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ELECTROPHORETIC MOBILITIES OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES SUBFRACTIONATED BY PARTITIONING IN TWO-POLYMER AQUEOUS PHASE SYSTEMS

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Summary

Mixtures of aqueous solutions of dextran and of poly(ethyleneglycol) form immiscible two-phase systems suitable for the separation (by partitioning) of cells based on subtle differences in their surface properties (Walter, H. (1977) in Methods of Cell Separation (Catsimpoolas, N., ed.), Vol. 1, pp. 307—354, Plenum Press, New York). Human peripheral blood lymphocytes were subjected to countercurrent distribution (a multiple-extraction procedure) in a charged phase system which yields fractions of cells enriched with respect to different surface markers (B-, T- and F_c-receptor-bearing) and of different size (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). The electrophoretic mobilities of cells in these fractions were measured by means of analytical particle electrophoresis (Bangham, A.D., Flemans, R., Heard, D.H. and Seaman, G.V.F. (1958) Nature 182, 642—644). The bulk of B-lymphocytes had not only the lowest partition coefficient (i.e., lowest affinity for the top, positively charged phase) but also the lowest electrophoretic mobility, T-lymphocytes had a higher partition coefficient and mobility, and the population comprising most of the F_c-receptor-bearing and largest lymphocytes had the highest partition coefficient but an intermediate mobility. The lymphocytes with highest mobil-

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ity resolved by countercurrent distribution have yet to be characterized.

The results with large and F_c-receptor-bearing lymphocytes again emphasize that the charges measured by cell electrophoresis and by partitioning are not necessarily the same. They also have as an important, practical consequence that populations enriched with respect to F_c-receptor-bearing lymphocytes should be obtainable by a simple one- or two-step extraction process.

Aqueous solutions of dextran and of poly(ethyleneglycol) when mixed above certain concentrations give rise to liquid, immiscible two-phase systems with a poly(ethyleneglycol)-rich top and a dextran-rich bottom phase. Such phases can be buffered, made isotonic and are suitable for the partitioning of cells [1,2]. Depending on the polymer concentrations and on the ionic composition and concentration chosen it is possible to select, to a great extent, whether charge-associated or lipid-related membrane surface properties will be reflected by the partition coefficient (i.e., the relative affinity of the cells for the top or bottom phase or their adsorption at the interface) [2].

Although both dextran and poly(ethyleneglycol) are non-ionic polymers some salts (e.g., phosphate) distribute themselves unevenly between the two phases [3]. An electrostatic potential difference between the phases results [4] with the top phase, in the case of phosphate, being positively charged with respect to the bottom. Cells (having a net negative surface charge) when added to such systems interact with the phase charge and the resulting cell partition coefficient is determined, largely, by charge-associated surface properties [2,5].

Human peripheral blood lymphocytes have recently been subfractionated by countercurrent distribution (a multiple extraction procedure) using a charged two-polymer aqueous phase system [6]. The lymphocytes remain viable after separation (approx. 90%) and the cells rosetting with sheep erythrocytes respond to T-cell mitogens. Countercurrent distribution clearly not only reveals the heterogeneity of the total human peripheral blood lymphocyte population but also shows that subpopulations of cells that rosette with sheep erythrocytes have complement, or that F_c-receptors are composed of additional subpopulations as well. The bulk of complement-receptor-bearing cells have the lowest partition coefficient (i.e., lowest affinity for the positively charged top phase), cells rosetting with sheep erythrocytes an intermediate partition coefficient and F_c-receptor-bearing cells the highest partition coefficient. The largest lymphocytes are among the subpopulation having the highest partition coefficient and do not respond to T-cell mitogens.

Since the membrane charge reflected by partitioning and by cell electrophoresis is not necessarily the same [2], we have examined the relative electrophoretic mobilities of human peripheral blood lymphocytes subfractionated by countercurrent distribution to gain further information on the surface properties of these cells. It was found that the bulk of B-lymphocytes have not only the lowest partition coefficient but also the lowest electrophoretic mobility, T-lymphocytes have a higher partition coefficient and electrophoretic mobility, and the population comprising F_c-receptor-bearing and largest lymphocytes have the highest partition coefficient but an intermediate electrophoretic mobility. The subpopulation of cells, obtained by countercurrent distribution,

with the highest electrophoretic mobility has yet to be characterized.

