Cytoplasmic Motions, Rheology, and Structure Probed by a Novel Magnetic Particle Method

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ABSTRACT The motions of magnetic particles contained within organelles of living cells were followed by measuring magnetic fields generated by the particles. The alignment of particles was sensed magnetometrically and was manipulated by external fields, allowing non-invasive detection of particle motion as well as examination of cytoplasmic viscoelasticity. Motility and rheology data are presented for pulmonary macrophages isolated from lungs of hamsters 1 d after the animals had breathed airborne γ-Fe₂O₃ particles. The magnetic directions of particles within phagosomes and secondary lysosomes were aligned, and the weak magnetic field produced by the particles was recorded. For dead cells, this remanent field was constant, but for viable macrophages, the remanent field decreased rapidly so that only 42% of its initial magnitude remained 5 min after alignment. A twisting field was applied perpendicular to the direction of alignment and the rate at which particles reoriented to this new direction was followed. The same twisting was repeated for particles suspended in a series of viscosity standards. Based on this approach, the low-shear apparent intracellular viscosity was estimated to be 1.2–2.7 × 10³ Pa·s (1.2–2.7 × 10⁴ poise). Time-lapse video microscopy confirmed the alignment of ingested particles upon magnetization and showed persistent cellular motility during randomization of alignment. Cytochalasin D and low temperature both reduced cytoplasmic activity and remanent-field decay, but affected rheology differently. Magnetic particles were observed in association with the microtubule organizing center by immunofluorescence microscopy; magnetization did not affect microtubule distribution. However, both vimentin intermediate filaments and f-actin reorganized after magnetization. These data demonstrate that magnetometry of isolated phagocytic cells can probe organelle movements, rheology, and physical properties of the cytoskeleton in living cells.
in the local environment of the particle. We describe the results of magnetometric measurements made on isolated lung macrophages for the purpose of detecting intracellular motion. To analyze the possible structural basis for magnetometrically sensed motion, we evaluated the effects of magnetization on the organization of the cytoskeleton. The combined magnetometric and morphological studies suggest that specific cytoplasmic components may be responsible for the type of motions probed with this system.

MATERIALS AND METHODS

Pulmonary macrophages were tagged with $\gamma$-Fe$_2$O$_3$ (magnetite) particles by having hamsters (n=16) breathe airborne particulates generated by a previously described technique (38). Briefly, iron pentacarbonyl vapors were oxidized under controlled conditions to produce an aerosol of magnetite particles at a concentration of $\sim 300 \mu g/liter$ (aerodynamic mean diameter = 0.7-$\mu m$, aggregates of $\gamma$-Fe$_2$O$_3$ with 0.1-$\mu m$ diameter subunits). Some of the inhalated material deposits on lung surfaces and almost all of the particles retained in alveoli are ingested by macrophages within 12-24 h (31-33). 1 d after inhalation exposure, pulmonary macrophages were harvested by multiple saline washes of the lungs. Each hamster was anaesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt), after which the trachea was exposed and cannulated with PE-190 tubing attached to an 18-gauge syringe needle. The lungs were lavaged 13 times with 3-ml aliquots of normal saline. The lack of divalent cations promotes detachment of macrophages from the alveolar surface, and gentle massage of the chest further increases cell yield (39).

Each hamster was anesthetized because of a high content of mucus, surfactant, and cell debris; the remaining washes were pooled and the macrophages were pelleted by centrifugation (100 g, 10 min). Cells were counted and sized by Coulter counter (Coulter Electronics Inc., Hialeah, FL), and the yield of cells lavaged from Syrian hamster lungs was 4-8 $\times$ 10$^6$ macrophages per animal with a viability of 85-94% as determined by dye exclusion. The particle content of macrophages was $\sim 20 \mu g$ Fe$^{2+}$/per 10$^6$ cells, as measured by magnetometry.

Cells were resuspended in Roswell Park Memorial Institute 1640 medium containing 0.2% bovine serum albumin (BSA) and 25 mM HEPES buffer at 37°C. The macrophages were divided into separate portions, and each aliquot of 10$^6$ cells was allowed to adhere to the bottom of 1.5-ml glass vials over a period of 1 h. The cells in each vial were incubated for an additional 1/2 h under one of the five following conditions: (a) 37°C, control; (b) 37°C and 20 $\mu M$ cytochalasin D; (c) 37°C and 0.2% dimethyl sulfoxide; (d) 37°C and 0.2% dimethyl sulfoxide plus 10% Formalin; (e) 37°C and 10% Formalin. Motion of the intracellular particles was then magnetometrically measured for the attached macrophages in each vial.

