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CORRELATION BETWEEN PHAGOCYTIC AND MEMBRANE SURFACE PROPERTIES REFLECTED BY PARTITIONING OF HUMAN PERIPHERAL BLOOD MONOCYTES IN TWO-POLYMER AQUEOUS PHASES

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Summary

Human peripheral blood monocyte-enriched fractions (identified by staining for peroxidase and by sizing) were obtained by velocity sedimentation at unit gravity of peripheral blood mononuclear cells. They were then fractionated by countercurrent distribution (a multiple-extraction procedure) in a charged Dextran/poly(ethylene glycol) aqueous phase system. The monocytes remained viable after the separation (order of 90%). Cells obtained from different cavities along the extraction train were tested for their ability to phagocytize latex particles. With increasing partition coefficient (presumably higher charge-associated membrane properties) the ratio of monocytes that phagocytized to monocytes that did not phagocytize increased appreciably. When, however, monocytes were permitted to phagocytize particles prior to countercurrent distribution, an increase in partition coefficient was associated with an appreciable decrease in the above-specified ratio. Control experiments indicate that the observed change in partitioning behavior cannot be ascribed to an alteration in size and/or density of the monocytes as a function of phagocytosis. It may be due to the internalization of charged surface groups during phagocytosis.

We conclude that there is a correlation between the surface properties of monocytes (as reflected by partitioning) and their ability to ingest particles. Furthermore, an alteration in the surface charge-associated properties of monocytes as a consequence of phagocytosis is indicated by the cells' reduced partition coefficient.

Introduction

Aqueous solutions of Dextran and of poly(ethylene glycol) when mixed above certain concentrations give rise to liquid, immiscible two-phase systems with a poly(ethylene glycol)-rich top and a Dextran-rich bottom phase [1]. Such systems, being aqueous, can be buffered and rendered isotonic and have been found to be extremely mild to cells partitioned in them [1—3]. Changes in polymer concentrations and salt composition and concentration have marked effects on the physical properties of the phases [1] which, in turn, interact with different cell surface components. As a consequence, one can choose, to some extent, whether charge-associated or lipid-related membrane surface properties will predominate in determining the cells' partition coefficient, K (i.e., the relative affinity of cells for the top or bottom phase or their adsorption in the interface) [4,5].

In the work reported here we have subjected human peripheral blood monocyte-enriched cell populations to countercurrent distribution in a charged phase system [1]. The nature of the distribution curve reflects the great heterogeneity of monocytes. Monocytes obtained from different parts of the extraction train were tested for their ability to phagocytize latex particles. With increasing K (presumably higher surface charge) the ratio of monocytes that phagocytized to monocytes that did not phagocytize increased appreciably. When, however, monocytes were permitted to phagocytize latex particles prior to countercurrent distribution an increase in K was associated with an appreciable decrease in the above ratio. These data clearly show that the surface properties of monocytes (as reflected by partitioning) correlate with their ability to phagocytize latex particles. Furthermore, the results indicate that charge-associated properties of monocytes change as a consequence of phagocytosis.

Materials and Methods

Reagents. Hypaque-M, 90%, was purchased from Winthrop Labs., NY. Ficoll 70 and 400 and Dextran T500 (lot Nos. 3936 and 5556) were obtained from Pharmacia Fine Chemicals, NJ. Hanks' balanced salt solution, Eagle's minimum essential medium with Earl's salts, and fetal calf serum were from Grand Island Biological Company, NY; 7.3 μm latex reference particles from Particle Information Service, OR; 1.1 μm latex particles (10% solids) from Dow Chemical Co., IN; poly(ethylene glycol) 6000 and poly(ethylene oxide) WSR-205 from Union Carbide, NY; 0.45 μm filter units from Nalgene Labware Div., NY; 3-amino-9-ethylcarbazole was from Sigma Chemical Co., MO; all other chemicals used were of reagent grade.

Preparation of human peripheral blood monocyte-enriched fractions by velocity sedimentation at unit gravity. Human peripheral blood mononuclear cells were isolated from 100 ml of defibrinated blood obtained from presumably hematologically normal individuals by layering the blood (diluted 1 : 2 with saline) on Hypaque-Ficoll 400 [6] and centrifuging at $1732 \times g$ for 10 min [7]. The cells collected from the top of the Hypaque-Ficoll cushion were washed with Hanks' balanced salt solution containing 2% fetal calf serum (heat-inactivated). The washed cells (containing less than 1% granulocytes) were

resuspended in Hanks' solution +2% heat-inactivated fetal calf serum and an aliquot taken for electronic cell counting (see below). A volume corresponding to between 1.2 and $1.5 \cdot 10^8$ cells was then mixed with an equal volume of 0.4% poly(ethylene oxide) in Hanks' solution and layered on a Ficoll 70 gradient in a Bont velocity-sedimentation apparatus at 4—5° C [7,8]. Separation by velocity sedimentation at unit gravity is determined primarily by cell size and was carried out as previously described in detail [3,7]. Cells in the gradient were collected in fractions after a 3 h sedimentation period as described by de Vries et al. [7].

