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Cellular requirements for the formation of EA rosettes by human monocytes

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Summary. The binding of sensitized red cells to Fc receptors in human monocytes was studied by evaluating the effects of various pharmacological reagents and other treatments on EA rosette formation. Cytochalasin B and 2-deoxyglucose inhibited rosette formation in a dose-dependent manner. Sodium azide and incubation at 4° also inhibited rosette formation, while at 37° increased numbers of RBCs bound to the monocytes. The microtubular poisons, vinblastine and colchicine at high concentrations resulted in decreased adherence of monocytes and inhibition of rosette formation, while at low concentrations of colchicine, enhanced rosette formation was sometimes observed. Contrary to the effects on rosette formation, binding of [^{125}I] IgG to monocyte monolayers was not altered by treatment of the monocytes with drugs. Magnesium ions were required to promote monocyte adherence, but both magnesium and calcium were needed for the best rosette formation. We conclude that the formation of EA rosettes is dependent not merely on binding of IgG to the Fc receptor but requires metabolically active monocytes, an intact cytostructure and suitable environmental conditions (temperature and cation concentration).

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INTRODUCTION

The presence on human monocytes and tissue macrophages of Fc binding sites has been demonstrated by the formation of rosettes with appropriately sensitized red cells (Lobuglio, Cotran & Jandl, 1967; Abramson, Lobuglio & Cotran, 1970b). Several characteristics of this interaction have been shown: (1) the binding site of the cytophilic gamma globulin has been localized to the CH3 domain of IgG (Okafor, Turner & Hay, 1974; Ciccimara, Rosen & Merler, 1975) and is restricted to IgG of subclasses 1 and 3 (Abramson, Gelfand, Jandl & Rosen, 1970a). (2) Following phagocytosis the monocyte is capable of regenerating Fc receptors (Schmidt & Douglas, 1972). (3) Activation of macrophages by mineral oil results in an increased percentage of cells forming rosettes (Rhodes, 1975).

The functional importance of the Fc receptor is in facilitating elimination of immune complexes, phagocytosis and antibody dependent cytotoxicity. In man the phenomenon of rosetting is of pathophysiological significance in immune haemolysis. This is evident by the rapid elimination of sensitized red cells by the reticulo-endothelial system (Schreiber & Frank, 1972), the presence of erythrophagocytosis

and the altered shape of the red cell following interdigitation with the phagocytic cell membrane, which results in loss of red cell viability (Swisher, 1972). We have studied some of the regulating effects on Fc receptor function in an entirely human system. Concurrent evaluation of binding of monomeric IgG as compared to rosette formation enabled the monocyte cellular requirements for fixation of red cells to be studied. Since several of the conditions used to test this affected the adherence of the monocytes, our assay system quantified simultaneously the amount of rosetted red cells by chromium labelling and the number of adherent monocytes by DNA analysis. The effects of the various experimental conditions on the morphology of the rosettes was also evaluated and assisted in the interpretation of the results. Our studies indicate that rosette formation is a complex cellular event, which probably occurs subsequent to the binding of IgG to its receptor.

MATERIALS AND METHODS

Monocyte monolayers

Heparinized blood from healthy donors (10 units of heparin/ml) was centrifuged at 1000 *g* for 3 min, after which the buffy coat and plasma were removed. The white cell suspension was layered onto Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N. J.) in 50 ml plastic centrifuge tubes and centrifuged at 400 *g* for 20 min. The mononuclear cells at the interface were harvested and washed three times in Hanks's Balanced Salt Solution (HBSS). The cells were then counted and resuspended in a HBSS solution with 10% sterile heat-inactivated foetal calf serum (FCS). Cell concentration was adjusted to $5–10 \times 10^6/mm^3$ and 0·4 ml of this suspension was pipetted into each well (16 mm diameter) of tissue culture trays (Linbro Scientific Inc., Hamden, Conn.). The trays were gently rocked for 45 min at 37° to facilitate adherence of monocytes and were then vigorously washed three times with HBSS to remove nonadherent cells. Quantification of the cell number in each assay was assessed by measuring the DNA content of the monocyte monolayer (see below).

