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Chemotactic factor-induced polarization, receptor redistribution, and locomotion of human blood monocytes

L. N. ISLAM & P. C. WILKINSON *Department of Bacteriology and Immunology, Western Infirmary, Glasgow*

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SUMMARY

The locomotor response of human blood monocytes to chemotactic factors was studied using a polarization assay on cells in suspension and by filming locomotion on albumin-coated glass. Cells in optimal (5×10^{-9} M) but uniform concentrations of *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) polarized well and showed a 'persistent random walk' type of locomotion, whereas in supraoptimal concentrations (10^{-7}M), the cells took erratic paths and polarized poorly, suggesting that monocytes cannot develop an anteroposterior polarity if hit by ligand molecules at many points on the cell surface simultaneously. Monocyte polarization in chemotactic factors at 37° was transient and was gradually lost after 15–20 min. Likewise, the ability to form Fc rosettes after this time was gradually lost, suggesting loss of functional receptors from the cell surface with time. In optimally polarized cells, Fc rosettes were frequently localized at the head of the cell. This localization also was lost with time. Using pure chemotactic factors (FMLP, C5a, leukotriene B_4) we found, as reported earlier (Cianciolo & Snyderman 1981), that polarization was restricted to a subpopulation (approximately 60% of cells) that responded to multiple attractants. However, 80–90% of monocytes polarized in response to combinations of any of the above pure attractants with candida-activated serum. This suggests that the subpopulation that lacks receptors for classical chemotactic factors nevertheless has locomotor capacity and can respond to undefined factors in activated serum, and that the great majority of blood monocytes is motile if appropriately stimulated.

INTRODUCTION

Although the locomotion of monocytes across endothelium into tissues is obviously important, not only in inflammation but also in the delivery of macrophages to the numerous tissues in which they normally reside, there have been few studies of the locomotor behaviour of monocytes other than studies of chemotaxis using filter or agarose assays. The assay of morphological polarization following addition of an attractant has been used widely in the study of neutrophils (Smith & Hollers, 1980; Keller, Zimmermann & Cottier, 1983; Haston & Shields, 1985) and lymphocytes (Wilkinson, 1986), but little in the study of monocytes. Cianciolo & Snyderman (1981) used this assay to show heterogeneity in the human blood monocyte response to attractants. About 60% of the cells responded to FMLP, chemotactic lymphokines or activated serum, but combinations of all three did not increase this percentage, suggesting the presence of a responsive receptor-bearing population and a remainder of unresponsive cells. We have re-examined these observations here and suggest that, while there are indeed two populations, if appropriate conditions are used practically all of the monocytes in blood can be stimulated into locomotor activity.

We have also studied polarization and locomotion of monocytes at optimal and supraoptimal concentrations of attractant (FMLP) to explore the idea, previously elaborated based on experiments with blood neutrophils (Shields & Haston, 1985), that in uniform, optimal concentrations of attractant, polarization and subsequent locomotion are efficient because the attractant concentration is sufficiently low for a cell to establish an anteroposterior polarity by responding to an initial stimulus at one pole, but that, at high concentrations, ligand binding occurs simultaneously at many points and the cell therefore fails to establish a polarity or to move efficiently. Experiments to test this in monocytes are described here. In neutrophils, polarization is accompanied by asymmetrical distribution of functional receptors to the cell head (Walter, Berlin & Oliver, 1982; Wilkinson, Michl & Silverstein, 1980). Here we report on the relationship between polarization of monocytes and the distribution of Fc receptors, measured by rosetting, on the cell surface, and point out differences in the maintenance of polarity and receptor asymmetry between monocytes and neutrophils.

Correspondence: Dr L. N. Islam, Dept. of Bacteriology and Immunology, Western Infirmary, University of Glasgow, Glasgow G11 6NT, U. K.

MATERIALS AND METHODS

Materials

Morpholinopropane sulphonic acid (MOPS), FMLP, glutaraldehyde (Grade II) and α-naphthyl acetate were obtained from Sigma, Poole, Dorset. Hanks' balanced salt solution (HBSS), Ca^{2+}-, Mg^{2+}-free salt solution (CMFS), RPMI-1640, fetal calf serum (FCS), sheep red blood cells (SRBC) and lymphocyte separation medium were from Flow Labs, Rickmansworth, Herts. Leukotriene B_4 (LTB_4) was from Miles Labs Ltd, Slough, Bucks. Platelet-activating factor (PAF) was from Novabiochem., Läufelfingen, Switzerland. Human serum albumin (HSA) was from Behringwerke, Marburg, FRG. Purified hog complement peptides C5a, C5a des Arg (C5adA), C3a and C3a des Arg were kindly provided by Dr B. Damerau, University of Göttingen, FRG (Damerau *et al.*, 1980). Rabbit anti-sheep red blood cell antibody was from Difco Labs, Detroit, MI.

