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ADHESION, PHAGOCYTOSIS AND CELL SURFACE ENERGY

THE BINDING OF FIXED HUMAN ERYTHROCYTES TO RAT MACROPHAGES AND POLYMETHYLPENTENE

DONALD F. GERSON [a,*], CHRISTIAN CAPO [b], ANNE MARIE BENOLIEL [b] and PIERRE BONGRAND [b]

[a] *Basel Institute for Immunology, Basel, CH-4005, (Switzerland) and* [b] *Laboratoire d'Immunologie, Hopital de Sante Marguerite, B.P.29, 13274 Marseille Cedex 2 (France)*

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Fixed human erythrocytes were used as model particles for the study of adhesion and phagocytosis by rat peritoneal macrophages. Erythrocytes were fixed with various concentrations of glutaraldehyde or tannic acid, or were treated with neuraminidase. Adhesion and phagocytosis of these cells were measured. In addition, the surface energy of these erythrocytes and macrophages was estimated by the contact angle technique. Free energies of adhesion, based on the cell surface energies, were correlated with both adhesion and phagocytosis.

Introduction

Phagocytosis of foreign microorganisms by macrophages is a crucial first-line of defense against infection. Many bacteria first adhere to the surface of a macrophage, and are then engulfed by it. Some relatively successful pathogens have a hydrophilic capsule, do not adhere, and are not phagocytized, unless opsonized by pathogen-specific antibody [1–3]. A lack of biochemical specificity (in the sense of antibody), extends to the ingestion of a variety of synthetic particles, and is a significant aspect of phagocytosis.

The generality of this process has led to studies of the physical properties of the surfaces of both macrophages and the particles they ingest. Mudd et al. [4] describe extensive early investigations of the physical properties of the cell surface which would be expected to be involved in phagocytosis: surface charge and surface free energy. Zeta potentials are estimates of surface charge obtained from electrophoretic mobility measurements. Phagocytosis did not correlate well with zeta potentials. In contrast, surface free energy considerations led to a physical model of phagocytosis which can be tested, and was qualitatively confirmed by early workers [4,5].

Methods developed at that time for determination of the surface free energy of cells in aqueous media were ingeneous, but difficult to execute, limiting further study [6]. An advance in methodology developed by Neumann and co-workers [7], allowed Van Oss, Neumann and others to make quantitative studies of the relations between surface free energies and phagocytosis which confirmed and extended the older work [8].

One limitation of the methods used by Van Oss and colleagues was that both macrophages and bacteria had to be air-dried prior to measurement, thus producing irreversible changes in the cell

* Present address, and address for reprint requests: Genex Corporation, 16020 Industrial Dr., Gaithersburg, MD 20877, U.S.A.

surface. We have investigated methods of determining surface free energies of living cells in aqueous media [9–12], and have been able to estimate these surface energies for a variety of cell types. The studies reported here are an application of these new methods to the relation between phagocytosis and surface free energies in aqueous media.

Materials and Methods

Reagents. Glutaraldehyde was purchased from Eastman Kodak (Rochester, NY, U.S.A.) or Fluka AG (Buchs, Switzerland), tannic acid was from Merck (F.R.G.), neuraminidase (crystallized) was from Sigma (St. Louis, MO, U.S.A.), agarose (Indubiose A 37) was provided by Industrie Biologique Francaise (Clichy, France), RPMI 1640 medium and fetal calf serum were from Gibco (Glasgow, U.K.). Fluorocarbon liquid, FC-48, was obtained from Sigma (St. Louis, MO, U.S.A.), washed three times with concentrated sulfuric acid, and washed six times with distilled water. Polymethylpentene strips were obtained in a convenient and sterile form as the microscope slide component of Costar Leighton tubes (3393).

Macrophage monolayers. Macrophages were obtained by washing the peritoneal cavities of untreated Wistar rats (Evic Ceba, Blanquefort, Gironde, France) with 10 ml RPMI 1640; 500-μl aliquots were deposited on ethanol-washed glass coverslips in petri dishes (Falcon 3001 F, 35 mm diameter, Becton, Dickinson and Co.) and unbound cells were washed out after 30 min at room temperature.

Erythrocytes. Human erythrocytes (group O, Rhesus negative) were obtained from healthy donors. They were washed three times in 0.9% saline before use.