Erythrocyte suspensions

Human erythrocytes, type $0Rh^+$ were extensively washed and labelled with ^{51}Cr. A human IgG preparation (1%) with anti-D antibody activity was used to sensitize the erythrocytes. Details of radioactive labelling and sensitization were as previously described (Abramson *et al.*, 1970a). Erythrocytes (E) or sensitized erythrocyte suspensions (EA) were the negative and positive controls respectively in all experiments. Detection of adequate sensitization was performed by Coombs test. Red cell suspensions of appropriate concentrations were used in each experiment, to achieve a final concentration of 4%.

Rosetting assay

Red cell suspensions were added to the monolayer and incubated for 2 h on a gentle rocker at room temperature to reduce the amount of phagocytosis. The monolayers were then washed four times with HBSS. Special care was taken in aspirating the HBSS to prevent disruption of the monolayer. The plates were then studied under an inverted microscope and the degree of rosetting was evaluated qualitatively. Viability studies using trypan blue exclusion were done directly on the plates. Distilled water (0·5 ml) was then added to each well in the plates, which were then rocked overnight. The radioactivity of this lysate was counted in a gamma counter, and the same solution was subsequently used for DNA quantification.

In experiments where the effect of the various pharmacological agents was tested, monolayers were incubated for 1 h at 37° in the presence of each drug dissolved in 0·25 ml HBSS, and the red cell suspension was then added and allowed to incubate for an additional 2 h. Sensitized red cells incubated in the presence of the various drugs did not agglutinate or lyse spontaneously nor was there an altered Coombs reactivity. In preliminary experiments where the drug was washed off prior to addition of the red cells variable rates of recovery from the drug inhibitory effect were noted, therefore incubation in the presence of the drug for the entire duration of the experiment was chosen as optimal. When testing for inhibition of rosette formation by various proteins, the monolayers were incubated with the protein solution at 37° for 15 min prior to addition of the red cell suspension. To test the effects of temperature, culture dishes were maintained at the appropriate temperature for 30 min prior to addition of the red cell suspension and rosetting was done at this temperature. The effect of divalent cations was studied by incubating the cultures in varying concentrations of calcium and magnesium in Krebs's

ringer phosphate (KRP) buffer for 30 min and then the red cell suspension made up in KRP without calcium or magnesium was added to the monolayer.

All experiments were done in triplicate and control triplicates of untreated monocytes were set up on each plate. The average percent counts (amount of rosettes) and average percent DNA (quantity of monocytes) of each triplicate was calculated using the control of each plate. The corrected percent rosettes was calculated from these values.

Fluorometric assay

Fluorometric assay of DNA was performed with minor modifications as previously described (Einstein, Schneeberger & Colten, 1976). In brief, 20 μl of a 1 mg/ml solution of polyriboguanadilic acid (Collaborative Research, Inc., Waltham, Ma.) 100 mM Tris, 10 mM EDTA, pH 7·4 and 900 μl of ice cold absolute ethanol were added to each sample to be tested and kept for 18 h at −20°. Each sample was washed twice in absolute ethanol with high speed centrifugation (12,000 *g*, 5 min; Beckman Microfuge) between washes. The supernatants were removed and the pellet dried at 100°. Twenty-five μl of deionized water and 25 μl of recrystallized 3,5-diaminobenzoic acid dihydrochloride (0·26 g/ml of deionized water, Gold Label, Aldrich Chemical Co., Milwaukee, Wisconsin) were added to each sample. The samples were incubated for 30 min at 60° in a water bath and 0·5 ml of 1 N HCl was added to each tube. The relative fluorescence was determined in a Perkin-Elmer MPF-3 fluorescence spectrophotometer (excitation 380 to 420 nm, emission 500 nm). A standard curve was constructed with known amounts of calf thymus DNA (Sigma Chemical Co., St. Louis, Missouri).

