FERRITIN CONJUGATES AS SPECIFIC MAGNETIC LABELS

Implications for Cell Separation

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ABSTRACT Concanavalin A coupled to the naturally occurring iron storage protein ferritin is used to label rat erythrocytes and increase the cells' magnetic susceptibility. Labeled cells are introduced into a chamber containing spherical iron particles and the chamber is placed in a uniform 5.2 kG (gauss) magnetic field. The trajectory of cells in the inhomogeneous magnetic field around the iron particles and the polar distributions of cells bound to the iron particles compare well with the theoretical predictions for high gradient magnetic systems. On the basis of these findings we suggest that ferritin conjugated ligands can be used for selective magnetic separation of labeled cells.

INTRODUCTION

Methods for separating cells according to subtle differences in their surface properties have many applications in studies of cells and tissues. The rapidly expanding use of fluorescence-activated cell sorters exemplifies this point. Cell surface-specific separation techniques using magnetic forces have much promise, and convenient ways of modifying the magnetic properties of specific cells and tissues deserve attention.

Red cells have been magnetically separated from lymphocytes, granulocytes, and other components of whole blood after simple chemical treatments that convert diamagnetic oxygenated Hb (Fe^{II}) to paramagnetic deoxy-Hb (Fe^{II}) or met-Hb (Fe^{III}) (Melville et al., 1975; Owen, 1978). Owen et al. (1979) have separated T cells from peripheral blood lymphocytes by allowing the T cells to form rosettes with paramagnetic sheep red blood cells and passing the mixture through a column packed with steel wool in a 70 kG (gauss) field.

Due to its electron opaque core, ferritin (in the form of covalent ferritin-antibody conjugates) is used extensively in electron microscopy. Because the core is also highly paramagnetic, similar ferritin conjugates can potentially be used as magnetic labels for selected cell types.

In this report we describe the use of ferritin to increase the magnetic susceptibility of cells. Concanavalin A (Con A) conjugated- and cationized-ferritin were used to make rat erythrocytes paramagnetic. The treated cells were then attracted to a magnetized iron filing, demonstrating that ferritin conjugated with ligands for cell surface receptors can be used to retain cells with specific surface markers. This technique shows promise in the design of magnetic cell separation systems.

METHODS

Preparation of Ferritin-labeled Rat Erythrocytes

Whole blood was collected in heparinized tubes by heart puncture of chloroformed adult male Sprague-Dawley rats. The blood was diluted 3:1 in isotonic phosphate-buffered saline (PBS, pH 7.2) containing 2 mM Ca^{2+}, 1.5 mM Mg^{2+}, and 0.2% bovine serum albumin, and centrifuged at 2,000 rpm for 10 min at room temperature. Serum and buffy coat were removed by gentle aspiration and the red blood cell pellet then resuspended and rinsed three times in 20 vol of the same buffer. The final pellet was resuspended at 10^8 cells/ml in PBS containing 0.02% NaN_3 and stored for up to 7 d at 4°C.

Erythrocytes in PBS were then incubated (10^6–10^7 cells/ml) with a ferritin-Con A conjugate (Miles-Yeda Catalogue no. 79-008; Miles Labs, Inc., Elkhart, IN) for 30 min at room temperature. The mole ratio of Con A/ferritin in the conjugate was 0.3, and the concentration of Con A ranged from 15–100 μg/ml. Labeled cells were diluted 15-fold with PBS and harvested by centrifugation as above. The pellet was resuspended by vortexing and rinsed once, then diluted 10^6 cells/ml in PBS. Alternatively, cells were incubated with cationized ferritin (no. F7879; Sigma Chemical Co., St. Louis, MO) at 200 μg/ml in PBS for 30 min at room temperature, rinsed as before, and diluted to a convenient concentration with PBS. Slide chambers were then filled directly with this stock suspension of ferritin-labeled erythrocytes.