Characterization of the mononuclear cells in the different fractions obtained from the velocity-sedimentation apparatus. Volumes corresponding to about 10^5 cells were taken from the different velocity-sedimentation fractions (16 in all) and Cytospin (Shandon Southern Instruments, PA) slides prepared. These were stained for myeloperoxidase activity [9] and 200—300 cells were counted on each slide. As previously reported by de Vries et al. [7], upper gradient fractions contained virtually monocyte-free lymphocytes while the lower ones contained increasing quantities of monocytes (up to about 80%) contaminated with decreasing quantities of lymphocytes which, though smaller, are also found in the monocyte fractions (see Results and Discussion). To have enough cells in the monocyte-enriched population for the experiments to be described, fractions in the different experiments were pooled so as to have not less than 40% and often as much as 70% monocytes.

Electronic cell counting and sizing. Aliquots of cells were counted on an Electrozone Celloscope (Particle Data, IL) operating on the Coulter Principle. 76- or 120-μm orifice tubes were used. Two drops of a red cell lysing agent (American Hospital Supply) were added to samples so that any contaminating erythrocytes would not be counted. The Celloscope is also attached to a 128-channel analyzer, an oscilloscope to display cell-size distributions and an X-Y plotter (Houston Instrument, TX). Relative cell sizes could thus be electronically obtained.

Monocyte phagocytosis of latex particles. Monocytes, washed two to three times with Hanks' solution +2% heat-inactivated fetal calf serum, were suspended in Eagle's modified medium with Earl's salts containing 20% heat-inactivated fetal calf serum at a final concentration of 10^6 cells/ml. A suspension of 1.1 μm latex particles (10% solids) was diluted 1 : 10 with the above-mentioned medium +20% heat-inactivated fetal calf serum and added to the monocyte suspension using 5 μl/ml cell suspension. The cells were then incubated at 37° C for 35 min with agitation on a multi-purpose rotator at 10 rev./min. After incubation, cells were washed twice with Hanks' solution +2% heat-inactivated fetal calf serum.

Preparation of Dextran-poly(ethylene glycol) aqueous phase systems. Two different phase systems prepared as described previously were used [1]. Both reflect charge-associated membrane surface properties and were selected as described by Walter [1]. Phase system 1 contained 5% (w/w) Dextran, 4% (w/w) poly(ethylene glycol) 6000, 240 mosM sodium phosphate buffer, pH 7.4, 30 mosM NaCl and 5% heat-inactivated fetal calf serum. Phase system 2 contained the same polymer concentrations but 150 mosM sodium phosphate buffer, pH 7.4, 130 mosM NaCl and 5% (w/w) heat-inactivated fetal calf serum.

Phase system 1 was used in our countercurrent distribution experiments. Since the electrostatic potential difference between top and bottom phases of system 2 is lower than that of system 1 [10], only cells of high charge-associated membrane surface properties will partition into the top phase of system 2. Hence, phase system 2 lends itself to better examination of cells having the highest K values in phase system 1, a purpose for which it was used in the single-tube partition experiments described below (see also discussion of data in Table I). Phase systems were mixed and filtered through a 0.45 μm filter unit, put into a sterile separatory funnel and permitted to come to 4—5°C in the cold-room. The system was then mixed again and allowed to settle. Top and bottom phases were separated.

Partitioning of monocytes before and after phagocytizing latex particles. Monocytes which had phagocytized latex particles, preparation A (see procedure above), or which had not phagocytized latex particles (but had been treated identically with the exception that the incubation was without latex particles), preparation B, were partitioned at 3—5°C in duplicate. Each cell preparation was suspended in 2 ml of top phase of system 2 and then mixed with 2 ml bottom phase. The phases were allowed to settle for 30 min. At the end of this time the top phase (containing those cells which preferred the top phase and hence had higher K values) was removed. The cells from the top phase were washed and preparation B was now permitted to phagocytize latex particles while preparation A was treated identically but without latex particles. Cells were washed again, Cytospin slides prepared, stained for peroxidase and counted for monocytes with particles as a percent of total monocytes present.