Pharmacological reagents

The following drugs were employed: colchicine, 2-deoxyglucose, sodium azide (Sigma Chemical Co., St. Louis, Missouri), cytochalasin B (ICI Research Lab., Cheshire, England), cycloheximide, ethylene-diamine-tetraacetic acid (EDTA), ethylene-glycol-tetraacetic acid (EGTA) (Eastman Chemical Products, Inc., Rochester, N. Y.), vinblastine sulphate (Eli Lilly Co., Indianapolis, Inc.). All drugs and chemicals were made up in stock solutions of HBSS, pH 7·4 and stored at 4°. Cytochalasin B was initially kept in dimethylsulphoxide (DMSO) at a concentration of 0·1%.

Proteins and iodination

Protein fractions were obtained as previously described (Franklin, 1960). The labelling of IgG with ^{125}I was done by a modification of the McFarlane procedure (McFarlane, 1958). The amount of binding of the radiolabelled IgG was determined on monolayers treated in the same way as in the rosetting experiments, except that the [^{125}I] IgG solution was substituted for the red cell suspension.

Phase contrast and electron microscopy

Monocyte monolayers plated on glass cover slips, were treated with colchicine and vinblastine for 1, 2½ and 4½ h. The cultures were then fixed with 2% purified glutaraldehyde (Polysciences, Warrington, Pa.) in 0·1 M cacodylate buffer, pH 7·2 containing 0·1 M sucrose and $4{\cdot}5 \times 10^{-3}$ M $CaCl_2$ for 15 min at 4°. Cells were washed in 0·15 M cacodylate buffer and were stained with toluidine blue. The cell preparations were coded, randomized, and their cell diameters were measured by two independent observers with an eye piece, equipped with a micrometer disc.

Duplicate cultures of monolayers in 35 mm petri dishes, (Falcon Plastics, Oxnard, Calif.) were set up at the same time and under identical conditions as the drug studies in the culture trays. At the end of the experiment the monolayers were fixed, embedded and sectioned as previously described (Einstein *et al.*, 1976). For the initial evaluation of the purity of the monocyte population endogenous peroxidase was identified cytochemically using the incubation medium of Graham and Karnovsky (Graham & Karnovsky, 1966).

Statistical calculations

Student's *t* tests, analysis of variance (f test) and correlation coefficients were used for analysing the data.

RESULTS

Assay system

The number of rosetted red cells (measured by chromium counts) was directly proportional to the number of adherent monocytes (measured by the DNA content of the adherent cell layer) (Fig. 1). Similar graphs could be drawn on each set of experiments. Rosetting was inhibited by IgG (95%) and Fc (94%) and not by albumin or Fab confirm-

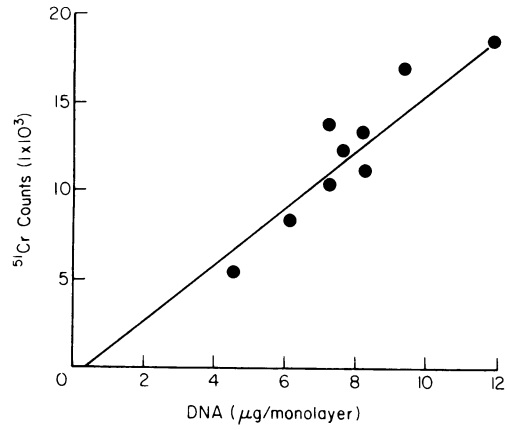

Figure 1. Relationship between ^{51}Cr counts (number of bound RBCs) and DNA content/monocyte monolayer (number of adherent cells) r = 0·82.

ing that binding of IgG to monocytes occurred through the Fc fragment. The number of rosettes formed varied with the degree of sensitization of the red cells. The amount of the antibody used in the assay was the minimal amount to achieve maximal rosetting.

Ultrastructural analysis showed that the adherent cells were primarily monocytes since they contained peroxidase positive granules characteristic of monocytes and more than 95% phagocytosed latex particles. The number of adherent cells forming rosettes with at least three attached RBCs varied from 60–90%. The adherent monocytes had an irregular ruffled plasma membrane and prominent microtubules within the cytoplasm. No extensive spreading of the cells was observed in these short-term cultures. At the site of attachment of the erythrocytes to the monocytes, the surface of the latter showed numerous elongated microvilli, which intertwined with finger-like projections on the erythrocytes. There were focal areas of close contact between the monocyte and the erythrocyte membranes in which the intervening 100 Å space contained slightly electron dense material. Erythrocyte shape was often markedly distorted due to this interaction. Various stages of erythrophagocytosis were evident throughout. Focal areas of close membrane opposition with intervening electron dense material persisted even in those areas where portions of the RBC were drawn into the monocyte interior.