Test substances

FMLP was prepared as a stock solution of 10^{-2}M in DMSO and stored at −20°. Fresh peptide solution at different dilutions was made up every day in HBSS + 10 mM MOPS, pH 7·4 (HBSS-MOPS). A stock solution (10^{-5}M) of LTB_4 was prepared in cold HBSS-MOPS and dilutions in the same medium were prepared and used on the day of breaking the ampoule. All solutions of LTB_4 were kept at 4° until used, as the substance is highly susceptible to decomposition/isomerization. A stock solution of PAF was prepared at 10^{-5}M in HBSS-MOPS and kept at 4°. Different batches of alkali-denatured human serum albumin (adHSA) were prepared as described previously (Wilkinson & Bradley, 1981). Activated human serum (AHS) was prepared by incubating (1 hr at 37°) fresh human serum with freshly cultured and washed *Candida albicans* blastospores at $5–6 \times 10^6$ spores/ml of serum (*C. albicans* was isolated from clinical specimens and maintained in culture in Sabouraud-dextrose agar, Oxoid Ltd, Basingstoke, Hants). Similar activation was obtained either with ethanol-killed (70% in saline) or live spores. At the end of the incubation period, the spores were removed from serum by two centrifugations at 800 *g* for 5 min. This candida-activated human serum (AHS) was stored at −20° until used.

Cells

The mononuclear cell fraction from normal human venous blood (heparinized) was obtained by centrifugation on lymphocyte separation medium. Monocytes were purified by adherence to a BHK cell-microexudate-coated flask followed by detachment using 3·5 mM EDTA as described by Ackerman & Douglas (1978). This detached most of the adherent cells, which were collected in a siliconized glass tube and washed three times with RPMI-FCS. Use of siliconized glass rather than tissue-culture plastic was important to minimize loss of monocytes due to adherence. The cell pellet was finally suspended in HBSS-MOPS containing 20 mg/ml HSA at 10^6 cells/ml for the polarization assay. This cell preparation contained >98% viable cells. Monocytes were identified by staining for non-specific esterase (Mueller *et al.*, 1975). The cells purified on a BHK microexudate-coated surface consisted of >96% monocytes, the remainder being lymphocytes. Purification on BHK-microexudate-coated surfaces was found to give better yields than other methods, including Percoll or Nycodenz centrifugation, or coating flasks with gelatin-plasma or with FCS (Islam, 1986).

Polarization assay

Monocytes in siliconized glass tubes were incubated with and without chemotactic factors for various times at 37°. Then the cells, without being allowed to cool, were fixed with 2·5% glutaraldehyde for 10 min, washed twice and resuspended in the remaining droplet of fluid after decanting the washing medium. The proportion of polarized cells was estimated by counting at least 300 cells from each preparation using a ×40 phase-contrast objective. The percentage of monocytes polarized was calculated as

$$\frac{\%\text{ total cells polarized}}{\%\text{ esterase-positive cells in the initial cell suspension}} \times 100.$$

Cells that showed ruffling at one edge or a polarized morphology with constriction ring and/or blebs were scored as responding. This distinction was straightforward for most of the cells in the population using chemotactic factors at optimal concentrations. However, in supraoptimal concentrations, cells, though deviating from a spherical shape, did not show a clear anteroposterior polarity. These cells often protruded pseudopodia at several points giving a multipolar appearance. These cells could be distinguished from well polarized cells by measuring the length-breadth ratio.

Measurement of length-breadth ratios

The images of 100 randomly chosen monocytes from different preparations were projected onto paper and their outline traced using a *camera lucida* drawing tube fitted to a Wild M 20 microscope (× 100 objective). The longest axis of each cell was measured and the results expressed as mean cell length ±SD (μm). The perpendicular distance at the midpoint of the 'length' axis was measured to obtain the breadth of each cell. The length : breadth ratio gave a quantitative expression of polarity.

