PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 159, 75–79 (1978)

Enhanced Granulocyte Mobility Induced by Chemotactic Factor in the Agarose Plate (40287)

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Recently, Keller *et al.* proposed a definition of terms related to the locomotion of leucocytes and other cells (1). In their proposal, cell responses to chemotactic (and/or chemokinetic) factor are classified into two types of reactions, namely, chemokinesis and chemotaxis. Although the definition of these two types of reactions is clear, it is not easy to demonstrate these reactions separately *in vitro*. Using several kinds of methods, some workers evaluated chemokinesis and chemotaxis (2–4), but it is not certain as yet whether these two types of reactions can be recognized as two essentially separated phenomena.

In this study we analyzed chemokinesis separately from chemotaxis in the agarose plate.

Materials and methods. Leucocyte preparation. Heparinized adult blood was mixed with a one-tenth volume of a 2% methyl cellulose solution (Nakarai Chemicals, Japan) and was allowed to settle at room temperature for 30 min. The leucocytes in the supernatant were sedimented by centrifugation at 250*g* for 10 min at 4°, and the cells were washed in ice-cold Hanks solution. The mononuclear cells were then separated from the leucocyte preparation by a modification of the method of Böyum (5). The leucocyte suspensions in Hanks solution were layered on top of Ficoll (Pharmacia)-Hypaque (Winthrop) solution (24 parts 9% Ficoll + 10 parts 33.9% Hypaque) and centrifuged at 250*g* for 45 min at 4°. The mononuclear cell layer from the top of the gradient and the Ficoll-Hypaque solution was gently aspirated and discarded, and the cell button was resuspended in 0.2 ml of Hanks solution. Contaminating erythrocytes were disrupted by hypotonic shock. After washing twice with Hanks solution, the cells were resuspended in Medium 199 (Gibco) containing 10% heat inactivated fetal calf serum at 1×10^8 cells per ml. For chemokinesis assays, chemotactic factor was added to the medium at a final concentration of 10%. The purified granulocytes contained less than 1% of other cells including platelets.

Chemotactic factor preparation: Bacterial chemotactic factor was produced by overnight growth of *Escherichia coli* in heart infusion broth at 37°. The culture broth was passed through a 0.22 μm filter and the filtrate was then stored at −70° until use.

Assay of leucocyte mobility. This was performed by a minor modification of Nelson's method (6). The agarose plate was prepared by placing 3 ml of 1% agarose (Behringwerke) solution in Medium 199 containing 10% heat inactivated fetal calf serum into 35 mm × 35 mm Falcon plastic dishes. When chemokinesis, namely the enhancement of random mobility by chemotactic factor was assayed, the chemotactic factor was added uniformly to agarose. After the agarose gelled, 3 mm × 3 mm wells were made by a stainless steel punch in the agarose plate, and 10 μl of cell suspensions and chemotactic factor were placed as shown in Fig. 1. After various periods of incubation in a 5% CO_2 incubator at 37°, the distance the cells moved was measured under an inverted microscope with an ocular grid. Four measurements were averaged from the margin of the well to the line of migration in four quadrants of each well. All experiments were carried out in duplicate or triplicate.

Preparation of cells for morphological examination. After incubation, the cells were fixed with agarose in place by flooding the plates with 4 ml of 10% formalin for 48 hours. After fixation, the agarose was gently removed, and the cells were stained by Giemsa solution.

Results. Random mobility and chemokinesis in the agarose plate. The random mobility and chemokinesis of normal adult granulocytes assayed by the agarose plate method are shown in Table I. Granulocytes stimulated by 10% *E. coli*-derived chemotactic factor added uniformly in agarose showed a 2.6 ±

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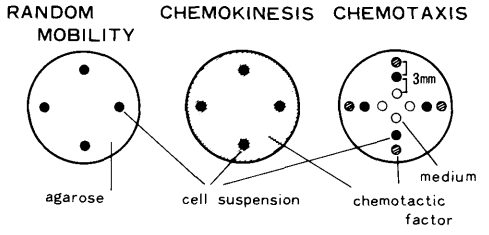

FIG. 1. Agarose plate method employed in the experiments. Three types of granulocyte mobility were measured in this method. The oblique lines means the presence of chemotactic factor derived from *E. coli.*

0.3 ($M \pm$ SE) fold enhanced mobility compared to nonstimulated granulocytes. Microscopic appearance of the preparations stained by Giemsa solution is shown in Figs. 2 and 3. Cells moving under the influence of chemotactic factor tend to be more irregular in outline than those moving without the influence of factor, and formation of blebs or pseudopodium-like structures can be observed. There is no regular orientation of cell axis.

