALS AND METHODS

Materials

4- to 16-wk-old CBA mice from departmental stocks were used throughout.

Human serum albumin (HSA) was obtained from Behringwerke, Marburg, FRG. Hanks' balanced salt solution (HBSS), RPMI 1640, newborn calf serum, and fetal calf serum (FCS) were purchased from Flow, Irvine, Scotland. HEPES and morpholinopropane sulphonate (MOPS) were obtained from Sigma Chemical Co. (St. Louis, MO). Casein and denatured HSA were prepared as described by Wilkinson (24) and Wilkinson and Allan (25). Na^{51}Cr was obtained from Radiochemical Centre, Amersham, Buckinghamshire, England.

Labeling of cells and subsequent assays were performed in RPMI 1640 supplemented with 20 mM HEPES, 1% glutamine, and 100 µg/ml penicillin/streptomycin. For all other procedures, HBSS buffered with 10 mM MOPS, pH 7.2 was used.

Methods

Small lymphocytes were obtained by gently teasing apart the brachial, axillary, and inguinal lymph nodes of CBA mice and preparing a cell suspension by standard methods.

Cells were labelled with Na^{51}Cr at 50 µCi per 5 × 10^7 cells/ml in RPMI 1640 + 10% FCS for 30 min at 37°C. Cells were washed three times to remove excess unbound label.

Bovine fibronectin was purified by affinity chromatography as described by Yamada and Olden (27). The preparations of fibronectin were tested for their ability to promote adhesion and spreading of baby hamster kidney (BHK) fibroblasts and polyoma-transformed BHK fibroblasts (our own unpublished data with Dr. J. Dysart and Dr. J. Edwards, Department of Cell Biology, Glasgow University) over the concentration ranges used in our experiments. Purity of the specimens was tested by PAGE (by J. Gilmour, Department of Bacteriology and Immunology, and Dr. J. Dysart, Department of Cell Biology) and showed two major bands of 220–240 kdaltons.

Type 1 collagen was extracted from rat tail tendons as described by Elsdale and Bard (10). Purity of the collagen was tested with PAGE (J. Gilmour) and showed that no fibronectin or other major impurities were detectable.

For preparation of substrata, Decon-cleaned (Decon Laboratories Ltd., Brighton, England) 13-mm cover slips were incubated in 0.5 ml of coating solution at 37°C for 30 min in Repli dishes (Sterilin Ltd., Teddington, England). Cover slips were washed by dipping through beakers of HBSS and placed in 0.5 ml of RPMI 1640 in Repli dishes. A detailed description of the treatment of the collagen coats is provided in Results.

Three-dimensional hydrated collagen lattices were prepared as described by Elsdale and Bard (10), who found that aqueous collagen that is a sol at acid pH will form a gel as the pH is increased to 7.0 and the osmolarity is adjusted to physiological strength. 2-ml aliquots of collagen were adjusted as described (10), pipetted into 3-mm petri dishes, and gels were allowed to set for 1 h at 37°C. For ^{51}Cr experiments, where many replicates were required, gels were cast in wells in tissue culture trays by adding 0.5 ml of collagen solutions.

Cellulose ester filters of various pore sizes were obtained from Millipore Co., Bedford, MA. Filters of 13-mm diameter were punched out and were coated with protein in the same way as the cover slips described above.

Confusion can arise from using the word "adhesion" to describe what may be several methods by which cells attach to a substratum. There is no direct method available to estimate cell-substratum adhesion and, in fact, the assay used in this paper is properly described as a distraction assay (21, 23), in that it measures the number of cells remaining attached after the cover slip is washed by a standard procedure. A major disadvantage is that weak adhesions may be broken by the shear forces introduced by the washing procedure. In spite of this, comparisons can be made between variously modified substrata (21, 23). The method was used as follows: 3 × 10^6 cells in 1 ml of RPMI 1640 were pipetted onto the cover slips or filters in Repli dishes, or on top of the collagen gels. These were incubated

at 37°C (or 4°C when stated) for 90 min. At the end of the incubation period, the samples were washed by dipping through an air/medium interface 15 times in a standardized fashion (21) and either placed directly in tubes for counting of gamma emission (for ^{51}Cr-labeled cells) or fixed in 2.5% glutaraldehyde in HBSS and stained with methyl-green pyronin for visual counting. Filters were fixed in 70% alcohol and stained, dehydrated, cleared, and mounted as previously described (24). ^{51}Cr assays were performed in quadruplicate. Visual studies were done in triplicate with six fields counted in each replicate under × 400 magnification.

