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Human neutrophil aggregation and increased adherence to human endothelial cells induced by heat-aggregated IgG and immune complexes

Y. HASHIMOTO & E. R. HURD *Department of Internal Medicine, Rheumatic Diseases Unit, The University of Texas Health Science Center at Dallas, Dallas, Texas, USA*

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SUMMARY

Several types of IgG-dependent phagocytic stimuli independent of complement were investigated for their property to induce human polymorphonuclear neutrophil leucocyte (PMN) aggregation and adherence to human endothelial cells (EC) in culture. A Coulter counter method was employed for the detection of cell aggregation. Aggregated IgG, ovalbumin–anti-ovalbumin (OV anti-OV) immune complexes (both insoluble and soluble) and opsonized latex particles induced a significant degree of PMN aggregation which was detectable as early as 2 min after exposure of PMN to these stimuli. This aggregation was dependent on divalent cations (Ca^{++}, Mg^{++}). The same phagocytic stimuli furthermore significantly increased adherence of PMN to cultured human EC and serum-coated plastic. Controls consisting of native IgG, and OV anti-OV complexes prepared from $F(ab')_2$ antibody failed to induce either aggregation or increased adherence of PMN. These data suggest that exposure of PMN to IgG-dependent phagocytic stimuli induces increased adhesiveness of PMN and that interaction between the Fc-receptor of PMN and the Fc-portion of phagocytic stimuli is essential for this effect.

INTRODUCTION

In recent studies, several chemotactic factors have been shown to cause polymorphonuclear neutrophil leucocyte (PMN) aggregation (Craddock *et al.*, 1977; O'Flaherty, Kreutzer & Ward, 1977) and altered adhesiveness to foreign surfaces (Fehr & Dahinden, 1979; Smith *et al.*, 1979a) and to cultured endothelial cell (EC) surfaces (Hoover, Briggs & Karnovsky, 1978; Smith Lackie & Wilkinson, 1979b). In other recent studies, a possible association between degranulation of PMN and either aggregation (Lackie, 1977) or PMN adhesiveness to an artificial surface (Gallin, Wright & Schiffman, 1978) has been suggested. On the other hand, the regulating mechanisms for PMN adherence and their relationship to phagocytosis has not been well studied. In the present study, we have investigated the influence of several Fc-dependent phagocytic stimuli on both PMN aggregation and adherence to human EC and plastic surfaces.

MATERIALS AND METHODS

Preparation and ^{51}Cr-labelling of human PMN

Human PMN from normal healthy donors were separated from the heparinized blood by

Correspondence: Eric R. Hurd, MD, Department of Internal Medicine, Rheumatic Diseases Unit, The University of Texas Health Science Center, 5323 Harry Hines Boulevard, Dallas, Texas, 75235, USA.

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Ficoll–Hypaque gradient centrifugation, dextran sedimentation and hypotonic lysis of red blood cells (Böyum, 1968). For aggregation studies, PMN were suspended in Hanks' balanced salt solution (HBSS) at a concentration of 5×10^6/ml.

For adherence experiments, PMN were suspended in HBSS at a concentration of 10^7/ml and incubated with 100 μCi/ml sodium chromate (New England Nuclear, Boston, Massachusetts) at 37°C for 60 min. The labelled cells were washed three times, passed through sterile gauze to remove large cell clumps and resuspended in HBSS at a concentration of 5×10^6/ml. The cells contained 97% PMN and viability was approximately 90% by the trypan blue dye exclusion test.

Phagocytic stimuli

Human aggregated IgG. Human Cohn fraction II (Sigma Chemical Co., St Louis, Missouri) was purified by DEAE cellulose (DE52 Whatman Biochemicals Ltd, UK) column chromatography and suspended in phosphate-buffered saline (PBS), pH 7·3, at a concentration of 50 mg/ml and heat-aggregated at 63°C for 20 min. This was fractionated by Sephadex G-200 column chromatography, and the first peak was collected for use as aggregated IgG. For control studies, purified IgG was centrifuged at 100,000 *g* for 60 min to remove aggregates and supernatant was used as 'native' IgG.