Loading the countercurrent distribution apparatus. Monocyte-enriched fractions ($1.5—2.5 \cdot 10^7$ cells) were obtained from the velocity-sedimentation apparatus. Depending on the experiment (see captions to figures) they were either washed with Hanks' solution +2% heat-inactivated fetal calf serum, then with top phase of phase system 1 and finally suspended in 4 ml of top phase; or one aliquot (i.e., one-half the available cells) was permitted to phagocytize latex particles (as outlined above) while the other aliquot was treated identically but incubated without latex particles. After these procedures, cells were washed as above and suspended in 4 ml top phase. The thin-layer countercurrent distribution apparatus (Buchler Instruments, NJ) used [11] consists of two circular Plexiglas plates with 120 concentric cavities and a bottom phase capacity of 0.7 ml. The bottom plate is a stator and the top a rotor plate. When only one cell preparation was to be subjected to countercurrent distribution, cavities 0—3 each received 0.5 ml of bottom phase and 0.9 ml of 'load mix' (i.e., cells suspended in top phase, see above). When the distributions of two cell preparations (e.g., monocytes which had been permitted to phagocytize latex particles and monocytes which had not) were to be compared, one population was loaded as above and the other was loaded in an analogous manner but in cavities 60—63. By carrying out 60 transfers we were able to carry out countercurrent distribution on two preparations at the same time and in the identical phase system on opposite sides of the plate without overlap. All other cavities (i.e., those not already loaded with load mix) received 0.6 ml of bottom phase and 0.8 ml of top phase to assure a stationary interface (see Ref. 12 for full

details). The fractionation was carried out at 4—5° C. The automatic cycle consists of shaking for 25 s, a settling time of 6 min (7.5 min in one control experiment, see Results and Discussion) followed by a transfer. After 60 transfers, cells were collected directly into plastic centrifuge tubes, kept in the cold and pooled in batches of six after adding 0.6 ml Hanks' solution +2% heat-inactivated fetal calf serum to each tube to convert the two-phase system into a single homogeneous suspending medium. They were then centrifuged at $400 \times g$ for 15 min. The supernatant solution was discarded and the cells after additional washing were finally suspended in 0.6 ml Hanks' solution +2% heat-inactivated fetal calf serum. Cells were analyzed as described below.

Analysis of monocytes from different parts of the countercurrent extraction train

A. Electronic cell counting. Aliquots of the cell suspensions obtained from different parts of the countercurrent extraction train were electronically counted.

B. Phagocytosis of latex particles after countercurrent distribution. Based on the counts obtained, cells were suspended at a final concentration of 10^6 cells/ml in Eagle's minimum essential medium +20% heat-inactivated fetal calf serum. Additional pooling of cells near the ends of the distribution was generally necessary to have adequate quantities with which to work. Cells from different parts of the extraction train which had not been permitted to phagocytize prior to countercurrent distribution were then allowed to phagocytize latex particles in a manner analogues to that described above. Cells which had phagocytized latex particles prior to the fractionation were treated in a fashion identical to that of the cells permitted to phagocytize after countercurrent distribution, except that the incubation in Eagle's minimum essential medium +20% heat-inactivated fetal calf serum was now without latex particles.

C. Preparation of slides, staining and counting. After washing the cells twice with Hanks' solution +2% heat-inactivated fetal calf serum, Cytospin slides were prepared from each fraction and stained for myeloperoxidase. Cells (200—300) were counted on each slide for: (a) relative percentages of monocytes and lymphocytes; (b) monocytes containing any latex particles as a percentage of total monocytes; and (c) monocytes containing two or more latex particles as a percentage of total monocytes.

D. Monocyte size distributions. Aliquots of the pooled cell fractions from the countercurrent extraction train were electronically sized as described above using 7.3 μm polystyrene reference particles. Mean cell diameters were calculated both for monocytes and lymphocytes from the size distribution plot assuming the cells to be spherical.

E. Cell viability. As one indicator of viability, aliquots of cells from the countercurrent distribution were tested for their ability to exclude trypan blue.

Presentation of data. In Figs. 1—3 and 5 we show the countercurrent distribution curves obtained. Plotted is the number of cells found in the distribution of monocytes and lymphocytes under the countercurrent distribution curve of the entire cell population. These were obtained by multiplying the percent of monocytes and lymphocytes, respectively (from slides stained for myeloperoxidase), by the total number of cells in the corresponding cavities. In Figs. 1B

and 5B we also indicate the relative mean cell diameters of monocytes and lymphocytes associated with different parts of the distribution. Cell diameters (in μm) were calculated from electronic size distributions as indicated above. In Figs. 2 and 3 we show in addition to the distribution of total cells and of monocytes, the number of monocytes having phagocytized latex particles as a percentage of total monocytes along the extraction train. Separately counted and plotted are all monocytes having particles and monocytes having two or more latex particles. In Fig. 4 we present tracings of the electronic size distributions of monocytes before and after phagocytosis of latex particles.

