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Human monocyte functional heterogeneity: monocyte fractionation by discontinuous albumin gradient centrifugation

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Summary. Human monocytes subserve many roles in the immune response. It is not clear, however, whether this functional heterogeneity reflects the action of different monocyte subpopulations. We separated human blood monocytes into distinct populations using a discontinuous (15–35%) serum albumin gradient technique. We examined if any of a number of monocyte functions were preferentially expressed by these five monocyte subsets. Monocytes in the 25% and 30% albumin fractions possessed more Fc (IgG) and C3 receptor activity than did monocytes in either of the 15, 20 or 35% fractions. In addition, monocytes isolated in the more dense albumin fractions were enriched for the capacity to support pokeweed mitogen-induced B-cell differentiation. All gradient fractions were equally capable of binding Raji cells and inhibiting Raji cell incorporation of [^{3}H]thymidine. These data indicate that fractionation of monocytes by a discontinuous albumin gradient is an effective method to enrich for those monocytes with certain functional characteristics.

INTRODUCTION

The macrophage exerts diverse biological functions. In man, characterization of the functional properties of this cell have generally focused on the blood monocyte because of its ready accessibility. These studies have shown that the monocyte contributes to both the afferent and efferent limb of the immune response. For example, monocytes by presenting antigen to T and B lymphocytes are requisite for the induction of immune responses. They elaborate several biologically active products, some of which subserve immunoregulatory activity, whereas others are directly involved in the inflammatory response (Unanue, 1980; Rosenthal, 1980). As effector cells, monocytes detect and destroy infectious agents and foreign cells primarily utilizing surface determinants, such as Fc (IgG) and C3 receptors (Frank, Schreiber & Atkinson, 1975). This versatility of action suggests that the blood monocyte, perhaps like the lymphocyte, represents an heterogeneous population with functionally distinct subsets.

To explore this question, we have separated monocytes into subsets which are distinguished by their ability to fractionate on a discontinuous albumin gradient. These subsets were then analysed for functional capabilities including expression of Fc (IgG) and C3 receptors, support of pokeweed mitogen (PWM)-driven B-cell activation, and recognition of a human lymphoblastoid cell line. The results indicate that monocytes isolated by fractionation on a 15–35% discontinuous albumin gradient show differential expression of Fc and C3 receptor activity and of their ability to support PWM-induced B-cell differentiation.

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MATERIALS AND METHODS

Preparation of blood mononuclear cells

Peripheral blood mononuclear cells were obtained from heparinized blood by ficoll-hypaque density gradient centrifugation. The cells were suspended in RPMI 1640 medium (Microbiological Associates, Walkersville, MD) supplemented with 10% foetal calf serum containing penicillin–streptomycin 1% v/v and glutamine 2% v/v.

Preparation of adherent cells

Mononuclear cells were adhered to plastic tissue-culture dishes to remove adherent cells as previously described (McDermott & Schreiber, 1978; Schreiber & Parsons, 1977). Following ficoll-hypaque density gradient centrifugation, mononuclear cells were suspended to $1{\cdot}5 \times 10^6$/ml in warmed RPMI 1640 supplemented with 10% heat-inactivated foetal calf serum, 2 mM glutamine, (100 U/ml) penicillin–streptomycin (1% v/v) and 50 μg garamycin. Corning (Corning Glass Works, Corning, NY) T150 tissue culture flasks were pretreated with this culture medium for 15 min at 37° and then 40–50 ml of the suspended cells were layered onto the flasks. After the cells were adhered for 1 hr at 37°, the non-adherent cells were gently decanted, and loosely adherent cells were removed by washing vigorously with warmed Hanks's balanced salt solution (Gibco Laboratories, Grand Island, NY). Cold phosphate-buffered saline without calcium or magnesium, but supplemented with 5% heat-inactivated foetal calf serum, was then added to each flask. Adherent cells were recovered following gentle perturbation and scraping of tissue culture plates with a rubber policeman and suspended at appropriate concentrations in cold RPMI 1640. More than 90% of the cells were esterase positive and able to ingest latex particles. These adherent cells are termed monocytes.

