MOTILITY OF THE LIMULUS BLOOD CELL

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SUMMARY

The sole cell type (the amoebocyte) found in the coelomic fluid of the horseshoe crab, Limulus polyphemus can be stimulated to become motile by extravasation or trauma. Motility was studied using time-lapse microcinematography and direct microscopic examination of cells in tissue culture and in gill leaflets isolated from young animals. Phase-contrast and Nomarski differential-interference contrast optics were employed. Both in culture and in the gills, motile cells showed 2 interconvertible morphological types: the contracted cell, which was compact and rounded and had a relatively small area of contact with the substratum, and a flattened form with a larger area of contact. In both morphological types, motility involved the protrusion of hyaline pseudopods followed by flow of granular endoplasm forward in the pseudopod. Cellular motility in vivo (in the gill leaflet) was morphologically identical to that displayed in tissue culture. In culture, motility was unaffected by the nature of the substratum: cells were indistinguishable on fluid (paraffin oil) or solid (glass) substrata or on hydrophobic (paraffin oil, siliconized glass) or hydrophilic (clean glass) surfaces. Cells migrated and spread on agar surfaces. Cell motility was unaffected by high concentrations (100 μg/ml) of the microtubule-depolymerizing agent colcemid and was abolished by cytochalasin B at 1 μg/ml.

INTRODUCTION

The active, pseudopod-directed motility of animal tissue cells is important for a number of processes including embryonic morphogenesis (Trinkaus, 1976), wound healing (Radice, 1977), inflammation (Marchesi, 1970) and intercellular invasion (Armstrong, 1977a). Most of our knowledge of tissue cell locomotion has come from studies of cells in tissue culture migrating on glass or plastic surfaces (Abercrombie, 1973; Goldman, Pollard & Rosenbaum, 1976). Indeed, most tissues and organisms are too large and opaque for direct microscopic observation of cell migration in situ. It is, thus, important in those situations where it is possible to observe cell locomotion in vivo to compare motility in vivo with that displayed in tissue culture. Previous studies have suggested that although motility in vivo and in culture is probably basically similar, the morphology and locomotion of vertebrate tissue cells can be profoundly affected by the nature of the substratum in culture (Elsdale & Bard, 1972; Harris, 1973) and marked differences are apparent in comparisons of cells in culture and the same cells in vivo (Bard & Hay, 1975; Bard, Hay & Meller, 1975). The present report presents the results of a study that compared the details of cellular motility of the motile blood cells of the horseshoe crab Limulus polyphemus on a variety of culture substrata and in the organism.

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MATERIALS AND METHODS

Glassware was rendered endotoxin-free by incubation at 180 °C for 4 h. The motility of amoebocytes on glass was studied by time-lapse microcinematographic and real-time observation of cells emigrating from aggregates of amoebocytes explanted into thin double-cover-slip chambers as described previously (Armstrong, 1977). Limulus serum or sterile, endotoxin-free 3 % NaCl (Travenol Laboratories, Deerfield, Ill.) buffered at pH 7.8 with 2 mM NaHCO₃ served as the culture medium. The motile behaviour of amoebocytes was studied in vitro in single gill leaflets dissected from small (25–40 mm width) individuals. Isolated gill leaflets were placed on microscope slides in MBL-formula artificial seawater and a coverslip was placed over the preparation. The walls of the gill leaflets of young animals are thin and very transparent and allow use of oil-immersion objectives. The amoebocytes remained actively motile for several hours in the preparation described above if the coverslip was sealed to prevent evaporation. All observations were made at room temperature.

RESULTS

Motility in vitro

The blood of Limulus contains a single cell type, the granular amoebocyte (Fig. 1). Although not adhesive in the intact organism, the cells develop ability to aggregate with each other to construct multicellular masses (‘amoebocyte tissue’) following extravasation. When 1–2-mm fragments of amoebocyte tissue were explanted into the double-cover-slip chamber used in these studies, cells emigrated from the explant onto the glass (Fig. 2). The morphology of the cells during their initial migration on the glass was as a compact, rounded cell with abundant cytoplasmic granules. These cells protruded hyaline, granule-free pseudopods which were thick, with blunt or pointed tips (cf. Figs. 5, 9). Forward movement of the trailing portion of the cell was accompanied by the flow of cytoplasmic granules into the pseudopod. The trailing portion of the cell appeared to be constricted, resembling the uropod of vertebrate lymphocytes and granulocytes (Fig. 5). Moving cells showed thin processes (‘retraction fibres’) that connected the uropod with residual sites of attachment to the substratum or the other cells.

After a period of time in culture (hours or days), the contracted cells flattened on the glass (Figs. 2, 3). The initial flattening of granular cells was reversible, but once degranulation occurred, the cells flattened irreversibly (Fig. 2). The reversibly flattened granular cells were still actively motile, though less so than the unflattened cells (Fig. 3). Once degranulation and irreversible flattening occurred, the cells showed very low levels of motile activity of lamellar processes and translocated either very slowly or were completely stationary.

