ADHESION OF LEUKOCYTES TO ENDOTHELIUM: ROLES OF DIVALENT CATIONS, SURFACE CHARGE, CHEMOTACTIC AGENTS AND SUBSTRATE

R. L. HOOVER,* R. FOLGER†, W. A. HAERING,* B. R. WARE‡ and M. J. KARNOVSKY*

*Department of Pathology, Harvard Medical School, 25 Shattuck St, Boston, MA and ‡Department of Chemistry, Harvard University, Cambridge, MA, U.S.A.

SUMMARY

The attachment of polymorphonuclear leukocytes (PMN) to the endothelial lining of blood vessels is an important initial event in the acute inflammatory reaction. Experiments were carried out to examine some of the parameters of this adhesive interaction and the extent to which the substrate (endothelium) plays a role in controlling the process. Using a monolayer collection assay for adhesion, we found a requirement for divalent cations. Mn²⁺ produces maximal adhesion followed by Zn⁺⁺ > Ni²⁺ > Mg²⁺ > Ba²⁺ > Ca²⁺. Pretreatment of either the endothelium or PMN with chemotactic agents (zymosan-activated serum, a bacterial filtrate, C₅a and formyl-methionyl-leucyl-phenylalanine) causes an increase in the attachment of PMN to endothelial monolayers. Modulations of net surface charge, as measured by laser Doppler electrophoresis, are not correlated to alterations in adhesion. Mn²⁺, which produces maximal adhesion, reduces the net surface charge to the same extent as Ca²⁺, which has the smallest effect on adhesion. Similarly, reductions in net surface charge are not responsible for the increases in adhesion due to the chemotactic agents because there are no differences in electrophoretic mobilities between cells treated with either chemotactic agents or their appropriate controls. Scatchard analysis of the binding of the chemotactic tripeptide, formyl-methionyl-leucyl-phenylalanine, indicates the presence of high-affinity binding sites on the surface of the endothelium. The results suggest a role for the endothelium in mediating the adhesive interaction with the PMN.

INTRODUCTION

An important component of the acute inflammatory response is the attachment of granulocytes to the endothelial lining of blood vessels. The mechanisms underlying this event are ill-understood. The adhesion of polymorphonuclear leukocytes (PMN), particularly to artificial substrates, has been studied extensively (Penny, Galton, Scott & Eisen, 1966; Smith, Hollers, Patrick & Hassett, 1979; MacGregor, Macarak & Kefalides, 1978). With procedures now available for culturing endothelial cells, however, previous in vivo studies can now be done in vitro (Lackie & DeBono, 1977; MacGregor et al. 1978; Hoover, Briggs & Karnovsky, 1978), permitting the study of what role each cell type plays in the interaction.

1 Present address: P.O. Box 1346, Pittsburgh, P.A., U.S.A.
2 Present address: Department of Chemistry, Syracuse University, Syracuse, N.Y., U.S.A.
Many factors have been studied in order to determine the adhesive interaction between the PMN and endothelium. In *in vivo* and *in vitro* studies, Thompson, Papadimitriou & Walters (1967) and Atherton & Born (1972, 1973) observed that divalent cations were required for adhesion. Atherton & Born (1972) further demonstrated *in vivo* that *E. coli* culture filtrate and neuraminidase treatment could increase the adhesion of PMN to vessel walls. O'Flaherty, Craddock & Jacob (1978a), Hoover *et al.* (1978) and Smith *et al.* (1979) have demonstrated that specific complement components and the formyl-peptide analogues, which are chemotactic for PMN, increase the adhesion of PMN to endothelial cells. Chenoweth & Hugli (1978) found specific receptors for the chemotactic complement components on human PMN, while earlier Williams, Snyderman, Pike & Lefkowitz (1977) had demonstrated the presence of receptors for the chemotactic formyl-tripeptides. Hoover *et al.* (1978) have also shown that if the endothelium is pretreated with the chemotactic agents, the attachment of PMN is increased. Likewise, Smith *et al.* (1979) have shown that treatment of an artificial substrate with chemotactic agents increased theattachment of PMN. The data suggest that the substratum (endothelium) as well as the PMN can be affected in a manner which influences their adhesive interactions.