Density gradient separation of adherent cells

Thirty-five percent bovine serum albumin (Miles Laboratories, Enkhardt, In) was diluted in RPMI 1640 to prepare 15, 20, 25 and 30% fractions. One millilitre of each dilution of albumin was carefully layered into a sterile nitrocellulose tube (Beckman Instruments, Fullertown, CA) to form a discontinuous albumin gradient. The 15% layer was mixed with the adherent cells ($1–2 \times 10^7$), and this suspension was carefully layered on top of the gradient. The gradient was placed in a Beckman L5-50 ultracentrifuge (Beckman SW 50.1 swinging bucket rotor) and centrifuged at 8000 r.p.m. at 4° for 30 min. The cells localized at each gradient interface were removed with a pasteur pipette and washed once with cold RPMI 1640. Cells were >90% viable in the 15–30% fractions and >80% viable in the 35% fraction as determined by trypan blue exclusion. The cells in fractions 15–30% were >90% esterase positive and the cells in the 35% fraction were 80–85% esterase positive. More than 90% of the cells in the 15–30% fractions and >80% of the cells in the 35% albumin fraction were able to ingest latex particles (1·0 μM) and resembled monocytes when stained with Wright's Giemsa and examined by light microscopy. Cells from each fraction (>80%) were able to adhere equally to tissue culture plates (Falcon Plastics, Oxnard, CA) after being washed. The percentages of the cells in each fraction were: 15% fraction, 6 ± 2%; 20% fraction, 8 ± 1%; 25% fraction, 12 ± 1%; 30% fraction, 22 ± 3%; 35% fraction, 48 ± 3%. Monocyte recovery following albumin gradient centrifugation was 80 ± 5%.

Non-adherent cells

Mononuclear cells that were not adherent to the tissue culture flasks were washed in RPMI 1640 and readherence to new T150 flasks effected to remove residual adherent cells. In selected experiments non-adherent cells were passed over a Sephadex G-10 column to deplete residual adherent cells further. Scrubbed nylon fibres (Associated Biomedic Systems, Buffalo, NY) were placed in the tip of a 30-ml syringe. Sephadex G-10 (Pharmacia, Piscataway, NJ) equilibrated in phosphate-buffered saline pH 7·4 was autoclaved at 110° for 40 min and stored in the cold. Just before use the buffer was aspirated and the Sephadex G-10 was resuspended in the culture medium and a 5×25 cm column poured. Seventy million non-adherent cells in 3 ml culture medium were loaded onto the column at room temperature and 20 ml of effluent collected. These non-adherent cells were then washed and resuspended in RPMI 1640.

Assessment of Fc (IgG) and C3 receptor activity

Mononuclear adherent cell Fc and C3 receptor activity was assessed by a modification of established methods (Schreiber & Parsons, 1977). Adherent cell monolayers were prepared on tissue culture plates (Falcon Plastics, Oxnard, CA) for 1 hr at 37° following albumin gradient separation. One millilitre of 10^5 adherent cells was employed from each gradient fraction. The Fc receptor activity of these adherent

cells was assessed using D-positive human erythrocytes radiolabelled with [^{51}Cr]-sodium dichromate (New England Nuclear, Boston, MA). These erythrocytes were then quantitatively sensitized at 37° with IgG anti-D antisera in the presence of 0·01 M EDTA so as to carry 7200 IgG anti-D per erythrocyte, as determined by the radiolabelled antiglobulin test (Cines & Schreiber, 1979). Human erythrocytes were also sensitized with subagglutinating concentrations of human IgM antierythrocyte cold agglutinin and exposed to a dilution (1:10) of autologous serum as a source of human C3 (Schreiber, Herskowitz & Goldwein, 1977). These C3-coated erythrocytes are immune adherence positive and are coated with C3b as assessed using monospecific antisera (Cines & Schreiber, 1979). Radiolabelled IgG- or C3-coated erythrocytes were incubated at 37° for 2 hr with the adherent cell monolayers; mononuclear cell-bound erythrocytes were determined by lysing the washed monolayers with H_2O and measuring the radioactivity of the lysate (Schreiber & Parsons, 1977). Amount of lysate radioactivity (mononuclear adherent cell-bound erythrocytes) is proportional to Fc (IgG) and C3 receptor activity (Schreiber & Parsons, 1977). Fc and C3 receptor activity of adherent cells from each individual following albumin gradient separation were expressed as percentage of total activity (sum of lysate radioactivity from all five gradient fractions):

