Enhanced expression of human monocyte complement (C3b) receptors by chemoattractants

Clin. exp. Immunol. (1980) **39**, 768–775.

Enhanced expression of human monocyte complement (C3b) receptors by chemoattractants

ELIZABETH J. GLASS & A. B. KAY *Department of Pathology, University of Edinburgh, Medical School, Teviot Place, Edinburgh*

(*Accepted for publication* 23 *August* 1979)

SUMMARY

The capacity of various leucoattractants to enhance, or unfold, receptors for complement (C3b) on human blood monocytes has been studied. A number of recognized monocyte chemoattractants including casein, supernatants from *C. parvum* 10390 and from human lymphocytes (cultured either in the presence or absence of phytohaemagglutinin) and the formyl-methionyl peptides, F-Met-Leu-Phe, F-Met-Met-Phe and F-Met-Phe, increased the percentage of monocytes which formed rosettes with IgM-sensitized sheep erythrocytes coated with complement. Comparable results were achieved irrespective of whether purified components or whole serum was used as a source of complement. In contrast, there was no significant increase in EAG (Fc) rosettes with those doses of casein which gave enhancement of C3b receptors. A small degree of complement receptor enhancement was observed with histamine but the unformylated peptides, Met-Leu-Phe and Met-Met-Phe, were without apparent effect. Maximal receptor enhancement was obtained at 30 min but when the leucoattractant was removed, enhancement was reversible, returning to normal values in approximately 120 min. Monocyte complement receptor enhancement increased with temperatures between 0°C and 37°C.

These data (1) confirm and extend our previous findings on leucoattractant-induced enhancement of complement receptors on human monocytes; (2) indicate that the phenomenon may have potential as a clinical test for monocyte function both in health and disease.

INTRODUCTION

We recently reported that complement receptors on human eosinophils were enhanced, or unfolded, following incubation with various pharmacological mediators to which they migrate in chemotaxis and/or chemokinesis. A number of eosinophilotactic agents, including the eosinophil chemotactic factor of anaphylaxis (ECF-A) tetrapeptides (Val-Gly-Ser-Glu and Ala-Gly-Ser-Glu), histamine and one of its major catabolites, imidazoleacetic acid, selectively enhanced receptors for complement (C3b and C4) on human eosinophils in a dose- and time-dependent fashion (Anwar & Kay, 1977a; Anwar & Kay, 1978). Later, in a short report we established that human neutrophils and monocytes undergo a similar enhancement of complement receptors following incubation with substances to which they are known to migrate *in vitro* (Kay, Glass & Salter, 1979). This suggested that complement receptor enhancement by leucoattractants may be a general biological phenomenon which might provide a mechanism whereby the degree of adhesion of phagocytic cells to opsonized particles was increased.

For these reasons it seemed important to provide further information on leucoattractant-induced complement receptor enhancement by various cell types and in the present report the monocyte has been studied in detail. Thus, we have confirmed and expanded our original observations by studying the

Correspondence: Professor A.B. Kay, Department of Clinical Immunology, Cardiothoracic Institute, Fulham Road, London SW3 6HP.

0099-9104/80/0300-0768 $02.00

effect of leucoattractants on both complement and IgG receptors, by undertaking time course experiments over a broad concentration range, examining a number of agents which promote monocyte migration and determining whether the phenomenon is reversible and temperature-dependent.

MATERIALS AND METHODS

Materials. Materials were obtained as follows. Casein (BDH Chemicals Limited, Poole, Dorset); phytohaemagglutinin (PHA-P, Wellcome Reagents Limited, Beckenham, Kent); *C. parvum* 10390 supernatant was a gift from Dr W. McBride (Department of Bacteriology, Medical School, University of Edinburgh); histamine acid phosphate (BDH Chemicals Limited, Poole, Dorset); F-Met-Leu-Phe was a gift from Dr Derek Hudson (Royal Postgraduate Medical School London), F-Met-Met-Phe, Met-Leu-Phe and Met-Met-Phe were gifts from Miles Laboratories (Stoke Poges, England); F-Met-Phe was obtained from Sigma Chemical Company (St Louis, Missouri, USA); preservative-free heparin (Evans Medical, Liverpool); Ficoll and Hypaque (Pharmacia, Uppsala, Sweden).