Immune complexes. Ovalbumin (OV) was purchased from Miles Laboratories Inc., Elkhart, Indiana. Anti-OV antiserum was produced in a New Zealand albino rabbit. This serum was purified by 37% ammonium sulphate precipitation, followed by passage over an OV-coupled Sepharose 4B affinity column. IgG anti-OV was subsequently prepared by purifying the anti-OV over a DEAE ion-exchange column. Insoluble immune complexes were prepared at equivalence by incubating equal volumes of OV (300 μg/ml) and anti-OV (1·7 mg/ml), washed twice and resuspended in PBS. Four-fold and nine-fold antigen excess complexes were also prepared by incubating excess antigen with the same volume of anti-OV employed to prepare insoluble complexes. IgG anti-OV was digested with 2% w/w pepsin at 37°C for 24 hr. The resulting $F(ab')_2$ solution was dialysed against PBS and further purified on a Sephadex G-150 column. This $F(ab')_2$ anti-OV showed no precipitation line by immunodiffusion when checked against goat anti-rabbit Fc antibody. Insoluble immune complexes utilizing the $F(ab')_2$ anti-OV were prepared in the same manner as described above.

Latex particles. Simple latex particles were obtained from DIFCO Laboratories, Detroit, Michigan, and latex particles coated with denatured IgG were purchased from Calbiochem–Behring Corporation, La Jolla, California. Both types of particles were washed twice in PBS before use.

Divalent cations

The influence of the divalent cations, Ca^{++} and Mg^{++}, on the aggregation of PMN induced with 40 μg/ml of insoluble OV anti-OV immune complexes was studied. Studies were performed using Ca^{++} and Mg^{++}-free buffer and Ca^{++} and Mg^{++} containing buffer at a 1 mM concentration. The effect of Mg^{++} and Ca^{++} individually was determined using two concentrations for each cation, i.e. 1 and 0·1 mM.

Coulter counter assay

The Coulter counter assay in this study was performed with a slight modification of the methods described in detail previously (Lackie, 1974; O'Flaherty *et al.*, 1977). The Coulter counter utilized was model ZBI equipped with a volume channelyzer. The aperture and amplification were set at values of 0·5 and 4 respectively; the lower threshold was set at 35 and the upper at infinity. At these conditions, the recorded mean particle volume of the control cell suspension was near 270 μm^3 and any particle smaller than 175 μm^3 was excluded from the counts. The volume channelyzer was set to divide the volume size range from 175 to 675 μm^3 into 100 intervals and the frequency of each of the intervals was recorded on an X–Y recorder.

Aggregation assay

All experiments were performed at 37°C unless otherwise described. One millilitre of PMN suspension was added to a polypropylene 12 × 75 mm culture tube containing a small stirring bar.

This tube was allowed to stand in a small 37°C water bath equipped with a magnetic stirrer and heater, and the bar was spun at a moderate speed. After warming the PMN to 37°C for 10 min, 100 μl of PMN suspension was removed and the exact initial particle number was detected. Within 30 sec, 100 μl of various reagents prewarmed to 37°C was added to the PMN suspension and the particle number at each time period (1, 2, 5, 10, and 20 min) was determined. Particle numbers were corrected for the slight decrease induced by the added reagents. The decrease in particle number at each time period reflected the degree of aggregation of PMN at that time and all the results were expressed as per cent decrease in particle count.

Culture of endothelial cells (EC)

A slight modification of the technique of Jaffe *et al.* (1973) was used. EC were obtained from umbilical cord veins by use of 0·2% collagenase and cultured in RPMI 1640 supplemented with 25% fetal calf serum, penicillin (200 units/ml), streptomycin (100 μl/ml) and fungizone (0·25 μg/ml). The cell suspension was divided equally among the 24-well (16 mm diameter each) tissue culture plate (Costar, Cambridge, Massachusetts) and incubated at 37°C in the presence of 5% CO_2 and 100% humidity. The cells were fed twice a week and usually became confluent in 4–7 days.