$$\% \text{ Fc or C3 receptor activity of each gradient fraction} = \frac{\text{radioactivity of gradient lysate fraction}}{\text{total radioactivity of gradient lysate fractions}} \times 100$$

Several investigators have observed that mononuclear cells and macrophages may be 'armed' to recognize target cells by preincubation with anti-target cell antibody (Alexander *et al.*, 1978; Parillo & Fauci, 1977). We assessed the ability of human monocytes and monocyte subpopulations to be armed with specific antibody. In these experiments, IgG anti-D antibody was employed with human D-positive erythrocyte targets isolated by elution from IgG anti-D sensitized erythrocytes with glycine hydrochloride-NaCl buffer (Williams & Chase, 1968). A 50% suspension of heavily sensitized anti-D coated erythrocytes in 0·15 M NaCl was incubated with an equal volume of glycine hydrochloride-NaCl buffer, pH 3·0, at 0° for 1 min. The cells were sedimented, the supernatant antibody harvested and the pH adjusted to 7·3 with 0·5 M Tris (hydroxymethyl) aminomethane buffer. The eluted IgG antibody was diluted with Hanks's balanced salt solution and 160 μg in 1 ml incubated with replicate monolayers of adherent monocytes for 30 min at 37°. The monolayers were washed, 1 ml of unsensitized erythrocytes (5×10^7) applied and sedimented for 5 min at room temperature (Gomez *et al.*, 1982) and the average number of erythrocytes bound to monocytes (red blood cell/monocyte) quantified visually after staining with Wright's Giemsa (McDermott & Schreiber, 1978). We observed that monocytes can be 'armed' (Alexander *et al.*, 1978; Parillo & Fauci, 1977) with IgG anti-D antibody to bind unsensitized human erythrocyte targets. However, this recognition process was not as efficient as when the erythrocytes were sensitized with IgG anti-D antibody prior to incubation with monocytes. Monocyte monolayers preincubated with IgG anti-D antibody and washed bound $2{\cdot}1 \pm 0{\cdot}5$ red blood cell/monocyte. However, if the erythrocyte targets were first sensitized with the same concentration of IgG anti-D antibody and washed prior to exposure to virgin monocytes, erythrocyte binding was more pronounced: $3{\cdot}9 \pm 0{\cdot}8$ red blood cell/monocyte.

Assessment of B-cell differentiation

Cultures of peripheral blood mononuclear cells (PBM), non-adherent cells and adherent cells were established in 0·2 ml in 96-well flat-bottom tissue-culture plates (Linbro, Flow Laboratories, Hamden, CN). The total number of cells cultured was kept constant at 1×10^5. Cultures were incubated in the presence or absence of optimal concentrations of PWM for 6 days at 37° in a humidified atmosphere containing 5% CO_2 and 95% air. B-cell differentiation was signalled by the appearance of immunoglobulin secreting cells (IgSC) in 6-day cultures. IgSC were enumerated by a protein A reverse haemolytic plaque assay as previously described (Levinson *et al.*, 1981). Briefly, 25 μl of sheep erythrocytes (40% suspension) which had been coated with protein A were mixed with a mononuclear cell suspension (100 μl appropriately diluted in Hanks's balanced salt solution) and with 250 μl of a 1·12% agarose (Seaplaque, Microbiological Associates, Bethesda, MD) at 43°. This mixture was then plated in a 5-cm plastic tissue-culture dish precoated with a layer of 2·5% agarose (Seakem, Microbiological Assoc., Walkersville, MD). The plates were incubated in a humidified atmosphere (5% CO_2/95% air) at 37°, and after 30 min, 1 ml of appropriately diluted sheep red blood cell-preadsorbed anti-Ig serum (Cappel Laboratories, Downingtown, PA) with specificity for human IgG, IgM and