*Preparation of EA, EAC*1423b *and 'EAC'*, This has been described elsewhere (Anwar & Kay, 1977b; Anwar & Kay, 1978) and was briefly as follows. Dextrose–gelatin–veronal buffer ($DGVB^{2+}$ pH 7.4 was used for washing sheep erythrocytes (E) during sensitization and coating with complement. The buffer was prepared by mixing equal volumes of isotonic Veronal-buffered saline (containing 0·0015 M Ca^{2+} 0·0005 M Mg^{2+} and 0·1% gelatin-Veronal buffer GVB^{2+}) with 5% dextrose in water containing the same concentration of Ca^{2+} and Mg^{2+}. The IgM and IgG fractions of rabbit antisera to sheep red cells (A) were prepared by Sephadex G-200 gel filtration (Shevach *et al.*, 1972). The IgG fraction was purified further by DEAE-52 anion exchange chromatography (Fahey & Terry, 1978). Either IgG or IgM was used in the sensitization of E for the preparations of EA^{rab}_{G} or EA^{rab}_{M} respectively. The antibody concentration of EA^{rab}_{M} selected was that which gave optimal sensitization for haemolysis but no agglutination. For the preparation of EA^{rab}_{G}, the antibody concentration was diluted so as to give approximately 30% monocyte rosettes. Functionally pure human complement components were added sequentially to EA^{rab}_{M} to prepare C3b coated cells (EAC1423b). The amounts were as follows: 400 effective molecules of C1, 400 of C4, 50 of C2 and 400 of C3. This amount of C4 was insufficient to give EAC14 rosettes with monocytes (Anwar & Kay, 1978). In some experiments fresh human AB serum was also used as a source of complement ('EAC'). Equal volumes of EA^{rab}_{M} at a concentration of 1×10^8 cells/ml and human AB serum, diluted with $DGVB^{2+}$, were mixed and resuspended in the same buffer to give a final concentration of 1×10^8 red blood cells/ml. The dilution of fresh serum was adjusted to give between 20 and 30% monocyte rosettes and was usually between 1 in 600 and 1 in 1000.

Monocytes. Blood from healthy donors was drawn into plastic tubes containing 10 units of preservative-free heparin/ml. Monocytes were separated on Ficoll–Hypaque cushions according to the method of Böyum (1968). The separated cells were washed twice in Dulbecco's PBS (modified) (pH 7·2–7·4), containing preservative-free heparin (2 units/ml) and resuspended in medium 199, pH 7·4, to a concentration of 1×10^2 monocytes/ml.

Lymphocyte supernatants. A cell suspension containing up to 90% lymphocytes was obtained using defibrinated blood as described by Böyum (1976). The separated cells were resuspended to a concentration of 3×10^6 lymphocytes/ml in medium 199. Under sterile conditions, and with added penicillin and streptomycin, 9 μl of PHA-P (0·5) μg/ml) were added to 3 ml of the mononuclear cell suspension. The cells were incubated for 3 days at 37°C in a humidified atmosphere containing 5% CO_2. After incubation the supernatants were harvested by centrifugation, aliquoted and stored at −70°C until use. The unstimulated lymphocyte supernatants were prepared in an identical fashion with the exception that PHA-P was added at the end of the 3-day incubation period.

Complement (EAC) and IgG (EA_G) rosettes. Equal volumes of monocytes (1×10^6/ml) and various concentrations of the leucoattractants under study or medium alone as a control, were mixed and incubated in a shaking water bath usually at 37°C for 30 min. The cells were then washed twice in medium 199 and the numbers readjusted to their original concentration in the same medium. A portion (0·1 ml) of EAC or EA_G (1×10^8/ml) was added to 0·1 ml for 10 min at 4°C and the pellets incubated at 37°C for 20 min for EAC and 0°C for 30 min in the preparation of EA_G. The pellet was resuspended gently in medium 199 containing 1% formal-saline and smears prepared on clean glass slides in duplicate. These were dried quickly in air, fixed in methanol and stained with May–Grünwald/Giemsa or fixed in 2·5% glutaraldehyde and stained using α-naphthyl acetate histochemistry according to the method of Yam, Li & Crosby (1971). Comparison of these two methods by four independent observers revealed no statistical significance in differentiating monocytes from lymphocytes.