There is also evidence for cation specificity in the cell adhesion process. For example, Rabinovitch & DeStefano (1973a, b) found that Mn$^{2+}$ was the most effective in promoting the spreading of macrophages and fibroblasts; and similarly, Martz (1980) showed that Mg$^{2+}$ and Mn$^{2+}$, but not Ca$^{2+}$ and Sr$^{2+}$, supported optimal adhesion during the prelytic attachment of immune cytolytic thymus-derived lymphocytes to specific ascites tumour target cells. While there is some evidence for a cation requirement for the interaction of PMN with endothelium (Beesley *et al.* 1978; Hoover *et al.* 1978), there is little information concerning cation specificity.

Cell surface charge can also regulate the formation of contacts between cells (Curtis, 1962; Weiss & Harlos, 1971). Reduction of the repulsive electrostatic interactions between like-charged cells could permit the attractive van der Waals forces to promote cell contact and thus could alter cell adhesiveness. Brown, Holbrow & Collins (1977) observed significant reductions in the electrophoretic mobilities (surface charge) and increases of PMN infiltration from patients with rheumatoid arthritis, which has an inflammatory component. Similarly Gallin, Durocher & Kaplan (1975) indicated that a reduction in cell surface charge was associated with an increase in PMN chemotaxis in response to C$5a$, which is also known to increase PMN attachment (Fehr & Jacob, 1977; Hoover *et al.* 1978).

Using the monolayer collection assay for adhesion (Walther, Ohman & Roseman, 1973) and laser Doppler electrophoretic light scattering, we have examined the effects of chemotactic factors and divalent cations on endothelial cells and PMN with regard to the PMN–endothelium adhesion and electrophoretic mobility. Based on our results we conclude that alteration of the net surface charge does not mediate the effects on adhesion due to chemotactic agents and divalent cations. We also show that endothelial cells have receptors on their surfaces for a specific chemotactic agent and that the binding of the agent increases the attachment of PMN.
MATERIALS AND METHODS

Cell preparation

Venous blood was collected from healthy volunteers into 10-ml tubes containing 250 U. heparin/tube. The PMN were isolated by dextran sedimentation and density gradient centrifugation. Six per cent dextran in phosphate-buffered saline, pH 7.2 (PBS) was mixed with whole blood at a ratio of 1:3, and allowed to stand for 40 min. The PMN-rich supernatant was removed and the cells were washed once in Hanks' balanced salt solution buffered with HEPES (15 mM), pH 7.4 (HH). The PMN suspension was then layered over Ficolliso-paque and centrifuged at 400 g for 30 min. The pellet was removed and contaminating RBCs eliminated by hypotonic lysis. (One millilitre of distilled water is titrated with the cells for 20 s, immediately followed by the addition of 15 ml of HH.) The PMN were then washed 3 times in HH. Viability was assessed by trypan blue exclusion and PMN characterization by Wright's stain. All analyses were concluded within 5 h to ensure healthy PMN.

For the adhesion assay, PMN were labelled with 51 Cr as described by Gallin, Clark & Kimball (1973). The final pellet of PMN was suspended at a concentration of 10^7 cells/ml in 1-2 ml of HH to which 51 Cr as sodium chromate (sp. act. 200-500 Ci/g chromium; New England Nuclear, Boston) had been added (0.1 ml/ml of cell susoension). After incubation at room temperature for 1 h, the cell suspension was rinsed in HH before use in the adhesion assay. Experiments were carried out which showed that during the assay, the 51 Cr released from PMN and subsequently picked up by endothelium represented less than 1.5 % and did not significantly contribute to the results observed.

Endothelial cells were isolated from calf aorta according to the methods of Booyse, Sedlak & Rafelson (1975). Clones of endothelial cells were obtained by plating a dilute suspension of cells (10^4/ml) in 100-mm Petri dishes. When distinguishable, the clones were removed with trypsin/EDTA (0.025%/0.5 mM) and plated into CoStar cluster dishes (no. 3524). It was then possible to subculture the cells into larger tissue culture plates once the cell number increased (near confluency). Cultures were not used beyond the 10th passage. Endothelial identification was monitored by detection of factor VIII antigen, as outlined by Booyse et al. (1975) and by electron microscopy. Viability was assessed by trypan blue exclusion.