To avoid the problem of shear force in this method, lymphocytes were also allowed to settle onto coated cover slips forming the bottom of a chamber. After 90-min incubation, the chamber was sealed and inverted, allowing the unattached cells to fall. The attached lymphocytes were then counted. No difference in results was found between this method and the previous one using shear force to remove unattached lymphocytes, and so for ease of handling and reproducibility the former method was employed.

For the assay of invasion of collagen gels by lymphocytes, 1.5 × 10^7 cells in RPMI 1640 + 10% FCS were allowed to attach to and infiltrate collagen gels for 1–24 h. Gels were fixed for 1 h in 2.5% glutaraldehyde in HBSS. The distance that the leading two cells had migrated into the gel was measured using the calibrated micrometer of the inverted microscope under × 200 magnification. Six randomly chosen fields on duplicate gels were counted.

Lymphocytes were filmed with a Nikon phase-contrast inverted microscope with an attached 16 mm movie camera using 10× and 20× objectives at 1 frame/4 s on Kodak Plus-X reversal film. Films were analyzed with a stop-action cine-projector that projected the film sequences onto drawing paper, thus allowing frame-by-frame analysis. Additional information was obtained by using high-powered (40× objective) phase-contrast and differential interference contrast optics. Still photographs were printed from time-lapse films.

RESULTS

2-D Substrata

We began by investigating lymphocyte adhesion to the surfaces of glass cover slips using both visual and distraction assays. The substrata were modified by coating with a variety of proteins known to influence locomotion and adhesion of other cell types. Results for these modified substrata were compared with those for clean glass, which will bind a substantial number of lymphocytes. Lymphocytes failed almost completely to attach to cover slips coated with serum, fibronectin (shown in Fig. 1), casein, or denatured HSA. Time-lapse cinematography of lymphocytes that had settled on the substrata showed that they were unable to move on them.

3-D Substrata

COLLAGEN: Collagen can easily be made to form 3-D gels in vitro (10, 20). The gels can be air-dried to form a flat 2-D surface of collagen fibers that can, in turn, be rehydrated to regain their 3-D structure (9).

Five collagen substrata were used that should be chemically identical but topographically different. These were:

(a) Wet collagen coats. The collagen that coats clean glass rehydrates to form a 3-D gel upon immersion in physiological medium.
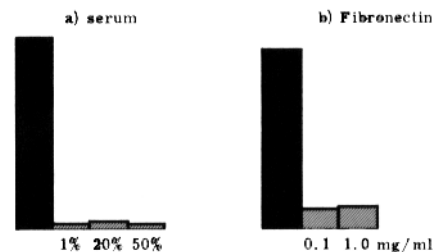

FIGURE 1   Lymphocyte adhesion to serum- and fibronectin-coated glass cover slips expressed as the percentage radioactivity associated with the coated cover slips compared with that associated with clean glass, which is taken as 100% (black columns).

(b) Dried collagen coats. As for *a* but air-dried to form a 2-D surface after rehydration to a gel (as described in *a*).

(c) As for *b* but rehydrated again by immersing the air-dried coat in 2% acetic acid followed by physiological medium.

(d) As for *c* but dried again after rehydration.

(e) Hydrated collagen gels cast in tissue culture dishes.

We also prepared coats from collagen heated to 80°C for 30 min, since heating destroys its capacity to form a gel. Lymphocyte attachment was measured using distraction assays with ^{51}Cr-labeled lymphocytes and by visual inspection.

