Extracellular pressure stimulates macrophage phagocytosis by inhibiting a pathway involving FAK and ERK

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Shiratsuchi, Hiroe, and Marc D. Basson. Extracellular pressure stimulates macrophage phagocytosis by inhibiting a pathway involving FAK and ERK. Am J Physiol Cell Physiol 286: C1358–C1366, 2004.—We hypothesized that changes in extracellular pressure during inflammation or infection regulate macrophage phagocytosis through modulating the focal adhesion kinase (FAK)-ERK pathway. Undifferentiated (monocyte-like) or PMA-differentiated (macrophage-like) THP-1 cells were incubated at 37°C with serum-opsonized latex beads under ambient or 20-mmHg increased pressure. Pressure did not affect monocyte phagocytosis but significantly increased macrophage phagocytosis (29.9 ± 1.8 vs. 42.0 ± 1.6%, n = 9, P < 0.001). THP-1 macrophages constitutively expressed activated FAK, ERK, and Src. Exposure of macrophages to pressure decreased ERK and FAK-Y397 phosphorylation (77.6 ± 7.9%, n = 7, P < 0.05) but did not alter FAK-Y576 or Src phosphorylation. FAK small interfering RNA (SiRNA) reduced FAK expression by >75% and the basal amount of phosphorylated FAK by 25% but significantly increased basal macrophage phagocytosis (P < 0.05). Pressure inhibited FAK-Y397 phosphorylation in mock-transfected or scrambled SiRNA-transfected macrophages, but phosphorylated FAK was not significantly reduced further by pressure in cells transfected with FAK SiRNA. Pressure increased phagocytosis in all three groups. However, FAK-SiRNA-transfected cells exhibited only 40% of the pressure effect on phagocytosis observed in scrambled SiRNA-transfected cells so that phagocytosis inversely paralleled FAK activation. PD-98059 (50 μM), an ERK activation inhibitor, increased basal phagocytosis (26.9 ± 1.8 vs. 31.7 ± 1.1%, n = 15, P < 0.05), but pressure did not further increase phagocytosis in PD-98059-treated cells. Pressure also inhibited ERK activation after mock transfection or transfection with scrambled SiRNA, but transfection of FAK SiRNA abolished ERK inhibition by pressure. Pressure did not increase phagocytosis in MonoMac-1 cells that do not express FAK. Increased extracellular pressure during infection or inflammation enhances macrophage phagocytosis by inhibiting FAK and, consequently, decreasing ERK activation.

During infection or inflammation, monocytes and macrophages are recruited to the inflammatory site and are critical to innate host defense mechanisms. Tissue pressure is often altered in association with inflammation or infection. Mechanical stimuli, such as pressure, are known to modulate cellular morphology and function in other cell types (6, 27, 31, 60). We therefore hypothesized that changes in extracellular pressure during inflammation or infection regulate macrophage phagocytosis through modulating the focal adhesion kinase (FAK)-ERK pathway.

A few recent reports suggest that physical forces such as extracellular pressure and repetitive strain may alter other aspects of macrophage functions. Pressure (40–130 mmHg) increases monocyte migration in a dose-dependent manner and enhances scavenger receptor expression in macrophages (47). Macrophages produce proinflammatory cytokines in response to very high cyclic pressure (~1,000 mmHg), the combination of cyclic strain and 75-mmHg pressure, or high pressure (>70 mmHg) combined with stimulation by endotoxin (36, 66). Increased pressure (40–90 mmHg), but not cyclic strain, has also been shown to increase the uptake of aggregated IgG by mouse macrophage J774.16 cell line (34, 38). The mechanisms or intracellular signals responsible for this effect have not been defined. However, these pressures are higher than what are often observed in edematous tissue (17, 39). Furthermore, the effect of physical forces, such as pressure or cyclic strain on particle phagocytosis by macrophages, involves more complex mechanisms than the simpler binding of aggregated IgG to Fc receptors expressed on the macrophage surface. Phagocytosis requires recognition of a ligand, adherence, plasma membrane mobility, actin filament polymerization, formation of the phagocytic cup, and ingestion (1, 35). We therefore examined the effects of constant low pressure (20 mmHg) on macrophage phagocytosis using undifferentiated (monocyte-like) and PMA-differentiated (macrophage-like) human THP-1 monocyte cells as a model. THP-1 cells have many characteristics of human monocytes and macrophages (14, 55, 65). These cells can be differentiated into a macrophage-like phenotype in a manner resembling the conversion of human monocytes to macrophages in many aspects (3, 5, 56). Differentiated THP-1 cells are therefore a common model for the study of human macrophage biology (10, 15, 19).