Adhesion assay

The adhesion test was essentially the monolayer collection assay described by Walther et al. (1973). One-half millilitre of 51 Cr-labelled PMN (2 x 10^6/ml) was added to a confluent monolayer of endothelial cells and incubated at 37 °C for 30 min in the appropriate test medium. The unattached PMN were then aspirated off and the monolayers washed once with HH. The monolayers and adherent 51 Cr-PMN were lysed with the addition of 0.5 ml of 1 M NH4OH for at least 2 h. The samples were placed in scintillation vials with Biofluor (New England Nuclear, Boston, MA) and counted in a Beckman Model LS-7000 liquid scintillation counter. Due to variability in 51 Cr labelling and PMN physiology, experiments performed on different sets of cells were difficult to compare directly; therefore, adhesion measurements were normalized to controls by dividing counts from treated samples by counts from control samples for the same set of cells.

Cell electrophoresis

PMN and endothelial cells were suspended in a low ionic strength (0.015 M) buffer (0.01 M HEPES, 0.01 M NaCl, pH 7.5) to which sorbitol was added at 4.5 % w/v to make the solution isotonic (290 ± 4 mOs/kg). The conductivity of the buffer was 12.1 ± 0.2 mho/cm and its viscosity was 1.14 ± 0.01 cp (1.14 ± 0.01 x 10^-3 N s m^-1) at 20 °C. Due to the increased viscosity of this buffer, the measured electrophoretic mobilities were corrected to the viscosity of pure water.

The electrophoretic light-scattering technique and the apparatus used in this study have been well described in prior publications (Ware, 1974; Smith & Ware, 1978). The cells were washed 3 times in low-ionic-strength buffer and were suspended at a final concentration of 6-2
1-2 x 10^6 cells/ml. The suspensions were injected into an electrophoretic chamber, which had been pretreated with methyl cellulose to reduce electro-osmosis (Vanderhoff, Micale & Krumrine, 1977). The velocities of the cells were determined by measuring the Doppler shift in frequency of the laser light scattered from the moving cells. These experiments were performed using a helium-neon laser (λ = 632.8 nm); the scattering angle was 57.7°. Doppler spectra were calculated in real time by a SAICOR model 51B (Honeywell; Hauppauge, N.Y.) spectrum analyser. The electrophoretic mobility is defined as the velocity of a particle per unit electric field. Data are displayed as a histogram of mobilities, where the y-axis intensity is presumed to be proportional to the number of cells moving with a given mobility. Because of the large radius of the cells under investigation, the width of the histogram is not affected by diffusion and may be taken to be an indication of the electrophoretic heterogeneity of the population. Average mobilities were determined by measuring the mid-points of the respective electrophoretic peaks at the peak half-heights.

Chemotactic factors

Chemotactic factors used in this study were trypsin-activated C5 (C5a), zymosan-activated serum (ZAS), Escherichia coli culture filtrate (BF) and formyl-methionyl-leucyl-phenylalanine (fMet-Leu-Phe). C5 (Cordis Labs, Miami, Fla.) was activated by the method of Goldstein, Hoffstein, Gallin & Weissmann (1973). 100 U. of C5 (CH50 units) were incubated with trypsin (0.5 μg/ml in Tris buffer, pH 7.3) at 37 °C for 10 min. Trypsin activity was then inhibited by addition of 10 μg of soybean trypsin inhibitor (SBTI). The C5 control (100 U.) received a mixture of 0.5 μg trypsin and 1.0 SBTI. Fresh normal serum was incubated with zymosan (1 mg/ml) for 15 min at 37 °C (Ward, Cochrane & Muller-Eberhard, 1965). The ZAS was then centrifuged to remove the zymosan. A portion of fresh normal serum (NS) was set aside for use as the control. Formyl-methionyl-leucyl-phenylalanine (Sigma Co., St Louis MO) in PBS was prepared at a concentration of 10^-8 M, which has been shown previously to affect chemotaxis (Schiffman, Corcoran & Wahl, 1975) and PMN aggregation (O'Flaherty & Ward, 1978). Bacterial filtrate was prepared from E. coli conditioned medium containing equal portions of Bactotryptose phosphate broth, Sabourand-dextrose broth and brain-heart infusion (Difco Labs, Detroit, MI). The E. coli were pelleted by centrifugation, and the supernatant fractionated and concentrated using the Amicon Micro-Ultrafiltration system (Amicon, Lexington, MA) with UMO5 and UM2 membrane filters. The low-molecular-weight fraction (500-1000 Daltons), which has been shown to be chemotactic, was used as the bacterial filtrate. Fresh medium was used as the control.