Then we assayed the two types of granulocyte mobility as a function of time (Fig. 4). Cells under the influence of chemotactic factor moved rapidly up to 3 h, after which time no marked increase in distance was observed. On the other hand, in the absence of the bacterial factor movement of granulocytes increased linearly. However, even after 19 hours these cells had not moved as far as those stimulated by chemotactic factor.

Relationship between the concentrations of chemotactic factor and the degree of chemokinesis. As shown in Fig. 5, granulocyte mobility increased linearly in proportion to the concentration of chemotactic factor. With more than 2.5% of chemotactic factor the stimulation of migration diminished. Furthermore with more than 10% of chemotactic factor, granulocyte mobility tended to decrease, although a significantly enhanced mobility could be observed when compared against random mobility.

Granulocyte mobility under a concentration gradient of chemotactic factor. First we examined the influence of a negative concentration gradient. In order to form a negative concentration gradient of chemotactic factor in the area surrounding the well, granulocyte suspension in medium containing 10% chemotactic factor was placed in an agarose well which did not contain chemotactic factor. As shown in Table I, granulocyte mobility under a negative concentration gradient of chemotactic factor increased significantly when compared to mobility without factor.

Next we examined the influence of a positive concentration gradient toward the center

TABLE I. GRANULOCYTE MOBILITY UNDER VARIOUS CONDITIONS OF CHEMOTACTIC STIMULUS.[a]

Gradient	Distance (Mean ± SD)
No factor (random mobility)	22.9 ± 6.3 (n = 9)
Negative gradient	33.0 ± 7.5 (n = 5)
Uniform gradient (10% chemokinesis)	54.5 ± 5.5 (n = 17)
Positive gradient (chemotaxis)	69.4 ± 13.4 (n = 17)

[a] Expressed as × 40 mm.

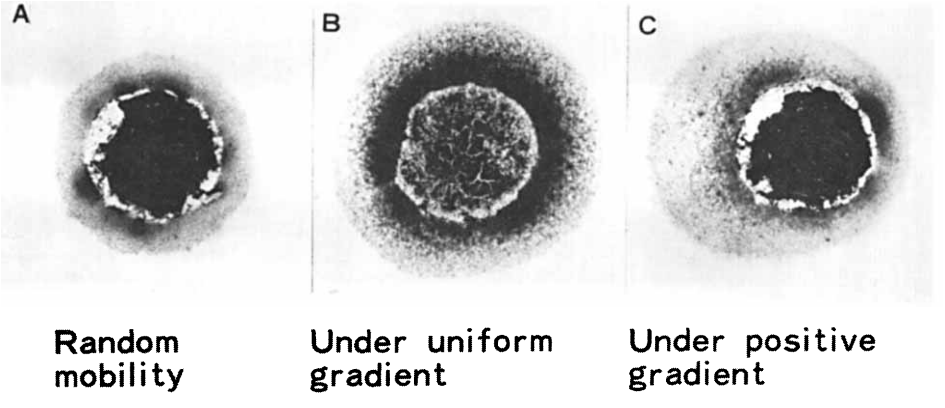

FIG. 2. Three types of granulocyte mobility after three hour culture. Cells were stained by Giemsa solution. A: random mobility, B: chemokinesis, C: chemotaxis, chemotactic gradient was made at the left side.

FIG. 3. Morphologies of random mobility (A) and chemokinesis (B). Chemokinesis was measured under 10% chemotactic factor. Cells were stained by Giemsa solution. Cells mobilizing under the influence of chemotactic factor tend to be more irregular in outline than cells mobilizing without the influence of factor. × 400.