RESULTS

Casein enhanced the percentage of monocyte C3b rosettes in a dose-dependent fashion (Fig. 1) irrespective of whether 'EAC' or EAC1423 were used as indicator cells. In contrast there was a small but insignificant increase in the percentage of EAG (Fc) rosettes. With the highest dose of casein the mean values for enhancement of Fc receptors was 13% whereas with 'EAC' and EAC1423 the increase was 92% and 100% respectively.

Enhancement of complement receptors by casein was also dependent on the time of incubation.

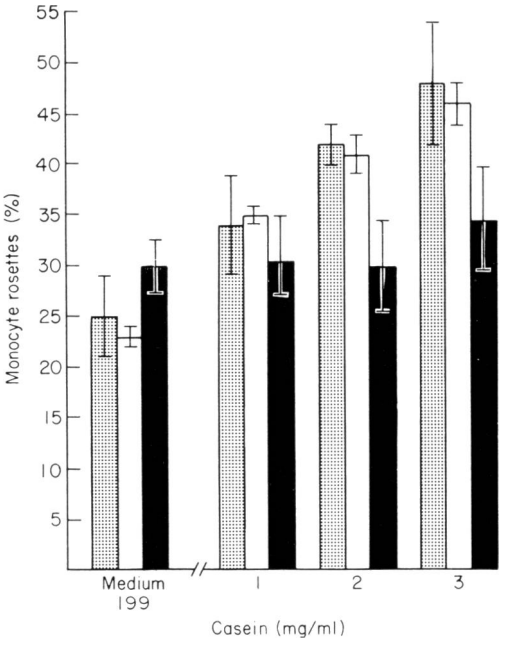

FIG. 1. The effect of casein on monocyte C3b and IgG rosettes. (□) EA_M1423, (▨) 'EAC', (▨) EA_G. Each column represents the mean ± s.e.m. of three experiments. Casein was dissolved in distilled water and the pH raised to 11 with 1 N NaOH. The pH was then adjusted to 7·4 with 1 N HCl. Nine parts of the casein solution were then added to one part of 10-times concentrated medium 199 with HEPES buffer, pH 7·4.

Maximal effects were observed at about 30 min after which there was no further increase (Fig. 2). Similar results were obtained irrespective of whether 'EAC' or EAC1423 were used as indicator red cells. A small but insignificant increase in Fc receptors was also observed in this time course study and was maximal after approximately 60 min. By 120 min this small increase had returned to the original value.

As the dose–response and time–course studies were essentially similar using either 'EAC' or EC1423, 'EAC' were used as indicator cells in further experiments.

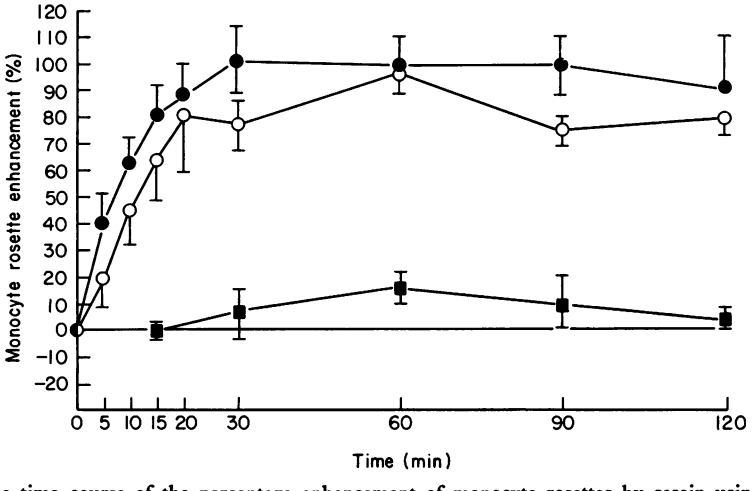

FIG. 2. The time–course of the percentage enhancement of monocyte rosettes by casein using sensitized erythrocytes coated with complement or IgG. (●—●) 'EAC', (○—○) EA_MC1423, (■—■) EA_G. Each point represents the mean ± s.e.m. of three experiments. The casein was prepared as in Fig. 1.