The PMN and endothelial cells (10^6 cells/ml) were treated with the chemotactic factors and controls as follows: BF, 2 % in HH, and its control, 2 % of unincubated bacterial media in HH; C5a and C5, 2 U./ml in HH; ZAS and NS, 10 % in HH and fMet-Leu-Phe, 10^-8 M in HH. In all experiments, the cells plus agent or control were incubated for 30 min at 37 °C and used at the quantities indicated above unless otherwise stated in the text.

Analysis of chemotactic receptors on endothelium

Scatchard plots were constructed for determining the presence and the number of chemotactic receptors on endothelium. Low-passage calf aortic endothelium was plated into CoStar multiwell dishes and various concentrations of labelled fMet-Leu-Phe (New England Nuclear; Boston, MA) added. Radioactive counts were made on the cells (bound) and on the supernatant (unbound). Non-specific binding fractions were analysed according to the methods of DeMeyts, Bianco & Roth (1976).

Divalent cations

Divalent-cation-free HH (15 mM, pH 7.4) was prepared by treating Ca++-, Mg++-free HH with ion exchange resins to eliminate any trace quantities of divalent cations according to the methods of BioRad (1980). Basically, divalent-cation-free HH was reconstituted in equal volume of Chelex 100 (analytical grade chelating resin, 100-200 mesh, sodium form, BioRad Lab, Richard, CA) which had been prepared previously and stored at 4 °C in 0.1 M
NaH$_2$PO$_4$ at neutral pH. One part of this resin solution was then added to 10 parts of divalent-free medium and stirred overnight at 4 °C. The mixture was centrifuged and the supernatant used as our divalent-cation-free HH. All procedures involving the resin solution were carried out in plastic containers to prevent cation contamination from glass. Using this medium as the control, divalent cations (Mn$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Mg$^{2+}$, Ba$^{2+}$ and Ca$^{2+}$) were then added at 1 mM concentrations and tested for their effects on the adhesion and the electrophoretic mobilities of the PMN and endothelial cells.

RESULTS

Cell purity and viability

The PMN cells are 90% pure as determined by Wright's stain. The endothelial cells and PMN were 95% viable as determined by trypan blue exclusion.

PMN-endothelial monolayer adhesion

Table 1 presents the results of adhesion experiments in which either PMN or endothelial cells were treated with chemotactic factors. The chemotactic factors enhanced adhesion of PMN to endothelium to varying degrees when compared to controls. ZAS enhanced adhesion to a greater extent than the other chemotactic factors. The effect of ZAS was equal, irrespective of whether the PMN or the endothelial cells were treated. All other chemotactic factors, except for fMet-Leu-Phe (10$^{-6}$ M), had a greater effect on adhesion when the endothelial cells were treated.

The adhesive response of PMN to endothelial cells increased with increasing quantities of C$\alpha$ (Figs. 1, 2). When PMN were treated (Fig. 1), the increase in adhesion appeared to level off; however, when the endothelial cells were treated (Fig. 2), adhesion continued to increase with increasing doses of C$\alpha$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PMN</th>
<th>Endothelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanks' balanced salt solution</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Bacterial filtrate (2 %)</td>
<td>1.18 ± 0.04*</td>
<td>1.31 ± 0.03*</td>
</tr>
<tr>
<td>C$\alpha$ (2 U./ml)</td>
<td>0.98 ± 0.13</td>
<td>1.03 ± 0.04</td>
</tr>
<tr>
<td>Zymosan-activated serum (10 %)</td>
<td>1.12 ± 0.04*</td>
<td>1.22 ± 0.04*</td>
</tr>
<tr>
<td>f-Met-Ala (10$^{-4}$ M)</td>
<td>1.67 ± 0.10</td>
<td>1.62 ± 0.13</td>
</tr>
<tr>
<td>f-Met-Leu-Phe (10$^{-4}$ M)</td>
<td>1.80 ± 0.09</td>
<td>1.92 ± 0.11</td>
</tr>
</tbody>
</table>

Values are based on the percentage of cells attaching under control conditions in HH (11.7 ± 2.1). Each experimental condition was repeated at least 3 times with duplicates in each experiment.