Ferromagnetic particles become magnetized when a strong external magnetic field is applied, and they produce a new field after the external field has been removed. This new field is called the remanent field and is maintained indefinitely in magnitude and direction unless the individual particles rotate. Thus, when a magnetic field is applied briefly to intracellular particles, the magnetic directions of these particles are aligned, and they produce a remanent field, proportional to the total mass of particles. The remanent magnetic field decreases only if the particle orientation becomes randomized away from the initial direction of alignment. During observation of cells with a microscope, a permanent magnet was used as a magnetic field source so as to produce a visual signal, i.e., alignment of the long axis of the magnetic particles along the field lines of the magnet.

For the magnetometric measurements of motility and apparent viscosity, alignment of the ingested particle magnetic moments was produced by a brief pulse (10 $\mu s$) of a homogeneous 0.1 T (tesla) [$=1000$ G (gauss)] magnetizing field. This was preferable to using a permanent magnet, as done in previous magnetometric studies (4, 14, 15, 37), because, as we have shown here, application of the permanent magnet physically rotates the particles and enhances magnetic particle aggregation. The short duration of the 10-$\mu s$ pulse does not permit particle movement even in a fluid with viscosity as low as water.

The weak remanent field produced by the cell-associated particles was detected using the apparatus shown in Fig. 1, which incorporated the noise-reduction and signal enhancement capabilities necessary to detect weak remanent fields ($\sim 4$ nT [$= 40 \mu G$] in the presence of much larger environmental magnetic fields ($\sim 10^6$ nT [$= 0.1$ G]). The remanent magnetic field was sensed with a Förster 1.107 fluxgate magnetometer (Förster Instruments, Inc., Corapolis, PA) with two pairs of low-noise field and gradient probes connected in a second-order gradiometer configuration (see Fig. 1). All four probes were oriented parallel to field lines from the cells and internalized particles to optimize their sensitivity to this magnetic field; but perturbing fields from distant sources produced signals of opposite polarity in each probe pair, yielding minimal output. The pulse magnetization was along the direction indicated by the arrows on the outer probes: this aligned the magnetic domains of the particles, and the probes could sense the remanent field. The cells and the vial containing them were then turned about a vertical axis at $\pm 10-13$ rotations per second. This rotation was part of the signal-enhancement scheme and produced only a small centrifugal force on the cells ($\sim 1/2$ g). A phase-sensitive amplifier combined the magnetometer signal with a reference signal synchronized to cell vial rotation and amplified only those signals in synchrony with sample rotation.

The implementation of these techniques made the apparatus optimally sensitive to the local magnetic field from the cells and served to cancel magnetic noise from outside sources.

Intracellular apparent viscosity was probed by first magnetically aligning the ingested particles with a brief pulse, and subsequently applying a magnetic field along the axis of cell vial rotation, i.e., perpendicular to the direction in which the particles had been aligned by magnetization. The magnitude of this right-angle field (2.5 mT = 25 G) was much smaller than the pulsed magnetizing field and did not alter the intrinsic magnetization of the particles. However, the magnetic particles behave as compass needles and slowly reorient toward the "twisting" field, and the amount of remanent field the fluxgate probes sense decreases. The remanent field signal can be related to the time course of particle rotation, and the rate at which particles rotate is determined by both the strength of the twisting field and the apparent viscosity of the medium which retards particle rotation. To calibrate these measurements, we suspended, in viscosity standards, samples of the same particles that were used to load the cells (Dow Corning dimethylpolysiloxane 200 fluid, Dow Corning Corp., Midland, MI) ranging from 60 Pa·s to 600 Pa·s (1 Pa·s = 10 poise) and these particles were twisted in the same manner as intracellular particles. This procedure served to correct for the fact that particle clumps contained non-magnetic volume which contributed to hydrodynamic size but not to remanent magnetic polarization. Particles were also mixed with and embedded in Epoxy and were thin-sectioned and examined in a transmission electron microscope.