Treatment with glutaraldehyde or tannic acid. Erythrocytes were suspended at 4% hematocrit in isotonic pH 7.2 sodium phosphate buffer containing glutaraldehyde or tannic acid at various concentrations as indicated below and were incubated overnight at room temperature on a rotatory agitator (Rotoshake, A. Kühner AG, Basel). Then they were washed six times in phosphate buffer before being stored at 4°C.

Neuraminidase treatment. Erythrocytes were suspended at 4% hematocrit in pH 5 RPMI 1640 medium containing neuraminidase for 60 min at 37°C.

Binding assay. Peritoneal cell monolayers were incubated for 60 min at room temperature in 4 ml of RPMI 1640 containing $1 \cdot 10^7$ erythrocytes. Then unbound erythrocytes were washed out and monolayers were microscopically examined with a $100\times$ oil-immersion lens. The binding index (b.i.) was defined as the number of bound erythrocytes per peritoneal cell. The ratio of bound cells to the total cells falling on each peritoneal cell was defined as the binding efficiency (b.e., Eqn. 1).

$$\text{b.e.} = \text{b.i.}/\text{number of erythrocytes falling on each peritoneal cell} \quad (1)$$

The binding efficiency is essentially an apparent equilibrium constant for bound vs. available cells. The binding efficiency was calculated as follows: spread macrophages were modeled as flat disks of about 7.5 μm radius and erythrocytes as spheres of 3.5 μm radius. The number of particles encountering a given macrophage was thus equal to the number of particles contained in a cylinder, the height of which was the thickness of the medium layer in the dishes and the section area was that of a circle of radius: $7.5\ \mu\text{m} + 3.5\ \mu\text{m} = 11\ \mu\text{m}$. It was calculated that an average of four erythrocytes fell on each peritoneal adherent cell.

Phagocytosis. In some cases, after binding, cell monolayers were incubated 30 min at 37°C in fresh RPMI 1640 containing 10% heat-inactivated (56°C, 30 min) fetal calf serum. Then they were stained with May-Grünwald-Giemsa stain and examined microscopically. The phagocytosis index was defined as the mean number of ingested erythrocytes per cell. The phagocytosis efficiency was the ratio, phagocytosis index/binding index, and is an apparent equilibrium constant for phagocytized vs. bound cells.

Binding strength determination. Our experimental procedure was fully described in previous papers [13–16]. Briefly, $5 \cdot 10^6$ rat peritoneal cells were incubated with $1 \cdot 10^7$ test particles in 600 μl of RPMI 1640 for 10 min at room temperature. Cells were then centrifuged (2 min, $450 \times g$) and resuspended by gentle inversion of test tubes. Aliquots were examined in a Neubauer hemocy-

tometer for determination of the binding index. Cell-particle conjugates were then subjected to a laminar shear flow (mean shear rate: 113000 s^{-1}) and the binding index was measured again. The resistance index was defined as the ratio:

$$\text{resistance index} = \text{b.i. (after shear)}/\text{b.i. (before shear)} \quad (2)$$

The resistance index was thus the fraction of bound particles that remained adherent to the macrophages after shear.

Electrophoretic mobility. We made use of the method described by Van Oss et al. [17], as previously reported [16]. Briefly, the inside of capillary tubes was coated with 3% agarose (to make electro-osmotic backflow negligible), then they were filled with an isotonic solution (43.2 g/l glucose and pH 7.2 phosphate buffer; ionic strength: 0.055) containing $1 \cdot 10^7$ particles per ml. The tubes were plugged with agarose and bridged to a power supply. The cells were subjected to an electric field (13.3 V/cm) and examined with a Zeiss microscope at 100× magnification with a micrometer-bearing eyepiece. The time needed for a particle to progress 0.12 mm was measured. In each experiment, the mean of 20 separate measurements was calculated.

Cell surface energy determination. Interfacial free energies at the cell surface in 0.9% NaCl were determined by the contact angle method [10,11]. Cells suspended in 0.9% NaCl were collected on 0.45 μm pore diameter Millipore filters to a density of approx. 10^4 cells/mm^2. The filter bearing the cells was cut in half and placed in a bath of 0.9% NaCl located at the focal point of the contact angle measuring device described earlier [10,11]. Drops of fluorocarbon liquid, FC-48, having a diameter of about 0.1 mm, were allowed to fall on the surface of the cells and settle to an equilibrium position. Contact angles were then measured through the drops. Use of a fluorocarbon liquid permits use of an equation-of-state for solid/liquid interfacial free energies [7,11,18–20]. The interfacial free energies at the cell surface, calculated in this manner, are thus consistent with other measurements of solid/liquid interfacial free energies. Since the values reported here are estimates of cell/aqueous interfacial free energies, they will be referred to as measures of hydrophobicity, and the larger the cell/aqueous interfacial free energy, the more hydrophobic the cell. This use of the term is generally, but is not completely, in agreement with other authors [21].