In further studies, we explored the potential contribution of three intracellular signal molecules, Src, FAK, and ERK, to the effects of pressure that we had observed. All three kinases have been shown to be activated by some physical forces in some other cell types (46, 53, 60, 64). In addition, Src is activated by interaction with integrin (2) and has been shown to mediate phagocytosis through integrins (13). Although a relationship between FAK and ERK and integrin-mediated phagocytosis in macrophages has not previously been established, FAK and ERK are activated by integrin cross-linking after phagocytosis occurs (35, 45). We used pharmacological inhibitors to probe the roles of Src and ERK in mediating the effect of pressure and a specific small interfering RNA (SiRNA) to inhibit FAK.

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We took advantage of the observation that another monocyte line, MonoMac-1, expresses very little FAK to further probe the role of FAK in mediating the pressure effect.

**MATERIAL AND METHODS**

**Antibodies and chemicals.** Antibodies against total ERK, phospho-ERK, and phospho-Src were purchased from Cell Signaling Technology (Beverly, MA). Anti-total FAK, anti-total Src, and anti-pY397-FAK antibodies were obtained from BD Transduction Laboratories (Lexington, KY). Santa Cruz Biotechnology (Santa Cruz, CA), and BioSource International (Camarillo, CA), respectively. Fluorescence-labeled latex beads (2.0 µm) were obtained from Polysciences (War-lington, PA). P22, a Src family kinase inhibitor, and PD-98059, a MEK inhibitor that prevents ERK activation, were obtained from Cabiochem (Santa Cruz, CA).

**Cells and cell cultures.** The human monocytic cell line THP-1 was obtained from the American Type Culture Collection (Rockville, MD), and MonoMac-1 cells were a gift from Dr. G. Vanham (Institute of Tropical Medicine, Laboratory of Immunology, Antwerp, Belgium). THP-1 cells were maintained in RPMI 1640 (GIBCO, Grand Island, NY), supplemented with 10% FBS (Sigma, St. Louis, MO), L-glutamine (200 mM), and 2-mercaptoethanol (5 × 10⁻⁵ M) (Sigma). MonoMac-1 cells were maintained in RPMI 1640 supplemented with 10% FBS, L-glutamine (200 mM), nonessential amino acids, and 1 mM sodium pyruvate.

THP-1 cells (5 × 10⁵ cells per 35-mm dish) were differentiated by stimulation with PMA (50 ng/ml, final concentration) for 3 days to obtain a macrophage-like phenotype, which closely resembles human monocyte-derived macrophages, as previously reported (3, 5, 10, 15, 56).

**Assay for phagocytosis.** Fluorescence-labeled latex beads (2.0 µm) were opsonized with 10% unheated FBS for 60 min at 37°C before the experiments. PMA-differentiated or unstimulated THP-1 or Mono-Mac-1 cells (1 × 10⁶) were mixed in 1 ml of tissue culture medium supplemented with 10% FBS in a 35-mm petri dish with opsonized latex beads at the multiplicity ratio of 1:5 and incubated for 2 h at 37°C. One set of dishes was placed in a calibrated pressure box that was described previously (6, 53, 60). The box was prewarmed to 37°C for 1 h before each study to prevent fluctuations in internal pressure caused by temperature shifts of the pressurizing gas. The pressure inside the pressure box was set at 20 mmHg, and the pressure box was sealed and monitored every 15 min. The second set of dishes was placed inside the same incubator but under ambient pressure conditions. After 2 h, macrophage monolayers were washed vigorously with PBS to remove extracellular beads, fixed with methanol for 10 min, and then counterstained with methylene blue. Monocytes were washed with PBS and fixed with 1% paraformaldehyde for 10 min. The uptake of fluorescent latex beads was counted under a fluorescence microscope. The number of intracellular latex particles was determined by counting fluorescent beads within cells. Four to six hundred consecutive monocytes or macrophages were counted in each culture. A previously described scoring method (31) was utilized to calculate the number of latex beads phagocytosed by macrophages or monocytes and the phagocytic index. In brief, cells were separated into the following categories: cells with no beads, cells with 1 bead, cells with 2–5 beads, cells with 6–10 beads, and cells with >10 beads. Percent phagocytosis was calculated as the total number of cells with at least one bead as a percentage of the total number of cells counted. The phagocytic index was calculated as 100 × [number of cells with 1 bead + (3.5 × number of cells with 2–5 beads) + (8 × number of cells with 6–10 beads)] ÷ [20 × number of cells with over 10 beads]/total cells counted.

