Cytotaxins Alter the Sedimentation Behavior of Human Granulocytes

Abstract. *Human granulocytes from the peripheral blood of healthy donors were subjected to transient gravity sedimentation analysis in Ficoll density gradient columns (37°C) containing different concentrations of* Escherichia coli *endotoxin-activated serum and medium 199. A dramatic serum concentration-dependent dispersion of the cells based on changes in sedimentation velocity was observed as a function of time, using a new optical scanning instrument. The phenomenon was virtually abolished in the presence of cytochalasin B, a known inhibitor of cellular chemotaxis. The width (second statistical moment) of the sedimenting cell distribution increased in a sigmoid fashion as a function of time regardless of cytotaxin concentration. This indicates that a slow and nonlinear response of the granulocytes to the cytotaxins occurs. This new kinetic method should be useful in examining an alternate manifestation of the chemoresponsiveness of phagocytic cells and of cell interactions in general.*

The in vitro response of human granulocytes to specific chemical substances in their environment is relevant to understanding their function in host resistance, assessing their potency in transfusion therapy, and aiding in the diagnosis of certain diseases (*1*). One of the major areas of physiological and clinical interest concerns the locomotion of granulocytes induced in vitro in the presence of certain chemical substances called cytotaxins, or chemoattractants. These factors can cause migration of the cells in the direction of increasing concentration of the attractant (chemotaxis) or change the speed and turning frequency of the cells moving at random (chemokinesis), or both (*2*). Different manifestations of these phenomena are measured by migration of the cells through micropore filters, migration in agarose plates, cinematographic recording of movement in special microchambers, and other similar methods, which have been reviewed recently (*2*).

In seeking alternative means of assessing objectively and dynamically the chemoresponse of granulocytes not strictly associated with locomotion, we explored the possibility that the reaction of cytotaxins with receptors on the granulocyte surface may alter the sedimentation behavior of the responding cells, which depends on cell volume, density, shape, and deformability (*3*). This search was aided by the recent development of a computerized optical scanning instrument (*4*), which is capable of measuring precisely and at periodic intervals several statistical parameters from the migrating distribution of a cell population subjected to gravity sedimentation in a shallow density gradient (*5*). Briefly, the density gradient is formed in cylindrical quartz tubes and the cell sample, usually containing 1.5×10^6 cells, is placed on top of the gradient. The tubes are scanned vertically by a light beam at regular time intervals and the light intensity is measured by a photometer. The light transmission signal is digitized and processed by a computer to estimate the statistical moments of the cell distribution as a function of time (*6*).

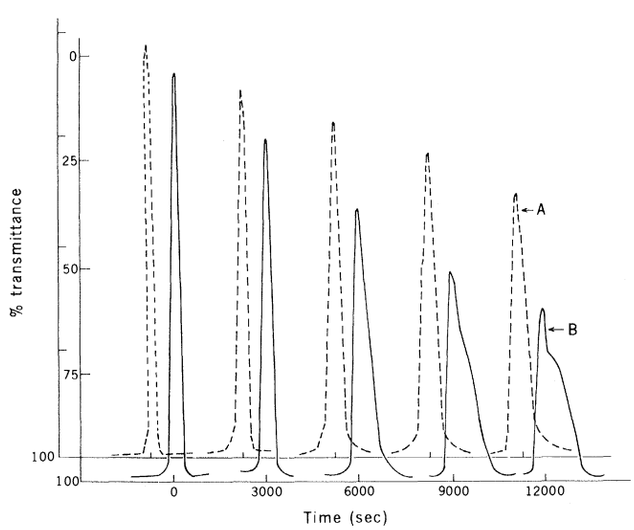

Fig. 1. Transient gravity sedimentation analysis of human granulocytes in the absence (A) and presence (B) of 2.3 percent *E. coli* endotoxin-activated serum. Note the broadening of the migrating cell distribution in the presence of cytotaxins.

We now report that human granulocytes in the presence of widely varying concentrations of *Escherichia coli* endotoxin-activated serum exhibit a time-dependent and transient alteration of their sedimentation behavior in Ficoll-medium 199 density gradient columns. The response depends on the concentration of cytotaxin in the medium and is abolished by the incorporation of cytochalasin B, an inhibitor of chemotaxis (*7*). These kinetic phenomena are described below in detail.