Free energies of adhesion, ΔG_a, were determined as follows. The interfacial tension between FC-48 and 0.9% NaCl was 50.0 ± 0.4 erg/cm^2. This, the contact angle (θ) for a given cell layer, and Eqns. 3a and 3b (Young's equation for contact angles) were used to calculate the interfacial free energies between the cell layer and either 0.9% NaCl (γ_{13}) or FC-48 (γ_{23}) [7,11,18,19]. The validity of the this type of equation-of-state for contact angles formed by hydrophobic liquids was recently confirmed [20].

$$0.5(\gamma_{12} + \gamma_{13} - \gamma_{23})/(\sqrt{\gamma_{12}\gamma_{13}}) = \exp[\gamma_{23}(0.00007\gamma_{13} - 0.01)] \quad (3a)$$

$$\gamma_{13} = \gamma_{23} + \gamma_{12}\cos\theta \quad (3b)$$

where γ_{12} = FC-48/0.9% NaCl interfacial tension (50.0 erg/cm^2); γ_{23} = FC-48/cell surface interfacial free energy (erg/cm^2); γ_{13} = 0.9% NaCl/cell surface interfacial free energy (erg/cm^2); θ = contact angle (degrees).

The cellular interactions which are addressed in this study are diagrammed in Fig. 1. Erythrocytes, after various treatments, were exposed to rat macrophages; then, (a) the fraction of strongly adhering (binding) cells was determined, and (b) the fraction of phagocytized cells was determined. Each step has a corresponding apparent equilibrium constant, and a corresponding change in interfacial free energy (ΔG_1 and ΔG_2, Fig. 1, respectively). For the adhesion step, the change in interfacial free energy per unit area of contact, $\Delta G_a \equiv \Delta G_1$, is expected to be related to the binding

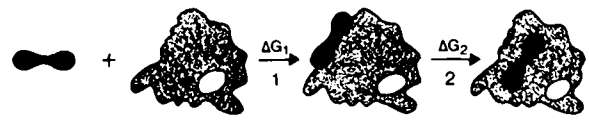

Fig. 1. Diagram of the phagocytic process: 1. An erythrocyte and macrophage come into contact and form a stable adhesion with an associated change in surface free energy, ΔG_1; 2. Engulfment then occurs, and is associated with another change in surface free energy, ΔG_2.

efficiency by Eqns. 4a and 4b (see Refs. 9–11).

$$\Delta G_a = \gamma_{EM} - \gamma_E - \gamma_M \quad (4a)$$

$$\Delta G_a = -\left[a \log(K_{eq}) + b\right] \quad (4b)$$

where: ΔG_a = free energy of adhesion (erg/cm^2); γ_{EM} = erythrocyte/macrophage interfacial free energy (erg/cm^2); γ_E = erythrocyte/0.9% NaCl interfacial free energy (erg/cm^2); γ_M = macrophage/0.9% NaCl interfacial free energy (erg/cm^2); a = empirical parameter having to do with factors affecting the interfacial free energies such as area of contact; b = empirical parameter having to do with other factors such as surface charge; K_{eq} = apparent equilibrium constant: for binding to macrophages this is the binding efficiency, b.e., Eqn. 1, and for phagocytosis, this is the phagocytosis efficiency.

Eqn. 4b defines the expected relation between the binding efficiency and the free energy of adhesion for cells contacting one another in 0.9% NaCl. Since we have measured only the surface free energy and since other factors, such as surface charge, may be important in determining adhesion, there may be deviations from these expectations. Allowance for such deviations is made by including the empirical parameters a and b in Eqn. 4b. These parameters may not be the same in different types of situation.