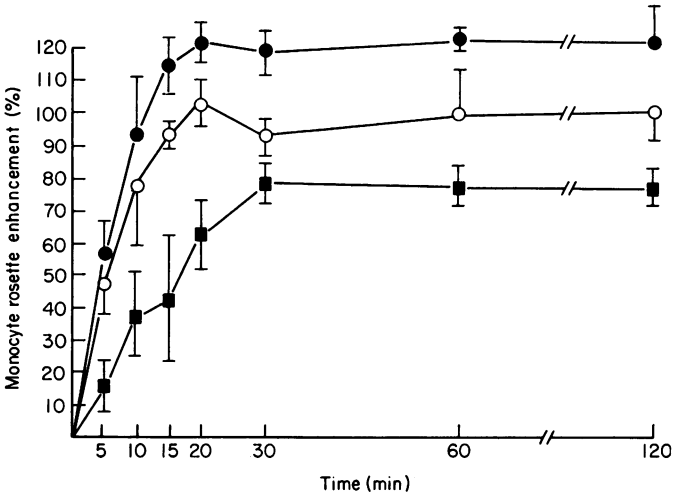

FIG. 3. The time–course of monocyte complement receptor enhancement by increasing concentrations of casein. (●—●) 3 mg/ml casein, (○—○), 2 mg/ml casein, (■—■) 1 mg/ml casein. Each point represents the mean ± s.e.m. of three experiments. The indicator red cells were 'EAC' (prepared with whole serum as a source of complement).

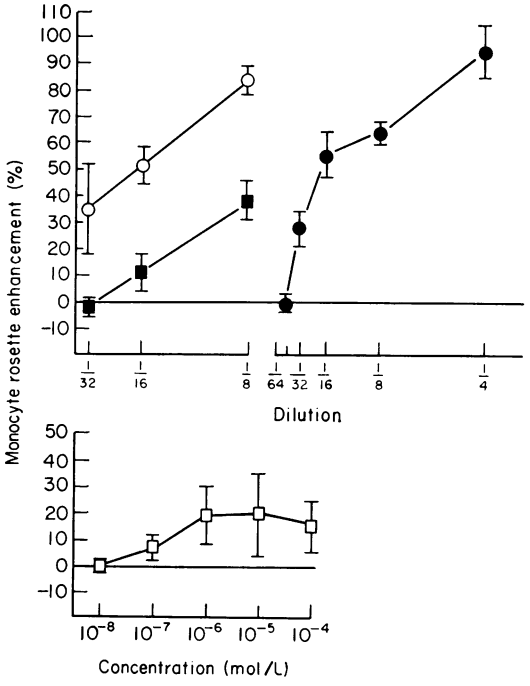

FIG. 4. The effect of various leucoattractants on the expression of monocyte complement receptors. (●—●) *C. parvum* 10390, (○—○) 'stimulated', (■–■) 'unstimulated', (□–□) histamine. Each point represents the mean ± s.e.m. of three experiments, except for histamine in which four experiments were performed. The *C. parvum* 10390 supernatant was prepared from formaldehyde-killed *C. parvum* organisms which had been washed twice in 0·9% NaCl, lyophilized and reconstituted in distilled water. The reconstituted material had a concentration of 7 mg/ml dry weight which represented 3×10^{11} organisms. The indicator red cells were 'EAC' as in Fig. 3.