* Values significantly different from controls, $P \leq 0.01$. 

Table 1. Adhesion of PMN to endothelial monolayers after treatment with chemotactic agents
Divalent cations were observed to promote the process of attachment, with relative efficacy in the order Mn$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Mg$^{2+}$, Ba$^{2+}$, Ca$^{2+}$. The data are presented in Table 2.
**Cell electrophoresis**

The increase in adhesiveness of cells treated with chemotactic agents may be partially due to changes in surface charge as reflected in alterations of electrophoretic mobilities. This is suggested by our results, which show that neuraminidase treatment reduces the electrophoretic mobilities (charge) of the PMN and endothelial cells by 45 and 43%, respectively (Figs. 3, 4) while concomitantly increasing adhesion (Hoover et al. 1978). Based on this, we have carried out experiments to see whether the adhesion increases due to chemotactic agents can be correlated to changes in electrophoretic mobilities. With all the chemotactic agents tested, we found both a reduction in the electrophoretic mobilities of the PMN and endothelial cells and an increase in adhesion when compared to untreated cells that had been incubated

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adhesion value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (No divalent ions)</td>
<td>1.00 ± 0.0 (10)</td>
</tr>
<tr>
<td>Mn**</td>
<td>1.95 ± 0.15 (6)</td>
</tr>
<tr>
<td>Zn**</td>
<td>1.85 ± 0.06 (2)</td>
</tr>
<tr>
<td>Ni**</td>
<td>1.68 ± 0.07 (3)</td>
</tr>
<tr>
<td>Mg**</td>
<td>1.64 ± 0.10 (5)</td>
</tr>
<tr>
<td>Ba**</td>
<td>1.25 ± 0.19 (2)</td>
</tr>
<tr>
<td>Ca**</td>
<td>1.22 ± 0.10 (5)</td>
</tr>
</tbody>
</table>

Values are compared to controls with no divalent cations (6.2% ± 0.9). Numbers in parentheses represent the number of experiments, each having at least 2 replicates.

All values are significantly different from controls, $P \leq 0.05$.

![Fig. 3. The electrophoretic mobility distribution of PMN before (----) and after (------) neuraminidase treatment (1 U./ml containing 10^6 cells, 30 min, 37 °C).](image)
R. L. Hoover and others

Fig. 4. The electrophoretic mobility distribution of endothelium before (-----) and after (----) neuraminidase treatment (1 U./ml containing 10⁶ cells, 30 min, 37°C).

in a basic salt medium. However, these values are the same as those obtained from cells treated with media that had no effect on adhesion. For example, Figs. 5 and 6 illustrate the effects of zymosan-activated serum (a chemotactic agent) and normal serum on the electrophoretic mobility of PMN and endothelium. In both instances, zymosan-activated serum reduces the surface charge, but likewise, so does normal serum — showing no significant difference between the 2 treatments. Table 3 gives the electrophoretic mobilities (± s.e.) of cells treated with other chemotactic agents.

Fig. 5. The electrophoretic distribution of PMN after treatment (15 min, 37°C) in control conditions (-----), 10% normal human serum (-----), and 10% zymosan-activated human serum (----).
Fig. 6. The electrophoretic distribution of endothelium after treatment (15 min, 37 °C) in control conditions (---), 10% normal human serum (----), and 10% zymosan-activated serum (-----).

<table>
<thead>
<tr>
<th>Table 3. The effects of chemotactic agents on the electrophoretic mobilities (cm²/V.s x 10⁻⁴) of PMN and endothelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Hanks' balanced salt solution</td>
</tr>
<tr>
<td>C5 (2 U./ml)</td>
</tr>
<tr>
<td>C5a (2 U./ml)</td>
</tr>
<tr>
<td>Normal serum (10%)</td>
</tr>
<tr>
<td>Zymosan-activated serum (10%)</td>
</tr>
<tr>
<td>Bacterial filtrate control (2%)</td>
</tr>
<tr>
<td>Bacterial filtrate (2%)</td>
</tr>
<tr>
<td>fMet-Leu-Phe (10⁻⁸ M)</td>
</tr>
</tbody>
</table>

In all cases, the chemotactic agents and the respective non-chemotactic medium reduce the surface charge. In general, it appears that the reduction in electrophoretic mobilities of PMN is greater than that of endothelial cells.