Western immunoblotting. THP-1 macrophages were incubated under ambient or increased pressure (20 mmHg) conditions for 30 min, rinsed once with cold PBS, and lysed with lysing buffer. Protein concentrations in cell lysates were measured by using bicinchoninic acid protein assay kits (Pierce, Rockford, IL). Equal amounts of protein were loaded in each lane. Cell lysates were resolved under reducing conditions on 10% SDS-PAGE and then transferred to nitrocellulose membranes. After blocking with 5% bovine serum albumin in Tris-buffered saline with 0.1% Tween 20, the membranes were incubated with antibodies against activated and total forms of protein overnight at 4°C, washed three times with 0.1% Tween 20-Tris-buffered saline, and then incubated for 60 min with 2,000:1 peroxidase-conjugated anti-rabbit IgG. The membrane-bound peroxidase activity was detected using ECL Plus Western blotting detection kits (Amersham, Arlington Heights, IL). Chemiluminescent images were captured and analyzed by using a Kodak Digital Science Image Station 440CF. All blots were studied within the linear range of exposure.

**Transfection of FAK SiRNA.** To inhibit FAK protein expression, THP-1 cells were transfected with SiRNA. The SiRNA duplex, 5’-GCAUGUGGCUUGAUAGGAdTdT/tdTTCUACCAG-GACGUAAACCU-5’, directed toward the mRNA target, 5’-AAGGCAUGUGGCUUGUA/GGA-3’, was synthesized by Dharmacon (Lafayette, CO). A scrambled RNA duplex (5’-GCGCGGCUUUGAAGAUCGdtdTd/tTTCGGCAAAACAUCCUAAGC-5’) was purchased from Dharmacon and used as a control. THP-1 cells (4 × 10⁵ cells per 60-mm petri dish) were stimulated with PMA (50 ng/ml) for 2 days before transfection. Transfection of duplex SiRNAs was performed using Oligofectamine (Gibco, Gaithersburg, MD), according to the manufacturer’s protocol. Twenty-four hours after transfection, cells were used for Western analysis and for assays of phagocytosis. Transfection efficacy was measured by transfecting Cy3-conjugated SiRNA targeted to luciferase (Upstate USA, Charlot-tsville, VA) and counting fluorescence-positive THP-1 cells by using a fluorescent microscope 24 h after transfection.

**Statistical analysis.** The significance of differences between groups was calculated by the Student’s t-test or paired t-test as appropriate. Ninety-five percent confidence was set a priori as the desired level of statistical significance.

**RESULTS**

**Effects of extracellular pressure on monocyte cell phagocytosis.** We first examined the effect of increased extracellular pressure on latex bead phagocytosis by human monocytic THP-1 cells. PMA-differentiated (macrophage-like) and unstimulated (monocyte-like) THP-1 cells were incubated with serum-opsonized fluorescence-labeled 2.0-µm latex beads (5 beads/cell) under ambient pressure or 20- or 100-mmHg increased pressure conditions at 37°C for 2 h. Exposure to pressure failed to modulate phagocytosis by unstimulated monocyte-like THP-1 cells (Fig. 1, A and B). Pressure increased both percent phagocytosis and the phagocytic index of PMA-differentiated macrophage-like THP-1 cells in a dose-dependent fashion (Fig. 1, C and D). Although 100-mmHg increased pressure induced a greater increment of phagocytosis in THP-1 macrophages than 20-mmHg-increased pressure, we chose to further study the effects of low pressure (=20 mmHg) because it more closely approximates tissue pressures associated with edema (17, 20, 39). Latex bead uptake by PMA-differentiated THP-1 cells was higher than in unstimulated cells, and increased pressure significantly augmented PMA-stimulated THP-1 macrophage phagocytosis (29.9 ± 1.8 vs. 42.0 ± 1.6%, n = 17, P < 0.001).