Effect of temperature on rosette formation

At 4° there was virtually total inhibition of rosette formation; there was no loss of viability and adherence of the monocytes to the plates was maintained. At 37° there was an increase in the number of rosettes formed compared to room temperature; however, fewer monocytes remained adherent to the plates (Table 1). At 4° cytoplasmic microtubules though present were reduced in number and were primarily seen in the central portions of the cells. At 37° microvilli were especially prominent while an increased number of monocytes had phagocytosed

Table 1. Effect of temperature on rosette formation and monocyte adherence

Exp.	Temperature	^{51}Cr c.p.m.	t*	*P*	DNA (μg)	t*	*P*
1	4	803 ± 244	4·86	<0·05	10·36 ±1·61	1·61	0·24
	20†	15,124 ±6,096	—	—	12·23 ±1·20	—	—
	37	16,322 ±1,417	0·39	0·89‡	4·57 ±0·46	10·29	<0·05
2	4	2,043 ± 798	40·55	<0·001	16·38 ±4·04	2·45	0·14
	20	24,760 ± 623	—	—	22·48 ±1·49	—	—
	37	21,485 ±1,286	4·00	0·1‡	5·87 ±1·13	15·41	<0·01
3	4	417 ± 348	8·49	<0·01	4·43 ±0·74	1·32	0·32
	20	24,406 ±5,518	—	—	5·63 ±2·37	—	—
	37	28,387 ±2,034	0·96	0·42‡	1·63 ±0·07	4·43	<0·05

* Calculated by comparison to controls at room temperature.

† 20° = room temperature.

‡ Not significant. (However when chromium counts are corrected for amount of DNA, there are significantly increased numbers of RBC's binding to the monocytes.)

1–2 red cells; large numbers of RBC bound to the monocytes produced florid rosettes.

Metabolic inhibitors

2-deoxyglucose inhibited rosette formation in a dose-related manner, while adherence of the monocytes and viability were not affected (Table 2). Sodium azide also inhibited rosette formation; however, at high concentrations significant numbers of monocytes were killed and subsequently washed off the plates (Table 2). Blunting of microvilli formation was noted on morphological analysis of cells treated by metabolic inhibitors. Cycloheximide in high doses and DMSO had no effect on the amount of rosetting or adherence of monocytes (Table 2).

Drugs affecting microfilaments and microtubules

Cytochalasin B had a clear dose-related effect on the number of red cells binding to the monocytes (Fig. 2). There was no effect on adherence of the monocytes to the plates. Ultrastructural examination

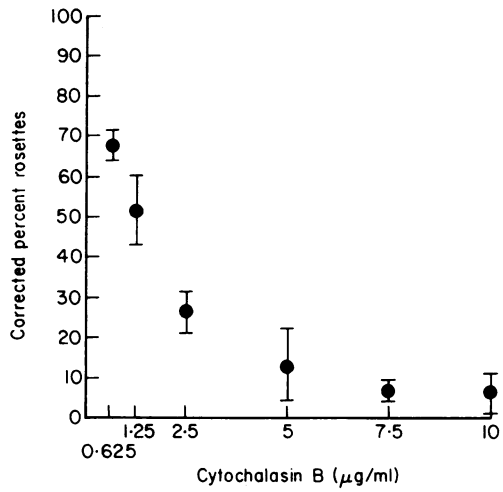

Figure 2. Dose-related inhibition of EA rosette formation by cytochalasin B. The ^{51}Cr counts are corrected for the DNA content in each monolayer. The data represent the means of at least three different experiments, each of which was done in triplicate.