Effects of drugs on motility

The motile amoebocyte was immobilized by cytochalasin B at 1 μg/ml but was insensitive to colcemid at 1, 10 and 100 μg/ml. The potency of the colcemid preparation was validated by demonstrating that, consistent with the report of Zimmerman & Zimmerman (1967), it inhibited the first mitotic division of fertilized Arbacia eggs at 10 μg/ml. In colcemid, both the speed of locomotion and the morphology and behaviour of motile cells was normal (Fig. 4). Granule flow was unaffected.
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Fig. 1. *Limulus* blood cells in a freshly excised gill leaflet. The oval fully granular cells in the centre of the field are unactivated whereas some of the cells along the margins of the field have already undergone degranulation. One is protruding a pseudopod (arrow). × 1309.

Fig. 2. *Limulus* amoebocytes in culture, showing the 3 morphological states shown *in vitro*: the contracted cell (*c*), the flattened granular cell (*g*), and the flattened degranulated cell (*d*). The plane of focus is the glass-culture medium interface. The pseudopods of the 2 contracted cells lie out of focus, above the substratum (arrows). × 867.

Substrate requirements for motility *in vitro*

The contracted amoebocyte was capable of migrating and flattening on a variety of substrata that do not support locomotion or spreading of vertebrate tissue cells, including siliconized glass, agar (Figs. 5, 6), and paraffin oil (Fig. 7) with either *Limulus* plasma or 3% NaCl as the culture medium. Rates of motility on paraffin oil were similar to those of cells migrating on glass. Amoebocytes also migrated through the gel (Levin, 1967) that formed when the contents of the amoebocyte granules were released from the cells into an environment containing bacterial endotoxin. (In most experiments, the cultures were maintained free of endotoxin to prevent the formation of gel.)
Fig. 3. Sequential views of the same field of a culture containing principally flattened granular amoebocytes at 5-minute intervals. Some of the more rapidly moving cells are identified by number in successive pictures. The contracted cells (1, 2) move more rapidly than the flattened cells (3, 4). ×517.
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The active motility of the blood cells could be observed in situ in isolated gill leaflets maintained on a microscope slide (Fig. 8). The blood cells in the isolated gills were best observed with a Plan 100/1.25 objective and Nomarski differential interference-contrast optics. Under these conditions, the details of motility could be observed almost as well as with cells in culture. In the intact organism, nearly all of the amoebocytes are in the immotile, unactivated state. Amoebocytes were observed in the intact animal as they circulated in the capillaries of the abdominal spines using transmitted illumination. Such cells were oval, lacked pseudopods and were non-adhering. This was also true of the blood cells contained in the gill leaflets just after isolation (cf. Figs. 1, 8) or in gills of animals that had been preserved by injecting the intact animal with 4% formaldehyde-seawater prior to dissection of the gill leaflets for observation under the microscope. Apparently the trauma of dissection of the gill leaflet induced a variable fraction of the blood cells in the gills to transform into the motile state. Some of these cells apparently were stimulated to aggregate as well, since nodules of amoebocyte tissue were present in the isolated gills. The transformed amoebocytes in isolated gills displayed the full range of morphological states that were present in vitro (e.g. the compact, rounded state (Fig. 9), the flattened granular state, and the flattened degranulated state (Fig. 10)). The only discernible differences in morphology were those observed with the degranulated cells: although degranulated cells often were as flattened as were degranulated cells in vitro (cf. Fig. 10), some were spindleshaped, being attached only at either end of the cell (Fig. 11).

In the isolated gills, the transformed granular amoebocytes migrated over the inner surfaces of the walls of the gill leaflets and over the vertical pillars (Fig. 8) that prevent upper and lower gill surfaces from coming together. Morphologically, cell motility was identical to that observed in vitro, being accomplished by the protrusion of hyaline pseudopods followed by the flow of granular cytoplasm into those pseudopods (Fig. 9). The amoebocytes migrating in the isolated gill leaflet could be viewed not only from above but also from the side when they moved over the pillars that keep the lumen of the gill leaflet open. Observation of cells from the side revealed that hyaline pseudopods often were protruded initially from the antero-dorsal surface into the fluid-filled space of the gill and only later were lowered to contact the substratum (Fig. 12).

DISCUSSION

Several processes are involved in the locomotion of animal tissue cells, including extension of the pseudopods (Abercrombie, Heaysman & Pegrum, 1970a), retraction of the trailing portions of the cell (Trinkaus, Betchaku & Krulikowski, 1971), the making and breaking of adhesions with the substratum (Abercrombie & Dunn, 1975), and a continual reorganization of both plasma membrane and the cytoskeleton (Abercrombie, Heaysman & Pegrum, 1970b). During locomotion, the Limulus amoebocyte extended pseudopods as hyaline, granule-free processes that were later
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invaded by granule-filled endoplasm. Endoplasmic flow was involved with forward movement of the trailing portions of the cell. The existence of extensive cytoplasmic flow distinguishes the motility of the amoebocyte from that of many of the vertebrate tissue cells that have been extensively studied in tissue culture, in which cytoplasmic flow is an insignificant feature of locomotion (Abercrombie, 1961). In this regard, the Limulus amoebocyte resembles the vertebrate leukocyte (Armstrong & Lackie, 1975; Zigmond, 1978). The lack of sensitivity of locomotion of the amoebocyte to pharmacological doses of antimicrotubule agents is shared by vertebrate leukocytes (Bammann, Norberg & Rydgren, 1974; Edelson & Fudenberg, 1973; Ramsey & Harris, 1973). Vertebrate fibroblasts lose the polarized distribution of lamellar pseudopods upon exposure to anti-microtubule agents with a concomitant reduction in locomotion of the cell (Goldman, 1971; Vasiliev et al. 1970).