The interfacial free energy between the cells in contact, γ_{EM}, is calculated numerically from Eqn. 3a by substitution of γ_E for γ_{12} and γ_M for γ_{13}. This assumes that intercellular contact does not change the interfacial free energies of the cells at the point of contact. While structural changes may occur, it is not yet possible to measure directly the interfacial free energy between contacting cells, and so this estimation procedure appears to be appropriate at this time. No attempt has been made to use the observations of an earlier study [22] to determine the area of contact. The resistance index, Eqn. 2, is a measure of the force required to separate cells which have made adhesive contact. Unfortunately, even in well-defined physical systems, the force required for separation is poorly correlated with the free energy of adhesion, probably because it is so heavily dependent on the mechanical properties of the cell surface [23,24].

The transition from adhesion to phagocytosis may well depend on the viscosities or rigidities of the membranes of both the phagocyte and the ingested particle. Clearly, if both are rigid phagocytosis cannot occur. If both are completely fluid, engulfment may be expected to depend on surface energies, as has been shown for physical systems [25]. Phagocytosis of normal and fixed erythrocytes by macrophages lies between these extremes. That erythrocyte rigidity may influence engulfment is suggested by an electron microscopic study by Tizard et al. [25].

Results and Discussion

Comparison of fixation and neuraminidase treatment

Various experiments were performed to compare the relative effects of surface charge and adhesiveness on binding and phagocytosis efficiencies, and to test the consistency of the methods for measuring binding and phagocytosis. In 18 separate experiments, macrophage monolayers were incubated with various numbers of glutaraldehyde-treated erythrocytes (0.25% glutaraldehyde) in order to determine the influence of the particle concentration on the binding efficiency and the phagocytosis efficiency. The correlation coefficient between the total number of erythrocytes (n) and the binding efficiency was not significantly different from zero ($r = -0.17$, $P = 0.5$) when n was varied between $1 \cdot 10^7$ and $4 \cdot 10^7$. Further, the phagocytosis efficiency was not significantly correlated with the binding index ($r = -0.27$, $P = 0.34$). Hence, it is concluded that the particle binding was low enough that saturation effects could be neglected.

Human erythrocytes were treated with tannic acid (0.1 mg/ml), glutaraldehyde alone (0.25%), neuraminidase alone (10 μg/ml), or neuraminidase and then glutaraldehyde. Then they were deposited on macrophage monolayers and incubated 60 min at room temperature. Unbound erythrocytes were then washed out and binding was recorded, or cells were warmed at 37°C and incubated 30 min for phagocytosis. In other experiments, a mixture of erythrocytes and peritoneal cells was centrifuged and conjugates were

counted before being subjected to a laminar shear flow ($G = 113000\ s^{-1}$) and counted again. Finally, erythrocytes were assayed for electrophoretic mobility in a pH 7.2 buffer of ionic strength 0.055. The results of this survey are given in Table I. For this wide range of types of treatment, there is no clear correlation between either binding efficiency or phagocytosis efficiency and any of the physical parameters which were measured. The greatest binding efficiency was with neuraminidase and glutaraldehyde treated erythrocytes, and the least binding efficiency was for the untreated controls. These extremes correspond with the extremes of both the resistance index and the contact angle measurements. Binding efficiency is also poorly correlated with phagocytosis efficiency, emphasizing that these are distinct processes as suggested in Fig. 1.

Effects of fixation

Erythrocytes were fixed with various concentrations of either glutaraldehyde or either glutaraldehyde or tannic acid, and binding, phagocytosis and surface properties were measured. The effects of glutaraldehyde concentration on binding efficiency and phagocytosis efficiency are given in Fig. 2. Neither binding nor phagocytosis is appreciable with fresh erythrocytes. As the concentration of glutaraldehyde is increased from 0.01% to 1.0%, binding efficiency rises from 0.01 to 0.54, and phagocytosis efficiency rises from approximately zero to 0.1.

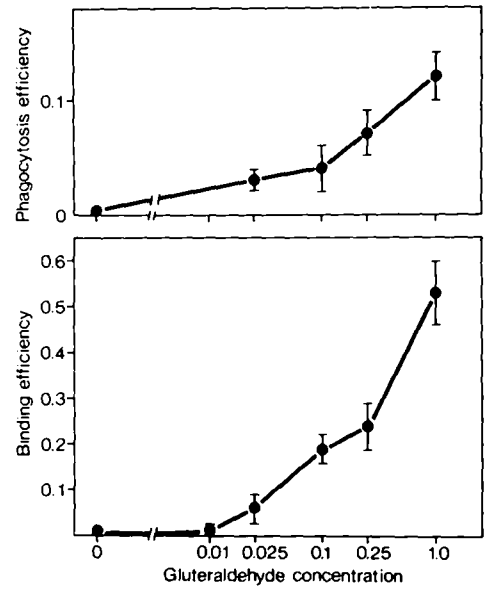

Fig. 2. Binding and phagocytosis efficiencies of gluteraldehyde (%, v/v) fixed human erythrocytes. Indicated ranges are one standard error of the mean.