Preparation of antibody-coated red cells

Sheep red blood cells (SRBC) were washed thrice with HBSS-MOPS and 0·2 ml of the packed cells were resuspended in 10 ml of a subagglutinating dilution (1:200) of rabbit anti-SRBC antibody in HBSS-MOPS and incubated for 30 min at 37°. Then the red cells were washed three times and resuspended in 10 ml of HBSS-MOPS at 4°.

Fc-rosetting assay

Monocytes in HSA (20 mg/ml)/HBSS-MOPS ± FMLP (5×10^{-9}M) were incubated on glass coverslips for various times at 37°. Then the cells were immediately incubated with antibody-coated SRBC at 4° for 1 hr, after which the coverslips were rinsed in cold HBSS-MOPS and fixed with 2·5% glutaraldehyde, rinsed and mounted in HBSS-MOPS for inspection. A cell with at least three bound red cells was scored as Fc-rosetted and 300 cells were counted from each preparation to estimate the percentage of Fc-rosetted monocytes.

Time-lapse filming for monocyte locomotion

The filming chamber used for visual assays of monocyte locomotion was a rectangular aluminium slide ($76 \times 26 \times 1·5$ mm) with a hole of 20 mm diameter drilled out of the centre. The details of filming assays with these chambers were described previously (Allan & Wilkinson, 1978). A clean 22 mm glass coverslip was attached to the bottom of the chamber with silicone grease. Monocytes, suspended at 4×10^5 cells/ml in 100

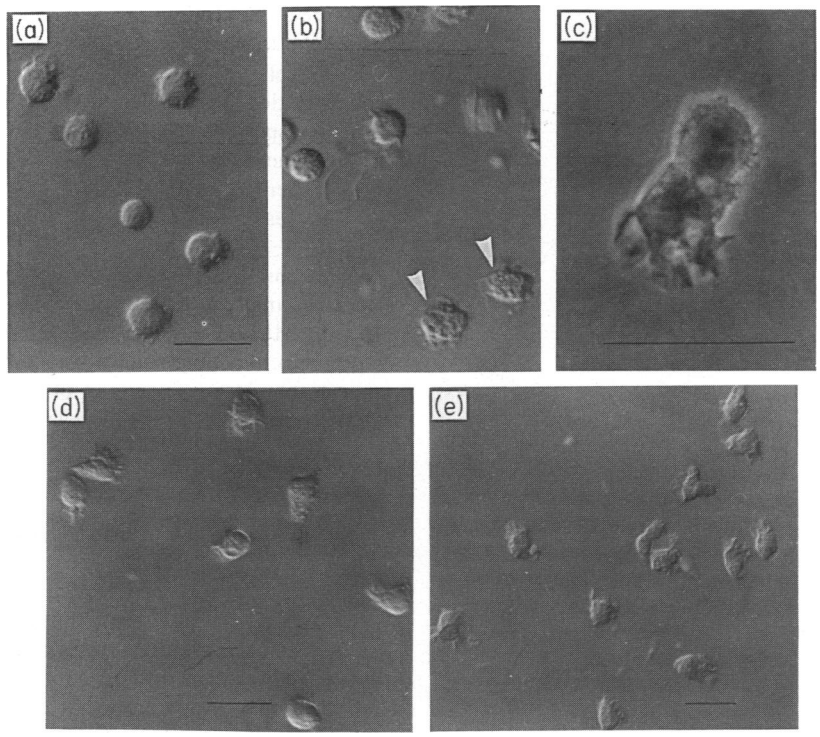

Figure 1. Monocytes fixed in suspension after 20 min at 37° in: (a) HSA/HBSS-MOPS; (b) 10^{-7} M FMLP (note the multipolar morphology of some monocytes, arrowed); (c, d) 5×10^{-9} M FMLP; (e) a mixture of FMLP at 5×10^{-9} M and AHS at 10% v/v. Photographs (a) and (b) are at same magnification. (c) High-power phase-contrast photograph. (a, b, d, and e) Differential-interference-contrast photographs. Bars = 20 μm.

mg/ml HSA/HBSS-MOPS containing FMLP at 10^{-7}M or 5×10^{-9} M, were added in sufficient volume to fill the chamber completely (0·5 ml). Then the chamber was sealed with another coverslip. Filming was carried out at 37° starting 10 min after setting up the chamber and the cells were filmed at 15 frames per min.