Particle rotation as a method of probing intracellular apparent viscosity has advantages over experiments that pull magnetic spheres within cells (17, 28, 29, 43); considerably smaller particle clusters (of the same size as organelles) can be used, and the particles are twisted while in one area of the cell. No translocation across cytoplasmic structures is required. Hydrodynamic corrections for effects of particle size and shape and for effects of cell boundaries are less severe than when using particle translocation (20).

For video microscopy, macrophages adherent to glass coverslips (37°C, 1 h) were inverted on a drop of culture medium and sealed on a glass slide with Vaseline. Cells and their internalized particles were placed on the stage of a Zeiss Photomicroscope III maintained at 37°C and were viewed under bright field, phase-contrast, or Nomarski differential-interference contrast optics. Nomarski
prominent in the perinuclear area. Frequently, particle-containing organelles were situated in proximity to the Golgi complex (Fig. 3C), and bundles of 10-nm filaments were evident in this region. The size distribution of particle clusters in cells not magnetized with the permanent magnet was determined from electron micrographs, and the average cluster diameter was calculated to be 0.64 ± 0.30 (SD) μm (41).

Cytoskeletal Organization
To evaluate effects of the particle twisting that accompanies application of the permanent magnet, we performed fluorescence microscopy on cells before and after magnetization. Organization of the cytoskeleton was visualized using indirect immunofluorescence staining for tubulin- and vimentin-containing structures and using phalloidin to stain filamentous actin. The indirect immunofluorescence staining pattern with anti-tubulin is characterized by the presence of many fibrillar structures, presumably microtubules, radiating from a single microtubule-organizing center adjacent to the nucleus (Fig. 4, A and B). As noted earlier, ingested particles accumulate in the cell center near the microtubule-organizing center. Upon particle alignment with the permanent magnet, no change in the pattern of anti-tubulin staining was apparent (Fig. 4, C and D) since cells on the same coverslip exhibited identical patterns regardless of the presence of aligned particles. In contrast, actin microfilaments, and to a lesser extent vimentin intermediate filaments, were both modified in distribution as a result of magnetization. Cells not treated with the permanent magnet and labeled with rhodamine phalloidin exhibited a generally diffuse cytoplasmic staining and well-delineated areas of stain which coincided with membrane ruffles (Fig. 5, A and B). Although phalloidin stain was

RESULTS

Particle Behavior in Living Cells

The magnetic iron oxide particles were resolved in living cells under bright field and Nomarski optics. As shown in Fig. 2, A and B, variable numbers of particles were found within single cells. In heavily loaded cells, the particles accumulated in a perinuclear region, whereas a more random particle disposition was seen in less loaded cells. Application of a permanent magnet caused particle alignment within cells (Fig. 2, C and D) without affecting cellular translocation and ruffling activity. After removal of the magnet, cytoplasmic movements continued, and within 3–5 min alignment of the particles was randomized (Fig. 2, E and F). Subsequent to magnetization, the particles tended to remain aggregated in chains, usually in a perinuclear position.

Time-lapse videotape recordings of cells maintained at 37°C showed alignment of the particles within 10–20 s after the magnet was applied, and, upon removal of the magnet, a gradual loss of alignment. The cells remained quite active during and after magnetization as evidenced by formation and retraction of pseudopods, persistent membrane ruffling, and a limited degree of translocation along the substrate. Occasionally, a polymorphonuclear leukocyte was seen among the lung macrophages. The neutrophils exhibited considerably faster locomotory movement than macrophages. Replay of video recordings showed that alignment with the permanent magnet caused maghemite-containing organelles within a single cell to link up in chains parallel to magnetic field lines. After removal of the magnet, these chains would bend and twist, but generally did not break up. Cells with only a few, small ingested particles showed continued translational movements of particle-containing organelles during and after magnetization. Particles in formalin-fixed cells could be magnetically oriented, but, in contrast to living cells, the alignment produced was stable and unchanging.