Human peripheral blood mononuclear cells were isolated from 100 ml of defibrinated blood from presumably hematologically-normal individuals by the method of Böyum [7]. The preparation of human peripheral blood lymphocytes, virtually free of monocytes, by velocity sedimentation at unit gravity of the mononuclear cells has previously been described in detail [6,8]. Lymphocytes used in the experiments to be described contained fewer than 0.5% monocytes.

Two different phase systems were used. Phase system 1 contained 5% (w/w) dextran T500 (lot No. 5556, Pharmacia Fine Chemicals, NJ), 4% (w/w) poly(ethyleneglycol) ('Carbowax 6000', Union Carbide, NY), 240 mosM sodium phosphate buffer, pH 7.4, 30 mosM NaCl and 5% (w/w) heat-inactivated fetal calf serum (Grand Island Biological Company, NY). Phase system 2 contained the same polymer concentrations but 150 mosM sodium phosphate buffer, pH 7.4, 130 mosM NaCl, and 5% (w/w) fetal calf serum. Phase system 1 has a higher electrostatic potential difference between the phases than phase system 2 [2]. Phase system compositions suitable for countercurrent distribution of lymphocytes were selected as described by Walter [2]. The phases were filtered through a 0.45 μm filter unit (Nalgene Labware Division, NY) and put into a sterile separatory funnel. After reaching cold-room temperature (4—5° C) they were mixed again and permitted to equilibrate. Top (poly(ethyleneglycol)-rich) and bottom (dextran-rich) phases were then separated.

An automatic countercurrent distribution apparatus with circular Plexiglas plates and 120 concentric cavities as described by Albertsson [9] was used. Bottom phase capacity was 0.7 ml. Between 2 and $4 \cdot 10^7$ lymphocytes were suspended in 4 ml of top phase ('load mix') of the system to be used. Cavities 0 to 3 each received 0.5 ml of bottom phase and 0.9 ml of 'load mix'. All other cavities received 0.6 ml of bottom and 0.8 ml of top phase. Cells were loaded in this manner to obtain a stationary interface [1]. The cycle of operation consisted of shaking for 25 s followed by a settling time of 6 min and a transfer. 119 such cycles (mixing, settling, transfer) were completed.

Cells from each cavity were collected directly into plastic centrifuge tubes and kept in ice. To convert the two-phase system into a single phase, 0.6 ml of isotonic NaCl solution was added to each tube. Adjacent tubes were pooled in batches of ten, the cells centrifuged at $400 \times g$ for 15 min and the supernatant solution discarded. Cells were washed twice in isotonic NaCl solution and were finally suspended in 0.6 ml of aqueous 0.15 M NaCl with pH adjusted to 7.2 ± 0.1 using 0.15 M $NaHCO_3$ (standard saline) [5]. Aliquots of these cell suspensions were then analyzed as described below.

To obtain the distribution curve of lymphocytes after countercurrent distribution, cell suspensions were counted using an Electrozone Celloscope (Particle Data, Chicago, IL), operating on the Coulter Principle, after lysing any red blood cells present wtih Zapoglobin (Coulter Electronics, Garden Grove, CA). Our celloscope is also fitted with a 128-channel analyzer, an oscilloscope to display cell volume distributions and an X-Y plotter. Cells from the different cavities were sized as previously [6] using 7.3 μm poly(styrene) reference particles (Particle Information Service, Grants Pass, OR). Mean cell diameters were calculated from the cell volume distribution plots assuming the cells to be spherical.

Electrophoretic mobilities were measured in a cylindrical chamber apparatus at 25°C using Ag/AgCl electrodes [10]. Cell samples (prepared as previously described) from the cavities of the countercurrent distribution apparatus were examined in standard saline.

The electrophoretic mobilities of human peripheral blood lymphocytes subfractionated by countercurrent distribution in dextran-poly(ethyleneglycol) aqueous two-phase systems have been measured. Fig. 1 shows the results obtained using a system with a high potential difference between the phases [2] in which cells with lower partition coefficients (i.e., those cells to the left) can best be visualized. It was found that cells with the lowest partition coefficient have the lowest electrophoretic mobility, cells with an intermediate partition coefficient have the highest electrophoretic mobility and those cells with the highest partition coefficient have an intermediate electrophoretic mobility. From previous work [6,11] it is known that the cells having the lowest partition coefficient comprise the bulk of B-lymphocytes: those with an intermediate partition coefficient are predominantly T-lymphocytes and the cells with highest partition coefficient contain most of the F_c-receptor-bearing cells. A summary of these data is shown by the lines under the graph in Fig. 1, the