Film analysis

Films were projected onto drawing paper using a stop-action 16 mm cine projector. Monocyte outlines were drawn at the first frame, and after each subsequent 20 frames (80 seconds) the cell centre was marked. These points were joined to give a cell track. The angle of turn between succeeding steps was measured from each track. The cell tracks were analysed to give the mean values for speed, diffusion coefficient and persistence time for the whole population. This was greatly simplified with the help of a graphic digitizing tablet (Summagraphics Ltd, Bit pad one, Fairfield, CT) linked to a BBC computer and using a program written by Dr J. M. Lackie, Department of Cell Biology, University of Glasgow. Details of this type of analysis and the program used are published elsewhere (Lackie & Burns, 1983).

RESULTS

Monocyte polarization

Control monocytes maintained carefully in suspension by preparation on siliconized glass remained spherical for up to 2 hr at 37° in HBSS-HSA. Addition of chemotactic factors caused ruffling within 30 seconds and within a few minutes a typical anteroposterior polarization with leading edge and constriction rings was evident (Fig. 1c & d). As previously reported by Cianciolo & Snyderman (1981), FMLP induced time- and dose-dependent polarization. In our experiments, the optimum concentration and time were 5×10^{-9}M and 20 min. Under these conditions 67·5 ± 5·5% of monocytes were polarized ($n = 9$). At this concentration of FMLP the cells showed good polarization (mean cell length 13·3 ± 0·5, c.f. 11·2 ± 0·3 μm for spherical cells, mean length:breadth ratio (1·57 ± 0·2). However, using supraoptimal concentrations of FMLP (10^{-7}–10^{-6}M) polarization was poor with irregular cell outlines. This 'multipolar' appearance is shown in Fig. 1b. Mean cell length at 10^{-6} M FMLP was 11·2 ± 0·2 μm; length:breath ratio 1·26 ± 0·1, and very few cells responded.

Monocyte locomotion in uniform optimal and supraoptimal concentrations of FMLP

Monocytes were allowed to adhere to HSA-coated glass coverslips and their locomotion was filmed in an optimal (5×10^{-9}M) and a supraoptimal uniform (10^{-7}M) concentration of FMLP. The concentration of HSA required to coat glass for optimal monocyte locomotion is high (50–100 mg/ml). Representative tracks are shown in Fig. 2. At optimal FMLP concentration, the well-polarized cells moved in smooth tracks with a tendency to persist in a given direction and to make narrow turns (mean angle of turn, 43°). However, at high FMLP concentration the poorly polarized cells showed erratic paths with frequent turns (mean angle of turn, 73°; FMLP 5×10^{-9}M, c.f. 10^{-7} M; Mann–Whitney U-test, $P < 0{\cdot}0001$). The true speed (S) and the 'persistence time' (P) of these cells was calculated by procedures detailed by Wilkinson *et al.* (1984). In the presence of

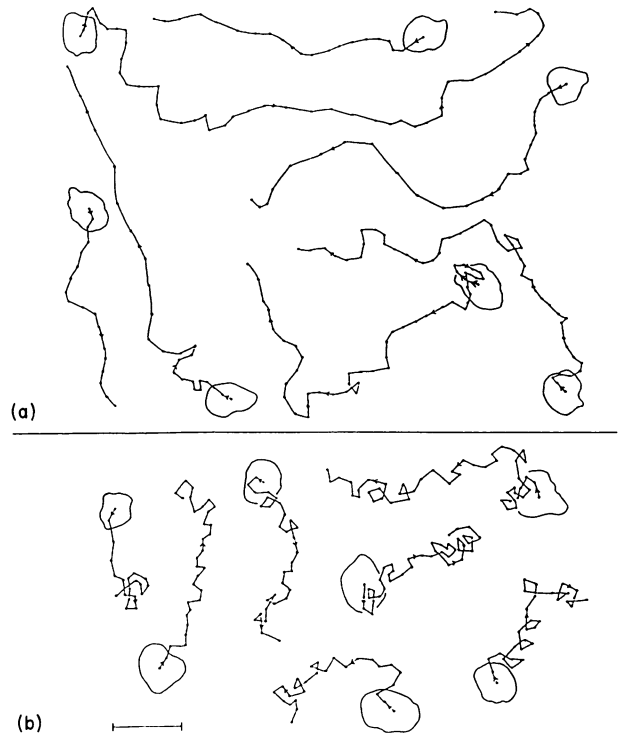

Figure 2. Tracks of monocytes moving on glass coverslips coated with HSA at 100 mg/ml in the presence of a uniform concentration of FMLP. (a) 5×10^{-9} M FMLP; (b) 10^{-7} M FMLP. Note the cells showed more erratic paths with more frequent turns in (b) than in (a). Bar = 20 μm.