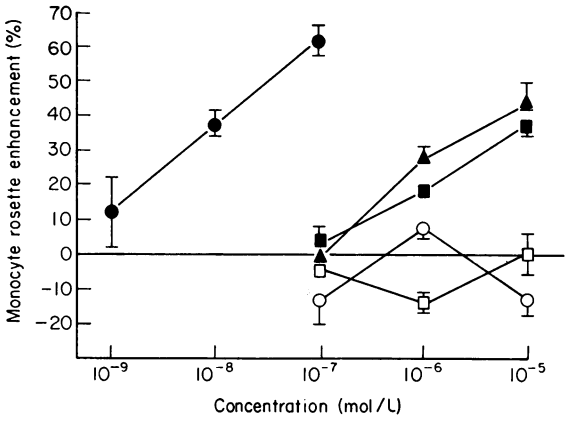

FIG. 5. The effect of formyl-methionyl peptides on the expression of monocyte complement receptors. (●—●) F-Met-Leu-Phe, (○—○) Met-Leu-Phe, (■—■) F-Met-Met-Phe, (□—□) Met-Met-Phe, (▲—▲) F-Met-Phe. Each point represents the mean ± s.e.m. of three experiments. The peptides were dissolved in dimethyl sulphoxide (DMSO) at a concentration of 10^{-2} mol/l and further diluted in medium 199 to the required concentrations. At the highest dose used (10^{-5} mol/l) the concentration of DMSO was approximately 10^{-2} mol/l and at this concentration had no effect on cell viability as determined by trypan blue exclusion and locomotion *in vitro* through micropore filters (Kay, Glass & Salter, 1979). The indicator red cells were 'EAC' as in Fig. 3.

The effect of increasing doses of casein on the rate and degree of enhancement of monocyte complement receptors is shown in Fig. 3. The degree of enhancement and the rate at which enhancement occurred was dependent on the concentration of casein. Increasing the concentration of casein from 1 mg/ml to 2 mg/ml increased the rate of monocyte rosette enhancement three-fold. Further increase in the concentration of casein up to 3 mg/ml had no apparent effect on the rate of complement receptor enhancement.

Other recognized monocyte chemoattractants were tested for their capacity to enhance monocyte complement receptors. Supernatants from lymphocytes stimulated with PHA increased the numbers of monocyte rosettes in a dose-dependent fashion (Fig. 4). In contrast, unstimulated lymphocyte supernatants had significantly less effect when tested at comparable dilutions. A leucoattractant elaborated

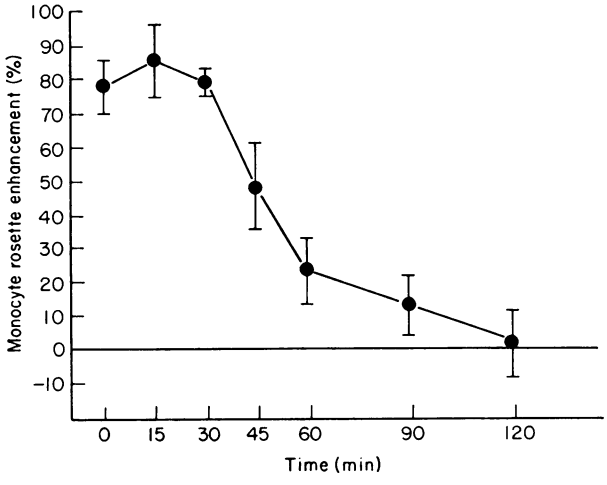

FIG. 6. Reversibility of monocyte complement receptor enhancement. Each point represents the mean ± s.e.m. of three experiments. Monocytes were pre-incubated with 3 mg/ml of casein for 30 min at 37°C, centrifuged and the cells resuspended in medium 199 to the original volume and incubated at 37°C for various time intervals as shown. The indicator red cells were 'EAC' as in Fig. 3.