The effects of tannic acid concentration on binding efficiency and phagocytosis efficiency are given in Fig. 3. Both binding and phagocytosis increase with fixative concentration, and by 10 mg/ml reach levels comparable to those achieved

TABLE I

BINDING AND PHAGOCYTOSIS OF TREATED ERYTHROCYTES BY RAT PERITONEAL MACROPHAGES

The binding efficiency is the probability for an erythrocyte to be bound by a macrophage on which it has fallen. The indicated range for all measurements is one standard error of the mean of 4–50 determinations. The phagocytosis efficiency is the probability that a bound erythrocyte be ingested by a macrophage after warming. The resistance index is the fraction of macrophage-conjugated erythrocytes remaining bound after shearing. The contact angle is the contact angle of FC-48 droplets on a cell layer in 0.9% NaCl.

Erythrocyte treatment	Binding efficiency	Phagocytosis efficiency	Resistance index	Electrophoretic mobility (μ/s V/cm)	Zeta potential (mV)	Contact angle (degrees)
Glutaraldehyde	0.28 ±0.03	0.10±0.06	0.66±0.08	−1.47±0.06	−18.9	148.5±4.7
Neuraminidase	0.19 ±0.04	1.16±0.10	0.53±0.05	−0.41±0.01	−5.3	161.4±2.2
Neuramindase+ glutaraldehyde	0.92 ±0.14	0.38±0.10	0.79±0.05	−0.58±0.05	−7.5	144.6±2.1
Tannic acid	0.38 ±0.05	0.10±0.02	0.46±0.06	−1.56±0.04	−20.0	160.0±2.5
Untreated	0.009±0.002	~0.00	~0.00	−1.32±0.05	−16.9	163.7±2.3

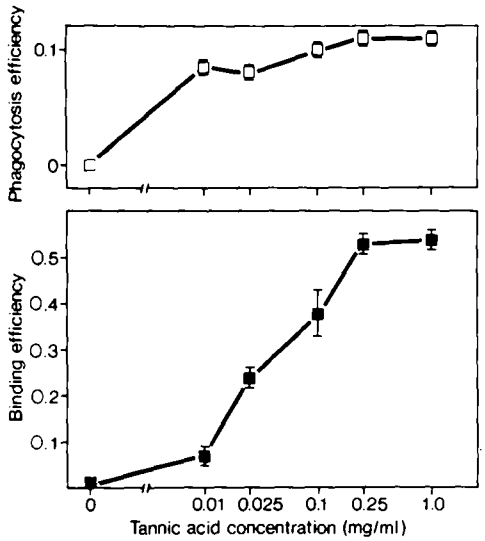

Fig. 3. Binding (■) and phagocytosis (□) efficiencies of tannic acid (mg/ml) fixed human erythrocytes. Indicated ranges are one standard error of the mean.

with 1% glutaraldehyde. Phagocytosis efficiency of tannic acid-treated erythrocytes is less dependent on the concentration of fixative than was the case for glutaraldehyde-fixed erythrocytes. The resistance index was essentially constant at 0.50 ± 0.10 for tannic acid concentrations from 0.1 to 1.0 mg/ml.

Contact angles of FC-48 on layers of cells treated with glutaraldehyde or tannic acid are given in Fig. 4. In this measurement system, the lower the contact angle, the more hydrophobic the erythrocyte surface and the greater its interfacial free energy at the aqueous inteface. As concentration is increased, both fixatives increase the relative hydrophobicity of the erythrocyte surface. Glutaraldehyde fixation results in a more hydrophobic cell surface over these concentration ranges.

Effects of neuraminidase

Treatment of erythrocytes with neuraminidase to remove sialic acid residues reduces cell surface charge (Table I), as expected, moderately increases binding efficiency and markedly increases phagocytosis efficiency. For the concentrations tested, phagocytosis efficiency is essentially unity, is constant, and is much higher than the phagocytosis efficiencies of erythrocytes fixed with glutaraldehyde or tannic acid. Binding efficiency is, however, lower than is seen following treatment of the erythrocytes with fixatives alone (Fig. 5, lower). When neuraminidase is followed by glutaraldehyde (0.25%) fixation, binding efficiency becomes greater than that obtained with either single treatment (Fig. 5, lower). Neuraminidase has a small effect on the contact angle of FC-48 on cell layers, and thus slightly increases cell surface hydrophobicity.