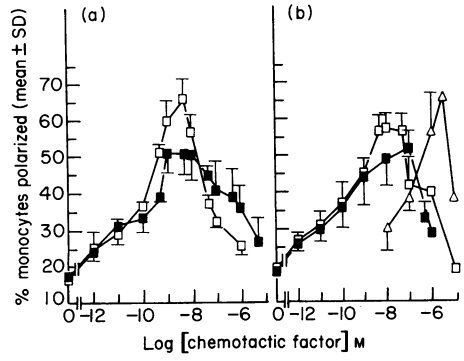

Figure 3. Dose–response curves for monocyte polarization in the presence of various chemo-attractants. Monocytes were fixed in suspension with 2·5% glutaraldehyde after exposure to (a) pure factors: FMLP for 20 min (□, $n=7$), leukotriene B_4 for 10 min (■, $n=6$); (b) complement peptides: C5a (□, $n=3$), C5adA (■, $n=4$) and C3a (△, $n=4$), each for 10 min. Higher doses of the complement peptides were not used more than once due to shortage of the samples.

100 mg/ml HSA, the speed of cells in 5×10^{-9}M FMLP was $4{\cdot}51 \pm 0{\cdot}46$ μm/min ($n=20$), and in 10^{-7}M FMLP $3{\cdot}98 \pm 0{\cdot}16$ μm/min ($n=19$). Thus raising the concentration of FMLP above optimum does not cause much reduction in cell speed. The speeds of these monocytes are considerably slower than those reported earlier for neutrophils under similar conditions by Shields & Haston (1985). The 'persistence time', which is a measure of the straightness of path, as defined by Dunn (1983), was $1{\cdot}11 \pm 0{\cdot}24$ min for cells in 5×10^{-9} M FMLP and $0{\cdot}54 \pm 0{\cdot}06$ min for cells in 10^{-7} M FMLP. Thus cells in a supraoptimal concentration of FMLP, although they were moving at about the same speed as cells in an optimal concentration of FMLP, would be expected to disperse much less effectively because of their higher frequency of turn. This is borne out by calculations of the diffusion coefficient $R(=2S^2P)$ for the two populations: R was $45{\cdot}15 \pm 0{\cdot}1$ μm²/min in 5×10^{-9} M FMLP and $17{\cdot}1 \pm 0{\cdot}01$ μm²/min in 10^{-7}M FMLP. As well as this, the proportion of translocating cells in 5×10^{-9}M FMLP was higher (34%) than in 10^{-7}M FMLP (17%). All these factors would lead to a much more effective dispersal of monocytes in optimal than in supraoptimal concentrations of FMLP (and to much more effective accumulation of the cells at a source if a gradient were present).

Polarization response of monocytes to purified attractants

Figure 3 shows dose–response curves for percentages of monocytes polarized in suspension in FMLP and in the presence of a number of chemo-attractants not previously studied using this assay. Other than FMLP, none of these induced >60% polarization of blood monocytes. The active concentrations of these factors are similar to those for neutrophils. Note that C3a has activity only at high concentrations, as was reported previously for neutrophils (Damerau *et al.*, 1980). PAF and C3a desArg (not shown) had low activity. Alkali-denatured HSA (not shown) had polarizing activity that varied from batch to batch. The most active sample at 1·7 mg/ml ($2{\cdot}5 \times 10^{-5}$M) gave 64·5% polarized monocytes. These findings suggest that, as previously reported, not more than 60–70% of human blood monocytes respond to pure chemotactic factors. Monocytes also showed polarization in *C. albicans*-activated human serum. The optimal concentration of serum was 10%, at which 61·5% of the cells were polarized. Control preparations of *C. albicans* spores (living or dead) had no activity in polarizing monocytes in the absence of serum.