Motility of the Limulus amoebocyte was surprisingly unaffected by the nature of the substratum. The morphology, spreading and motility of vertebrate tissue cells are markedly different on hydrophobic and hydrophilic surfaces (Harris, 1973; Ivanova & Margolis, 1973) and these cells are unable to spread and move on fluid surfaces such as paraffin oil (Harris, 1973). In contrast, the Limulus amoebocyte appeared to be unaffected in morphology or motility by any of the surfaces used in the present study.

Systematic comparisons of the motility of tissue cells under various in vitro and in vivo circumstances are very few in number, due in large measure to the limited number of situations where cells can be observed in vivo. Certain embryonic tissues, including whole teleost (Armstrong, 1978; Trinkaus, 1973), and sea-urchin (Gustafson & Wolpert, 1967) embryos, the chick embryo cornea (Bard & Hay, 1975), and the frog tadpole tailfin (Billings-Gagliardi, 1977; Clark, Clark & Rex, 1936; Radice, 1977) have proven thin and transparent enough for the study of cell motility in vivo. In adult organisms, the test cells of the tunicate (Izzard, 1974) and the motile blood cells in the wing veins of the cockroach (Arnold, 1961) have been subjected to study. Only in the study of Bard & Hay (1975) have detailed comparisons been made of cell behaviour in culture and in vivo. They reported that the motility of corneal mesenchyme cells differed in the 2 situations. Cells which showed broad leading lamellae when migrating on glass exhibited thin pseudopods in the cornea. Ruffling activity was readily apparent in vitro but was absent in situ.

Fig. 4. Emigration in vitro of cells from an amoebocyte tissue fragment in the presence of 100 μg/ml colcemid. Both the numbers of cells emigrating, the distances of movement and the morphologies of individual cells were the same as control culture. × 259.

Fig. 5. Contracted cells migrating on agar. The cells are indistinguishable from cells migrating on clean glass. u, uropod. × 1309.

Fig. 6. Flat granular cell on agar. × 1309.

Fig. 7. Cells migrating on siliconized glass and paraffin oil. A large droplet of paraffin oil occupies the centre of the field. Cell morphology is identical on the 2 substrata. Cells migrated freely and without pause from glass to oil (arrows) and from oil to glass. × 206.
The present study compared the motility of the *Limulus* amoebocyte *in vivo* (in the isolated gill leaflet) with its performance on a glass surface in tissue culture. The range of cellular morphologies was the same in culture and in the gill leaflet. The observations indicated further that at the morphological level, the motility of the compact, rounded amoebocyte was indistinguishable in the 2 situations. Thick hyaline pseudopods were protruded from the anterior-dorsal surface and subsequently were lowered to contact the substratum. After protrusion, the pseudopods were invaded by endoplasm. Active cytoplasmic streaming accompanied motility in both situations. Observations of cells in the gill leaflet provided an opportunity to observe cells in side view. It was observed that pseudopodial protrusion was often accomplished without contact between pseudopod and substratum, with these pseudopods being lowered to bring them into contact with the substratum only after full extension had been accomplished. This observation is interesting because it can account for the ability of cells to overlap each other even though contact paralysis of pseudopodial extension apparently operates in these cells (Armstrong, 1977a) and because it suggests that the forces that extend the pseudopod are not those of a passive spreading (haptotaxis) onto local areas of high-energy surface (Carter, 1967, 1970), since the pseudopod was extended into the plasma-filled space and was not in contact with the substratum.

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REFERENCES


Fig. 8. Low-magnification view of cells in the isolated gill leaflet of a young *Limulus*. The gill has been freshly isolated and most of the cells are unactivated. The upper and lower epithelia of the gill leaflet enclose a blood-filled space and are kept apart by pillars of connective tissue (p). × 206.

Fig. 9. Contracted cells in isolated gill leaflets are morphologically and behaviourally indistinguishable from cells *in vitro*. u, uropod × 1309.

Fig. 10. Flattened degranulated cell (d) in an isolated gill leaflet. The cell shows ruffling activity at its margins (arrow). × 1309.

Fig. 11. Spindle-shaped degranulated cell (s) in an isolated gill leaflet. × 1309.
Fig. 12. Sequential views of an amoebocyte migrating in an isolated gill leaflet viewed from the side. Hyaline pseudopods are protruded from the antero-dorsal surface (A, D) and are subsequently lowered to contact the substrate (E, E, F). The plane of focus was changed slightly between frames E and F. × 1432.
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