**Potential role of Src and related kinases.** Src family kinases have previously been shown to mediate particle uptake through complement receptors expressed on phagocytic cells (1, 35). Serum-opsonized latex beads are phagocytosed via the com-
We therefore next examined the effect of pressure on FAK activation in THP-1 macrophages. THP-1 macrophages constitutively expressed both Y397 and Y576 phosphorylated FAK. Exposure to pressure significantly inhibited FAK-Y397 phosphorylation (an autophosphorylation site) in THP-1 macrophages but had no effect on FAK-Y576 phosphorylation (a Src-binding site) (Fig. 3B).

To examine whether this downregulation of FAK activation by pressure might contribute to pressure-induced phagocytosis, FAK SiRNA was transfected into PMA-stimulated THP-1 macrophages. Using Cy3-conjugated luciferase SiRNA, we defined our transfection efficacy as 91.9 ± 4.0% (86.0–97.0%; n = 4). Total FAK protein expression by THP-1 cells transfected with FAK SiRNA was decreased by 70–90% compared with cells transfected with scrambled SiRNA or mock-transfected cells (Fig. 4A). Transfection of FAK SiRNA significantly increased basal phagocytosis (P < 0.05) compared with mock-transfected or scrambled SiRNA-transfected THP-1 macrophages (Fig. 4B). Exposure to increased pressure significantly inhibited FAK-Y397 phosphorylation in mock-transfected THP-1 macrophages.
fected and scrambled SiRNA-transfected THP-1 macrophages (Fig. 2C). However, FAK SiRNA-transfected cells exhibited markedly lower levels of Y397 phosphorylated FAK, and a further decrease in response to pressure was not detectable. Pressure increased phagocytosis in all three groups (Fig. 4B). However, phagocytosis by FAK SiRNA-transfected cells was increased only 26.8 ± 3.9% by pressure, substantially lower than the 68.6 ± 13% increase that we observed in the same experiments in cells transfected with scrambled SiRNA and then exposed to pressure. This difference was statistically significant (n = 5, P < 0.05).

Fig. 3. Studies of focal adhesion kinase (FAK) and pressure. A: induction of FAK protein expression in THP-1 cells after differentiation with PMA. Top: typical Western blot for total FAK, stripped and reprobed for GAPDH as a protein loading control. Bottom: graph summarizing densitometric analyses. Values are means ± SE of total FAK to GAPDH normalized to day 0 (unstimulated) data (n = 3). PMA stimulation dramatically increased total FAK protein expression in THP-1 cells. B: effect of pressure on FAK phosphorylation in PMA-differentiated THP-1 cells. Top: typical Western blots for Y397 or Y576 phosphorylated FAK, each stripped and reprobed for total FAK as a loading control. Bottom: values are means ± SE of the ratio of Y397 or Y576 phosphorylated FAK to total FAK, normalized to control. Open bars, ambient pressure; solid bars, increased pressure. Increased pressure inhibited FAK Y397 phosphorylation (*P < 0.05, n = 7) but did not affect FAK Y576 phosphorylation.