Construction of Slide Chambers

For visualization of the behavior of ferritin-labeled cells in high gradient magnetic fields, the cell suspension was introduced into a transparent chamber on the light microscope stage (see Poo et al., 1978). The initial, transient flow of the cell suspension through the chamber is driven by capillary forces. The chamber was constructed from a 1 × 25 × 75 mm glass microscope slide and a 22 × 40 mm glass coverslip held apart from the slide by two or more layers of double-coated tape (3 M no. Y9469). To create localized regions of high field gradient, spherical iron filings (no. 5308, 40 mesh; Mallinckrodct Inc., Science Products Div., St. Louis, MO) were placed inside the chamber.

Iron filings were held in place either by friction against the two glass surfaces or by a medial piece of transparent double-coated tape. The entire chamber was placed between the pole pieces of a 5.2 kG permanent magnet (Permag Corp., Hicksville, NY) and observed from below with an inverted microscope. Cell trajectories were recorded on video tape for later analysis.

THEORY

Ferritin is an organic iron compound consisting of a hydrated ferric-oxide micelle surrounded by a protein sheath formed from 20–24 subunits (Brady et al., 1968). The protein sheath has a molecular weight of 45 kdaltons, an inner diam. of 75 Å, and an outer diam. of 120 Å (Harrison, 1964; Bielig et al., 1966), while the iron core may contain as many as 2,000 iron atoms (Fischbach and Anderegg, 1965). The core is strictly paramagnetic, with an average magnetic susceptibility of 5,900 × 10^{-6} cgs emu/mol iron at 27°C (Michaelis et al., 1943).

Ferritin that is bound to a cell surface will increase the average magnetic permeability of the cell (μ_c). Otherwise similar cells differing in the amount of bound ferritin can, in principle, be partitioned on the basis of this difference.

The magnetic force on a cell ($\mathbf{f}_c$) depends on both the magnetic dipole moment ($\mathbf{m}$) induced by an imposed magnetic field ($\mathbf{H}_0$), and the spatial variation of the field $\mathbf{f}_c = \mu_0 \mathbf{m} \cdot \nabla \mathbf{H}_0$. For uniform H_0, the magnetic dipole moment of a cell that binds ferritin is a function of the amount of ferritin bound and colinear with the field $\mathbf{m} = 4\pi a^3 \mathbf{H}_0(\mu_c - \mu)/(\mu_c + 2\mu)$, where a is the cell radius and μ is the magnetic permeability of the medium.

High gradients in magnetic-field intensity exist near materials of high magnetic permeability placed in a magnetic field. We use spherical iron filings to provide both a high permeability and a convenient geometry for analysis. The magnetic force attracting a ferritin-loaded cell to an iron filing of radius R follows the field around the filing, which is the familiar dipole plus uniform field (Melcher, 1981)

$$H_r = H_0[1 + 2(R/r)^3] \cos\theta$$

$$H_\theta = -H_0[1 - (R/r)^3] \sin\theta.$$

The equations of motion of ferritin-labeled cells can be found from a simple model based on a balance between magnetic and viscous forces. The statement of force balance for cells of radius a in a fluid of viscosity η is

$$(6\pi\eta a)\mathrm{d}r/\mathrm{d}t = H_0^2[a^3 4\pi\mu_0(\mu_c - \mu)/(\mu_c + 2\mu)] (3R^3/r^4)[1 - 3\cos^2\theta - (R/r)^3(1 + 3\cos^2\theta)]$$

$$(6\pi\eta a r)\mathrm{d}\theta/\mathrm{d}t = -H_0^2[a^3 4\pi\mu_0(\mu_c - \mu)/(\mu_c - 2\mu)] (3R^3/r^4)(1 + R^3/2r^3)\sin 2\theta.$$

Fig. 1 shows the particle trajectories described by the equations of motion. The trajectories are perpendicular to contours of equal magnetic-field strength and towards increasing field. Cells are expected to be captured preferentially at the poles of the iron filing where the radial magnetic force is towards the filing and not captured at all at the equator, where the radial magnetic force is away from the filing.