Results and Discussion

Countercurrent distribution patterns of human peripheral blood monocyte-enriched cell populations and cell viability

Human peripheral blood mononuclear cells were obtained by centrifugation of blood layered over a Hypaque-Ficoll cushion as previously described [6]. The mononuclear cells were then separated on a Bont apparatus by velocity sedimentation at unit gravity [3,7], a procedure which yields a segregation based primarily on size [8]. Upper fractions contain virtually monocyte-free lymphocytes as determined by staining for peroxidase [9] while the lower fractions are monocyte-enriched but contain diminishing quantities of contaminating lymphocytes which, though smaller, are physically either carried down with the monocytes or mixed with the monocytes during collection of the velocity-sedimentation fractions. Monocyte-enriched fractions prepared in this manner, and containing between 40 and 75% monocytes, were subjected to countercurrent distribution in a phase system selected so as to reflect charge-associated properties. Figs. 1A, 2, 3, and 5A depict representative distribution curves obtained. The curves of monocytes and lymphocytes overlap to some extent with the bulk of the monocytes (again determined by myeloperoxidase staining) having a lower K than the bulk of the lymphocytes. The shape of the distribution curve depends on the relative quantities of monocytes and lymphocytes present. Thus, in Fig. 1A, we see a typical distribution when the ratio of monocytes to lymphocytes is about 1 : 1; Fig. 5A shows a distribution when this ratio is increased to almost 2 : 1.

Countercurrent distribution has previously been shown, with other cells, not to have deleterious effects on them [2,3,13]. Lymphocytes, after countercurrent distribution, can be cultured, respond to mitogens, undergo rosetting and form antibodies. Monocytes, in the current experiments, were found to have excellent viability, as indicated by the trypan blue exclusion test, being of the order of 90% viable. Furthermore, the monocytes phagocytized latex particles not only before but also subsequent to countercurrent distribution with the somewhat fewer cells able to phagocytize after countercurrent distribution being a function of the time elapsed during the fractionation and not due to the procedure per se (see discussion below).

Countercurrent distribution of monocyte-enriched fractions followed by phagocytosis of latex particles by monocytes in different parts of the extraction train

Cells obtained as outlined above by velocity sedimentation at unit gravity

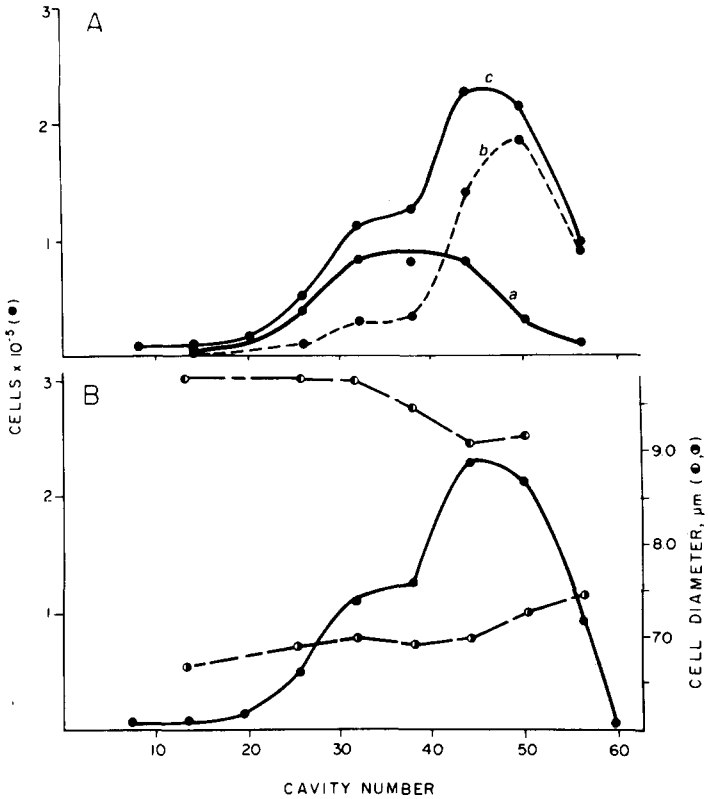

Fig. 1. Countercurrent distribution pattern of a human peripheral blood monocyte-enriched cell population obtained by velocity sedimentation of mononuclear cells as described in the text. A shows the distribution of monocytes (a), identified by myeloperoxidase staining and sizing, and of lymphocytes (b), under the curve of the entire cell population (c). The ratio of monocytes to lymphocytes was about 1 : 1. B depicts the mean sizes of monocytes (●) and of lymphocytes (◑) through the distribution. The phase system contained 5% (w/w) Dextran, 4% (w/w) poly(ethylene glycol), 240 mosM sodium phosphate buffer, pH 7.4, 30 mosM NaCl and 5% (w/w) fetal calf serum (heat-inactivated), 60 transfers were completed at 4—5°C with a settling time of 6 min and a shaking time of 25 s. For additional details see text.

were subjected to countercurrent distribution. The monocytes in different parts along the extraction train were then permitted to phagocytize 1.1 μm latex particles. Cytospin slides were prepared of each fraction, stained for myeloperoxidase to identify monocytes and counted for monocytes with latex particles as percentage of total monocytes present. In Fig. 2 we present results of such an experiment. It can be seen that the monocyte subpopulation with low K values (i.e., those cells to the left) contains relatively few monocytes that phagocytize latex particles (about 32%), and that with increasing K the percentage of monocytes with particles increases to 80% of the total monocytes present. We conclude that there is a correlation between the surface properties of monocytes, as discerned by partitioning, and their ability to phagocytize.