Adherence assay

The surfaces of EC were washed six times with HBSS supplemented with 10% heat-inactivated fetal calf serum. Next 0·9 ml of the ^{51}Cr-labelled PMN suspension and 100 μl of the reagent to be tested were mixed gently. Each 300 μl of the PMN suspension (approximately 1×10^6 cells) was transferred into the triplicate well with the confluent monolayer of EC. This was incubated at 37°C for 30 min. Non-adherent cells were aspirated and the surface of the well was washed twice with warm HBSS. Adherent cells were lysed with 0·1 N NaOH, collected in a tube and counted in a Packard auto-gamma scintillation spectrometer. Per cent adherence to EC was determined by calculating the percentage of the cells which were initially added to the well. In some experiments, PMN adherence to plastic tissue culture plates coated with heat-inactivated fetal calf serum was measured utilizing the same technique.

Lysosomal enzyme assay

β-glucuronidase release from PMN was detected by the method described by Fishman (1974). A 1-ml suspension of PMN (5×10^6 cells) was incubated with the reagents to be tested at 37°C and each 0·5 ml was transferred to a small tube containing 0·5 ml ice-cold PBS. This tube was immediately centrifuged at 700 ***g*** for 8 min and 0·5 ml of the supernatant was analysed for enzyme activity.

RESULTS

PMN aggregation

When the PMN cell suspension was stirred without reagent, the particle number never fell more than 10%. This constituted the control value. Fig. 1 summarizes the effects of various phagocytic stimuli on PMN aggregation.

Aggregated IgG. When heat-aggregated IgG was added to the PMN suspension at a final concentration of 1 mg/ml, the PMN aggregated quickly and the particle number decreased nearly 50%. The maximum decrease was observed between 2 and 5 min after the addition of aggregated IgG, and then the number of particles slowly increased (37% decrease at 20 min). Native IgG at the same concentration failed to induce a significant decrease in particle number. When the concentration of aggregated IgG was reduced to 0·1 mg/ml in final concentration (data not shown in the figure), the maximum decrease was 20% at 5 min and the number of particles increased slightly (16% decrease at 20 min).

Latex particles. In this procedure, the ratio of latex particles to PMN was adjusted to approximately 50:1. Opsonized latex particles induced a remarkable fall in particle number, i.e. a decrease of 75% by 10 min. Simple latex, in contrast, failed to induce a significant decrease in particle number.

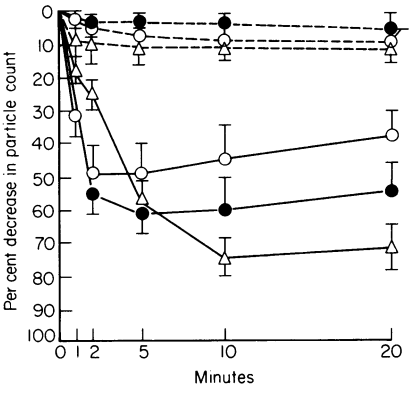

Fig. 1. Effect of phagocytic stimuli on PMN aggregation. All the data are shown as per cent decrease in particle count as an index of degree of PMN aggregation. Comparisons were done between the following reagents: aggregated IgG (1 mg/ml) (o——o) and native IgG (1 mg/ml) (o- - -o) ($n=5$); insoluble IgG complex (40 μg/ml) (•——•) and insoluble F(ab')$_2$ complex (40 μg/ml) (•- - -•) ($n=4$); opsonized latex particles (Δ——Δ) and unopsonized latex particles (Δ- - -Δ) ($n=4$). Vertical lines represent standard deviations.

Immune complexes. Insoluble immune complexes (final concentration 40 μg/ml) induced a rapid and striking fall in particle number. A decrease of 55% was reached as early as 2 min followed by a maximum decrease of 62% at 5 min and 58% at 20 min. On the other hand, insoluble immune complexes prepared with F(ab')$_2$ antibody at the same concentration (40 μg/ml) failed completely to induce aggregation. Figs. 2 and 3 show the further analyses of the effects of immune complexes. In Fig. 2, a dose-effect utilizing insoluble immune complexes is demonstrated. IgG immune complexes could induce significant aggregation with concentrations as low as 10 μg/ml. At this concentration a decrease of approximately 23% occurred at 5 and 10 min. Using a concentration of 20 μg/ml, the maximum decrease was 37%. At a concentration of 40 μg/ml, the maximum decrease was 62% at 5