Electron micrographs (Fig. 3, A–C) showed that the electron-dense γ-Fe₂O₃ particles were confined to membrane-bound organelles having the appearance of phagosomes or secondary lysosomes. The micrographs show cells which had either not been magnetized (Fig. 3, A and C) or cells which had been fixed after particle alignment with a permanent magnet (Fig. 3, B and C [inset]). Although the alignment and aggregation of particles within organelles was strikingly apparent in thin sections, it was difficult to define membrane boundaries in magnetized samples (Fig. 3B). Resin impregnation in such regions was often incomplete and the hard and abrasive particles resisted sectioning. Voids were created in the resin where particles fell out (Fig. 3, A and B). The linkage of individual particles into chains upon magnetization could distinctly be seen (Fig. 3, B and C [inset] arrows), and these aggregates were quite prominent in the perinuclear area. Although, particle-containing organelles were situated in proximity to the Golgi complex (Fig. 3C), and bundles of 10-nm filaments were evident in this region. The size distribution of particle clusters in cells not magnetized with the permanent magnet was determined from electron micrographs, and the average cluster diameter was calculated to be 0.64 ± 0.30 (SD) μm (41).
unevenly distributed or patchy in some untreated cells, there was little correspondence of the distribution of f-actin with the location of ingested particles. However, upon particle alignment, two changes in phalloidin staining were noted. The cell margin was regularly stained with areas of ruffling being the most intensely labeled (Fig. 5D). The most conspicuous alteration was the appearance of prominent patches of phalloidin staining which spatially coincided with the location of aligned particles (compare Fig. 5, C and D). The appearance of phalloidin patches was confined to cells containing particles, suggesting that as a result of alignment, local changes in the organization of f-actin were induced.

Previous studies have shown that cultured mouse macrophages form perinuclear coils of intermediate filaments (24). Perinuclear aggregates of ingested particles were typically located within intermediate filament coils in hamster lung macrophages (Fig. 5, E and F). As a result of particle alignment with the permanent magnet, the appearance of the intermediate filaments was altered in the sense that the bundles noted in magnetized cells were not as clearly demarcated, as evidenced by a diffuse perinuclear stain (Fig. 5, G and H). Staining intensity was not diminished in samples prepared from three different experiments suggestive of some degree of rearrangement of the filaments comprising the perinuclear bundles. Higher resolution images of this region, using electron microscopy, will be needed to clarify the organization of intermediate filaments in relation to particle alignment. It should be noted, however, that transmission electron micrographs of the perinuclear region do demonstrate intermediate filament aggregates associated with ingested particles (Fig. 3C).

Magnetometric Measurement of Motility and Viscosity

Figs. 6, A–C, are representative recordings of the remanent
FIGURE 3 Transmission electron micrographs of hamster alveolar macrophages containing maghemite particles. A illustrates a portion of a control (nonmagnetized) cell in which particles occupy numerous perinuclear vacuoles, including a densely stained secondary lysosome (arrow). In B, a portion of a macrophage subjected to magnetization for 2 min with a permanent magnet, showing the alignment of particle-bearing phagosomes into chains (arrows). The magnetization field lines were in the plane of the section and running vertically. The voids in A and B correspond to areas where particles fell out of the section. At higher magnification, as shown in C, maghemite particles are shown collected within a single membrane-limited phagosome; this phagosome is situated near the Golgi complex (GC) and a bundle of intermediate filaments (IF). The inset in C illustrates the alignment of particles within an endocytic invagination of the cell surface which bears a coated pit (arrow). A and B, ×19,000; C and inset, ×61,500. Bar, 1 µm (B); and 200 nm (C).

magnetic field after pulse magnetization from particle-containing cells incubated in the magnetometry apparatus shown in Fig. 1. In each graph, the upper curve (R) shows the spontaneous decrease of the remanent field in living cells with no external force applied; the lower curve (R + T) was produced when a twisting field of 2.5 mT (or 25 G) was applied perpendicular to the original direction of magnetization. First, we present the effects of spontaneous cell move-
FIGURE 4 Macrophage anti-tubulin staining patterns before and after application of a permanent magnet. Before magnetization, phase-lucent particles are located adjacent to nuclei as shown in A; the corresponding anti-tubulin pattern (B) illustrates many microtubules emanating from a single microtubule-organizing center (arrow). This pattern remains unchanged after magnetization (C) when particles exhibit alignment (D); the cell indicated by the arrow in D does not contain ingested particles. Bar, 10-μm divisions.

ments on the time course of the remanent field.