Phagocytosis of latex particles by monocyte-enriched fractions followed by countercurrent distribution

Monocyte-enriched fractions obtained by velocity sedimentation at unit

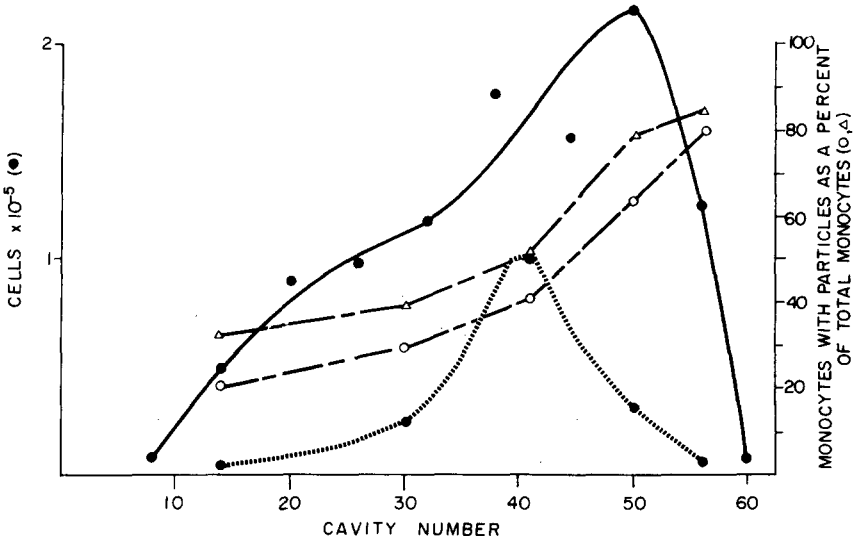

Fig. 2. Countercurrent distribution patterns of an aliquot of a monocyte-enriched cell population obtained and subjected to countercurrent distribution as in Fig. 1 (except that cells were incubated as described in the text so as to be treated in similar fashion to cells in the experiment to be described in Fig. 3). After countercurrent distribution, cells from cavities along the extraction train were permitted to phagocytize latex particles. Slides of cells were prepared, stained for myeloperoxidase activity and counted. ●———●, distribution of entire cell population; ●- - - - -●, distribution of monocytes; △— - —△, monocytes with any number of phagocytized particles as a percentage of total monocytes; ○— - —○, monocytes with two or more phagocytized particles as a percentage of total monocytes. For discussion and details see text.

gravity were permitted to phagocytize latex particles and were then subjected to countercurrent distribution. The run was analyzed as outlined in the previous section. Fig. 3 shows that in such an experiment the cell population with

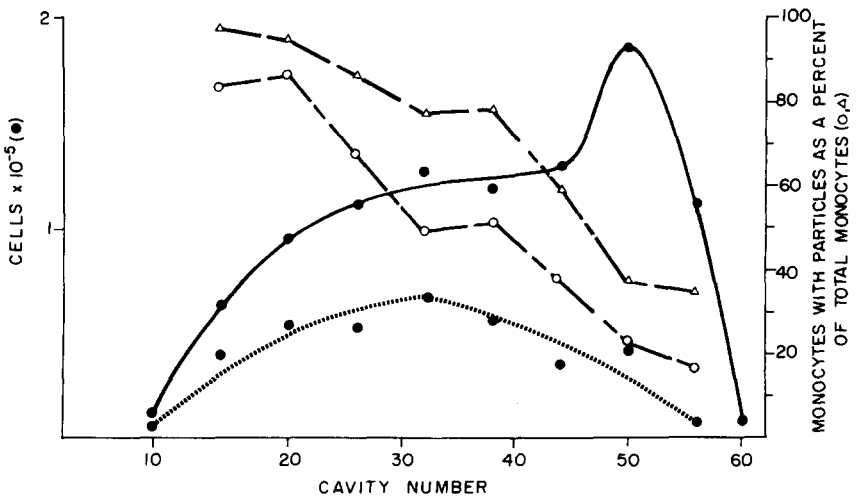

Fig. 3. Countercurrent distribution pattern of an aliquot of the same monocyte-enriched cell population as in Fig. 2 and run at the same time. Prior to countercurrent distribution, monocytes were permitted to phagocytize latex particles. Conditions, symbols and analysis as in Fig. 2.

lowest K (i.e., those cells to the left) contains the largest percentage of phagocytizing monocytes (about 85%). With increasing K the percentage of monocytes with particles decreases to about 25% of the total monocytes present. These results are thus opposite to those obtained when monocytes are permitted to phagocytize particles after countercurrent distribution (compare Figs. 2 and 3). There is thus a correlation between the ability of monocytes to phagocytize and their subsequent partition behavior.