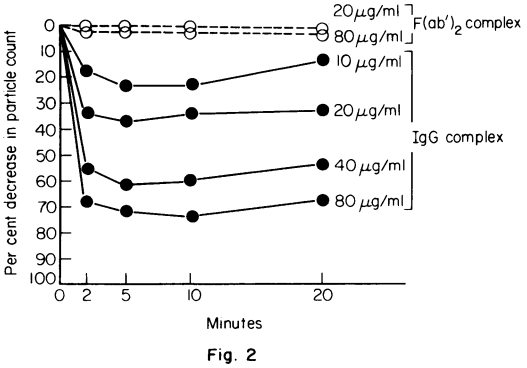

Fig. 2

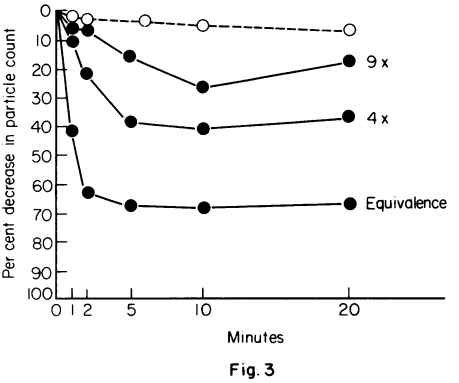

Fig. 3

Fig. 2. Dose-effects of IgG OV anti-OV and F(ab')$_2$ immune complexes on PMN aggregation. PMN aggregation induced by insoluble OV anti-OV immune complexes prepared with intact IgG (——) at final concentrations of 80 to 10 μg/ml and by complexes prepared with F(ab')$_2$ antibody (- - - -), 20 and 80 μg/ml. Values represent the mean of three experiments with similar results.

Fig. 3. Effect of size of OV anti-OV immune complexes on PMN aggregation. Comparison of the effects of OV anti-OV immune complexes prepared at Ag–Ab equivalence, four-fold antigen excess and nine-fold antigen excess. Complexes at equivalence were added at a final concentration of 60 μg/ml. Values represent mean of three experiments. PMN in HBSS without immune complexes ($n=5$) (o- - -o) showed no significant aggregation.

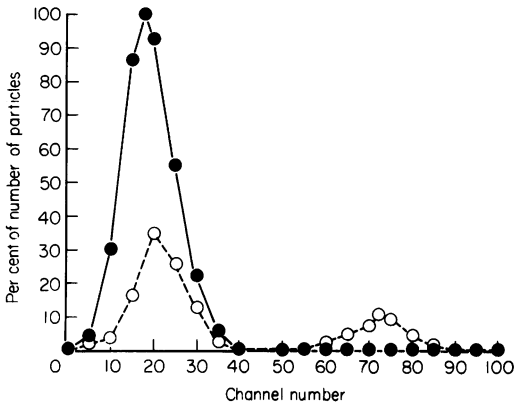

Fig. 4

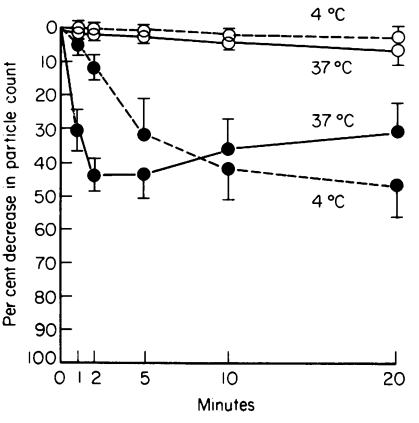

Fig. 5

Fig. 4. Profile of PMN aggregation induced by aggregated IgG. The distribution of volume sizes of a PMN suspension before (●——●) and 5 min after (o- - - -o) addition of 1 mg/ml aggregated IgG at 37 °C. The absolute volumes of the major peak to which aggregated IgG had not been added (——), the first (- - - -) and second peaks (- - - -) to which aggregated IgG had been applied, are 265, 270 and 535 μm^3 respectively. The second peak with the dotted line represents doublets, i.e. aggregates of two PMN. The aggregation of more than three PMN was not detected by the volume channelyzer because of the threshold setting used in these experiments. The actual particle number decreased from 4,500 to 2,200 in this particular experiment.