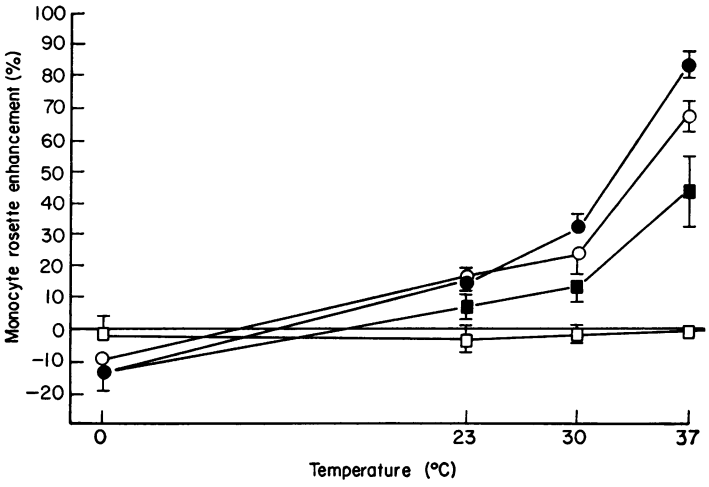

FIG. 7. The effect of temperature on monocyte complement receptor enhancement. (●—●) 3 mg/ml casein, (○—○) 2 mg/ml casein, (■—■) 1 mg/ml casein, (□—□) medium 199. Each point represents the mean± s.e.m. of three experiments. The indicator red cells were 'EAC' as in Fig. 3.

from *C. parvum* 10390 also had a similar effect. Histamine gave a slight increase in the percentage of monocyte complement rosettes at doses of 10^{-5} and 10^{-6} mol/l.

A number of formyl-methionyl peptides were also tested for their effects on monocyte complement receptors (Fig. 5). With F-Met-Leu-Phe activity was observed at 10^{-7} and 10^{-8} mol/l with little enhancement at 10^{-9} mol/l. F-Met-Met-Phe and F-Met-Phe were less active than F-Met-Leu-Phe but both gave enhancement at 10^{-5} and 10^{-6} mol/l but not at 10^{-7} mol/l. The unformylated peptides, Met-Leu-Phe and Met-Met-Phe, were virtually inactive.

Monocyte complement receptor enhancement appeared to be reversible (Fig. 6). Following 30 min incubation with casein the cells were washed and resuspended in medium 199. There was a gradual decrease in monocyte rosette enhancement and by 120 min there was no discernible difference from the controls. The experiments were reported with F-Met-Leu-Phe (10^{-7} mol/l) and gave virtually identical results.

Monocyte complement receptor enhancement was also temperature dependent as shown in Fig. 7. At 0°C there was no increase in the percentage of rosettes formed with any of the concentrations of casein used. However, at 23, 30 and 37°C, enhancement was observed and increased proportionally with the rise in temperature. The increase in monocyte complement receptor enhancement was particularly marked between 30°C and 37°C. The higher concentrations of casein gave correspondingly increased rosette formation at these temperatures.

DISCUSSION

In a recent short report we established that leucoattractants enhance complement receptors on human neutrophils and monocytes (Kay, Glass & Salter, 1979), and that these observations, taken together with previous studies on the eosinophil (Anwar & Kay, 1977a; Anwar & Kay, 1978) indicated that complement receptor enhancement by agents which promote cell locomotion might be a general biological phenomenon. In the present report we have studied details of complement receptor enhancement on the blood monocyte. It seemed important to extend our knowledge of complement receptors to human monocytes since cells of the monocyte/macrophage series are not only specialized phagocytic cells but also synthesize and secrete a variety of products into their immediate microenvironment. In addition, quantification of the degree of complement receptor enhancement might provide a further variable for studies of monocyte function both in health and disease.

The rosette method for identifying cell surface markers is a well established technique. However, a major disadvantage, which applies particularly to the present study is the difficulty in measuring the degree of binding between the monocyte membrane and individual indicator erythrocytes. For instance,

treatment with the various leucoattractants not only increased the percentage of monocytes having three or more adherent red cells but the red cell binding itself appeared far more 'firm' than that observed in the untreated controls. Techniques that facilitate measurements of the binding affinity are required to appreciate the full extent of the complement receptor enhancement phenomenon.

It should be noted that in the present experiments the amount of complement on the red cells was limited to give approximately 25–35% rosettes in the untreated (control) monocyte suspensions. Even when the amount of complement was increased or when optimal conditions were used for complement receptor enhancement, it was unusual to achieve greater than 75% monocyte rosettes. This suggested that there might be a population of monocytes which express complement receptors poorly. They may be either immature cells or possibly an as yet unidentified subset.