Analysis of experiments in which monocytes phagocytize latex particles after countercurrent distribution compared to those in which latex particles are phagocytized prior to countercurrent distribution

A preliminary conclusion, to be examined in greater detail below, is that with increasing K there are more monocytes which phagocytize particles (Fig. 2); while phagocytosis of particles alters the surface properties of monocytes in such a manner that their K subsequent to phagocytosis is decreased (Fig. 3). In line with this view is the finding that the mean distribution of monocytes containing latex particles is always slightly displaced to the left (i.e., to lower K values) when compared to the mean distribution of monocytes without particles from the same individual (e.g., Figs. 2 and 3).

The percentage of total monocytes which phagocytized latex particles under the conditions used ranged from 50 to 80% in different experiments. Relative increases (Fig. 2) or decreases (Fig. 3) of monocytes with particles, as a percentage of total monocytes, with increasing K are obtained irrespective of the mean percentage phagocytosis in a given experiment. However, the mean percentage of phagocytizing monocytes after countercurrent distribution (as in Fig. 2) was found to be (with the exception of one experiment in which there was no difference) of the order of 20% less than the mean percentage of monocytes which phagocytized prior to countercurrent distribution (as in Fig. 3). The time consumed by countercurrent distribution is such that phagocytosis by cells after this procedure always takes place on the day following preparation of cells; phagocytosis prior to countercurrent separation is always on the same day. Therefore, we examined whether the reduced mean percentage of phagocytizing cells after countercurrent distribution was a function of the time elpased or of the distribution procedure per se by allowing some monocytes to phagocytize on the day of cell preparation and some on the day following. Cells that had been kept refrigerated overnight contained between 15 and 20% fewer phagocytizing monocytes. It thus appears that the reduced number of phagocytizing monocytes is a function of the time elapsed rather than of countercurrent distribution. Irrespective of the cause for the reduced number of phagocytizing cells after countercurrent distribution, the results of such experiments (Fig. 2) may be influenced. Moreover, their interpretation with respect to phagocytosis prior to countercurrent distribution (Fig. 3) may be affected. The partitioning study presented in Table I was designed to address this problem.

Of two aliquots of a monocyte preparation taken, one was permitted to phagocytize latex particles. The cells in both aliquots were then partitioned in single tubes containing the charged phase system 2. Cells which preferred the top phase (i.e., those cells having higher K values) were removed in each case.

TABLE I

RELATION BETWEEN SURFACE PROPERTIES OF AND PHAGOCYTOSIS BY HUMAN PERIPHERAL BLOOD MONOCYTES AS REFLECTED BY THEIR PARTITION BEHAVIOR IN TWO-POLYMER AQUEOUS PHASES

Monocytes were partitioned in a two-polymer aqueous phase system either before or after permitting the cells to phagocytize 1.1 μm latex particles. The percentage of monocytes in the top phase (i.e., those monocytes with higher partition coefficient, K) which either had phagocytized (before partitioning) or did phagocytize latex particles (after partitioning) was determined. The phase system contained 5% (w/w) Dextran, 4% (w/w) poly(ethylene glycol), 150 mosM sodium phosphate buffer, pH 7.4, 130 mosM NaCl and 5% (w/w) heat-inactivated fetal calf serum. Partitioning was at 3—5°C. The percentages indicate that monocytes that have phagocytized latex particles prior to partitioning have a lower K than do cells capable of phagocytizing latex particles (i.e., after partitioning). See text for additional details and discussion.

	Expt. 1	Expt. 2
Percentage of monocytes in top phase which had phagocytized latex particles prior to partitioning	51, 50	49, 50
Percentage of monocytes in top phase which phagocytized latex particles after partitioning	80, 82	80, —

Slides were prepared of cells from the top phase which had phagocytized prior to partitioning; while the cells from the top phase of the population which had not yet phagocytized were permitted to phagocytize latex particles. Slides were then also prepared of these cells. Table I indicates that those monocytes which had phagocytized prior to partitioning preferred the top phase to a lesser extent than did monocytes which could but had not yet phagocytized particles (as shown by their phagocytosis after partitioning). These results, obtained on the same day, are thus similar to and supportive of the results depicted in Figs. 2 and 3 (i.e., the increase and decrease, respectively, in the number of monocytes with particles as a percentage of total monocytes, with increasing K).

While membrane surface properties are established as the major determinant of the K of cells in two-polymer aqueous phase systems [1], it is possible that, under given circumstances, the cells' size and density may have a major bearing. Increase in size and/or density would tend to lower the apparent K. We attempted, therefore, to gain some information on these cell parameters as a function of phagocytosis by monocytes.

Fig. 4 shows the electronic size distributions of monocytes before (top) and after (bottom) phagocytosis. The percentage of monocytes which phagocytized latex particles was 59%. The right-hand peak, in each distribution, is the monocyte population, the middle peak the lymphocytes and the small left-hand peak is due to debris and electronic noise. Since the size distributions are superimposable (with the monocytes, in this experiment, having an 8.9 μm diameter) it is clear that no change in size of monocytes is discernible as a function of phagocytosis. It should be mentioned that we have noticed a small, but reproducible, increase in monocyte size (but not lymphocyte size) in the course of the 35 min incubation during phagocytosis. For this reason, both preparations depicted in Fig. 4 were incubated, one without and the other with latex particles.