We found that increasing the dosage of C₅a, a potent chemotactic agent, increases the adhesiveness of PMN to endothelium regardless of which cell is treated; however, the electrophoretic mobilities do not significantly change with doses (Figs. 1, 2). Furthermore, there are no differences in the electrophoretic mobilities between cells treated with C₅a and C₅, which does not increase adhesion.

Finally, there is an apparent divalent cation specificity with regard to adhesion, i.e., Mn²⁺ causes more PMN to adhere to endothelium than does Ca²⁺ (Table 2); however, both cations reduce electrophoretic mobility of the cells to the same extent (Table 4).
Table 4. The effects of Mn^{2+} and Ca^{2+} (1 mM) on the electrophoretic mobilities (cm²/V.s x 10⁴) of PMN and endothelium

<table>
<thead>
<tr>
<th></th>
<th>PMN</th>
<th>Endothelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn^{2+}</td>
<td>1.20 ± 0.01 (4.6%)</td>
<td>1.13 ± 0.03 (4.2%)</td>
</tr>
<tr>
<td>Ca^{2+}</td>
<td>1.20 ± 0.01 (4.6%)</td>
<td>1.12 ± 0.01 (4.3%)</td>
</tr>
</tbody>
</table>

Values in parentheses represent % difference from controls.

**Binding analysis**

Scatchard plots made of the binding of [*H]*fMet-Leu-Phe to endothelium showed a curve indicating receptor sites of multiple affinities (K_{D1} = 3.0 x 10⁻¹⁰ and K_{D2} = 3.1 x 10⁻⁷ (Fig. 7)). When a large excess of 'cold' fMet-Leu-Phe was added to the incubation medium, binding of [*H]*fMet-Leu-Phe decreased by 90%, indicating specific receptor sites (DeMeyts et al. 1976). Nonspecific binding represented less than 1% of the total binding.

![Scatchard plot](image)

Fig. 7. A Scatchard plot of the binding of [*H]*formyl-methionyl-leucyl-phenylalanine to endothelium. K_{D1} = 3.0 x 10⁻¹⁰; K_{D2} = 3.1 x 10⁻⁷.
CONCLUSIONS

In this paper we have examined the adhesive interaction between polymorphonuclear leukocytes and endothelium and have tried to determine some of the factors affecting this interaction. We have shown that this adhesion is increased by divalent cations and that there are degrees of efficacy depending upon the cation used. We have also demonstrated a lack of correlation between charge reduction and increased adhesion by chemotactic agents, and a role for the substrate (endothelium) in modulation of PMN attachment.

Recently, Beesley et al. (1978) have shown that Mg\(^{2+}\) increased the attachment of PMN to endothelium better than Ca\(^{2+}\). In this study we have extended the list of divalent cations to show that not only is Mg\(^{2+}\) capable of promoting adhesion, but so are Ba\(^{2+}\), La\(^{3+}\), Ni\(^{2+}\), Zn\(^{2+}\) and Mn\(^{2+}\). Rabinovitch & DeStephano (1973a, b) have also found that macrophages spread more and fibroblasts adhere better in the presence of Mn\(^{2+}\) than other divalent cations. Many factors could account for this divalent cation preference. (1) If for some reason one ion is more readily bound to the cell surface due to the physical properties of that ion, it might reduce surface charge more effectively; thus, electrostatic repulsion would be lowered, allowing more cells to attach. This, however, does not seem to be the mechanism operating, since there appears to be no ranking of the ions by physical parameters; i.e., using cell electrophoresis, we could not distinguish between the least (Ca\(^{2+}\)) and the most (Mn\(^{2+}\)) effective ion. Collins (1966) working with embryonic cells and Armstrong (1966) using chick limb bud also found no correlation between increased cell adhesion and charge reduction due to different divalent cations. (2) There could be enzymes on the cell which require specific ions for activation. For example, Roseman (1970) and Webb & Roth (1974) have shown that there are glycosyltransferases on the surfaces of cells and that these may be involved in cell–cell adhesion. These enzymes also require Mn\(^{2+}\) for optimal activation. (3) The divalent cations may bind to specific proteins in the membrane, and in doing so cause conformational changes which contribute to a more adhesive surface.