Table 2. Effect of various drug treatments on rosette formation and monocyte adherence

Pharmacologic agent	No. of experiments	Concentration	Percent DNA of control	Corrected percent rosette formation
Colchicine	10	1×10^{-7} M	99·5 ±11·5	107 ±10·8
	7	1×10^{-6} M	51·3 ±16·6	83·9 ±56·3
	7	1×10^{-5} M	53·3 ±18·5	58·2 ±32·8
Vinblastine	4	1×10^{-6} M	59·6 ±19·6	88·1 ±13·1
	4	1×10^{-5} M	75·0 ±40·0	87·5 ±21·5
	9	1×10^{-4} M	69·5 ±30·0	56·0 ±26·8
2-deoxyglucose	5	1×10^{-1} M	110 ±17·2	35·9 ± 9·4
	4	5×10^{-2} M	115 ±12·8	48·9 ± 8·3
	7	1×10^{-2} M	107·9 ±22·7	93·9 ±24·2
Na Azide	3	1×10^{-3} M	102·6 ± 8·7	95·8 ± 7·8
	4	1×10^{-2} M	42·5 ±21·8	60·3 ±21·0
Cycloheximide	1	5 μg/ml	100	108
	1	10 μg/ml	85	102
	1	20 μg/ml	91	105
DMSO	2	1% v/v	105·5 ± 3·5	103·9 ± 6·8
EDTA	1	5×10^{-3} M	53	64·2
	1	5×10^{-2} M	69	65·3
	1	5×10^{-2} M	61	73·7
EGTA	4	1×10^{-3} M	92·5 ± 9·4	91·7 ±18·4
	3	1×10^{-2} M	71·5 ±15·5	72·9 ±14·6

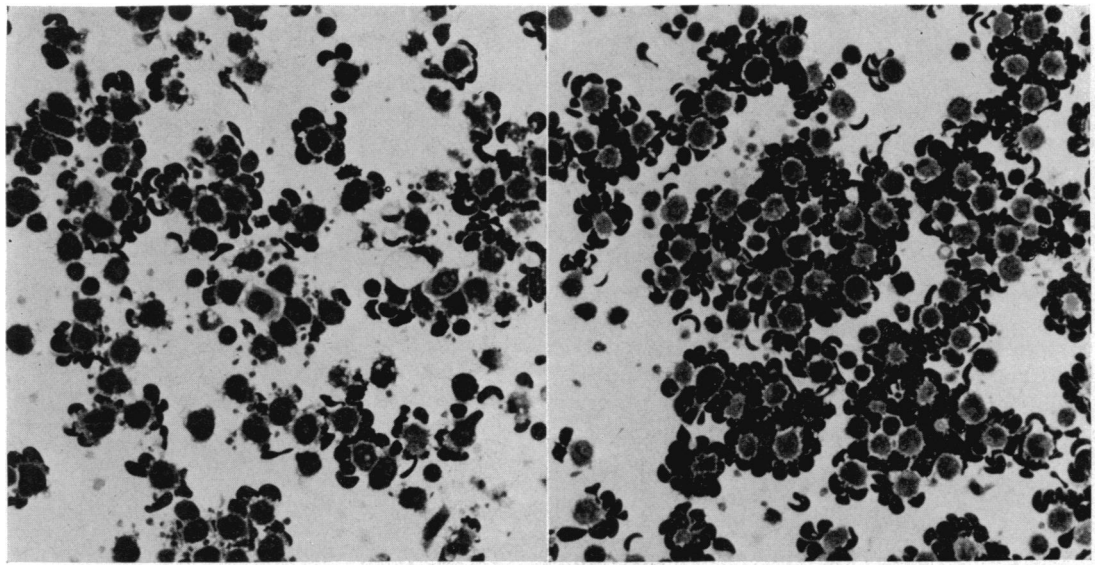

Figure 3 (a) One micron thick plastic section of human monocytes rosetted at room temperature with anti-D antibody sensitized erythrocytes. Toluidine blue stain. × 500. (b) Human monocytes treated with 1×10^{-6} M colchicine and rosetted with anti-D antibody coated erythrocytes at room temperature. There is a striking enhancement of rosette formation. Toluidine blue stain. × 500.

of these monolayers, however, failed to show disruption or aggregation of the microfilaments.