IgA was added. After 1 hr of incubation, the anti-Ig serum was replaced with 1 ml of appropriately diluted guinea-pig complement (Gibco Laboratories, Grand Island, NY) which had been preadsorbed with sheep red blood cells. The addition of complement results in lysis of the red blood cells surrounding Ig-secreting cells and formation of a plaque. Plaques were counted under magnification and the results expressed as IgSC/10^6 cultured cells.

Monocyte recognition of Raji lymphoblastoid cells

Mononuclear adherent cells have the capacity to recognize (bind) human lymphoblastoid cells such as Raji (Guerry *et al.*, 1978). We studied our mononuclear adherent cells for such binding as previously described (Guerry *et al.*, 1978). Raji cells were generously supplied by the University of Pennsylvania Cell Center and were >95% viable as assessed by trypan blue exclusion. Mononuclear cell monolayers were first pre-incubated with latex particles and washed. One millilitre of Raji cells (1×10^6/ml) in RPMI with 10% heated (56°) AB serum were then added to the mononuclear cell monolayers (1×10^5 cells) and incubated for 2 hr at 37° under 5% CO_2. The monolayers were then washed five times with RPMI, air dried and stained with Wright's Giemsa. Binding was examined by light microscopy by assessing the number of Raji cells attached to 200 latex-containing mononuclear cells. The data were expressed as the mean number of Raji cells per monocyte. The ingestion of latex particles by mononuclear adherent cells did not affect their ability to bind Raji (Guerry *et al.*, 1978).

Monocyte inhibition of Raji cell growth

We also explored whether mononuclear adherent cells differ in their ability to inhibit the incorporation of [^{3}H]thymidine into Raji lymphoblastoid cells. Mononuclear adherent cells (0·1 ml) were added in increasing concentrations (in quadruplicate) to flat-bottom Linbro plates (Flow Laboratories, Hamden, CT). Raji cells were added in a volume of 0·1 ml at a concentration of 5×10^5/ml. The buffer system was as indicated above for adherent cells, and effector (adherent cell):target cell (Raji) ratios ranged from 80:1 to 1:1. Adherent and Raji cells were incubated at 37° under 5% CO_2 in a humidified chamber for 24 hr. Tritiated thymidine was then added to each well and the incubation continued for 24 hr. Cells were then harvested (Otto Heller Co. Cell Harvester, Madison, WI) and the incorporation of [^{3}H]thymidine into Raji cells determined.

Percentage inhibition of [^{3}H]thymidine incorporation into Raji cells =

$$\left(1 - \frac{\text{c.p.m. incorporated into Raji + adherent cells}}{\text{c.p.m. incorporated into Raji}}\right) 100$$

Under the experimental conditions, adherent cells alone incorporated < 1% of the amount of [^{3}H]thymidine incorporated by Raji cells, in all experiments.

Statistical analyses were performed using the paired *t* test.

RESULTS

Fc and C3 receptor activity

The relative Fc (IgG) and C3 receptor activities of the monocyte (adherent cell) albumin gradient fractions are depicted in Fig. 1. The results were similar for both receptors. Monocytes that fractionated with the 25% or 30% albumin gradient fractions possessed significantly greater Fc and C3 receptor activity than did monocytes in the 15%, 20% or 35% gradient fractions ($P < 0{\cdot}005$). Unfractionated monocytes possessed Fc and C3 receptor activity which approximated the mean activity of all the fractions. Similar results were observed in three experiments in which monocyte albumin gradient fractions were preincubated or armed with IgG anti-D antibody. When the 30% and 35% monocyte fractions were incubated at 4° for 30 min with different concentrations of albumin, neither Fc nor C3 receptor activity was altered. These data