We have subjected two aliquots of monocytes to countercurrent distribution (data not shown) one without and the other after phagocytosis of latex par-

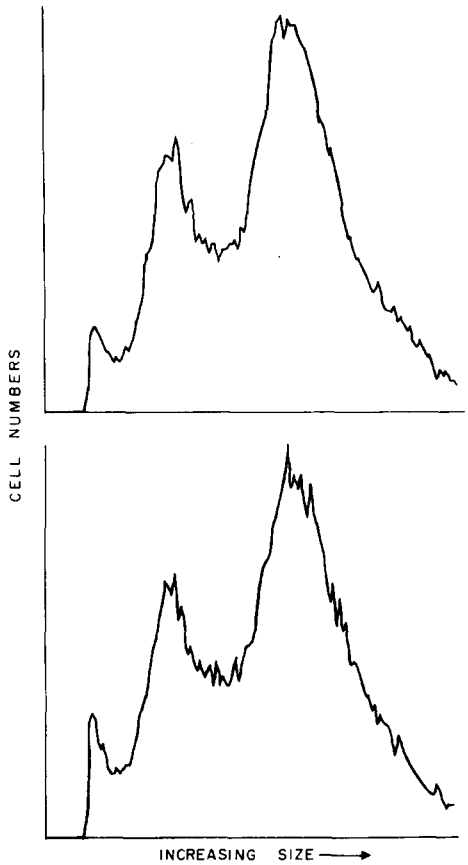

Fig. 4. Electronic sizing patterns of a human peripheral blood monocyte-enriched cell population before (top) and after (bottom) phagocytosis of latex particles. 59% of monocytes phagocytized particles in this experiment. Since the two patterns are strictly superimposable, no change in monocyte size is discernible as a consequence of phagocytosis. See text for further discussion.

ticles (73% of cells phagocytized) using an extended settling time (7.5 min) during the countercurrent distribution run. Extension of the settling time should result in markedly greater displacement of cells containing latex particles to the left (i.e., to lower apparent K values) if their size and/or density were increased. We found that no such difference exists between the distribution of monocytes which have and those which have not phagocytized latex particles. The slight displacement of the distribution of latex-containing cells to the left as compared to cells not having latex particles is no greater than when the more rapid settling time (6 min) is used and is, therefore, most probably due to changes in monocyte surface properties as a function of phagocytosis.

Relation between phagocytosis and monocyte subpopulations of different mean sizes

We have previously reported [3] that lymphocytes with high partition coefficients, K (i.e., cells with high affinity for the top, positively charged phase), are the largest lymphocytes in the population and are enriched with Fc receptor-

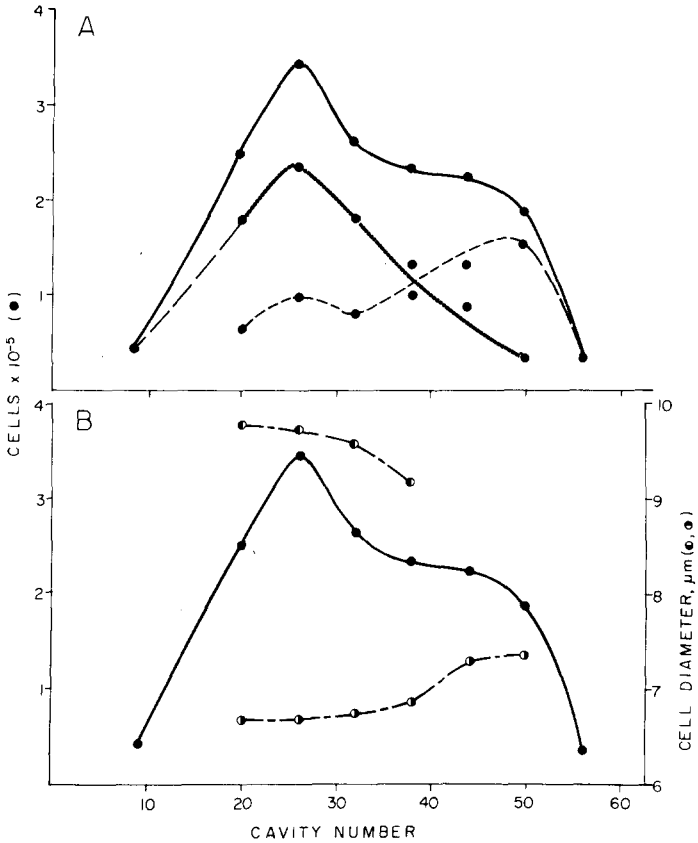

Fig. 5. Countercurrent distribution pattern of a human peripheral blood monocyte-enriched population (ratio of monocytes to lymphocytes about 2 : 1) after permitting monocytes to phagocytize latex particles. Symbols and analysis as in Fig. 1. Note that monocytes with higher partition coefficients still have a smaller mean size. See text for discussion.

bearing cells. In Fig. 1B we show that with increasing K monocytes have a smaller mean size. Since the separation by countercurrent distribution depends predominantly on the membrane surface properties of cells [1], it follows that monocytes of different mean size differ in surface properties. In addition, since with increasing K monocytes contain increasing percentages of monocytes capable of phagocytosis (Fig. 2), it appears that monocytes of smaller mean size, as resolved by countercurrent distribution, contain a greater percentage of phagocytizing monocytes.