Lymphocytes were not easily distracted from wet coats of collagen (a 3-D substratum) but they did not attach well to collagen coats that had been air-dried and thus made 2-D (Fig. 2, columns *C* and *DC* at 37°C). The numbers attaching were significantly increased when dried coats were rehydrated into 3-D gels. This was not a result of collagen dissolving and leaving bare glass because, when the process was repeated (i.e., the rehydrated gels were re-dried), the number of lymphocytes attaching was again reduced. Lymphocyte attachment to coats prepared from heated collagen was much lower than that to hydrated collagen (wet coats) although fibers of the heated collagen were clearly visible on staining the cover slips, indicating that heating had not converted the collagen to gelatin. Attachment to hydrated coats was much lower at 4°C than at 37°C, indicating that it required an active cell metabolism and was not the result of a passive trapping by the gel surface. Visual counting of lymphocytes attached to hydrated and air-

dried collagen gels on cover slips confirmed the results obtained by using ^{51}Cr-labeled lymphocytes and shown in Fig. 2.

To study the ability of lymphocytes to attach to and subsequently migrate into collagen gels, and its relation to collagen concentration, gels of different concentration were cast in tissue culture dishes. Lymphocyte attachment to the gel surface was measured both visually (data not shown) and by removing the whole washed gel from the dish and counting the total number of ^{51}Cr-labeled cells that had attached to and migrated into the gel. Lymphocytes attached and moved into collagen gels (Table I) at 37°C but this was reduced at 4°C and at room temperature, again indicating that passive entrapment was not responsible for the attachment.

Lymphocytes actively invaded collagen gels prepared over the limited concentration range that we found workable. The presence of serum had no effect on either attachment to or locomotion of the cells in collagen gels (Table I). Fig. 3 is a

TABLE I
Lymphocytes on Collagen Gels

Temperature	Concentration of collagen in gel, mg/ml			
	0.5	1.0	2.0	3.0
4°C	2.15	3.4	2.6	1.9
22°C	2.3	4.8	3.9	4.0
37°C + FCS*	10.18	10.10	13.3	10.42
37°C − FCS	10.9	11.3	17.6	9.2

Effect of collagen concentration, temperature, and presence of serum on lymphocyte numbers in collagen gels, using ^{51}Cr-labeled lymphocytes. The figures represent the radioactivity associated with washed collagen gels expressed as a percentage of the total radioactivity added, in this case 2,000 cpm/8 × 10^{6} lymphocytes on each gel.

* + *FCS* indicates 10% FCS in the medium added to the gel. Each figure is the mean of six replicates.
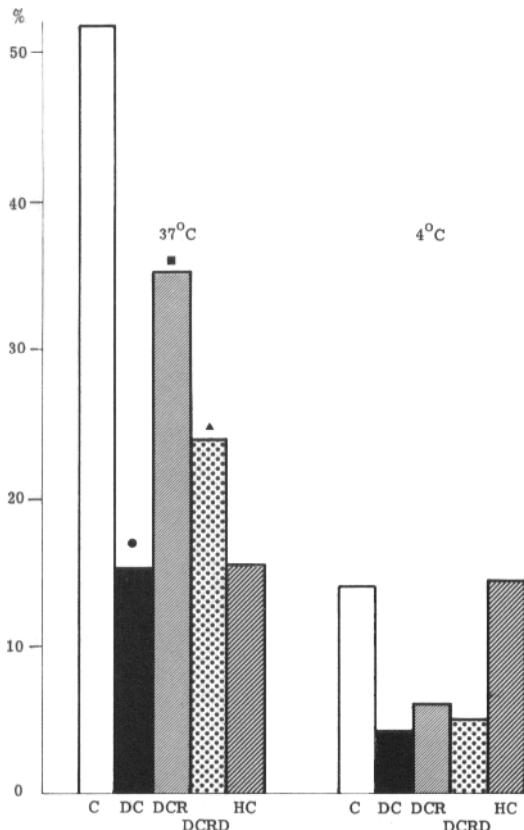