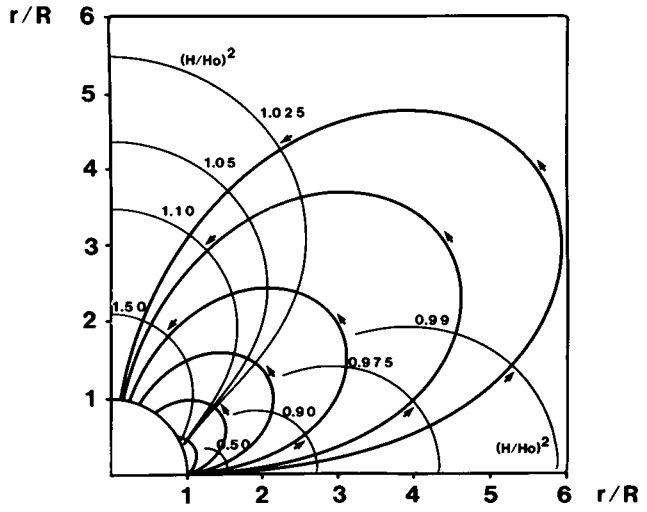

FIGURE 1 Paramagnetic particle trajectories near iron filing in uniform magnetic field. The imposed magnetic field has magnitude H_0 and a north-south axis. Contours of constant magnetic field intensity around the iron filing are labeled with the square of their normalized amplitude. The magnitude of the magnetic force on a particle is proportional to the gradient of $(H/H_0)^2$. Trajectories of paramagnetic particles are perpendicular to the constant field contours and towards increasing field intensity. Distance from the filing is normalized to the filing radius.

RESULTS

Magnetic Interactions with Ferritin-labeled Erythrocytes

Fig. 2 *c*, *d* shows ferritin-Con A and cationized ferritin-labeled erythrocytes bound to an iron filing. The distribution and density of bound cells is consistent with the surface magnetic force predicted by theory. The majority of cells are captured after the initial transient east-west flow. Once bound, however, cells are dislodged from the filing surface only by vigorous flow.

Cell trajectories lead to preferential capture at the poles of the iron filing; cells starting from positions near the equator are observed to move in a direction nearly tangent to the filing before capture. Cell velocities increase dramatically as the cells approach the filing surface, as is consistent with the dipole field around the filing falling off with the cube of the distance.

In the control experiment, illustrated in Fig. 2*b*, far fewer erythrocytes are bound to the iron filing. This suggests that erythrocytes are bound to the filing mainly by magnetic forces on the cell mediated by the presence of surface-bound ferritin. Background binding could be nonmagnetic or reflect the presence of methemoglobin (met-Hb) in the bound cells. The met-Hb content of circulating erythrocytes has been shown to rise with cell age (Waller et al., 1959) hence some paramagnetic cells are expected in the unlabeled population.