Combined effect of various chemotactic factors on monocyte polarization

In order to test whether the cell population that was polarized by one chemo-attractant could be induced to polarize further in the presence of additional chemo-attractants, monocytes were incubated with mixtures of chemo-attractants each at their optimum concentration. The results from several experiments are summarized in Table 1, which shows that when pure substances (FMLP, C5a, LTB_4) were combined together, the percentage of polarized cells did not exceed the number obtainable with the maximally effective concentration of any chemo-attractant tested alone. However, when fresh candida-activated human serum (AHS) was combined with any of the pure chemotactic factors, an additive effect was seen, with >80% monocyte polarization in most cases. With FMLP+AHS the effect was maximum and $85{\cdot}9 \pm 7{\cdot}3$% (SD) ($n=13$) of monocytes in the populations were polarized after 20 min at 37° (Fig. 1e). Control, unactivated sera did not give this additive effect with FMLP, C5a or LTB_4 (not shown).

Table 1. Effect of the combination of different chemotactic factors upon monocyte polarization in suspension.

% monocytes polarized (mean ± SD) with					
Single chemotactic factors		Combination of pure factors		Pure factors + AHS	
C5a	56·4 ± 4·2	C5a + FMLP	57·3 ± 5·7	C5a + AHS	71·5 ± 7·0
LTB_4	50·9 ± 5·5	C5a + LTB_4	49·5 ± 5·8	LTB_4 + AHS	75·4 ± 9·7
FMLP	65·7 ± 5·7	LTB_4 + FMLP	68·1 ± 9·0	FMLP + AHS	85·9 ± 7·3
AHS	61·5 ± 6·8	C3a + C5a	58·2 ± 8·7	FMLP + LTB_4 + AHS	87·6 ± 4·4
C3a	53·9 ± 11·9	C3a + FMLP	58·2 ± 2·9	FMLP + LTB_4 + C5a + AHS	85·4 ± 2·8

Factors were used at maximally effective concentrations: C5a, 10^{-8} M; LTB_4, 10^{-9} M; FMLP, 5×10^{-9} M; C3a, 10^{-6} M and AHS, 10% v/v. The optimum time of incubation for FMLP alone or its combinations was 20 min, for the other factors 10 min, at 37°.

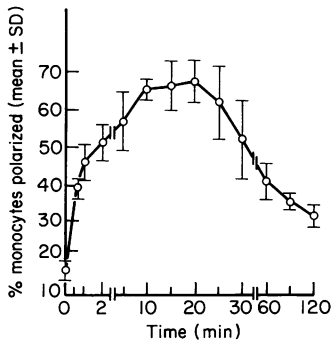

Figure 4. Time-course of polarization of monocytes exposed to an optimal dose of FMLP (5×10^{-9} M), $n = 9$.

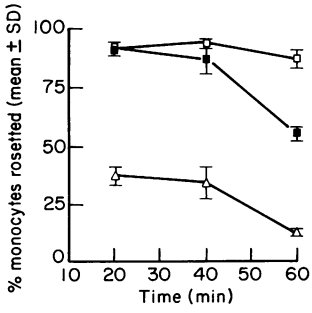

Figure 5. Effect of a uniform concentration of FMLP on the number and distribution of Fc rosettes in monocytes at different times. Total Fc rosettes in HSA alone (□); total Fc rosettes in FMLP + HSA (■); head-rosettes on locomotor cells in FMLP + HSA (△).

Time-courses for chemo-attractant-induced monocyte polarization

Figure 4 shows that polarization of monocytes increases rapidly during the first few minutes of incubation with attractants, but declines thereafter. Maximum polarization in LTB_4 and C5a was seen at 10 min (not shown) and in FMLP at 20 min. This early decline is not seen in neutrophils (Shields & Haston, 1985). Mononuclear phagocytes internalize membrane receptors rapidly and it is possible that loss of polarization with time might be due to loss of receptor numbers or of the ability to redistribute receptors. Receptor distribution has been difficult to visualize using chemotactic ligands, so we used an easily visualized ligand, i.e. antibody-coated sheep cells, the receptors for which (Fc receptors) are plentiful on human monocytes (Anderson & Looney, 1986).

Effects of chemo-attractants on numbers and distribution of Fc rosettes in monocytes

Monocytes adherent to HSA-coated glass coverslips were incubated at 37° with and without FMLP for times between 20 and 60 min, and were then chilled to 4° for Fc rosetting. FMLP caused polarization of the cells, which was maintained in many cells during the subsequent incubation at 4°. In both HSA alone and HSA + FMLP (5×10^{-9}M) > 90% of the monocytes formed Fc rosettes. In HSA this was true for all intervals between 20 and 60 min. However, in FMLP there was a gradual decline in the total number of cells with Fc rosettes from 91·3% at 20 min to 55% at 60 min (Fig. 5). This was also the case when only the polarized cells were considered. At all times, polarized cells formed fewer rosettes than rounded cells; and the proportion of rosette-forming polarized cells declined from 20 to 60 min (Figs 5 and 6). These results suggest a loss of functional Fc receptors from the cell surface as incubation with FMLP is continued.