FIGURE 2 Lymphocyte adhesion to glass cover slips coated with collagen (3 mg/ml). The columns represent radioactivity associated with collagen-coated cover slips expressed as a percentage of that associated with clean glass cover slips, which is taken as 100%. *C,* wet collagen coats; *DC,* dried collagen coat; *DCR,* dried coat rehydrated with 2% acetic acid; *DCRD,* dried coat, rehydrated and then re-dried; *HC,* heated collagen. *P* values between ● and ■ = 0.0004 and between ■ and ▲ = 0.002.
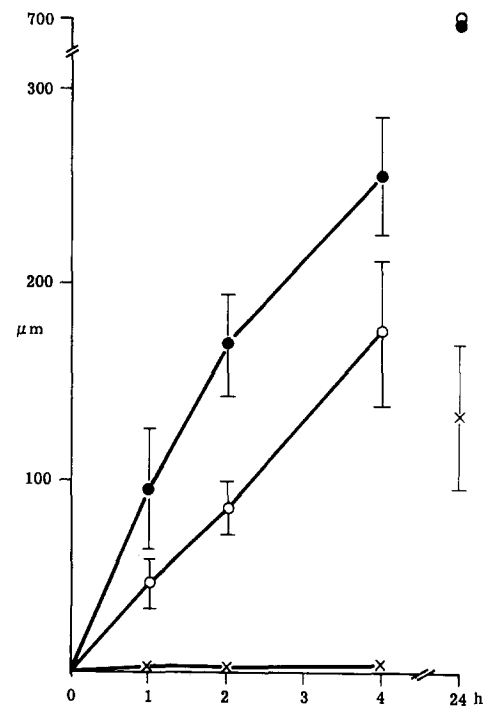

FIGURE 3 Lymphocyte migration into collagen gels. This shows the distance (measured in micrometers) migrated by lymphocytes into collagen gels over 24 h. ×, 3.0 mg/ml collagen; ●, 1.5 mg/ml collagen; ○, 1.0 mg/ml collagen. Bars, 1 SD from the mean of five fields counted. The leading-front method (24) was used to measure distance, i.e., the farthest distance from the surface reached by at least two lymphocytes in one field.

graph of the time-course of lymphocyte movement into gels at three different collagen concentrations. Gels of 1.0 mg/ml were more quickly infiltrated than those made at 1.5 mg/ml, and appreciable migration into the more dense gel (3.0 mg/ml) took much longer, with a lag period of >4 h. Once inside the gel, the rate of invasion was similar in the dense gel to that at the lower concentrations, suggesting that the gel surface at higher concentrations is less easily penetrated. Time-lapse cinematography of individual cells within gels of different concentrations indicated that invasion was an active process, with the lymphocytes pushing their way through the fiber network. Lymphocytes were never observed to translocate on the upper surface of the gel although they frequently had locomotor morphology.

There are various possible explanations for the greater attachment of lymphocytes to 3-D collagen gels than to 2-D collagen surfaces.

(a) Lymphocytes may adhere better to 3-D than to 2-D collagen. However, molecular adhesion forces should be similar for both since the two proteins are chemically identical, though a reversible denaturation on drying, followed by renaturation on rehydration, cannot be excluded.

(b) Lymphocytes may be passively trapped by 3-D collagen. This seems unlikely to provide an adequate explanation since attachment is reduced at 4°C.

(c) Lymphocytes may actively attach to the 3-D collagen by protruding pseudopods into gaps in the collagen lattice, which are no longer present once the collagen is dried. Thus, the increased attachment may be related not to differences in adhesion but to the capacity of lymphocytes to migrate into collagen gels. We explored possibilities a and c in two ways. Firstly, we used a different type of 3-D matrix, namely a serum-coated micropore filter. If explanation c is correct, lymphocytes should attach better to filters with pores wide enough for pseudopod insertion than to filters with pores too narrow for pseudopod insertion. Furthermore, the possibility of denaturation can be excluded in such an experiment. If explanation a were correct, lymphocytes should adhere equally well to all filters made of the same material whatever the pore size. Secondly, we examined the morphological changes occurring as lymphocytes moved in 3-D collagen gels. These experiments are described in turn below.