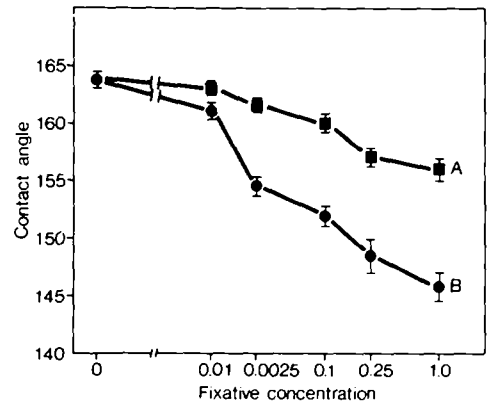

Fig. 4. Contact angles (degrees) of the fluorocarbon test fluid, FC-48, on layers of human erythrocytes fixed with either tannic acid (A, mg/ml) or glutaraldehyde (B, %, v/v).

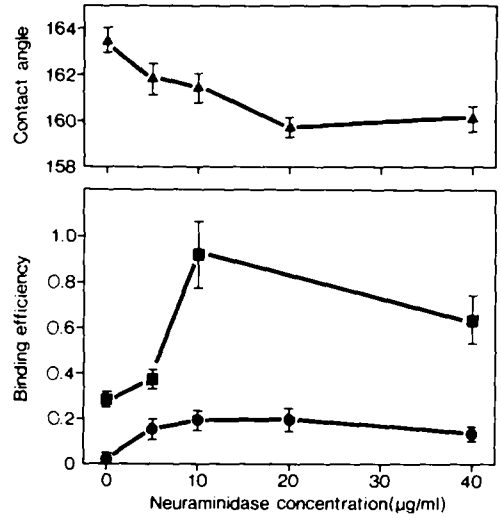

Fig. 5. Binding efficiency and contact angles (degrees) of the fluorocarbon test fluid, FC-48, on layers of human erythrocytes treated with neuraminidase (●, ▲), or neuraminidase followed by 0.25% glutaraldehyde (■).

Free energies of adhesion and binding

The contact angle data of Figs. 4 and 5 were used with Eqns. 3a and 3b to calculate the interfacial free energy between the cell surface and 0.9% NaCl. The contact angle of FC-48 on rat peritoneal macrophages in 0.9% NaCl was 147.0°, and the corresponding interfacial free energy was 0.8 erg/cm^2. This is close to the interfacial free energy of 2.1 erg/cm^2 for rabbit macrophages in Ringer's solution obtained by Shapiro and Harvey [27]. Interfacial free energies between treated erythrocytes and rat macrophages were estimated from cell-medium interfacial free energies with Eqn. 3a. Free energies of adhesion were calculated from Eqn. 4a.

To test the applicability of Eqn 4b to the binding of treated erythrocytes to macrophages, the log of the binding efficiency was plotted against the free energy of adhesion for each case (Fig. 6). For glutaraldehyde-treated erythrocytes, adhesion to rat macrophages closely follows Eqn. 4b with constant parameters, *a* and *b*. Erythrocytes initially treated with neuraminidase then with glutaraldehyde also fall on this line. For erythrocytes treated with various concentrations of tannic acid, the relation between binding efficiency and ΔG_a follows a smooth curve which intersects the curve for glutaraldehyde treated erythrocytes only at the point for fresh untreated cells. Neuraminidase, over the concentration range used, does not result in a graded series of either binding efficiency or free energy of adhesion, but neuraminidase treatment results in a binding efficiency and a free energy of adhesion close to that obtained with 0.25 mg/ml tannic acid. The curve for tannic acid-treated erythrocytes in Fig. 6 suggests that macrophages are being saturated at a relatively low binding efficiency. Also it is clear that the parameters of Eqn. 4b are quite different for glutaraldehyde compared to tannic acid or neuraminidase treated erythrocytes, suggesting that the macrophages may interact somewhat differently with these surfaces. This may be due to differences in the relative contributions of the polar and dispersive components of the interfacial free energies [28].