Fig. 5. Effect of temperature on PMN aggregation by aggregated IgG. Comparison of PMN aggregation by aggregated IgG, 1 mg/ml, at two different temperatures, 37°C (●——●) and 4°C (●- - - -●). Native IgG, 1 mg/ml, induced no significant aggregation at either 37°C (o——o) or 4°C (o- - - -o). Data represent mean of four different experiments.

min. Finally, using 80 μg/ml, the maximum decrease was 74% at 10 min. In contrast, as has been shown, insoluble $F(ab')_2$ complex failed completely to induce aggregation both at a concentration as low as 20 μg/ml and as high as 80 μg/ml. Fig. 3 shows the comparison among insoluble, four-fold antigen excess and nine-fold antigen excess IgG complexes. Insoluble immune complexes (final concentration 60 μg/ml) induced a rapid and striking fall in particle number as described. With four- and nine-fold antigen excess complexes, the maximum decrease was 41 and 26% respectively at 10 min, followed by recovery to 37% (four-fold excess) and 18% (nine-fold excess) at 20 min.

Evidence that the PMN were, in fact, aggregating rather than adhering to the walls of the tube is summarized as follows: (1) polypropylene rather than polystyrene tubes were used; this ensures against adherence of PMN to walls of the tubes; (2) all samples were periodically withdrawn and examined in the microscope to be certain that the degree of aggregation by the Coulter counter method correlated with that seen by microscopy; and (3) by use of the channelyzer, it was possible to observe the formation of aggregates. Fig. 4 demonstrates formation of doublets, i.e. aggregation of two cells.

Effect of temperature

This was performed utilizing aggregated IgG (1 mg/ml) as phagocytic stimuli.

If the experiments were performed at 4°C, as shown in Fig. 5, the particle number slowly but continuously declined reaching a 47% decrease at 20 min. It appeared from this that at 4°C PMN aggregate slowly but continuously over a 20-min observation period. In contrast, at 37°C, the aggregation occurred rapidly, and with time appeared to be at least partially reversible. Native IgG (1 mg/ml) did not induce significant aggregation at either 37 or 4°C.

Effect of divalent cations

The influence of divalent cations was studied to determine whether they were essential for the aggregation of PMN. The results are summarized in Table 1. In these experiments insoluble immune complexes were added to a PMN suspension at a final concentration of 40 μg/ml. If both

Table 1. Influence of divalent cations on PMN aggregation induced with insoluble OV anti-OV immune complexes (40 μg/ml)*

	Time (min)			
	2	5	10	15
Ca^{++}-, Mg^{++}-free	25·1	9·5	9·3	10·2
Ca^{++}, Mg^{++} 1 mM	59·3	61·7	59·9	55·2
Ca^{++} 1 mM	54·7	46·8	45·5	39·8
Ca^{++} 0·1 mM	47·0	35·2	15·8	13·1
Mg^{++} 1 mM	57·7	57·0	56·8	46·0
Mg^{++} 0·1 mM	54·5	45·1	37·3	30·8

* Values represent the mean of three experiments and demonstrate per cent decrease in particle number.

Ca^{++} and Mg^{++} were depleted, no significant decrease in particle count occurred except for a 25% decrease at 2 min. Both Ca^{++} and Mg^{++} independently induced significant PMN aggregation at a concentration of 1 mM. At this concentration, Mg^{++} induced a 57·7% decrease at 2 min and Ca^{++} induced a 54·7% decrease at the same time period. At a concentration of 0·1 mM, Mg^{++} appeared to maintain aggregation better than Ca^{++} at the 5-, 10- and 15-min time periods.

PMN adherence to endothelial cells in culture

Effect of aggregated IgG. As shown in Fig. 6, if PMN were suspended in protein-free HBSS, the per cent adherence of the PMN to EC was 10·8 ± 3·6%. When either human serum albumin (HSA) or native IgG was added at a final concentration of 1 mg/ml, slightly increased adherence was observed (approximately 20% as compared with the HBSS control). However, aggregated IgG at the same concentration induced a marked increase, i.e. an increase of 200% as compared with the HBSS control ($P < 0·001$) and 140% increase as compared with native IgG ($P < 0·01$).