MICROPORE FILTERS: We used cellulose ester filters of different pore sizes (from 0.22 to 8.0 μm) as an alternative 3-D fibrous matrix. Table II shows the results of experiments designed to investigate lymphocyte attachment to filter surfaces in the presence and absence of serum. Lymphocytes did attach to the surfaces of all filters in the absence of serum (a nonphysiological form of adhesion comparable to that on clean glass), but, when filters were pretreated with medium containing 10% FCS, attachment to the 0.22- and 0.45-μm (pore size) filters (which are too small to permit penetration by lymphocytes) was almost abolished. However, substantial numbers attached to 3- and 8-μm filters. We interpret this as meaning that, for lymphocytes, the 0.22- and 0.45-μm filters are essentially 2-D, in that the surface topography prevents insertion of pseudopodia. They can, however, use the 3- and 8-μm filters as 3-D matrices. Lymphocytes did not enter the 0.22- and 0.45-μm filters, but a small number (3% of the total adherent cells) entered the 3.0-μm filters, and over half of the cells attaching to the 8.0-μm filters penetrated the filter. These results, together with the collagen data, indicate that a surface that is nonadhesive in two dimensions will allow lymphocytes to attach and

TABLE II

Adhesion of Lymphocytes to Cellulose Ester Filters

Treatment of filter	Filter pore size	No. of cells adhering	% Cells migrating into filter	Distance migrated (mean ± 1 SD)
	μm	mean ± SD		μm
With serum				
	0.22	27 ± 8	0	—
	0.45	37 ± 11	0	—
	3.0	378 ± 161	3.2	36 ± 12
	8.0	451 ± 112	5.2	25 ± 10
Without serum				
	0.22	1,340 ± 129	0	—
	0.45	1,245 ± 171	0	—
	3.0	1,242 ± 94	0	—
	8.0	1,242 ± 66	1	10 ± 6

This shows the number of lymphocytes attached to, or within, the matrix of micropore filters either pretreated with medium containing 10% FCS or with medium alone. The figures are means of numbers in four microscopic fields from two replicate filters, counted at × 400 magnification. The distance migrated was measured by the leading-front method (24).
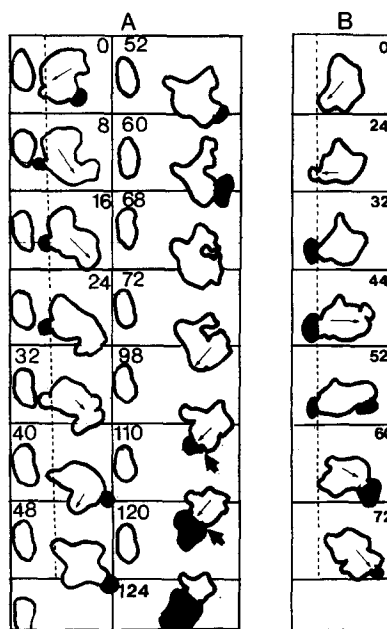

FIGURE 4 Columns A and B are two sequences traced from a time-lapse film of a lymphocyte moving through a collagen gel. The tracings are spatially arranged, allowing lines to be drawn through a fixed point in each frame (broken line). The time interval in seconds is shown in each frame. The lymphocyte in column A formed a pseudopodium toward the fixed object (stippled), after which the cell body moved past the extension, which remained fixed. After 110 s, the lymphocyte moved through the fixed constriction (marked with the large black arrowhead). Column B shows a lymphocyte extending a pseudopodium after 24 s and subsequently moving in the opposite direction. The pseudopodium is indicated by the broken line. Pseudopodia became phase dark (shaded areas) as they expanded behind the constriction.

then migrate if the shape allows them to obtain traction by inserting and expanding pseudopodia.