The capacity of casein to attract human monocytes in directional and random motion is well established (Keller & Sorkin, 1967) and, therefore, it seemed reasonable to use this agent in the majority of experiments. However, a number of other agents with chemotactic activity for monocytes gave comparable increases in the percentages of complement rosettes (Figs 4 and 5). These included supernatants from lymphocytes cultured in the presence of PHA (Snyderman *et al.*, 1972), a supernatant from *C. parvum* 10390 (Wilkinson, O'Neill & Wagshaw, 1973) and several formyl-methionyl peptides (Showell *et al.*, 1976). Snyderman *et al.* (1972) found a certain amount of monocyte chemotactic activity in supernatants from unstimulated lymphocytes. In the present study the highest concentration of the unstimulated supernatants also gave enhancement of monocyte complement rosettes. These observations all support the view that there is a direct relationship between cell locomotion and enhancement of complement receptors (Kay, Glass & Salter, 1979).

The apparent inability of casein and F-Met-Leu-Phe to alter receptors for IgG on the monocyte is in agreement with previous observations on the eosinophil in which it was found that chemoattractants affected the expression of complement but not Fc receptors (Anwar & Kay, 1977a; Anwar & Kay, 1978). The reasons for these apparent differences between complement and IgG receptors is not clear at this time but may be explained by the experimental procedure. For instance, optimal IgG rosettes are obtained at 0°C whereas 37°C is used for complement. Also, in the time course experiment (Fig. 2) a slight increase in Fc receptors was found but this was statistically insignificant. Although it seems likely that IgG receptors are not enhanced to the same degree as complement receptors, further studies using different techniques for studying cell surface markers (i.e. immunofluorescence) will be required before firm conclusions can be drawn.

We have considered the possibility that our observations might have been the result of unfolding of receptors for IgM (Fc), rather than for complement, in a similar way that *Vibrio cholerae* neuraminidase exposes cryptic sites for the Fc portion of IgM on rabbit and human monocytes (Haegert, 1979). It is unlikely that our present findings can be explained in this way since, at least with human neutrophils and eosinophils, receptor enhancement was observed with $EA_MC1423b$ and EA_MC14 but not with $EA_MC1423b'$ (i.e. C3b cells treated with the C3b inactivator (Law, Fearon & Levine, 1979)). Thus not all complement intermediates prepared with EA_M react in receptor enhancement (Anwar & Kay, 1978). Comparable experiments with monocytes using red cells coated with various complement components are in progress as are blocking studies with IgM.

Although we have drawn the general conclusion that agents which promote cell locomotion also enhance complement receptors, we cannot say with certainty whether this effect is related to their chemotactic and/or chemokinetic principles. It has been shown that casein (Keller & Sorkin, 1967), the F-Met peptides (Showell *et al.*, 1976) and *C. parvum* chemotactic factor (Russell *et al.*, 1976) promote directional migration as well as increasing the rate of cell movement. In order to establish that the chemotactic, rather than the chemokinetic, principle is required for complement receptor enhancement, further studies using substances which increase random migration, but are not chemotactic, are required. As discussed elsewhere (Anwar & Kay, 1978) possible explanations of the mechanisms of complement receptor enhancement include 'membrane unfolding', 'receptor externalization' and 'subunit association'. The generation of new receptors was considered unlikely since optimal enhancement, at least with casein, was achieved by 30 to 60 min.

Complement receptor enhancement on the monocyte (and also the neutrophil (E. J. Glass, unpublished observation)) appears to be both reversible (Fig. 6) and temperature-dependent (Fig. 7). Although these findings do not support any single mechanism (see above) they may be useful observations in our endeavours to provide insight into the mechanism of complement receptor enhancement. For instance, it is reasonable to suppose that during receptor enhancement there is an alteration in membrane fluidity which is both reversible (Fig. 6) and probably occurs in the upper temperature range, i.e. between 30°C and 37°C (Fig. 7). By the use of a combination of various techniques which might include cell membrane fractionation, the use of radiolabelled complement components and membrane fluidity probes we hope to develop further our knowledge of this phenomenon.

At the present time one of our principal interests relates to the possibility of complement receptor enhancement as a useful clinical laboratory test. For instance, although the response to histamine is usually small there is a marked enhancement of monocyte complement receptors by this agent in a few individuals (E. J. Glass, unpublished observation). The reasons for such variations between apparently healthy individuals are presently unclear but the possibility that it may be related to their atopic state is being explored. Also, the effect of tumour-derived products on complement receptor enhancement by normal monocytes is the subject of study in progress (Glass & Kay, 1979), details of which will be the subject of a separate report.