The same results were obtained, but to a lesser degree, by utilizing a lower concentration of

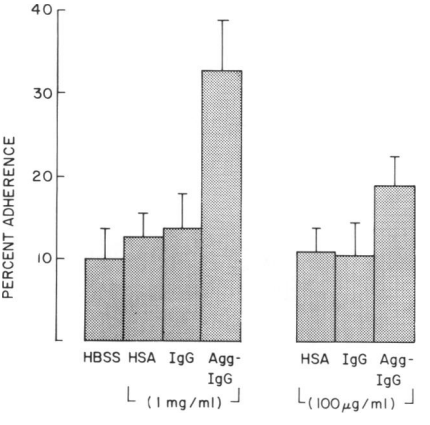

Fig. 6. Effect of aggregated IgG on PMN adherence to endothelial cells. The final concentrations of HSA, native IgG and aggregated IgG were 1 mg/ml (*left*) and 100 μg/ml (*right*). Data are shown as the mean ± s.d. of four different experiments. The difference between native IgG and aggregated IgG was statistically significant at both concentrations ($P < 0·01$ for 1 mg/ml and $P < 0·05$ for 100 μg/ml).

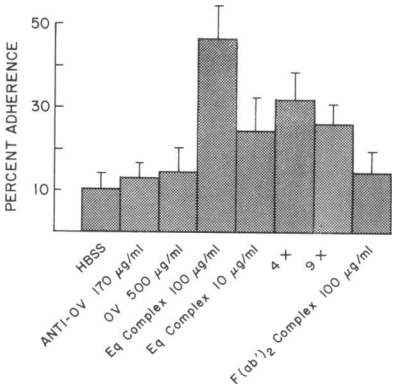

Fig. 7. Effect of OV anti-OV immune complexes on PMN adherence to endothelial cells. Each reagent was employed at the final concentration indicated. The final protein concentrations of four- and nine-fold antigen excess immune complexes were 320 and 470 μg/ml respectively. Data are shown as the mean $\pm$ s.d. of four different experiments. *P* values by Student's *t*-test for each reagent compared with the OV control are as follows: insoluble OV anti-OV complex, 100 μg/ml, $P < 0{\cdot}0005$, and 10 μg/ml, $P < 0{\cdot}05$; four-fold antigen excess complex, $P < 0{\cdot}001$; nine-fold antigen excess complex, $P < 0{\cdot}01$; $F(ab')_2$ insoluble complex, 100 μg/ml, $P > 0{\cdot}5$.

reagent (100 μg/ml). Aggregated IgG induced a 73% increase as compared with the HBSS control ($P < 0{\cdot}01$) and a 68% increase as compared with native IgG ($P < 0{\cdot}05$).

Effect of immune complexes. Fig. 7 shows a summary of the effect of immune complexes using various reagents. When either OV (500 μg/ml) or anti-OV (170 μg/ml) was present in the adherence assay system, the per cent adherence of PMN was slightly increased from the HBSS control, suggesting a non-specific effect of protein on PMN adherence. Immune complexes, however, induced a significantly increased amount of adherence. Insoluble complexes at final concentrations of 100 and 10 μg/ml induced $47{\cdot}1 \pm 8{\cdot}0\%$ ($P < 0{\cdot}0005$) and $24{\cdot}3 \pm 7{\cdot}8\%$ ($P < 0{\cdot}05$) adherence respectively. As indicated, these were significantly higher than the OV control ($13{\cdot}0 \pm 3{\cdot}1\%$). Four- and nine-fold antigen excess immune complexes at final protein concentrations of 320 and 470 μg/ml, respectively, also induced significantly increased adherence, i.e. $32{\cdot}6 \pm 5{\cdot}8\%$ ($P < 0{\cdot}001$) and $25{\cdot}2 \pm 5{\cdot}2\%$ ($P < 0{\cdot}01$) respectively, as compared with the OV control. Insoluble $F(ab')_2$ complexes, at the same concentration as the IgG complexes, in contrast, induced only $140 \pm 3{\cdot}5\%$ adherence which was not significantly different from the OV control. When serum-coated plastic was employed as the adhering surface, essentially the same results were obtained as shown in Fig. 8.