Lymphocyte Locomotion in Collagen Gels

Frame-by-frame analysis of time-lapse films of lymphocytes moving in gels indicated that they were using a novel method of locomotion. Pseudopodia were extended and then rapidly expanded, with a marked constriction between the original pseudopod and the cell body. This constriction was apparently used as an anchor for exerting either a pulling or pushing force that could work only if the anchor was held firmly by the substratum, which in this case appeared to be small gaps in the
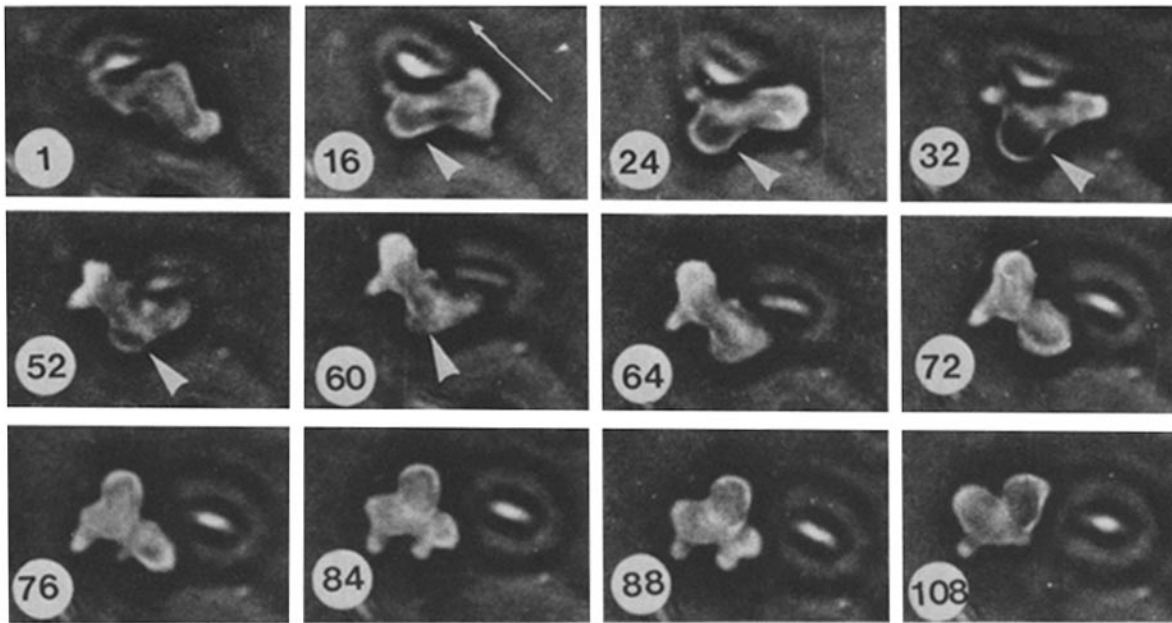
FIGURE 5   A sequence from a time-lapse film of a lymphocyte moving through a collagen gel enlarged to show the "anchoring" pseudopodia. The time interval (in seconds) is shown in each frame. The lymphocyte moved from right to left past a fixed object. The large extension indicated by arrowheads remains fixed while the lymphocyte moves laterally past it in the direction of the fine arrow. These photographs were printed directly from a positive image time-lapse film and are therefore negative images.

fibrous gel matrix. On occasion, the cell moved through the constriction ring but the ring itself remained quite clearly related to a gap in the gel structure. This is shown in Fig. 4, a series of cell outlines traced from a cine film. The important features are described in the legend to Fig. 4. Further evidence is provided by the sequential photographs in Fig. 5. These are 12 frames printed directly from a time-lapse film showing the morphological changes as the lymphocyte moved away from a fixed point.

DISCUSSION

We wish to suggest on the basis of the results presented above that lymphocyte locomotion is largely independent of adhesion to a substratum. Lymphocyte adhesion to a 2-D protein-coated surface is so poor that the cells have no means of generating locomotor force. For a cell supported by a 3-D protein matrix, locomotion may be possible even though the cell lacks the capacity to make strong adhesions with that matrix. This was shown using collagen, which as a 2-D surface supported neither adhesion nor locomotion of lymphocytes. The proposal that lymphocyte locomotion does not require an adhesive interaction is based partly on their behavior on plane substrata. On such substrata, areas of close contact, or adhesion, must be broken and reformed during locomotion. Lymphocytes make no effective contacts on glass coated with serum, fibronectin, and denatured proteins but they can attach to the surface of a 3-D matrix such as a collagen gel or the surface of a serum-coated cellulose ester filter, provided the filter pore size is large enough to admit pseudopods such as those shown in Figs. 4 and 5. That these surfaces are basically nonadhesive for lymphocytes has been demonstrated by drying down the collagen gel to remove the 3-D matrix without inducing a chemical change, by using coats of collagen heated to prevent gel formation, or by reducing the pore size of filters so that the pores are too small (0.22 and 0.45 μm) for insertion of sizable pseudopodia, in which case the lymphocyte behaves as if it

were on a 2-D rather than 3-D surface. As a result, the surface will not support lymphocyte attachment.