If cells were formalin-fixed, the remanent field was constant (data not shown), but for oxygenated macrophages at 37°C, the field decreased rapidly (Fig. 6A, curve R) to 42% of its initial value in 5 min. Since the magnetization of each particle is permanent, the spontaneous decrease in remanent field seen for living cells is due to random rotation of individual particles away from their original direction of magnetization as driven by active cytoplasmic rearrangements that move and reorient the particle-containing organelles. The remanent field decay is slowed by either cooling the cells to 10°C (Fig. 6B, curve R), or by incubation in 20 μM cytochalasin D for 1/2 h prior to magnetization (Fig. 6C, curve R). Dimethyl sulfoxide alone had no effect (data not shown). Video recordings confirmed that these treatments inhibit active movements within the cytoplasm.

The externally applied twisting force causes reorientation to occur more rapidly as evidenced by the increased decay rate for the remanent field (R + T curves). Moreover, a dramatic difference can be seen in the effect of a twisting force on each preparation; cooled cytoplasm resists shear, but cytochalasin D-treated cytoplasm does not (contrast the R + T curves in Figs. 6, B and C). Elastic recoil was also observed, as illustrated in Fig. 6D. Here, the twisting field was applied for 1 min during the interval between the two arrows. The yield upon force application and corresponding recoil after force removal is evident in the recording. The R + T curves for normal cells (e.g. Fig. 6A) were assumed to be the product of spontaneous reorientation and driven reorientation. The reorientation due to twist alone can then be generated by dividing the R + T curve by the R curve. By comparing the resulting curve to the curves obtained when twisting particles suspended in a series of viscosity standards, it was calculated that the apparent cytoplasmic viscosity for these particles within oxygenated cells at 37°C ranges from 1.2 kPa·s (12,000 poise) at shear rates of 0.003 s⁻¹ to 2.7 kPa·s (27,000 poise) at shear rates of 0.001 s⁻¹ (viscosity of H₂O at 37°C = 0.0007 Pa·s = 0.007 poise). The cytoplasm is undoubtedly a non-Newtonian viscous fluid in that the observed viscosity will depend on the rate of shear, the size of the moving object, and the history of shear. However, the shear rates applied here were comparable to observed spontaneous particle motions in cells in that remanent field decay rates were approximately doubled, and the particle size was comparable to the size of intracellular organelles (~0.3–0.9 μm diameter).

The intracellular particle clusters exist in a distribution of
FIGURE 5 Shown are corresponding bright field (left) and fluorescence (right) micrographs of alveolar macrophages before (A, B, E, and F) and after (C, D, G, and H) permanent magnet magnetization. Control cells exhibit perinuclear particles (A) while rhodamine-phalloidin staining (B) shows that F-actin is concentrated in the cortex and diffusely localized throughout the cytoplasm. A different field of cells is shown in C and D where particles have become aligned and phalloidin staining is prominent in central patches, which correspond to areas occupied by magnetic particles (arrows, D; compare with C). In E, central particle aggregates coincide in distribution with bundles of intermediate filaments demonstrated by indirect immunofluorescence with anti-vimentin antibody (F). After magnetization (G), aligned particles remain associated with foci of anti-vimentin staining (H) although the filament bundles are not well defined. Bar, 10-μm divisions.
sizes, and the larger ones contribute more to the remanent field in direct proportion to their larger mass (i.e., in proportion to the cube of their diameter). The effect on apparent viscosity measurement using rotation is minimal, however, since both the magnetic torque and viscous resistance to rotation increase as diameter cubed. Consequently, the effect of diameter on the observed magnetometric signal cancels out. Also, the effect of cell boundaries on particle rotation is small in that a 1-μm sphere rotating within a fixed 10-μm shell experiences a viscous drag only 0.1% larger than if it were in an infinite sea of material (9, 20). The effect of particle eccentricity up to a 5:1 ratio on the hydrodynamic diameter relevant to rotation is likewise small (<10%) (9).

It is important to remember that the maghemite particles are within phagosomes, so that the immediate environment of the particles is the vacuole interior (Fig. 3C). Since the exact site of shear is unknown, the measurements may be influenced by phagosomal contents. The magnetometrically measured apparent viscosity is an average over vesicles that may be variable in size, particle content, and protein content. The hydrodynamic diameter may be increased by the phagosome membrane and by attachment to other cell structures; these effects will make the measured viscosity larger than for freely rotating magnetic particles.