To examine this possibility, the binding of treated erythrocytes to polymethylpentene was studied. Polymethylpentene coverslips provide a

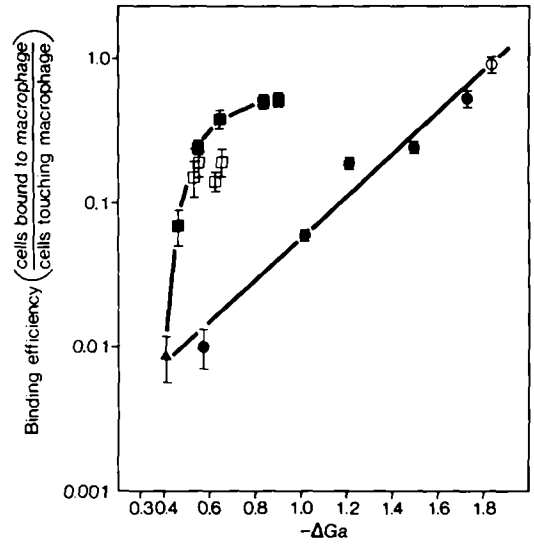

Fig. 6. Binding efficiency as a function of the free energy of adhesion (ΔG_a, erg/cm^2 of contact) for human erythrocytes treated with: glutaraldehyde (●), tannic acid (■), neuraminidase (□), neuraminidase then glutaraldehyde (○), or no fixative (▲). Indicated ranges are one standard error of the mean.

uniform, flat surface suitable for studies of cell adhesion [29]. The surface energy of polymethylpentene is almost entirely due to the dispersive component γ^d. The contact angle of FC-48 on polymethylpentene under 0.9% NaCl was 99.7°, and the corresponding interfacial free energy between polymethylpentene and 0.9% NaCl was determined to be 13.3 erg/cm^2 with Eqns. 3a and 3b. Free energies of adhesion between the various types of fixed erythrocyte and polymethylpentene were calculated by the same procedure as for adhesion to macrophages (see above). Binding of the cells was determined by allowing an erythrocyte suspension ($1 \cdot 10^6$ cells/ml 0.9% NaCl), to equilibrate in the Costar Leighton tube for 2 h, then washing by dipping three times in 0.9% NaCl. Cell density (cells/mm^2) was determined by counting the cells in ten identical fields, 2 mm apart along the length of the plastic strip.

The relation between the log of the cell density and ΔG_a is given in Fig. 7. Erythrocytes fixed with 1.0 to 0.025% glutaraldehyde bound significantly to polymethylpentene, in 0.9% NaCl, as expected from the results of Horisberger [30,31]. When binding is observed, the log of the binding is linearly

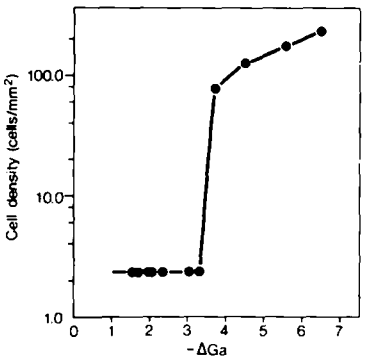

Fig. 7. Cell densities of fixed human erythrocytes on polymethylepentene as a function of the free energy of adhesion (ΔG_a, erg/cm^2 of contact).

related to ΔG_a, as expected from Eq. 4b. For all the other types of erythrocyte tested, binding was low and either equal to the binding of untreated erythrocytes (approx. 2/mm^2), or zero. Fixation with tannic acid did not increase erythrocyte binding to polymethylpentene. The lack of significant binding for all cases in which ΔG_a was more positive than -3.5 erg/cm^2 suggests that a minimum free energy of adhesion is required to retain cells under the washing condition used in these experiments. In previous studies, adhesion of bacteria to plastic surfaces also followed Eqn. 4b [32], but in that case there was no evidence for a sharp cut-off value of ΔG_a for adhesion.

These results suggest that glutaraldehyde fixation increases the dispersive component of cell surface energy to a greater extent than tannic acid fixation. Similar results were obtained when glutaraldehyde- and neuraminidase-treated erythrocytes were compared by partitioning in a benzene/0.9% NaCl two-phase system [16].

Free-energies of adhesion and phagocytosis

The process of phagocytosis, as depicted in Fig. 1, is not simply the passage of the phagocytized particle from the bulk medium into the cytoplasm of the phagocyte, since the phagocyte surrounds the particle with a membrane [33]. In terms of an analysis of the surface energy changes, the difference between the two possibilities is significant, since in the former case it would be necessary to know the interfacial free energy between the phagocytized particle and the cytoplasm of the phagocyte. However, if we proceed with the assumption that the second case not only predominates but always occurs, and that the particle is surrounded by a membrane; then the analysis is the same as for adhesion, except that the total area of contact is larger.