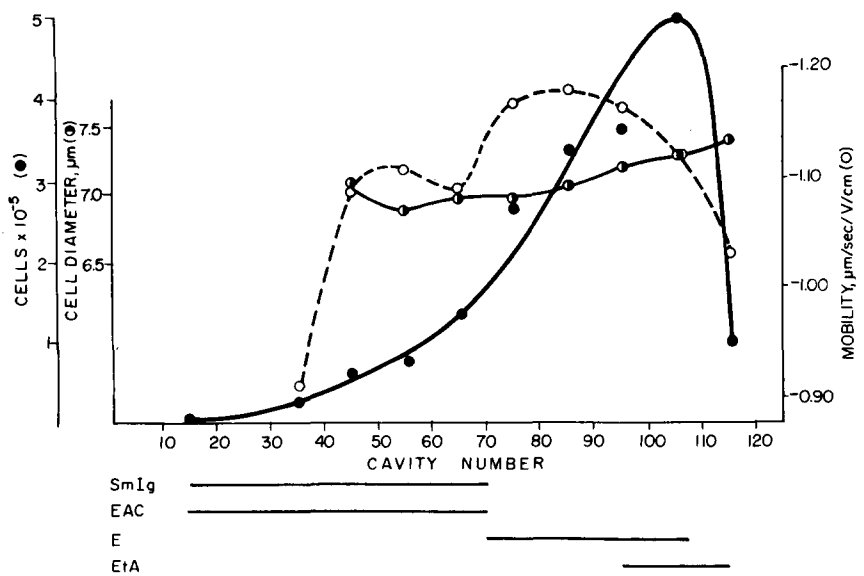

Fig. 1. Countercurrent distribution pattern of human peripheral blood lymphocytes in phase system 1 (see text for composition) having a high electrostatic potential difference between the phases. The number of cells found in the different cavities is plotted (●). In addition we show the mean electrophoretic mobilities (○) in μm/s per V per cm and mean diameter (⊙) in μm of lymphocytes through the distribution. Under the distribution curve of lymphocytes are drawn lines (from Refs. 6, 11) relating to different markers studied by rosetting and by fluorescent-labeled anti-human IgM and IgD. The lengths of the lines show the cavities in which the indicated markers are present to an extent greater than in the original, unfractionated lymphocyte population. SmIg, surface immunoglobulin-bearing (B-) lymphocytes (obtained with fluorescent-labeled antibody); EAC, complement-receptor-bearing (B-) lymphocytes (by rosetting with sheep erythrocyte-antibody (IgM)-complement complex); E, T-lymphocytes (by rosetting with sheep erythrocytes); and EtA, F_c-receptor-bearing lymphocytes (by rosetting with trypsinized sheep erythrocyte-antibody (IgG) complex). 119 transfers were carried out at 4—5°C with a settling time of 6 min and shaking time of 25 s. See text for additional details.

lengths of which indicate the cavities in which cells with indicated markers are present to an extent greater than in the original unfractionated lymphocyte population. In addition, the cell population with highest partition coefficient includes lymphocytes of largest mean size [6].

In Fig. 2 an analogous experiment is depicted in which a phase system of lower potential difference was used. Cells with highest affinity for the top phase (presumably highest charge-associated properties) can best be subfractionated and examined in this system. Again it is clear that cells with lowest partition coefficient have the lowest electrophoretic mobility and that with increasing partition coefficients the electrophoretic mobilities first increase and then, finally, decrease. We know that the lymphocytes with the highest partition coefficient contain cells with no response to T-cell mitogens [6] which also do not rosette with sheep erythrocytes (Ref. 12, at least not under conditions in which the bulk of T-lymphocytes do). It appears that most of the B-lymphocytes have the lowest, T-lymphocytes a higher and F_c-receptor-bearing lymphocytes an intermediate electrophoretic mobility whilst the highest electrophoretic mobility in the population (around cavity 85 in Fig. 2) belongs to lymphocytes which have yet to be characterized.