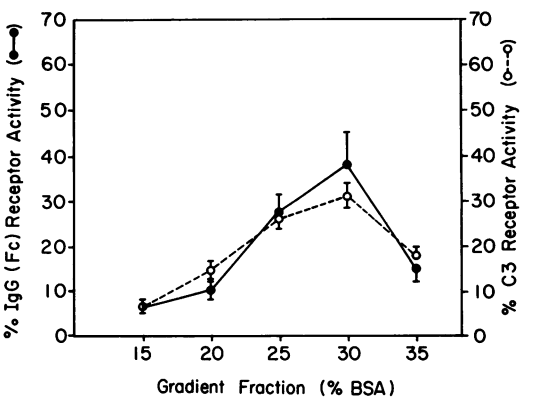

Figure 1. IgG (Fc) and C3 receptor activity of monocyte albumin gradient fractions. The relative percent monocytes binding IgG-coated human erythrocytes (●——●) and C3-coated human erythrocytes (O- -O) are shown for bovine serum albumin gradient fractions 15–35%.

indicate that fractionation of monocytes by discontinuous albumin gradients can enrich for those cells with substantially increased or decreased Fc and C3 receptor activity.

Support of B-cell differentiation

For these studies the 15% and 20% gradient fractions were pooled, as were the 25% and 30% gradient fractions. These fractions were compared with the 35% gradient fraction. Preliminary studies were carried out to determine culture conditions for demonstrating dependence of PWM-induced B-cell differentiation on monocytes. Previous studies indicated that cell number and configuration of the culture wells are important factors to be considered in experimental design (Knapp & Baumgartner, 1978). Monocyte dependence was readily apparent under the culture conditions used in these studies. In five experiments, less IgSC were generated in cultures depleted of adherent cells (<0·5% adherent cells) using the two-step technique described (Table 1). Moreover, IgSC responses were augmented by the addition of from 5–10% adherent cells.

Table 1. Response of mononuclear cell fractions to PWM

	IgSC/10^6 Cultured Cells				
Cells*	EXP 1	EXP 2	EXP 3	EXP 4	EXP 5
Unfractionated	5500	2205	20,460	4680	874
Non-adherent	875	300	4539	2850	180
Adherent	0	151	237	60	80
Non-adherent + adherent†	2625	1383	31,746	5300	780

* Cells (1×10^5) were cultured in replicate wells with PWM (1:1000) for 6 days whereupon IgSC were measured.

† 1×10^4 adherent cells were added to 9×10^4 non-adherent cells.

We next determined if albumin density gradient separated adherent cells differed in their capacity to support PWM-induced differentiation of B lymphocytes. Five experiments were performed (Table 2). Reconstitution of the IgSC response of non-adherent cells was consistently seen with the unfractionated adherent cells. In four of five experiments the 35% gradient fraction and in five of five experiments the 35% and/or the 25–30% gradient fractions were more effective than the unfractionated monocytes. In contrast, the 15–20% gradient fraction was less effective than the unfractionated monocytes ($P < 0.05$). The 15–20% gradient fraction provided no support of B-cell differentiation in two experiments (experiments 1 and 5). Thus, in all experiments the 35% and/or the 25–30% fractions were more effective in supporting PWM-induced B-cell differentiation than the 15–20% gradient fractions.

Table 2. Effect of adherent mononuclear cell populations on PWM-induced IgSC response of non-adherent cells

	Ratio: (non-adherent + adherent) IgSC / non-adherent IgSC†				
Adherent cells added*	EXP 1	EXP 2	EXP 3	EXP 4	EXP 5
Unfractionated	3·0	4·3	1·7	6·9	1·8
15%+20%	0·75	3·3	1·7	5·5	1·0
25%+30%	1·62	1·6	3·0	18·1	5·4
35%	14·50	5·1	3·0	4·6	3·5

* Replicate mixtures of 9×10^4 nonadherent cells plus 1×10^4 adherent cells were cultured with PWM for 6 days whereupon IgSC were measured.