Distribution of rosettes on polarized cells

Following polarization of monocytes in FMLP, there was a redistribution of Fc rosettes. Many cells still showed rosettes over the cell body, usually excluding the tail, but a substantial

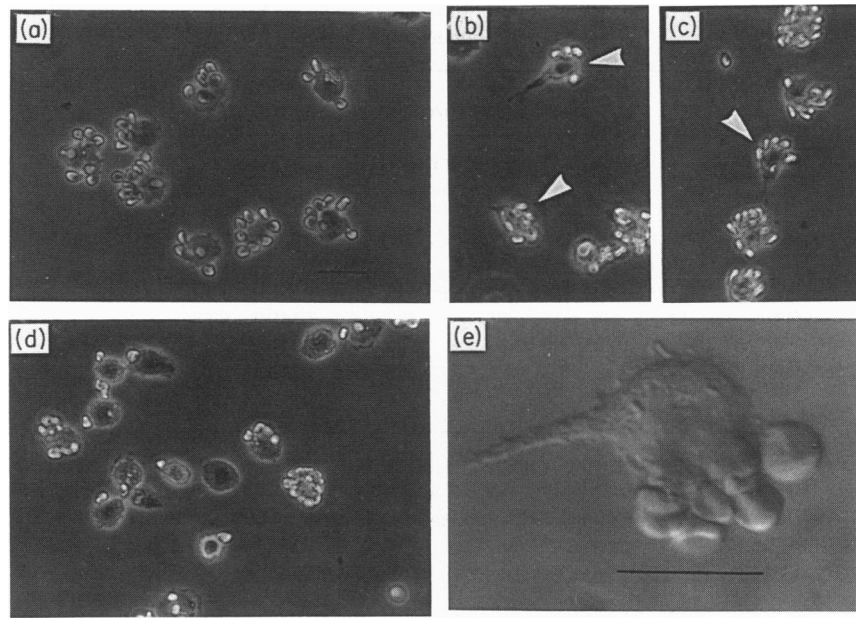

Figure 6. Monocytes rosetted with antibody-coated sheep erythrocytes (Fc receptor) after incubation in a uniform concentration of 5×10^{-9} M FMLP for various times. (a) No FMLP, after 60 min; (b, c) with FMLP, after 20 min (note rosettes formed in the head region of monocytes with locomotor morphology, arrowed); (d) FMLP after 60 min (note lack of rosette formation by many cells); (e) high-power differential interference-contrast photograph of head rosetted monocyte pre-exposed for 20 min in FMLP before incubating with sheep red cells; (a–d) phase-contrast photographs, each at same magnification. Bars = 20 μm.

proportion showed redistribution of rosettes to the head of the cell (Fig. 6 b,c). This proportion again was dependent on the time of incubation in FMLP. After 20 min incubation in FMLP, 37·5% of the rosetted cells showed their rosettes at the cell head. This figure declined to 12·2% after 60 min in FMLP (Fig. 5). If the cells were allowed translocate, the head rosettes were swept back towards the tail. As shown in Fig. 4, monocytes show maximum polarization in FMLP at 20 min and lose polarity thereafter. This loss of polarity is accompanied (i) by a loss of capacity to form Fc rosettes (Figs 5 and 6d) and (ii) by a drop in the proportion of cells with rosettes redistributed to the head-end.

DISCUSSION

Monocytes polarize and move within the first few minutes following stimulation with chemotactic factor. As shown for neutrophils (Shields & Haston, 1985), cells in optimal and uniform concentrations (5×10^{-9}M) of FMLP move rapidly and show persistent paths. At a supraoptimal concentration, persistence is poorer and the cells show erratic tracks. We suggest that this is because, at optimal FMLP concentrations (near the ED_{50}), ligand-activated signalling is slow enough to allow the cell to distinguish a point of first stimulation on its surface. The cell redistributes receptors to that point, which then becomes the head of the cell as the cell polarizes and subsequently moves. Anterior distribution of functional receptors then favours persistence of movement in that direction. If the ligand is presented in a concentration gradient, chance will determine that most cells polarize with their heads facing the gradient source. In consequence, they will move towards the gradient source. In other words, we do not consider that there is any *intrinsic* difference between chemotactic and chemokinetic responses. The response is determined by the environment (isotropic or anisotropic) in which the cells find themselves, and cell membrane and intracellular events are the same in both. In high (supraoptimal) concentrations, ligand binds rapidly at many points on the cell surface and the cell, in consequence, shows a multipolar shape instead of antero-posterior polarity and the persistence of locomotion is reduced. Consequently, chemokinetic and chemotactic responses are poor.