Electrophoretic mobilities of lymphocytes, unlike those of erythrocytes which are virtually invariant (-1.08 ± 0.02 μm/s per V per cm), are generally associated with fairly large standard deviations (approx. ±12%). These are due to the relative quantities of lymphocyte subpopulations present in the fraction examined as well as on the individual, immunological, etc. state of the donor [13]. The significance of differences in mobilities between adjacent sets of cavities was not always established due to the prohibitively large number of measurements that would be required. However, all experiments carried out showed the qualitative behavior illustrated in Figs. 1 and 2. A mean electro-

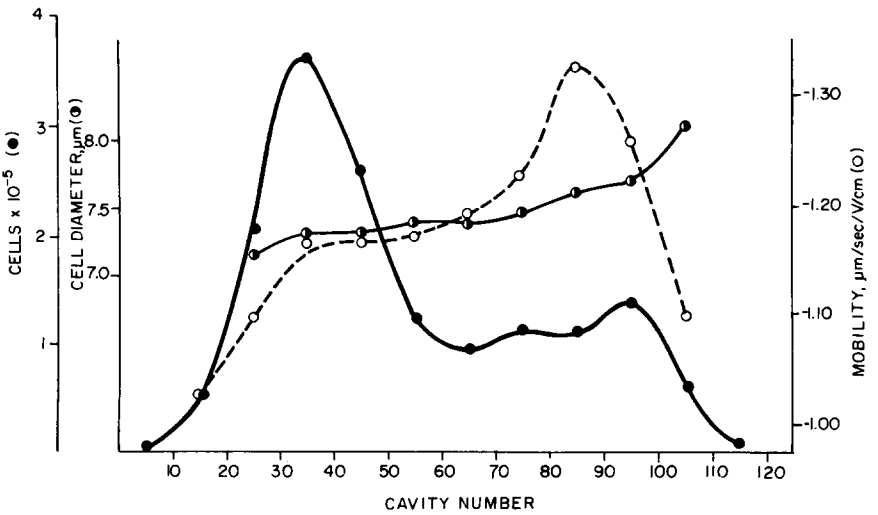

Fig. 2. Countercurrent distribution pattern of human peripheral blood lymphocytes in phase system 2 (see text for composition) having a low electrostatic potential difference between the phases. Graph symbols and conditions of countercurrent distribution as in Fig. 1. See text for discussion.

phoretic mobility of −1.05 has been reported for human peripheral blood lymphocytes with different individuals' cells having mean electrophoretic mobilities as low as −0.93 and as high as −1.14 [13]. Recent reports [14–17] indicate that 'normal' human B-lymphocytes have an electrophoretic mobility of −1.0 (or less) μm/s per V per cm, T-lymphocytes a mobility in excess of −1.15 and a lymphocyte population comprising F_c-receptor-bearing cells, 'null' cells and T-cells forming low-affinity rosettes with sheep erythrocytes has an intermediate mobility ranging from −1.0 to −1.15. Whilst the mean electrophoretic mobility values we obtained are somewhat higher, the sequence of increasing mobility of cell populations containing B-, F_c- and T-lymphocytes is the same (Fig. 1). In addition, countercurrent distribution reveals a lymphocyte subpopulation with a very high electrophoretic mobility (Fig. 2), the biological properties of which are not yet known. Finally, the mobility of the largest lymphocytes is lower than that of the smaller ones (Figs. 1 and 2), a result which agrees with a previous report [18] in which, however, due to their lower mobility, antibody-producing and large lymphocytes were thought to be synonymous.

The basis for the separation of cells in the charged phase systems used is most probably related to membrane charge-associated surface properties [2,5]. Yet it is clear that the cell population comprising F_c-receptor-bearing and the largest lymphocytes has an intermediate electrophoretic mobility but the highest partition coefficient. This result again emphasizes that the charges measured by cell electrophoresis and by partitioning are not necessarily the same [2]. One explanation for this observation is that the cell surface charge distribution of the F_c-receptor-bearing and large lymphocytes differ from those of the T-lymphocytes with respect to their ability to contribute to electrophoretic and partition behavior. Alternatively, the various subpopulations observed may be partially distinguished by a differential ability to bind one of the phase polymers, a phenomenon which can also affect the partition coefficient [5].

A practical consequence of the fact that F_c-receptor-bearing lymphocytes are among those having the highest partition coefficients in the human peripheral blood lymphocyte population is that one should be able to obtain populations enriched with respect to these cells by a simple one- or two-step extraction process [2]. The latter can be accomplished rapidly, without the addition of another cell population (as in the negative selection procedure of rosetting) and without centrifugation. The study of F_c-receptor-bearing lymphocytes should prove to be both of interest and importance since the quantity and properties of these cells correlate with some disease states (e.g., rheumatoid arthritis [17]).

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