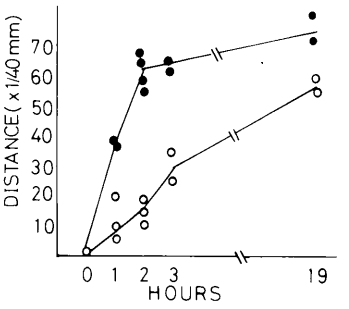

FIG. 4. The time-course of leucocyte mobility. Cells under the influence of 10% chemotactic factor (●) moved rapidly, whereas cells not under the influence of the factor (○) showed a gradual and linear increase.

well in which cell suspensions were placed. Cells exposed to a chemotactic gradient appear to extend further than those under any of the uniform concentrations tested (Table I and Fig. 5). This fact suggests that chemotaxis is also occurring in this condition.

Effect of preincubation with chemotactic factor on granulocyte mobility. Table II shows the effect of preincubation of granulocytes with Medium-199 containing 0, 2.5, or 10% of chemotactic factor at 37° for 1 hr, followed by washing with Hanks solution, and resuspension in Medium-199 containing heat inactivated 10% of fetal calf serum. The cells were then placed into the wells in the agarose plate with or without chemotactic factor. Preincubation with chemotactic factor of three concentrations had no influence on chemokinesis observed in agarose containing 5% of chemotactic factor. Furthermore granulocytes preincubated with 10% of chemotactic factor did not show enhanced mobility, namely chemokinesis, when the granulocytes were placed into the wells in agarose not containing chemotactic factor.

Discussion. Random mobility and chemokinesis could be observed separately by the agarose plate method. Granulocytes under a uniform concentration of *E. coli*-derived chemotactic factor moved at a significantly higher rate than in the absence of factor. Morphologically differences were also apparent. Cells showing chemokinesis tend to be irregular in outline, whereas those showing random mobility tend to be rounded.

Nelson *et al.* and Cutler reported that the distance the cells moved toward the outer well in which chemotactic factor was placed, was determined by chemotaxis of granulocytes (6–8). But from the results obtained in our experiments, we conclude that this distance may be based partially on chemokinesis.

The time course of cell mobility triggered by chemokinesis is analogous to that in response to chemotaxis as reported by Nelson *et al.* and Cutler (6, 7). Thus the distance of cells showing chemokinesis as well as those showing chemotaxis increases with the passage of time. In our experiments, there was a dose response relationship between chemo-

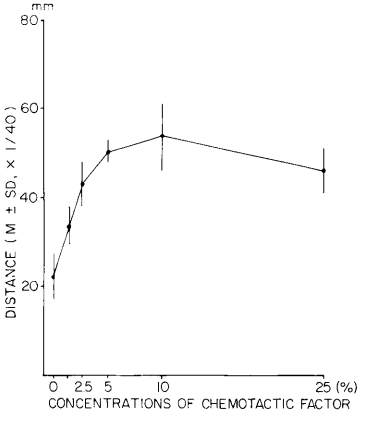

FIG. 5. Relationship between the concentrations of chemotactic factor and the degree of chemokinesis. Leucocyte mobility increased linearly in proportion to the concentrations of chemotactic factor under less than 2.5% of the factor.

TABLE II. EFFECT OF PREINCUBATION WITH CHEMOTACTIC FACTOR ON GRANULOCYTE CHEMOKINESIS.[a]

	Concentration of chemotactic factor with which cells were preincubated		
	0%	2.5%	10%
Mobility in agarose containing 5% factor (M ± SD)	50.9 ± 2.2 $n = 5$	51.3 ± 3.8 $n = 3$	53.4 ± 3.3 $n = 3$
Mobility in agarose containing no factor (M ± SD)	22.9 ± 6.3 $n = 9$		22.3 ± 11.7 $n = 3$

[a] Expressed as × 40 mm.