These findings resemble those reported earlier for neutrophils (Shields & Haston, 1985; Haston & Wilkinson, 1987). However, monocytes appear to lose polarity more rapidly than neutrophils, with a maximum at about 10–20 min. As they lose polarity, they also appear to lose surface receptors, judged by loss of ability to form Fc rosettes. Also, when the cells are well polarized, most of the Fc rosettes are at the cell head and, as polarity is lost, so head rosettes are lost. Since monocytes internalize membrane proteins more rapidly than neutrophils, it is possible that loss of polarity is due to internalization of chemotactic receptors with time. A related possibility is suggested by the study of Detmers *et al.* (1987) who showed, using immunogold labelling, that Fc receptors (FcR III) on resting human neutrophils exist as microclusters. This distribution is required for binding of IgG-coated particles. On treatment of the cells with phorbol esters, the clusters disaggregated and functional activity was lost. Thus our findings could be explained by a loss of the functional (clustered) state of Fc receptors on monocytes with time after treatment with FMLP, though this has not been tested. Whatever the cause, loss of polarity and loss of rosettes depend on active metabolism and are not observed at 20° (Cianciolo & Snyderman, 1981) or in the presence of 5×10^{-2} M deoxyglucose, a glycolytic inhibitor (unpublished observations of the authors). Loss of chemoattractant receptors may be pertinent to the fact that monocytes

become sessile during the first few hours of culture *in vitro*.

The question whether monocytes show intrinsic heterogeneity or whether heterogeneity is only manifest once they leave the bloodstream and differentiate into macrophages in specialized sites is unresolved, though the latter suggestion is the most favoured. Earlier studies of the chemotactic properties of monocytes suggested that a subpopulation (up to 60%) carried all the chemotaxis receptors and were motile, while the remainder were unresponsive (Cianciolo & Snyderman, 1981; Falk & Leonard, 1980). Also Van Epps & Chenoweth (1984) showed binding of fluoresceinated C5a to only 68% of blood monocytes by flow cytometry. Our results using pure chemotactic fctors, either alone or in mixtures, are in agreement with these findings. However, we found that mixtures of any one pure factor (FMLP, C5a, LTB_4) with candida-activated serum caused polarization of 80–90% of monocytes. A recent study using flow cytometry (Ohura *et al.*, 1987) suggests that 90% of human blood monocytes express receptors for C5a and FMLP. This suggests that most monocytes *are* motile and respond to attractants, but that a minority respond only to undefined factors in activated serum. This finding is slightly different from that of Cianciolo & Snyderman (1981) in as much as they did not find an increased percentage of polarized forms on combining a pure factor such as FMLP with activated serum. We activated serum with *C. albicans* (living or dead) whereas they used zymosan, which may account for this difference. In this regard, it is important to point out that candida cells or supernatants in the absence of serum had no polarizing activity, and that therefore we believe the additional factor to be a component of activated serum other than C5a.

These findings complement our earlier visual studies of heterogeneity of chemotactic and locomotor responses in mouse peritoneal macrophages (Wilkinson, 1982). In that study, 70% of macrophages in a thioglycollate-induced inflammatory exudate were motile and migrated to gradient sources (*C. albicans* spores), whereas resident macrophages were poorly motile and did not polarize; only 18% responded to chemotactic gradients. This suggests that newly recruited cells from the blood monocyte population are motile, but gradually lose responsiveness, possibly because of loss of receptors from the cell surface as shown *in vitro* here. Differences in expression of chemotactic activity in mononuclear phagocytes are more likely, in our view, to reflect differences in differentiation in different environments (as in lymphokine-driven acquisition of locomotor capacity by the U937 cell line: Pike *et al.*, 1980; Harris & Ralph, 1985) than differences in genotype.

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