† Non-adherent cells (1×10^5) were cultured alone as described.

Monocyte recognition of Raji cells

Incubation of Raji cells with mononuclear adherent cells led to close physical association between the two cell types. Frequent formation of rosettes was observed, as previously described (Guerry *et al.*, 1978). These adherent cells (monocytes) were fractionated by bovine serum albumin discontinuous gradient centrifugation and examined for their ability to bind Raji cells. There was no difference between the monocyte

Table 3. Monocyte subset recognition of Raji lymphoblastoid cells

Gradient fractions	Raji-bound/monocyte*
15%	1·2±0·5
20%	1·1±0·5
25%	1·0±0·4
30%	0·9±0·6
35%	1·1±0·6
Unfractionated	0·9±0·6

* Results represent Mean±SEM of Raji-bound per monocyte in 4 experiments.

Table 4. Inhibition of Raji cell growth*

Gradient fraction	Monocyte/Raji ratio				
	80:1	40:1	20:1	10:1	5:1
15%	—	—	12±3	8±2	8±2
20%	31±5†	11±3†	11±2	5±2	5±2
25%	25±5	10±3	9±2	9±2	9±3
30%	30±3	20±4	12±2	8±2	5±2
35%	32±4	18±4	4±2	9±2	10±3

* Results represent mean %±SEM of at least 3 experiments.

† Results represent a pool of the 15% and 20% gradient fractions.

fractions in their ability to express this recognition process (Table 3). Similar results were observed when the data were expressed as the number of monocytes binding one or more Raji cells.

Incubation of blood monocytes with Raji lymphoblastoid cells also resulted in inhibition of the incorporation of [^{3}H]thymidine into Raji cells. However, significant inhibition was only observed at high effector to target cell ratios (Table 4). There were no apparent differences among the monocyte fractions in expressing this activity.

DISCUSSION

Human peripheral blood monocytes serve several functions which range from antigen processing to effector mechanisms involved in the detection of foreign cells (Rosenthal, 1980; Frank, Schreiber & Atkinson, 1975; Schroer & Rosenthal, 1980). This suggests that monocytes may represent a heterogeneous population of cells with distinct functional subsets. We separated human monocytes (adherent, phagocytic peripheral blood mononuclear cells) into five different populations using a discontinuous bovine serum albumin gradient technique. In doing so, we observed heterogeneity of certain monocyte functions among these populations. Although the cells were morphologically similar, the monocytes in the 25% and 30% fractions possessed substantially more Fc (IgG) and C3 receptor activity than did the monocytes in the three other albumin fractions. Furthermore, these monocyte subpopulations with enhanced Fc receptor activity were also observed to be most effective in the IgG antibody-dependent cell-mediated cytolysis (ADCC) of tumour cell line targets (unpublished observation).

In addition, monocytes in the more dense albumin gradient fractions were enriched for the capacity to support PWM-induced B-cell differentiation (Table 2). Certain other monocyte effector functions, such as the ability of monocytes to bind Raji cells—a binding process mediated by a non-immunoglobulin, non-complement, trypsin-sensitive locus (Guerry *et al.*, 1978)—and the ability of monocytes to inhibit the incorporation of [^{3}H]thymidine into Raji cells were equally distributed among the five monocyte fractions (Tables 3, 4).

The ability of monocyte gradient fractions to support pokeweed mitogen (PWM)-induced B-cell differentiation was assessed to explore whether one or more monocyte subsets are particularly active in the inductive phase of the immune response. This is an important issue, since the support of B-cell differentiation is a major function of the human monocyte. Monocytes have been observed to have a dual effect (inhibition or enhancement) on the PWM-induced B-cell response (Knapp & Baumgartner, 1978; Gnelig-Mayling & Waldmann, 1981). It is not known whether these differential effects reflect the action of distinct monocyte subpopulations.