kinesis and the concentration of chemotactic factor, and the degree of chemokinesis was determined by the final concentration of chemotactic factor with which granulocytes came in contact. Thus cells coming in contact with higher concentration of chemotactic factor move more rapidly at random, and can increase their chances of coming in contact with chemotactic factor of higher concentration, because of the enhanced mobility. If this does not occur, namely when they move away from the chemotactic factor, the cells remain in the same location and may regain random movement regardless of whether they have been stimulated or not. Thus the number of granulocytes in the area of lower concentration of chemotactic factor decreases, whereas the number in an area of higher concentration increases. This hypothesis may be supported by another finding in the present experiments. Some enhancement of mobility under a negative concentration gradient of chemotactic factor, which can not be explained by the concept of chemotaxis, suggests that chemokinesis occurs also under a chemotactic gradient. As far as *E. coli*-derived chemotactic factor is concerned, besides a real chemotactic response (1–4, 9, 10), a chemokinetic response may also account for the effect of chemotactic gradient on trapping of cells at the site of inflammation.

The phenomenon of "deactivation" which was shown by Ward and co-workers' study concerning chemotaxis (11), could not be observed in chemokinesis induced by *E. coli*-derived chemotactic factor. It is uncertain whether "deactivation" can be observed also in chemokinesis induced by other chemotactic factor such as complement-derived factors. However, if such a phenomenon occurs in chemokinesis induced by some chemotactic (or chemokinetic) factor, the trapping of cells in an area where high concentration of chemotactic factor is present may be performed more effectively.

We believe that chemokinesis in addition to chemotaxis plays an important role in the defense mechanisms *in vivo*. Further investigation is required to better understand the basic mechanisms involved in the chemotactic (or chemokinetic) response of granulocytes.

The authors gratefully acknowledge the helpful advice of Dr. Paul G. Quie.

Summary. Human granulocyte mobility under various conditions of chemotactic stimulus was studied using the agarose plate method. Enhanced mobility was observed when granulocytes were incubated in the agarose plate containing chemotactic factor generated from *E. coli*. A dose response type relationship was observed between the degree of enhanced mobility and the concentrations of chemotactic factor in a range of less than 10%. The rate of mobility was rapid up to 3 hr, after which time it was very slow. Preincubation of granulocytes with chemotactic factor of various concentrations did not have any influence on granulocyte mobility assayed after preincubation. The degree of mobility tends to be determined by the final concentration of chemotactic factor coming in contact with granulocytes. Thus granulocytes under a negative concentration gradient also showed an enhanced mobility. On the basis of these findings, we propose the hypothesis that the accumulation of granulocytes at the site of inflammation can be in part explained by chemokinesis, i.e. enhanced random mobility.

1. Keller, H. U., Wilkinson, P. C., Abercrombie, M., Becker, E. L., Hirsch, J. G., Miller, M. E., Scott Ramsey, W., and Zigmond, S. H., Clin. Exp. Immunol. **27,** 377 (1977).
2. Keller, H. U., and Sorkin, E., Immunology **10,** 409 (1966).
3. Zigmond, S. H., and Hirsch, J. G., J. Exp. Med. **137,** 387 (1973).
4. Anderson, R., Glover, A., and Rabson, A. R., J. Immunol. **118,** 1690 (1977).
5. Böyum, A., Scand. J. Lab. Clin. Invest. Suppl. **97,** 21, (1968).
6. Nelson, R. D., Quie, P. G., and Simmons, R. L., J. Immunol. **115,** 1650 (1975).
7. Cutler, Jim E., Proc. Soc. Exp. Biol. Med. **147,** 471 (1974).
8. Nelson, R. D., Fiegel, V. D., and Simmons, R. L., J. Immunol. **117,** 1676 (1976).
9. Zigmond, S. H., J. Cell. Biol. **75,** 606 (1977).
10. Malech, H. L., Root, R. K., and Gallin, J. I., J. Cell Biol. **75,** 666 (1977).
11. Ward, P. A., and Becker, E. L., J. Exp. Med. **127,** 693 (1968).

Received February 22, 1978. P.S.E.B.M. 1978, Vol. 159.