Well defined effects of tubular disruption were more difficult to obtain because of wide individual variation in response to these drugs. At high concentrations ($>10^{-5}$ M) both colchicine and vinblastine clearly decreased the number of adherent cells and there was significant inhibition of rosette formation (Table 2). A frequent observation was that some cells had formed some very good rosettes, while most were devoid of attached red cells. At low concentrations of colchicine ($< 10^{-6}$ M) on some occasions enhancement of rosette formation was seen. Augmentation of rosette formation by colchicine by comparison to the control is clearly shown in Fig. 3. The opposite effects of colchicine at varying dosage are shown in Fig. 4. Increasing concentrations of the drug resulted in a gradual decrease in the number of monocytes attached to the plate. At low concentrations the number of RBCs binding to the monocytes are higher than in the control; this is especially marked when significant detachment of cells begins to occur. However, at high concentrations when the number of cells being

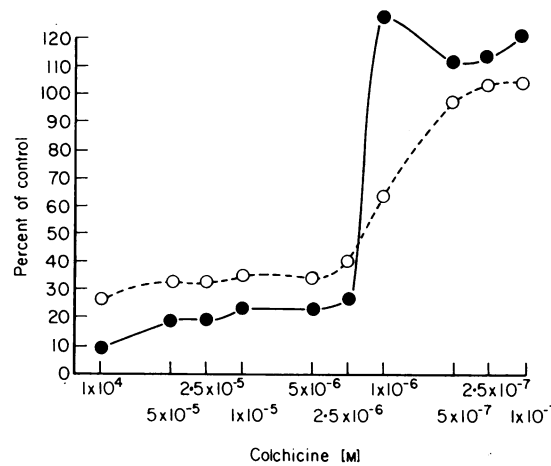

Figure 4. The data represent means of triplicate cultures at varying concentrations of colchicine in a single donor. The number of adherent cells (amount of DNA) decreases as colchicine concentration is increased. Increased rosette formation is seen at low concentrations. This is particularly prominent when altering of the monocyte occurs so that adherence is significantly reduced (10^{-6} in this donor). At high concentrations of colchicine inhibition of rosette formation is noted despite marked detachment of the adherent cells. (●) Red cells bound; (○) Monocytes attached to plate.

Table 3. Effect of tubular disruptive drugs on monocyte diameter

Drug	Concentration	Diameter (μm)	t*	*P*
Untreated	—	16·20 ±1·33	—	—
Colchicine	1×10^{-7}	14·12 ±1·84	7·75	<0·001
Colchicine	1×10^{-4}	14·12 ±1·49	9·23	<0·001
Vinblastine	1×10^{-4}	13·65 ±1·12	11·57	<0·001

* Compared to untreated monocytes. Sixty cell diameters were measured for the control and each of the drugs used.

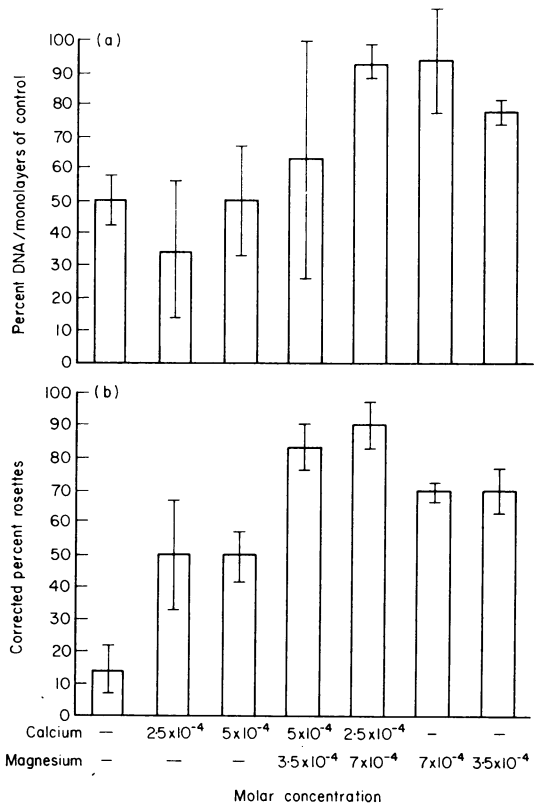

Figure 5. Rosette formation done at various calcium and magnesium concentrations. Increasing calcium concentrations are plotted from the left of the *y* axis and increasing magnesium concentrations are plotted from the right of the *y* axis so that concentrations approaching optimal concentrations are represented in the center of this axis. Results are examined as a percentage of optimal divalent concentrations of KRP buffer. (a) Increasing magnesium concentration promotes monocyte adherence, while the effects of calcium are minimal. (b) Both increasing magnesium and calcium concentrations promote rosette formation.

detached is not affected greatly, the chromium counts are markedly lower than in the control, even after correcting for the DNA content of the monolayer.