Fig. 4. Transfection with small interfering RNA (SiRNA) targeted to FAK into PMA-differentiated THP-1 cells. A: total FAK protein expression in mock-transfected cells, cells transfected with scrambled SiRNA, and cells transfected with FAK SiRNA incubated under ambient or increased pressure conditions. Top: typical Western blot for total FAK, stripped and reprobed for GAPDH as a loading control. Bottom: graph summarizing densitometric analyses, expressed as means ± SE of total FAK to GAPDH normalized to control mock-transfected cells. Total FAK protein in cells transfected with FAK SiRNA was significantly lower than FAK protein in mock-transfected cells or scrambled SiRNA-transfected cells (*P < 0.01, n = 7). B: effect of pressure on phagocytosis in mock-transfected cells, cells transfected with scrambled SiRNA, and cells transfected with FAK SiRNA. Values are means ± SE of percent phagocytosis. Transfection with FAK SiRNA significantly increased basal phagocytosis compared with cells transfected with scrambled SiRNA (P < 0.05, n = 5). Pressure significantly increased phagocytosis in all 3 groups (*P < 0.01, n = 5 compared with the corresponding controls). C: effect of pressure on FAK phosphorylation in mock-transfected cells, cells transfected with scrambled SiRNA, and cells transfected with FAK SiRNA. Top: typical Western blot for Y397 FAK phosphorylation, stripped and reprobed for total FAK and for GAPDH. Bottom: graph summarizing densitometric results expressed as means ± SE of the ratio of Y397 phosphorylated FAK to total FAK, normalized to control values in mock-transfected cells. Open bars, ambient pressure; solid bars, increased pressure. Note that results of FAK Y397 phosphorylation in FAK SiRNA-transfected cells are presented on a different scale for ease of depiction. Pressure significantly inhibited FAK Y397 phosphorylation in mock-transfected cells and scrambled SiRNA-transfected cells (*P < 0.05, n = 5).
Potential role of ERK. ERK plays a role in signal transduction from cell membranes to nuclei, is activated by pressure and other mechanical stimuli in other cell types (23, 57, 58, 62), and has been shown to be activated after phagocytosis in macrophages (22, 37). Little is known about the contribution of ERK activation to the regulation of particle phagocytosis. We therefore examined the effect of pressure on ERK activation. Pressure inhibited ERK phosphorylation in THP-1 macrophages (Fig. 5A). The effect of ERK inhibition on pressure-mediated phagocytosis was examined accordingly. THP-1 macrophages were pretreated with the MEK inhibitor PD-98059 (50 μM) for 60 min before exposure to pressure to inhibit activation of ERK. Pretreatment of macrophages with PD-98059 inhibited 80% of ERK phosphorylation (Fig. 5B) and significantly increased basal phagocytosis. Exposure to pressure did further augment phagocytosis in cells pretreated with PD-98059 (Fig. 5C). However, the effect of pressure on phagocytosis in PD-98059-treated cells was reduced by 50% compared with the effect of pressure in cells treated with DMSO vehicle (n = 5, P < 0.05). These data suggest that downregulation of ERK activation by pressure may contribute to pressure-mediated phagocytosis.

Pressure effects on ERK in THP-1 macrophages transfected with FAK SiRNA. We next sought to determine whether the effect of pressure on FAK might contribute to the effect of pressure on ERK. ERK activation in THP-1 macrophages transfected with SIRNA targeted to FAK was therefore examined. Exposure to pressure inhibited ERK activation in mock-transfected (n = 5, P < 0.05) and scrambled SiRNA-transfected THP-1 macrophages (n = 5, P < 0.03), but pressure did not alter ERK activation in FAK-SiRNA-transfected cells (Fig. 6).

Effect of pressure on MonoMac-1 phagocytosis. The pressure effect on phagocytosis by MonoMac1, another human monocytic cell line (68), was also tested. Expression of FAK in MonoMac-1 cells was below detectable levels by Western blotting, and stimulation with PMA did not induce further FAK expression in this cell line (data not shown). Pressure did not augment phagocytosis by these MonoMac-1 cells even after stimulation with PMA (6.99 ± 0.78 vs. 7.48 ± 0.84%, n = 6, P = 0.140).

**DISCUSSION**

The present study demonstrated that extracellular pressure stimulated macrophage phagocytosis in a dose-dependent manner. In particular, a relatively modest increase in extracellular pressure (20 mmHg above ambient) increased serum-opsonized latex bead phagocytosis by PMA-stimulated THP-1 macrophages. We found that FAK and ERK inhibition each promoted phagocytosis. Furthermore, the effect of pressure appears, at least in part, to be due to inhibition of FAK-Y397 autophosphorylation and consequent downstream inhibition of ERK activation by increased pressure, because exogenous inhibition of these signals mimicked pressure stimulation of phagocytosis and attenuated the further effect of pressure with combined treatment. Comparison with the PP2 data confirms that the cells were capable of even more active phagocytosis when stimulated by another agent directed at a pathway different from the pressure mechanism. Indeed, inhibition of Src activation by PP2 increased basal macrophage phagocytosis, but Src family kinases did not appear to contribute to the pressure effect.

Extracellular pressures within inflamed or infected tissues can vary substantially. For example, infection or edema in
closed compartments may increase tissue pressure by 5–80 mmHg (7, 49, 50), although edema formation in unconstrained inflamed or infected tissues may actually be associated with decreases in connective tissue interstitial fluid pressures by as much as 150 mmHg (63). Invasive fungal pathogens such as *Pythium insidiosum*, a potential lethal infectious pathogen for humans, exert pressures as high as ~200 mmHg (43). Macrophages moving from the capillaries into inflamed tissue are also exposed to capillary pressure elevations. In patients with colonic inflammatory bowel disease, for instance, colonic blood flow is increased two- to sixfold, causing capillary pressure to rise by 10–40 mmHg (17, 20). Activation or inhibition of macrophages by changes in extracellular pressure may, therefore, affect macrophage function in such settings.