This work was supported by the Cancer Research Campaign.

REFERENCES

ANWAR, A.R.E. & KAY, A.B. (1977a) The ECF-A tetrapeptides and histamine selectively enhance human eosinophil complement receptors. *Nature*, **269**, 522.

ANWAR, A.R.E. & KAY, A.B. (1977b) Membrane receptors for IgG and complement (C4, C3b and C3d) on human eosinophils and neutrophils and their relation to eosinophilia. *J. Immunol.* **119**, 976.

ANWAR, A.R.E. & KAY, A.B. (1978) Enhancement of human eosinophil complement receptors by pharmacologic mediators. *J. Immunol.* **121**, 1245.

BÖYUM, A. (1968) Isolation of leucocytes from human blood. Further observations. Methyl cellulose, dextran, and Ficoll as erythrocyte aggregating agents. *Scand. J. clin. Lab. Invest.* **21**, suppl. 97, 31.

BÖYUM, A. (1976) Isolation of lymphocytes, granulocytes and macrophages. *Scand J. Immunol.* **5**, suppl. 5, 9.

FAHEY, J.L. & TERRY, E.W. (1978) Ion exchange chromatography and gel filtration. *Handbook of Experimental Immunology* 3rd edn (ed. by D. M. Weir), Chapter 8. Blackwell, Oxford.

GLASS, E.J. & KAY, A.B. (1979) Monocyte complement receptors and lung cancer. *Br. J. Cancer*, **39**, 484 (abstract)

HAEGERT, D.G. (1979) Phagocytic peripheral blood monocytes from rabbits and humans express membrane receptors for IgM molecules: evidence that incubation with neuraminidase exposes cryptic IgM (Fc) receptors. *Clin. exp. Immunol.* **35**, 484.

KAY, A.B., GLASS, E.J. & SALTER, D.McG. (1979) Leucoattractants enhance complement receptors on human phagocytic cells. *Clin. exp. Immunol.* **38**, 294.

KELLER, H.U. & SORKIN, E. (1967) Studies on chemotaxis. VI. Specific chemotaxis in rabbit polymorphonuclear leucocytes and mononuclear cells. *Int. Arch. Allergy appl. Immunol.* **31**, 575.

LAW, S.K., FEARON, D.T. & LEVINE, R.P. (1979) Action of C3b-inactivator on cell-bound C3b. *J. Immunol.* **122**, 759.

RUSSELL, R.J., MCINROY, R.J., WILKINSON, P.C. & WHITE, R.G. (1976) A lipid chemotactic factor from anaerobic coryneform bacteria including *Corynebacterium parvum* with activity for macrophages and monocytes. *Immunology*, **30**, 935.

SHEVACH, E.M., HERBERMAN, R., FRANK, M.M. & GREEN, J. (1972) Receptors for complement and immunoglobulin on human leukaemic cells and human lymphoblastoid lines. *J. clin. Invest.* **51**, 1933.

SHOWELL, H.J., FREER, R.J., ZIGMOND, S.H., SHIFFMAN, E., ASWANIKUMAR, S., CORCORAN, B. & BECKER, E.L. (1976) The structure activity relationships of synthetic peptides as chemotactic factors and inducers of lysosomal enzyme secretion for neutrophils. *J. exp. Med.* **143**, 1154.

SNYDERMAN, R., ALTMAN, L.C., HAUSMAN, M.S. & MERGENHAGEN, S.E. (1972) Human mononuclear leucocyte chemotaxis: a quantitative assay for humoral and cellular chemotactic factors. *J. Immunol.* **108**, 857.

WILKINSON, P.C., O'NEILL, G.J. & WAGSHAW, K.G. (1973) Role of anaerobic coryneforms in specific and non-specific immunological reactions. II. Production of a chemotactic factor specific for macrophages. *Immunology*, **24**, 997.

YAM, L.T., LI, C.Y. & CROSBY, W.H. (1971) Cytochemical identification of monocytes and granulocytes. *Am. J. clin. Pathol.* **55**, 283.