Phase contrast microscopy of monolayers treated with colchicine and vinblastine showed fewer adherent cells, which were symmetrically rounded and their diameters were significantly reduced (Table 3). Similar results were obtained when the monolayers were incubated for longer periods of time. No microtubule formation could be discerned in preparations of either microtubular poisons. Rounded aggregates of paracrystalline material were present in the cytoplasm of vinblastine treated cells.

Effect of divalent cations

Incubation with EDTA or EGTA resulted in significant inhibition of rosette formation. EDTA decreased the number of adherent cells (Table 2). When the assay was done at varying cation concentrations magnesium was more important in determining adherence of the monocytes. Both calcium and magnesium were required for optimal rosette formation (Fig. 5a, b).

Binding of labelled IgG

The various treatments of the monocytes used did not significantly inhibit the binding of IgG to the monocytes (analysis of variance f=1·96) (Table 4). Nevertheless, cytochalasin B and incubation in the cold slightly decreased the amounts of IgG bound to the monocytes (Table 4).

Table 4. Effect of various treatments on the amount of binding of labelled monomeric [^{125}I] IgG to monocytes

Treatment	Dose	IgG Bound* % of Control
Colchicine	1×10^{-4} M	96·6 ±23·1
Vinblastine	1×10^{-4} M	86·8 ± 6·2
Cytochalasin	10 μg/ml	61·8 ±16·2
2-deoxyglucose	1×10^{-2} M	79·1 ±17·5
Na azide	1×10^{-2} M	89·6 ± 7·6
4°	—	60·2 ± 7·1
37°	—	85·1 ± 2·3

* Each experiment was done in triplicate and compared to control untreated cultures (100%) on the same plate. The percent binding has been corrected for the number of monocytes in each assay by use of DNA determinations. Each experiment was repeated three times.

DISCUSSION

These experiments have shown that formation of rosettes between sensitized erythrocytes and human monocytes requires suitable environmental conditions, metabolically active cells and an intact cytostructure. Our findings on the effects of divalent cations confirm that magnesium is essential for monocyte adherence (Rabinovitch, 1975), while the presence of both calcium and magnesium is necessary for optimal rosette formation. Similar effects of temperature and the requirement for magnesium but not calcium have been shown in the binding of EAC3b sensitized red cells to mouse peritoneal macrophages (Lay & Nussenzweig, 1968). Although previous studies on the modulation of both Fc and C3b function have now been reported in varying sources of macrophages from different animal species, several lines of evidence indicate that these results are not always comparable. First, guinea-pig pulmonary macrophages formed more rosettes than did guinea-pig peritoneal macrophages (Rhodes, 1975). Secondly, binding of cytophilic antibody is species specific (Abramson *et al.*, 1970b). Thirdly, varied effects of trypsin digestion (Rabinovitch, 1970) and microtubular poisons on the complement and Fc receptors (Atkinson & Parker, 1977b) indicate that the effects on one receptor are not necessarily applicable to the other. Therefore, to eliminate these variables all our studies were done on human monocytes in an homologous system free of added complement.