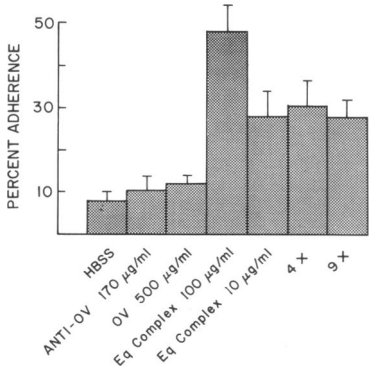

Fig. 8. Effect of OV anti-OV immune complexes on PMN adherence to serum-treated plastic. A plastic multiwell petri dish was incubated with medium containing 20% heat-inactivated fetal calf serum, washed and used as the adhering surface. The final concentration of each reagent was the same as that shown in Fig. 7.

Table 2. Release of β-glucuronidase from PMN exposed to immune complexes*

Reagent	Conditions of incubation		
	37°C, 5 min	37°C, 30 min	4°C, 30 min
HBSS control	1·9 ± 0·2	2·2 ± 0·3	2·3 ± 0·2
IgG complexes (60 μg/ml)	2·9 ± 0·4 ($P < 0·05$)†	8·2 ± 1·6 ($P < 0·005$)	2·4 ± 0·3 (n.s.)
$F(ab')_2$ complexes (60 μg/ml)	2·0 ± 0·3 (n.s.)	2·4 ± 0·4 (n.s.)	2·3 ± 0·3 (n.s.)

* Values represent percentage of total release obtained by treatment of PMN with 0·2% Triton X. Figures are the mean ± s.d. of four experiments.

† Student's *t*-test between experimental and control mean values.

n.s. = Not significant.

Lysosomal enzyme degranulation

In order to study the possible effect of the immune complexes utilized on PMN degranulation, β-glucuronidase release from PMN was determined adding both IgG and $F(ab')_2$ complexes at a final concentration of 60 μg/ml.

As shown in Table 2, at 37°C, IgG complexes induced a significant release of β-glucuronidase as early as 5 min ($P < 0·05$) and also at 30 min ($P < 0·005$) when compared with the HBSS control. $F(ab')_2$ complexes, on the other hand, failed to induce degranulation of PMN over the 30-min observation period. When the experiment was performed at 4°C, no significant differences were detected utilizing the three different reagents.

DISCUSSION

Altered adhesiveness of PMN is believed to be an important aspect of PMN function in acute inflammation. Nevertheless, the regulating mechanisms of adhesiveness and its possible relationship to other PMN functions have not been well documented. Recently, studies of the influence of chemotactic factors on PMN adhesiveness have been reported. Craddock *et al.* (1977) demonstrated PMN aggregation induced by complement-derived chemotactic factor (C5a). O'Flaherty *et al.* (1977) demonstrated the same type of aggregation and increased adherence to an artificial surface using a synthetic chemotactic peptide. In more recent studies, Fehr & Dahinden (1979) demonstrated increased adhesiveness of PMN stimulated by a high concentration of chemotactic factors and showed an inverse correlation between the effect of chemotactic factors on PMN locomotion and adherence. In rabbit studies, the same results were reported by Smith *et al.* (1979b) using cultured EC as the adhering surface.

In the present experiments, conducted in the absence of complement, we have demonstrated that phagocytic stimulation, especially if Fc receptor-mediated, also induces a marked increase of PMN adhesiveness. The Fc receptor on the human PMN membrane (Messner & Jelinek, 1970) is well known to play an essential role in the ingestion of opsonized materials such as sheep erythrocytes sensitized with IgG antibody (Scribner & Fahrney, 1976). From the results of the present study it would appear, therefore, that the interaction between the Fc portion of the IgG molecule and the Fc receptor of PMN is important and critical for the triggering of the increased adhesiveness demonstrated. The difference between the effect of native and heat-aggregated IgG may be explained by the difference in their capacity to stimulate membrane receptors as demonstrated by Weissmann, Brand & Franklin (1974).