The phagocytosis efficiency defined above is an apparent equilibrium constant between bound and phagocytized particles, and thus the applicability of Eqn. 4b can be tested by comparing the log of the phagocytosis efficiency to ΔG_a (Fig. 8). Each type of fixation results in a linear relation between log phagocytosis efficency and ΔG_a. Glutaraldehyde treatment results in the lowest phagocytosis efficiencies, but the most negative free energy changes. With increasing glutaraldehyde concentration, $-\Delta G_a$ increases monotonically, and is linearly related to phagocytosis efficiency. The phagocytosis efficiency of both tannic acid-fixed and neuraminidase-treated erythrocytes is relatively constant for each case, and thus is relatively independent of concentration and ΔG_a. These results indicate that when $-\Delta G_a$ and surface charge (Table I, REf. 34) are high, phagocytosis efficiency is low, but depends on ΔG_a. It appears that when

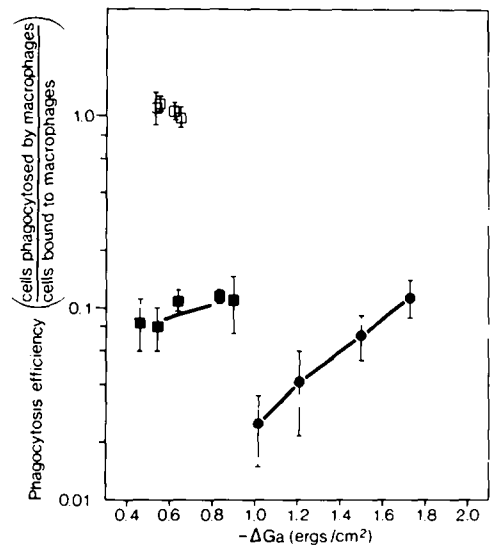

Fig. 8. Phagocytosis efficiency as a function of the free energy of adhesion per unit area of contact (ΔG_a) for human erythrocytes treated with: glutaraldehyde (●), tannic acid (■), or neuraminidase (□). Untreated erythrocytes have a phagocytosis efficiency of approximately zero.

the particle to be ingested is sufficiently charged, phagocytosis proceeds in accordance with the model described by Fig. 1 and Eqn. 4. However, when the charge on the particle to be ingested is reduced by neuraminidase, phagocytosis efficiency saturates at low negative values of ΔG_a. Normal erythrocytes are both charged and hydrophilic, and are neither bound nor engulfed by macrophages. Either reduced surface charge, or increased hydrophobicity, or both, result in increased binding and phagocytosis efficiencies.

The possibility of specific interactions between the surface of the macrophage and the fixed erythrocyte cannot be overlooked in light of the prevalence of putative receptor-ligand interactions thought to be involved in many examples of intercellular adhesion. One possible model for this type of adhesion is the specific lectin-sugar interaction. These could be enhanced in some way by fixation of erythrocytes with tannic acid or glutaraldehyde, or by removal of sialic acid residues to expose underlying monosaccharides in the glycocalyx [35].

Conclusions

The interactions of rat macrophages with glutaraldehyde or tannic acid-fixed human erythrocytes has been studied over a range of fixative concentrations. Corresponding measurements of the contact angle of a hydrophobic test fluid droplet on cell layers in 0.9% NaCl have allowed estimations of the free energy of adhesion between the cells. The observed binding and phagocytosis efficiencies of fixed erythrocytes to rat macrophages varies systematically with the free energy of adhesion, but depends on the type of fixation, as would be expected. Neuraminidase treatment, to reduce surface charge, increases both binding and phagocytosis efficiencies but does not affect the free energy of adhesion. These results lead us to believe that the cell surface energy is an important determinant of both binding and phagocytosis. The results also indicate that when surface charge is high, phagocytosis increases with increasingly negative free energies of adhesion, but that when surface charge is low, phagocytosis is efficient at lower negative free energies of adhesion. As expected from the very general free energy analysis presented above, we have found that high phagocytosis efficiencies can be achieved either by reducing the surface charge of relatively hydrophilic particles, or by increasing the hydrophobicity of more highly charged particles.

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