We (Hoover et al. 1978) as well as others (O'Flaherty et al. 1978a; O'Flaherty, Krentzer & Ward, 1978b; O'Flaherty, Showell, Becker & Ward, 1978c; O'Flaherty & Ward, 1978) have shown that chemotactic agents increase the adhesiveness of PMN. It has been suggested that C\(_5\)a, a potent chemotactic agent and adhesion promoter, reduces the charge on the PMN surface (Gallin et al. 1975). We also find this to be true; however, C\(_5\) also reduced the charge on the cell by the same degree but did not promote adhesion or chemotaxis. We also found the same result comparing normal serum to zymosan-activated serum. Normal serum and ZAS have the same effect on net surface charge but only ZAS increases adhesion. None of the agents studied which increased adhesion could be correlated to modulations in surface charge except for neuraminidase. This may be a special case because removal of sialic acid may expose other sugars which could act as ligands. Based on this, we conclude that reduction in net surface charge is not a major factor in this adhesion process.
During the acute inflammatory process, the number of PMN adhering to and emigrating through the endothelium increases (Grant, 1973). This may be due to the release of chemotactic agents into the bloodstream which make the PMN more adhesive, as suggested by the in vivo studies of O'Flaherty, Showell & Ward (1977) who infused chemotactic agents into an animal and produced neutropenia. We propose, however, that these agents are also altering the substrate (endothelium) in a manner that makes it more adhesive, thereby capturing and directing the PMN to the site of inflammation. This hypothesis is based on our results, which show that treatment of the endothelium as well as the PMN can increase adhesiveness, and the results of other investigators, who showed that substrate modulations can influence attachment and migration of fibroblasts (Carter, 1965; Harris, 1973) and leukocytes (Allan & Wilkinson, 1978). Dierich, Wilhelmi & Till (1977) who found that casein bound to a micropore filter influences leukocyte migration, even suggest that in vivo, migrating leukocytes may be crawling along tissue surfaces coated with chemotactants. It is also interesting to note that Allison, Smith & Wood (1955) have shown that a thermal injury to the rabbit ear produced an inflammatory response which increased the attachment of leukocytes. The important point is that the PMN adhered initially to the site of the blood vessel nearest the injury. If only the PMN were affected in this reaction, then it might have been expected to find PMN over the entire surface of the vessel; however, by binding to the endothelium, the chemotactic agents may have altered the substrate and made it more adhesive, thus directing the PMN to the site of injury.

It is well known that many events occur when chemotactic agents bind to the surface of the PMN: for example, superoxide production increases (Boxer et al. 1979), lysosomal enzymes are released (Goldstein et al. 1973) and cell shape changes (O'Flaherty et al. 1978). Any or all of these could be contributing to the effects observed. Unfortunately, there are no similar data available on what happens to the endothelium when chemotactic agents bind to the surface. Indeed, we find our conclusions concerning charge and adhesion equally applicable to experiments in which either the PMN or endothelial cells were treated under the same conditions.

Most studies on the effects of agents which influence PMN/endothelium interactions have been focused on the PMN. In this paper, we have presented evidence which shows that endothelium has specific receptors for the chemotactic agent (fMet-Leu-Phe) and that incubation of the endothelium with this agent increases the number of PMN attaching. The effect of the chemotactic agent binding to the endothelium and how it mediates an increase in adhesion are not yet known. We have also shown that there is no correlation between an increase in adhesion due to chemotactic agents and surface charge; and that the interaction between the PMN and endothelium is divalent cation-dependent, with Mn2+ being more effective than Zn2+ > Ni2+ > Ba2+ > Ca2+.

We want to thank Kay Cosgrove for her assistance in the preparation of this manuscript and Robert Rubin for his photographic assistance. This work was supported by Grants GM 23788 and HL 17747 from the National Institutes of Health. Ben Ware is an Alfred P. Sloan Research Fellow.
REFERENCES


(Received 10 March 1980)