Directional Movement of Cells as Affected by Aggregation and Dispersal

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SUMMARY

The effect of clumping and scattering upon the paths taken by polymorphonuclear leukocytes was studied in slide-coverlip preparations. Scattered cells moved at random. Clumped cells moved away from the location of the cluster. The evidence seemed to justify the conclusion that the cells were displaying negative chemotaxis to some substance diffusing from the cluster, presumably a metabolite. The possibility that the outward migration of neoplastic cells from the central tumor may depend upon a similar mechanism is presented.

The invasive propensity of cancer cells appears to depend primarily upon their reduced adhesiveness, which permits them to become detached individual units (3). The eventual penetration of surrounding tissue by the neoplastic cells, however, must depend in large measure upon their ability to move independently (4), as emphasized by Abercrombie and Ambrose (1).

Questions arise as to whether the outward dissemination of malignant neoplastic cells from the primary tumor is the result of random wandering, or a mechanically imposed directional response, or whether it might be a chemically induced polarity upon the motility mechanisms of the cells—negative chemotaxis.

Consideration of these possibilities led to an investigation of the effects of clumping and scattering on the directional movements of motile cells. The cells selected for this preliminary study were rabbit polymorphonuclear leukocytes, chosen because they are readily obtainable; move at a good speed; and display positive chemotaxis, negative chemotaxis, and random motion under appropriate conditions (6). They are also characterized, as are cancer cells, by a low degree of mutual adhesiveness and the ability to invade other tissues.

MATERIALS AND METHODS

The leukocytes were obtained by injecting 300 ml of 0.85% saline solution into the peritoneal cavity of the rabbit and withdrawing it 3–5 hr later. The accumulated leukocytes were recovered by low-speed centrifugation, washed 3 times in Earle’s physiologic salt solution, and then suspended in Difco TC Medium 199. A drop of the cell suspension was spread between a slide and coverslip and sealed to prevent evaporation. No plasma or other matrix was used because of the possibility that the cells would follow stress lines in the medium and that erroneous conclusions regarding their directional responses would be made. If care is taken not to agitate the centrifugated plugs of cells too vigorously when suspending them in the medium, cell clusters of varying size will remain in the final preparation. More thorough mixing of cells and medium results in well-scattered individual cells distributed throughout the microscopic fields.

The slide-coverlip preparation was placed on the stage of a microscope housed in an incubator at 37°C. The microscope, to which a still camera was attached, was equipped for dark-field illumination. A preliminary photographic exposure of the selected field provided a record of the positions of the cells at the start of the experiment. Thereafter, a continuous exposure of 15 min recorded the paths of the moving cells. By punching holes through the locus of each cell on prints made from the first exposure and then superimposing this perforated print over the print on which the paths were recorded, the initial position of each cell could be marked. A 3rd photograph at the conclusion of the 15-min period recorded the final position of the cells where this was desired. This method is a modification of that used by Harris (5) in his studies of monocytes.

In the experiments to be described, no chemotactically active substances were introduced into the preparations. Instead, the directional movement of uniformly scattered cells was compared with that of cells surrounding and emigrating from a cluster.

RESULTS

Fig. 1 is a photographic record of the paths of initially scattered leukocytes as they moved during a 15-min period. The original position of each cell is indicated by an open circle. It is apparent that the paths go in all directions. If the center of the photograph is arbitrarily selected as a point of reference, 11 cells moved toward it.
and 12 away from it; 3 neither approached nor departed a significant distance. This is obviously an example of random movement.

In Fig. 2, the paths of cells migrating from a cluster are shown. Examination of these paths reveals that the great majority of cells moved away from the cluster. Such a result might be due to the fact that, within the cluster, the cells are mechanically impeded from going in any direction other than outward. That is, their range of freedom is physically restricted in all other directions by collisions with adjacent cells. In other words, the cells might be behaving like diffusing gas molecules. However, if one examines those scattered cells that were well separated from the cluster at the start of the experiment, all but 2 of them also moved away from the location of the clusters.