We confirmed the observation that monocytes are required for optimal B-cell differentiation. Rigorous depletion of adherent cells using a two-step technique—adherence of monocytes to tissue-culture flasks followed by adherence of monocytes to Sephadex G-10—led to a reduction in the IgSC response (Table 1), an index of B-cell differentiation. This response was reconstituted with the addition of 5–10% autologous monocytes. Our studies indicate that monocytes in the more dense fractions (35% or 25–30%) are particularly effective in B-cell differentiation, when compared to either unfractionated monocytes or monocytes in the less dense (15–20%) gradient fraction (Table 2). Further studies are necessary to determine whether the reduced effectiveness of the monocytes in the 15–20% gradient fraction is due to the depletion of a subpopulation of monocytes especially effective in supporting B-cell differentiation or the relative enrichment of a subpopulation of monocytes that inhibits B-cell differentiation.

Other investigators have analysed peripheral blood monocytes for their functional heterogeneity. Raff *et al.* employed a 17–25% discontinuous albumin gradient and observed an increase in Fc receptor activity in a 17–23% fraction, as published in abstract form (Raff *et al.*, 1974). Studies using counterflow centrifugation have also separated monocytes according to

their functional surface receptor characteristics (Sanderson *et al.*, 1977; Yosaka *et al.*, 1981). These investigators observed that volumetrically larger monocytes released more superoxide to certain phagocytic stimuli and were more effective in stimulating the growth of stem cells into myeloid cells (Norris *et al.*, 1979). Such monocytes are also more responsive to chemotactic stimuli (Arenson, Epstein & Seeger, 1980; Cionciola & Snyderman, 1981). Differences among human monocytes in prostaglandin-E synthesis (Bankhurst *et al.*, 1981; Goldyne & Stobo, 1979; Picker *et al.*, 1980), and IgA (Fanger *et al.*, 1980) and IgE (Melewicz & Spiegelberg, 1980) surface receptors have also been observed.

Studies in animal models, including those using a similar discontinuous albumin gradient technique (Chapes & Tompkins, 1981) also suggest functional heterogeneity among cells of the macrophage lineage (Fishman & Weinberg, 1979). With regard to antigen-presenting properties, it now appears probable that only Ia^+ macrophages are active. For example, the ability of murine macrophages to present soluble antigen to antigen-primed T cells is restricted to an Ia^+ macrophage subset (Lee & Wong, 1980). Heterogeneity of Fc receptors on peritoneal macrophages have also been observed (Kovai, Laczko & Csabo, 1979). Furthermore, murine peritoneal macrophages isolated by discontinuous gradients of ficoll have been noted to be heterogeneous in their ability to alter the growth and viability of allogeneic tumour cells (Campbell, Shalley & Miller, 1980).

Our data suggest that isolation of human monocytes by discontinuous albumin gradient centrifugation can identify those monocytes that are particularly effective in certain functional assays. It is as yet unclear whether this monocyte heterogeneity reflects distinct subsets or differences in stages of maturation or activation. The availability of monoclonal antibodies directed at antigens on the monocyte surface should provide a further basis for identifying monocyte subsets which might differ in their functional properties, including those involved in antigen processing. Recent studies suggest that certain monoclonal antibodies directed at specific monocyte/macrophage determinants may select for human subpopulations required for antigen presentation to reactive T cells (Raff, Picker & Stobo, 1980). Nevertheless, the experience with the isolation of plasma proteins suggests that more than one preparative procedure is often necessary to achieve immunochemical and functional homogeneity. A similar approach will probably be useful in the isolation of functionally distinct subpopulations of cells, such as monocytes. The ability to isolate functionally distinct monocyte subpopulations may thus very well involve more than one preparative procedure. Furthermore, preparative techniques that enrich for monocytes with marked Fc and C3 receptor activity, and that depend upon the binding of monocytes to Fc (IgG)- and/or C3-coated substances or depend upon the binding of monocytes to antibodies directed at the receptor site, may alter the receptor or cell function. The discontinuous albumin gradient technique provides a method of significantly enriching for monocytes with substantial Fc and C3 receptor activity which does not depend upon these receptors for the isolation process.

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