Human granulocytes from healthy donors were isolated from peripheral blood samples by dextran settling followed by counterflow centrifugation in a Beckman JE-6 elutriator assembly (*8*). The final leukocyte preparation contained approximately 97 percent granulocytes and 3 percent monocytes. A shallow Ficoll (Pharmacia) density gradient (2.5 to 6.25 percent Ficoll 400) in medium 199 was formed in each of the six columns of the Transanalyzer instrument (*4*). The density gradients contained different concentrations of autologous serum activated by *E. coli* endotoxin (lipopolysaccharide B, Difco) (*8*), except in the control columns, which contained autologous serum only. In some experiments, cytochalasin B (Calbiochem) at a concentration of 5 μg per milliliter of gradient was also incorporated in the columns. It should be mentioned that the concentrations of activated serum and cytochalasin B were uniform throughout the gradient. The granulocytes (1.5×10^6 cells in 0.5 ml of 2 percent Ficoll solution in medium 199) were layered on top of the gradient. All solutions were maintained at 37°C throughout the experiment by means of circulating thermostated water. Gravity sedimentation of the cells was followed optically over 5 hours by repetitive scanning of each column at fixed time intervals.

Typical gravity sedimentation patterns as a function of time are shown in Fig. 1. There is increasing dispersion of the distribution of cells migrating through the columns containing cytotaxin in comparison to the control columns. The sedimentation distribution showed a strongly positive skewness toward the high-velocity cells. To obtain a measure of the dispersion, the variance of the asymmetric peak (second statistical moment, m_2) (*6*) was plotted against sedimentation time for different concentrations of activated serum (Fig. 2). In all the experiments done in the presence of activated serum, the m_2 values followed a sigmoid-type curve as a function of time (t). The same type of curve was obtained when

0036-8075/79/0831-0936$00.50/0

cytochalasin B was also incorporated, but was not obtained with the control granulocytes, which followed a straight-line relationship. This indicates that in the presence of endotoxin-activated serum, the sedimentation behavior of granulocytes changes with time. There is an initial delay of approximately 1 hour (3.6×10^3 seconds in Fig. 2) followed by a sharp increase in velocity dispersion, which reaches an inflection point at about 2.5 hours (0.9×10^3 seconds) and then decelerates. The dispersion coefficient D (*5*) was estimated from the slope of the straightest portion of the sigmoid curve by linear regression ($D = {}^1/_2\, m_2 t$). A log-log plot of D against concentration of activated serum was linear (Fig. 3). It is important to note that changes in D were observed even at serum concentrations 100-fold less than those normally used in routine chemotactic assays. The control granulocytes exhibited a D value of 0.24×10^{-5} cm²/sec, and in the presence of cytochalasin B and the maximum concentration of activated serum (2.3 percent), the D value was 0.37×10^{-5} cm²/sec, which was only slightly higher than that of the controls. Thus, cytochalasin B virtually, but not entirely, abolished the cytotaxin effect.

The physical changes more likely to be responsible for the observed phenomena are not obvious to us at present. Several possibilities exist, such as changes in cell density, volume, status of membrane rigidity, cell shape, and aggregation. The latter is less likely because the scanning patterns are not consistent with those obtained from aggregated cells, which produce a smeared migration pattern rather than discrete peaks.

In our judgment, the experiments described above are significant in the following respects. First, they show that human granulocytes exposed to cytotaxins undergo a very slow change of certain physical properties governing their sedimentation velocity. Second, cytochalasin B, an inhibitor of chemotaxis, virtually abolishes this effect. Third, very low concentrations of activated serum can produce a measurable change in D, suggesting that the method may be applicable to detect and probably quantitate cytotaxins in patients' serums using autologous or heterologous granulocyte preparations. Fourth, the migrating distributions are amenable to kinetic mathematical analysis and modeling, which may provide some insight into the mechanism of the phenomenon.

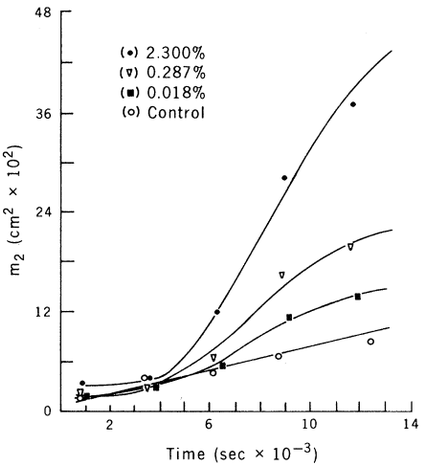

Fig. 2. Second moment (m_2) of the cell distributions at different concentrations of *E. coli* endotoxin-activated serum plotted as a function of sedimentation time. Note the sigmoid shape of the curves in the presence of activated serum and the straight-line relationship in the control.