It is interesting to note that when monocytes were permitted to phagocytize latex particles, the cells subjected to countercurrent distribution, and the run analyzed as in Fig. 1 (Fig. 5), we found that with increasing K monocytes still have a smaller mean size (even though decreasing percentages of phagocytizing monocytes are now associated with this cell subpopulation (see discussion of Figs. 2 and 3)). We believe this to be due to the small shift of the distribution curve of monocytes which have phagocytized to the left (i.e., to a lower mean K value), leaving those monocytes which have not phagocytized (and which also have a smaller mean size) on the right end of the distribution. This shift

also causes the increase in the percentage of monocytes that have phagocytized on the left end of the distribution (Fig. 3).

Conclusions

By use of an extremely sensitive method (partitioning in charged two-polymer aqueous phase systems), we have been able to fractionate human peripheral blood monocytes on the basis of their charge-associated surface properties into subpopulations differing in their ability to phagocytize latex particles. Furthermore, an alteration in the monocytes' surface properties as a consequence of phagocytosis is indicated by the cells' reduced partition coefficient.

Previous studies [14] have indicated that phagocytosis of latex particles by macrophages is accompanied by a decrease in plasma membrane as evidenced by a reduction in spreading, pinocytosis and phagocytosis. Investigations on the fate of membrane surface markers during phagocytosis indicate that several membrane transport carriers are not internalized during phagocytosis [15,16], suggesting that transport and phagocytic sites go their separate ways. Particularly interesting is the finding that concanavalin A binding is markedly reduced after particle uptake by mouse peritoneal macrophages [17]. 25—50% of concanavalin A receptors have been shown, in embryonic muscle cells, to be electrophoretically mobile (and, hence, charged) [18]. A reduction in charged surface groups as a function of phagocytosis would decrease the partition coefficient of monocytes which is in line with our findings.

The appreciable heterogeneity of monocytes clearly shown by the broad nature of the countercurrent distribution curves leads one to conjecture as to whether other biological functions of monocytes are carried on to differing extents by monocytes with different charge-associated surface properties. Investigations to test this possibility are underway.

Acknowledgment

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References

1 Walter, H. (1977) in Methods of Cell Separation (Catsimpoolas, N., ed.), Vol. 1, pp. 307—354, Plenum Press, New York
2 Brunette, D.M., McCulloch, E.A. and Till, J.E. (1968) Cell Tissue Kinet. 1, 319—327
3 Walter, H., Webber, T.J., Michalski, J.P., McCombs, C.C., Moncla, B.J. Krob, E.J. and Graham, L.L. (1979) J. Immunol. 123, 1687—1695
4 Brooks, D.E., Seaman, G.V.F. and Walter, H. (1971) Nat. New Biol. 234, 61—62
5 Walter, H., Krob, E.J. and Brooks, D.E. (1976) Biochemistry 15, 2959—2964
6 Böyum, A. (1968) Scand. J. Clin. Lab. Invest. 21, Supp. 97, 77—89
7 De Vries, J.E., Caviles, A.P., Bont, W.S. and Mendelsohn, J. (1979) J. Immunol. 122, 1099—1107
8 Bont, W.S., de Vries, J.E., Geel, M., van Dongen, A. and Loos, H.A.J. (1979) Immunol. Methods 29, 1—16
9 Kaplow, L.S. (1975) Am. J. Clin. Pathol. 63, 451
10 Reitherman, R., Flanagan, S.D. and Barondes, S.H. (1973) Biochim. Biophys. Acta 297, 193—202
11 Albertsson, P.-Å. (1970) Sci. Tools 17, 53—57
12 Albertsson, P.-Å. and Baird, G.D. (1962) Expt. Cell Res. 28, 296—322
13 Walter, H., Miller, A., Krob, E.J. and Ascher, G.S. (1971) Expt. Cell Res. 69, 416—424

14 Werb, Z. and Cohn, Z.A. (1972) J. Biol. Chem. 247, 2439—2446
15 Tsan, M. and Berlin, R.D. (1971) J. Exp. Med. 134, 1016—1023
16 Straus, D.C., Imhoff, J.G. and Bonventre, P.E. (1977) J. Cell. Physiol. 93, 105—115
17 Lutton, J.D. (1973) J. Cell Biol. 56, 611—617
18 Poo, M.-m., Poo, W.-J. and Lam, J.W. (1978) J. Cell Biol. 76, 483—501