Recently, several studies have been reported regarding the possible mechanism of increased adhesiveness of PMN. Lackie (1977) demonstrated a positive correlation between increased secretion of lysosomal enzymes and enhanced aggregation of rabbit PMN. Gallin *et al.* (1978) reported a possible association between degranulation and hyperadhesiveness of human PMN

utilizing chemotactic factors for the stimulation of PMN and emphasized the importance of a decreased negative surface charge in causing increased PMN adhesiveness (Gallin, 1980). More recently, Bockenstedt & Goetzl (1980) purified an acidic protein of the specific granules as a possible mediator of enhanced adherence. For these reasons we measured the lysosomal enzyme release from PMN and found a significant amount of degranulation as early as 5 min after exposure of the PMN to the same immune complexes that were utilized in both the aggregation and adherence studies. Thus the aggregation and increased adherence to EC observed at 37°C may be at least partially explained by this mechanism.

The dependence of PMN aggregation on the presence of divalent cations observed in these studies is not unexpected. The important role of these ions in the initiation of cell movement and cellular adhesiveness has been well documented (Gallin & Rosenthal, 1974; Lynn & Mukherjee, 1978); and, more interestingly, calcium ionophore has been shown to induce PMN aggregation (Lackie, 1977). These observations suggest that stimulation of the PMN membrane by phagocytic stimuli induces a divalent cation flux which leads to a conformational membrane change. The mechanism of the slow and gradual aggregation at low temperature (Fig. 5) is not clear. Simple mechanical bridging between cells mediated by phagocytic stimuli is a possible explanation, especially in the presence of insoluble aggregating stimulatory agents. One may suggest, therefore, that aggregation results mainly from increased adhesiveness of the membrane of the PMN as a consequence of the action of degranulating stimuli and that this adhesiveness may be enhanced by simple mechanical cell bridging.

With regard to the adherence studies, the question arises as to whether Fc receptors are present on the surface of EC. Although in a recent report, Shadforth, Cunningham & Andrews (1979) demonstrated Fc receptors on cultured EC surfaces from human umbilical cord, we could not confirm their results using various methods (unpublished observations). Thus PMN–EC bridging mediated by immune complexes does not appear to us to be a likely explanation for increased attachment of PMN to EC. We prefer to attribute this change to the increased adhesiveness of the PMN. The increased adherence to an artificial surface observed in these experiments would support this suggestion. Thus both aggregation and increased adherence to EC may primarily reflect the hyperadherent membrane of the PMN which is induced via stimulation of the Fc receptor and/or subsequent phagocytosis.

In consideration of the hypothesis that chemotactic factors stimulate PMN via membrane receptors (Aswanikumar *et al.*, 1976; Becker, 1976), it would appear theoretically possible that membrane perturbation induced by specific stimuli can cause hyperadhesiveness of PMN either as a result of degranulation or alternatively as a parallel phenomenon with degranulation and other functions derived from the activation of the 'secretory code' of PMN (Weissmann *et al.*, 1979).

As discussed by Fehr & Dahinden (1979), increased adhesiveness of PMN may contribute to trapping of PMN at the site of acute inflammation. The clinical significance of PMN aggregation induced by complement has been discussed elsewhere (Craddock *et al.*, 1977) and a possible association between *in vitro* adherence and *in vivo* margination has also been suggested (Fehr & Jacob, 1977). More interestingly, Sacks *et al.* (1978) have demonstrated that endothelial cell injury can be induced by 'activated' PMN. Circulating immune complexes are known to play an important role in certain diseases, e.g. systemic lupus erythematosus (Harbeck *et al.*, 1973) and Felty's syndrome (Andreis *et al.*, 1978), and the phagocytosis of these complexes by PMN has also been demonstrated (Hurd, Andreis & Ziff, 1977; Hurd, Jasin & Gilliam, 1980). It is suggested that in such clinical situations, aggregation and/or increased margination of PMN to EC *in vivo*, amplified by the presence of both immune complexes and activated complement components, may at least partly contribute to neutropenia or vasculitis, both of which are common manifestations of these diseases.

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