More generally, this work shows, apparently for the first time, that transient gravity sedimentation analysis (*5*) offers a new experimental approach to the kinetic study of cell interactions comparable to the method of analytical ultracentrifugation of macromolecules. For future experiments this system offers additional advantages because the liquid column allows incorporation of reagents and ligands at gradient concentrations of various shapes. The density of the medium can be made to approximate that of the cells, thus abolishing sedimentation and allowing chemotactic migration to occur. Finally, fractions of the migrating cells can be physically removed from the column and further analyzed by other methods.

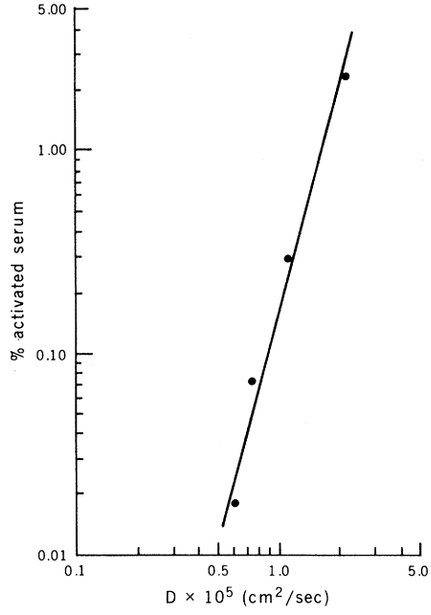

Fig. 3. Log-log plot of the apparent dispersion coefficient D (obtained by linear regression from the straightest portion of the curves of Fig. 2) as a function of the concentration of activated serum.

NICHOLAS CATSIMPOOLAS
Biophysics Laboratory, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 02139

SANFORD R. KURTZ
Department of Pathology, University Hospital, Boston University Medical Center, Boston, Massachusetts 02118

EUGENE M. SKRABUT
ANN L. GRIFFITH
Biophysics Laboratory, Department of Nutrition and Food Science, Massachusetts Institute of Technology

C. ROBERT VALERI
Naval Blood Research Laboratory, Boston, Massachusetts 02118

References and Notes

1. J. A. Bellanti and D. H. Dayton, Eds., *The Phagocytic Cell in Host Resistance* (Raven, New York, 1975); T. J. Greenwalt and G. A. Jamieson, Eds., *The Granulocyte: Function and Clinical Utilization* (Liss, New York, 1977); J. M. Goldman and R. M. Lowenthal, Eds., *Leucocytes: Separation, Collection and Transfusion* (Academic Press, New York, 1975).
2. For a discussion of terminology and assays, see P. C. Wilkinson and R. B. Allan, in *Leucocyte Chemotaxis: Methods, Physiology and Clinical Implications*, J. I. Gallin and P. G. Quie, Eds. (Raven, New York, 1978), pp. 1–24.
3. H. C. Mel, *Nature (London)* **200**, 423 (1963); E. A. Peterson and W. H. Evans, *ibid.* **214**, 824 (1967); R. G. Miller and R. A. Phillips, *J. Cell. Physiol.* **73**, 191 (1969).
4. N. Catsimpoolas, A. L. Griffith, J. M. Williams, A. Chrambach, D. Rodbard, *Anal. Biochem.* **69**, 372 (1975); N. Catsimpoolas and A. L. Griffith, in *Electrophoresis '78*, N. Catsimpoolas, Ed. (Elsevier–North Holland, New York, 1978), pp. 59–68.
5. N. Catsimpoolas, R. Rossi, A. L. Griffith, *Life Sci.* **18**, 481 (1976); N. Catsimpoolas and A. L. Griffith, *Anal. Biochem.* **80**, 555 (1977); S. N. Agathos, A. L. Griffith, R. Uauy-Dagach, V. R. Young, N. Catsimpoolas, *ibid.* **81**, 143 (1977).
6. N. Catsimpoolas and A. L. Griffith, in *Methods of Cell Separation*, N. Catsimpoolas, Ed. (Plenum, New York, 1978), vol. 2, pp. 1–65.
7. E. L. Becker, A. T. Davis, R. D. Estensen, D. G. Quie, *J. Immunol.* **108**, 396 (1972).
8. F. J. Lionetti, S. M. Hunt, P. S. Lin, S. R. Kurtz, C. R. Valeri, *Transfusion* **17**, 465 (1977).

9. We thank S. Cook, S. A. Tuttle, and R. Carciero for technical assistance. This work was supported by the U.S. Navy (Naval Medical Research and Development Command 61153N-MR0410201-0045 and Office of Naval Research contracts N00114-70-C-0111 and N00114-76-C-1155).

12 February 1979; revised 9 April 1979