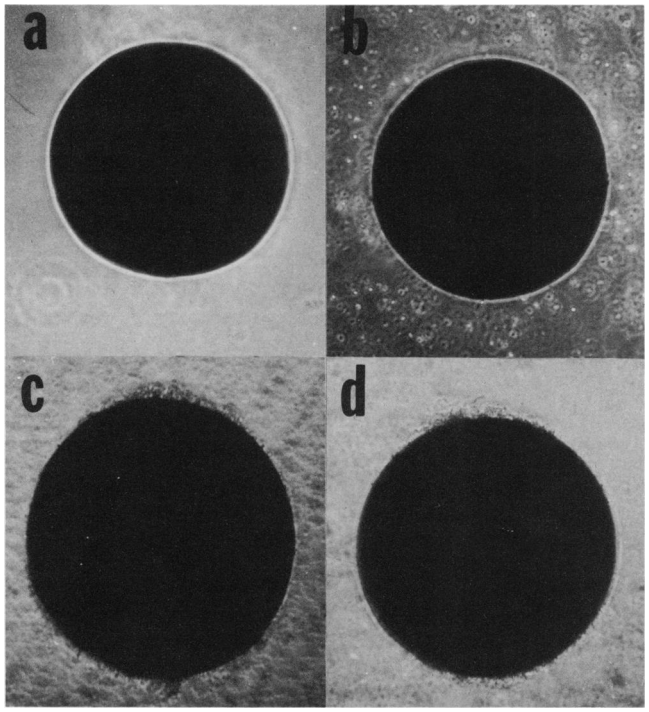

FIGURE 2 Light micrograph of ferritin-treated rat erythrocytes bound to an iron filing. The filing is in a magnetic field with a north-south axis. A suspension of erythrocytes is introduced at one end of the chamber and distributed via an east-west flow. (*a*) An iron filing before introduction of the cell suspension. The average diameter of the filings used is 250 μm. (*b*) Control rat erythrocytes. The filing is in a magnetic field with a north-south axis. Note that a few cells do bind to the particle although they contain no ferritin. This binding could be nonmagnetic, or due to oxidized haemoglobin in the cells. (*c*) Magnetic capture of erythrocytes labeled with Con A-ferritin. Note the clumping of the cells at the poles of the iron filing where the radial magnetic force is greatest. Few cells bind around the equator, where the radial magnetic force is directed away from the filing. Cells surrounding the filing have settled to the bottom of the microscope slide under the influence of gravity. (*d*) Magnetic capture of erythrocytes labeled with cationized ferritin.

DISCUSSION

In the present study we have demonstrated that cell-surface bound ferritin can impart a paramagnetic susceptibility to cells. The resulting susceptibility is significant enough that magnetic interactions can be used to remove the cells from suspension using fields of relatively low intensity. In principle, different cell types can be removed from suspension preferentially by utilizing ferritin carrying a specific antibody or other ligand with reactivity towards unique cell surface components. The technology for production of ferritin antibody conjugates is well developed (for reviews see Feteanu, 1978; Andres et al., 1973). In fact, because conjugates of ferritin with anti-immunoglobulin G are commercially available, an indirect labeling protocol using specific monoclonal antibodies in the first step should require no chemical synthesis.

Considering our results we suggest that the magnetic properties of ferritin conjugates may be exploited to purify cells differing little in bulk physical properties, such as volume, density, and surface charge; efforts are currently being made to test this possibility.

The prototype magnetic cell separation was performed by Rous and Beard in 1934 when they allowed Kupffer cells to phagocytose colloidal iron oxide particles and then captured the cells with a magnet; today carbonyl iron is routinely used to separate phagocytic cells from mixed populations (Lee, 1980). Other generalized techniques for magnetic separation of cells have been reported. Magnetic separation has been successfully performed via bound magnetic microspheres (Kronick et al., 1978; Molday et al., 1975, 1977; Molday and MacKenzie, 1982; Rembaum et al., 1982). Microspheres have an advantage in that the iron enclosed by the polymer sphere can be ferrimagnetic; hence, weaker fields will suffice for separation. However, application of this technique to separation has been limited by the difficulty of synthesizing the microspheres, and by their tendency to aggregate during protein coupling and cell labeling procedures (Molday and MacKenzie, 1982). Newly introduced iron-dextran reagents of even smaller size than the polymeric microspheres also reportedly have less tendency to self-associate (Molday and MacKenzie, 1982).

Because ferritin is commercially available, it provides a means for immunospecific cell separation without the added step of microsphere manufacture. In biochemical and/or electrophysiological characterization of magnetically separated cells, there should be some concern with alterations induced by the magnetizing agents themselves. In this respect, ferritin has the advantages of a relatively small size and benign chemical nature. Indeed, ferritin is of ubiquitous occurrence in mammals, where it functions as a soluble and nontoxic storage depot for excess iron. Block and Bothwell (1983) have recently shown that ferritin-antibody conjugates used to deliver ferritin to cells in culture are nontoxic when internalized; thus, internalization via antigenic modulation may offer an alternative mechanism for sequestration of ferritin by cells. A third consideration for all magnetic separations is the level of nonspecific binding and, since few cell types bear specific ferritin receptors (Granick, 1951), we can expect ferritin conjugates to have low background binding.

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