DISCUSSION

The study of cytoplasmic structure has commanded considerable attention in recent years owing to the widespread belief that the organized matrix of cytoskeletal fibers mediates many of the motile activities exhibited by cells. The historical and contemporary aspects of this endeavor have been thoroughly summarized recently (22, 26, 27). It is noteworthy, however, that few of these approaches directly address the physical properties of cytoplasm in relation to a particular motile activity. In this paper, we have presented results which suggest an alternative approach for probing cytoplasmic architecture which pertains to an organelle resident within the cytoplasm of macrophages. We have shown that alveolar macrophages readily engulf magnetic particles into phagosomal structures, and that subtle changes in the rotational mobility of particle-laden phagosomes can be magnetometrically detected. These signals can be used as an experimental indicator both of cytoplasmic activity and of the apparent cellular viscosity that retards motion of these organelles. Not only was it possible to estimate motion and viscosity for a discrete organelle with this technique, but it was found that the response of these organelles to an externally applied permanent magnet caused local alterations in the organization of the cytoskeleton that correlated well with other metabolic parameters of cell function. These findings are discussed with respect to other determinants of macrophage function and their potential application to a variety of problems in cell biology.

Macrophages have been widely studied because they are easy to obtain in large numbers and they exhibit pronounced...
locomotory and phagocytic activities. Previous studies have shown that macrophages express a dynamically changing array of contractile proteins whose organization is regulated under a number of conditions including spreading, migration, and phagocytosis. For example, macrophage spreading on artificial substrates is accompanied by the deployment of actin into the cell cortex, particularly in regions of ruffles where membrane activity is exaggerated (1). Moreover, spreading can be enhanced by phorbol esters which amplify changes in the organization of other cytoskeletal fibers such as microtubules and intermediate filaments. Specifically, it has been noted that phorbol esters induce intermediate filament extension and the appearance of actin patches (24). By selectively perturbing the macrophage phagosomes with an external magnetic field, we have shown that f-actin and, to a lesser extent, intermediate filaments, are subjected to local changes in organization that presumably reflect the association of this organelle with these components of the cytoskeleton. Further support for this interpretation derives from observations that (a) the particle-loaded phagosomes are normally situated within intermediate filament cables as evidenced in transmission electron microscopy and immunofluorescence microscopy (see Figs. 3 and 5), (b) cytochalasin D, which caps the ends of actin filaments (21), impairs particle motions (see Fig. 6 C), and (c) patches of actin appear upon particle alignment (see Fig. 5 D). Although more work is needed to define the spatial relationships of phagosomes with the cytoskeleton, these studies suggest that both actin filaments and intermediate filaments may be partly responsible for the organelle movement detected. The observation that cytoplasmic microtubules are not affected by forced particle alignment implies that these structures are less involved in the regulation of phagosome organization.

Microtubules have been widely implicated in the guidance and motility of cytoplasmic organelles, but their participation may vary in different cell types (30). However, several observations relevant to the macrophage cytoskeleton pertain here. It is noteworthy that the fusion of lysosomes with phagosomes appears to occur predominantly in the perinuclear region (8), and our own video microscopy experiments indicate that most of the particle-laden phagosomes we have examined are perinuclear and apparently are unable to move translationally. Although microtubules appear to be essential in orienting macrophage lysosomes (24) and in centripetal movement (3), microtubules are not necessary for phagosome formation or fusion with lysosomes (3). The complexity in regulation owes in part to the many interactions that can exist between actin and microtubules (26) and microtubules and organelles (35). Although it has been proposed on the basis of in vitro experiments that MAP-2 can mediate binding of actin to microtubules (26), it is likely that such ATP-sensitive linkages are not involved in maintaining phagosomes within a perinuclear location. Rather, we envision these organelles to be embedded within a meshwork of f-actin and perhaps intermediate filaments which provides a barrier to translational movement and thus maintains them in a perinuclear position. Measurements of apparent local viscosity would then be a reflection of the maintenance of this structural matrix and would differ considerably from the matrix in the cortex where activity and structural rearrangements would involve primarily actin and its associated proteins (1, 44). Since cultured macrophages readily endocytose maghemite particles, it should be possible to probe cortical cytoplasm directly as a nascent phagosome is formed and is translocated to the perinuclear region.