Cytochalasin B has primarily been used because of its reported disruptive effect on microfilament structures (Wessels, Spooner, Ash, Bradley, Luduena, Taylor, Wreen & Yamada, 1971). However, several other effects on cellular metabolism and transport have been reported, including inhibition of chemotaxis and phagocytosis (Becker, Davis, Estensen & Quie, 1972), inhibition of lymphocyte capping (dePetris, 1974), inhibition of antibody dependent cytotoxicity (Gelfand, Morris & Resch, 1975), enhanced antigen and mitogen induced lymphocyte transformation at low concentrations and inhibition at high concentrations (Hoffman, Ferguson & Simmons, 1977; Yoshinaga, Yoshinaga & Waksman, 1972). Recently cytochalasin B has been shown to inhibit EAC3b rosette formation (Atkinson, Michael, Chaplin & Parker, 1977a). A clear concentration-dependent inhibition of EA rosette formation without any effect on monocyte adherence was demonstrated. Similar results to ours have now been reported in rabbit alveolar macrophages (Atkinson & Parker, 1977b) and in a human system (Herskovitz, D-Guerry, IV, Cooper & Schreiber, 1977). The concentrations of cytochalasin B used were well within the range toxic to microfilaments; however, microfilament abnormalities were not readily apparent on ultrastructural analysis. Part of the effect of cytochalasin B may be due to the reported increase in negative charge induced by this drug (Mayhew & Maslow, 1974), thus promoting repulsion of cytophilic antibody; however, this would still not account for the marked inhibition of rosette formation at concentrations shown to have only slight impairment of IgG binding.

The actions of colchicine and vinblastine on rosette formation were investigated because of their known effect on disruption and inhibition of formation of microtubules (Ukena & Berlin, 1972). Interestingly, augmentation of rosette formation at low concentrations of colchicine was frequently found. At this dose a significant decrease in cell diameter occurred and the monocytes became rounded. These cell changes and the dosages at which they occurred were similar to those reported previously (Pesanti & Axline, 1975). It is conceivable that because of this change in shape, approximation of Fc receptors occurs, thus facilitating the binding of more RBCs. Nevertheless, at high concentrations of colchicine a clear inhibition of rosette formation occurred. Since no effect on binding of monomeric IgG was observed it would seem that the integrity of the microtubules is important in fixation of red cells to the monocyte plasma membrane. Contrary to our findings recent studies of colchicine and vinblastine on rabbit alveolar macrophages at identical concentration showed no effect on the formation of EA rosettes (Atkinson & Parker, 1977b).

Rosette formation is primarily dependent on a chemical bond between the cell receptor and its appropriate ligand. The greater the number of IgG molecules on the red cell, and/or the Fc receptors on the monocytes, the greater will be the chance of interaction and fixation of the erythrocyte. The binding of the relatively large red cell to the monocyte membrane is apparently facilitated by the formation of microvilli, which would increase the surface area available for attachment. Since blunting of the microvilli and decreased rosette formation were noted following treatment by metabolic inhibitors

and at 4°, presumably this phenomenon is dependent on active cellular metabolism and is induced by initial interaction of the receptor and its ligand. Recently in a mouse system 2-deoxyglucose was shown to have a selective effect on Fc and complement mediated phagocytosis; thus a direct effect on the receptor is also possible (Michl, Ohlbaum & Silverstein, 1976). Several studies have shown that the spatial distribution and natural movement of specific receptors within the bilipid plasma membrane is controlled by the cytoskeletal network of microtubules and microfilaments (Singer & Nicolson, 1972). Cytochalasin prevents this membrane aggregation of integral proteins, while colchicine at low concentrations promotes aggregation of receptors that are still linked to microfilaments.

We would propose that our findings lend support to these concepts. The sensitized erythrocyte which no doubt is an adequate multivalent ligand may promote aggregation of Fc receptors. The inhibitory effect of the cytoskeletal poisons would limit aggregation, without affecting binding of monomeric IgG, while colchicine at low concentrations would decrease the cell surface area and may disrupt microtubules without affecting microfilaments and thus promote aggregation. The decreased rosette formation noted in monocytes treated with metabolic inhibitors and in the cold could be analogous to the known inhibition of capping of IgG on B lymphocytes seen under similar conditions (Unanue, Karnovsky & Engers, 1973). Thus it is likely that impaired cell metabolism not only results in decreased microvilli formation, but may indirectly affect receptor movements within the membrane.

These studies have shown that there is no correlation between rosette formation and binding of IgG. Rosette formation is an active cellular event, while attachment of cytophilic IgG to the Fc receptor is probably a strictly chemical binding.

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