Our morphological observations on lymphocyte locomotion are very similar to those made several decades ago by workers who described lymphocyte locomotion in cell cultures, in plasma clots, and on plane glass (8, 13, 16, 17, 18). Lewis (15) observed that lymphocytes moving in liquid medium on a plane surface had a very tenuous hold on the substratum, and Harris (13) reported that even the few lymphocytes moving on a plane substratum could be removed by inverting the slide chamber. Lymphocytes can, however, be induced to adhere and to move, although poorly, if the substratum is coated with a ligand. For example, Fc-positive lymphocytes adhere and translocate on surface-bound antigen-antibody complexes (2), and phytohemagglutinin can be used to induce adhesion in the same way (26). A distinctive morphological feature of moving lymphocytes observed by workers cited above was the constriction ring, which remained fixed with respect to the environment as the cell moved through it. Lewis (15) suggested that the presence and position of the constriction was independent of the environment because it was also present in lymphocytes in fluid medium. However, de Bruyn (8) reported that two lymphocytes following an identical path produced constriction rings at identical points, e.g., a narrow gap in a network of fibrin. He concluded that in this case the constrictions were "indentations caused by external factors." This type of locomotion and the observations described in this paper have little in common with the classical description of fibroblast translocation over a plane substratum such as serum-coated glass (1). Comparable studies of fibroblasts moving *in situ* (4, 22) and in hydrated collagen lattices (4, 6, 7, 10) have demonstrated that adhesion of the leading part of the cell is probably important in gaining traction and that forward movement is accomplished by a flow of cytoplasm into the anterior margin of the cell or by a shortening of the extended process thus pulling the cell body forward. In fact, Grinnell and Bennett (11) have shown

by electron microscopy that fibroblasts can form adhesion plaques with collagen fibers in the absence of fibronectin. We would suggest, however, that lymphocytes, rather than gaining traction by adhering to individual collagen fibers, make use of the constriction points as anchors for forward movement. It could still be argued that very low affinity adhesions might provide traction, given a large enough area of contact. However, the morphological changes observed during locomotion through a 3-D matrix would suggest that the extending and particularly the expanding of the pseudopodia through gaps in the gel framework are instrumental in providing anchorage points during locomotion.

It should be pointed out here that we have found lymphocyte morphology to be a very unreliable guide as to the direction of locomotion in 3-D matrices. Many of the protrusions shown in Figs. 4 and 5 would be taken for uropodia. In fact, such protrusions may be either at the front or at the back of the cell. As observed on 2-D surfaces, the uropod is a rather permanent feature of the morphology of moving lymphocytes. In collagen gels, lymphocytes form protrusions that are much more transient.

The highly efficient locomotion of lymphocytes through 3-D lattices in vitro is likely to reflect their ability to migrate through complex tissues in vivo. It is possible that the architecture of such lattices might lead to accumulation in, or avoidance of, particular sites in tissues. For example, lymphocytes might move well through a lattice with apertures of an optimal size for a cell to squeeze through. However, if the pores become too wide, the cells might lose purchase and stop moving. In other words, the availability of anchorage points would control lymphocyte speed. This could lead to cell accumulation in lacunae. We are at present attempting to test this hypothesis by comparing lymphocyte behavior, i.e., speed and turning angles in collagen and fibrin gels of different densities. Furthermore, cells that respond to the spatial configuration of their surroundings may be particularly responsive to guidance in the axis of aligned fibrous tissue matrices (9, 10). The contribution of these factors remains to be explored.

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