These results are important in the interpretation of data obtained on the behavior of magnetic particles within intact organisms (4, 5, 7, 14, 15, 23). The phenomenon of remanent field decay after magnetization has been observed for magnetic particles retained in lungs of humans and animals, and has been termed “relaxation” (6). A number of speculations about the force driving relaxation have been put forward, including respiratory movements, surfactant and mucus flow, cardiac pulsations, particle diffusive motion, and cell motion. On the basis of a theoretical model of relaxation, Nemoto proposed that alveolar macrophage motility was the mechanism responsible for relaxation (23). Cytoplasmic activity was also implicated in the magnetometric study of Gehr et al. (14) in which magnetic particles were injected into the circulation and taken up by Kupffer cells in the liver. The correlation of videomicroscopy and magnetometry of isolated macrophages reported here provides more conclusive evidence that part of the remanent field decay observed for lungs in vivo can be attributed to intracellular motions within particle-containing pulmonary macrophages.

An earlier report on the relaxation phenomenon in isolated macrophages did not assess the contribution of Brownian movement to the motion detected (37). The application of a known external torque, as described here, can answer this question. From our estimates of apparent viscosity for control cells, we can calculate the contribution of Brownian movement to remanent field decay to be of the order of 9.2 × 10⁻⁴ min⁻¹ (11). However, relaxation is observed to proceed at a much faster rate (~7.8 × 10⁻³ min⁻¹) over the first fifteen minutes (see Fig. 6A). Thus, intracellular particles appear not to be driven primarily by thermal energy. Moreover, although various treatments have been found to influence cellular relaxation (37), an effect on active force generation in the cell could previously not be distinguished from an effect on intracellular apparent viscosity. From our viscosity results for 10°C and cytochalasin D–treated cells, it can be said that cooling the cells reduces motility by both disrupting force generation and increasing apparent viscosity (Fig. 6 B), whereas cytochalasin D treatment only disrupts force generation and does not increase viscosity (Fig. 6 C). The nature of the viscoelastic phenomenon (Fig. 6 D) needs further characterization, but it reinforces the idea that the cell interior is not a simple Newtonian fluid but can both dissipate and store energy associated with particle displacements.

Previous investigations of cell viscosity (Table I) have used particles ranging from a very large size (~100 μm) (29) to molecular probes of small size (~0.003 μm) (22, 40, 42). The larger particles have been observed optically, and the molecules have been monitored by techniques that essentially measure their diffusion mobility. We used particles comparable in size to cell organelles (~0.3–0.7 μm) in conjunction with a non-optical method that is easier to quantify for large numbers of cells. Furthermore, rather than examining reconstituted actin gels (44) or nonmammalian cells (17, 28, 29, 40, 43), our results apply to intact mammalian macrophages incubated at physiological conditions. It is clear from Table I that a wide range of apparent viscosities has been reported in a variety of cell systems. Some differences in results can be attributed to the non-Newtonian nature of the cytoplasm, i.e. as the shear rate goes down, the measured viscosity goes up. There also appears to be a trend that larger particles yield a larger relative viscosity. It must be kept in mind that all
measurements involving motion of a sphere in a non-Newtonian fluid are subject to difficulties in interpretation because even though the motion or rotation of the sphere in the fluid may be uniform, the shear rate is not, and changes with position on the surface of the sphere. In this type of situation, force (stress) and motion (strain) measurements cannot be unambiguously related to viscosity.

In our experiments, maghemite particles were twisted while contained in phagosomes. The possibility exists that particles can rotate within the phagosome, in which case the apparent viscosity is weighted by a contribution from the contents of the vacuole interior. If the phagosomal contents were of low viscosity, then our estimates of cytoplasmic apparent viscosity are too low, and should be considered only as a lower bound. However, we observed that cytochalasin D reduced apparent viscosity (Fig. 6C), and other studies have shown that the action of cytochalasin D is the disruption of cytoplasmic elements (21). This suggests that our method of examining viscosity is sensitive to structure in the cytoplasm. Moreover, the elastic recoil observed (Fig. 6D) is easier to understand as a property of cytoplasmic filaments rather than a property of cytoplasmic physical properties, and can provide a means of probing organization of the cytoskeleton.

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