In order to assess this dispersal response, in 12 preparations the paths of 182 cells that were initially located at least 5 cell diameters from the edges of the clusters, and from there outward a distance of 165 μ, were recorded. Comparison of the initial and final positions of these cells after a 15-min period revealed that, of the 182, 134 had moved away from the location of the cluster while only 36 had moved toward it and 12 had neither approached nor departed from the location of the cluster. All of these cells had been in an original position that permitted 360° freedom of movement. Such a response is interpreted as negative chemotaxis of the cells to the cluster.

A demonstration of this dispersing effect is presented in Figs. 3, 4. Fig. 3 shows the initial positions of the cells; a central cluster is surrounded by some scattered cells. Fig. 4 shows the same field 15 min later. The cells are now widely scattered, and the cluster has almost disappeared.

It is concluded from these observations that, under the circumstances described, randomly scattered leukocytes, in the absence of introduced chemotactic substances, move at random, remain dispersed, and avoid aggregation. Clustered cells disperse until they are widely scattered and thereafter remain dispersed.

DISCUSSION

The movement of cells away from the location of the cluster suggests that they are responding to a negatively chemotactic substance diffusing out of the cluster. Beard and Rous (2) interpreted the behavior of Kupffer cells cultivated in vitro on strands of filter paper as a negatively chemotactic reaction to each other.

From the observations reported here, it is tentatively concluded that negatively chemotactic metabolic substances accumulate in the vicinity of aggregated cells under the conditions pertaining in these experiments. Isolation and identification of these substances have not as yet been accomplished. Protein degradation products should be examined; it would be of interest, for example, to determine the effect on motility and directional movement of interference with protein synthesis of the cells, as by puromycin and actinomycin D. A difference in oxygen and carbon dioxide concentrations, within and beyond the aggregated cells, is another possible mechanism. Studies on the effect of pH are also indicated.

The results obtained in this preliminary study excite speculation. One wonders if a cell supposedly moving at random is not actually displaying negative chemotaxis to its own metabolites as the latter are excreted at different sites on its surface. Could it be that the presence of such chemotactically active metabolites at the cell surface constitutes the essential stimulus to cell motility itself? Would potentially motile cells move if their metabolites were constantly washed away?

Returning to a consideration of the invasiveness of cancer cells, the results obtained with leukocytes suggest the possibility that motile cancer cells may be forced to migrate outward from the location of the central mass of tumor; that is, they may be exhibiting negative chemotaxis to metabolic products accumulating in the neoplasm. The central necrosis characteristic of rapidly growing, poorly vascularized neoplasms may depend upon excessive accumulations of such substances, while cells at the periphery are stimulated to move away from somewhat lower concentrations.

Consideration of negative chemotaxis as a factor in the dissemination of neoplastic cells opens a field for further explorations into the problem of invasiveness of cancer cells. To compare the behavior of clumped and scattered tumor cells would necessitate experiments of much longer term than those reported here because of the more sluggish movement of neoplastic cells. This would demand a longer term of culture and, therefore, a different technical approach. Ascites tumor cells seem to offer possibilities in this regard.

REFERENCES

FIG. 1.—Paths of polymorphonuclear leukocytes in slide-cover-slip preparations, as recorded photographically, under dark-field illumination, over a period of 15 min. The open circles indicate the initial positions of the cells. It is apparent that the leukocytes have moved in a random manner. \( \times 307. \)

FIG. 2.—The paths of leukocytes as they emigrated from a centrally located cluster. Open circles indicate the locations of the cells that were at a distance from the cluster at the start of the 15-min recording period. Only 2 cells (marked with arrows in upper right and lower left corners of photograph) moved toward the cluster. All other leukocytes moved more or less directly away from the cluster. \( \times 307. \)
Fig. 3.—Distribution of leukocytes at the start of a recording period. A cluster is surrounded by scattered cells. × 307.

Fig. 4.—The same field as shown in Fig. 3 after the cells had moved for 15 min. The leukocytes are now widely dispersed, and the original cluster has almost entirely disappeared. × 307.
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