Monocytes and macrophages are sensitive to biomechanical stimuli, including cyclic strain, shear, and pressure, although forces of different nature or magnitude may yield different effects (27, 34, 38, 41, 52). Exposure of human monocyte-derived macrophages to cyclic high pressure (250–1,000 mmHg) induces tumor necrosis factor-α at 12 h and interleukin-1β and -6 at 24 h (36). Mechanical strain has been reported to induce class A scavenger receptor expression and immediately early response gene expression in human monocytic THP-1 cells (47). Shear stress increases THP-1 cell adhesion to human umbilical vein endothelial cells (54). Constant applied pressure (40–130 mmHg) increases migration of human monocyte cell line U937 cells (52). However, such forces are more consistent with those within the cardiovascular system. The effect of a constant lower pressure, such as 20 mmHg, on macrophage function has not yet been described and may be more relevant to the cell biology of inflamed tissue. Furthermore, the effect of mechanical forces on phagocytosis of particle antigens is unknown and would not necessarily parallel adhesion, migration, or cytokine release.

Higher pressures have been reported to enhance macrophage IgG complex uptake by mouse macrophage J774 cells (34). However, phagocytosis of particulate antigens requires much more complicated mechanisms than the binding of immunocomplex to the macrophage surface. These include recognition of a ligand, adherence, plasma membrane mobility, actin filament polymerization formation of the phagocytic cup, and ingestion. The event of particle phagocytosis stimulates activation of tyrosine kinases and induces further phosphorylation of tyrosine kinase associated with phagosome in macrophages. However, tyrosine kinases within the cell may also influence phagocytosis.

Macrophages contain Src and related Src family kinases. Src family kinases may facilitate phagocytosis by initiating actin polymerization and particle internalization (1, 35). Serum-opsonized particles are ingested through complement receptors or integrins. The nonreceptor tyrosine kinase Src family signals are activated by integrin cross-linking and mediate cell adherence and binding of phagocytic targets to cells. Phagocytosis by triple Src family kinase (Hck, Fgr, and Lyn) knockout macrophages is modest and delayed (12), and optimal internalization of gram-positive bacterium *Staphylococcus aureus* depends on expression of Src by fibroblasts (13). However, the role of Src in macrophage phagocytosis is likely to be more complex. Macrophages lacking Src have normal phagocytic capacity (21). Furthermore, a human monocyte transgenic mouse model has recently been described in which upregulated Src family kinases are associated with depressed macrophage phagocytosis (4), and expression of the Src kinase family member Fgr attenuates both Fc receptor-mediated and complement receptor-mediated phagocytosis in murine macrophages (18). These last reports are more consistent with our observation that inhibiting the Src family kinases by PP2 promoted THP-1 phagocytosis under either ambient or increased pressure conditions.

Src is activated by pressure in colon cancer cells (53), smooth muscle cells (61), and cardiomyocytes (46, 64). However, we were not able to demonstrate a change in Src phosphorylation in THP-1 cells exposed to pressure. Furthermore, our data suggest that neither Src nor related Src family kinases mediate the effect of pressure on THP-1 cell phagocytosis.

FAK is a multifunctional tyrosine kinase associated with signaling triggered by integrin cross-linking and expressed by a variety of cell types. Kharbanda et al. (25) demonstrated the induction of FAK expression in monocyte-derived macrophages stimulated with macrophage colony-stimulating factor, and others have also reported that FAK is expressed in human monocytes (40). Indeed, cross-linking the Fcγ receptors in human monocytes increases FAK protein tyrosine phosphorylation. Differentiation of bone marrow cells to a macrophage phenotype by granulocyte-macrophage colony-stimulating factor induces FAK expression in mice (29). Undifferentiated THP-1 cells expressed only a small amount of FAK, but FAK expression was dramatically increased in PMA-differentiated macrophage-like THP-1 cells. Because FAK expression in macrophage lineages varies with the agent used to induce differentiation (29), it is possible that MonoMac-1 cells might express FAK in response to some other differentiating agent. Alternatively, this cell line may simply lack FAK expression.

In the mouse macrophage J774 cell line, phagocytosis through low-affinity Fc receptors induces FAK activation (9), but little is known about the role of FAK in induction of macrophage phagocytosis. FAK activation has been shown to promote integrin-dependent phagocytosis by another cell type,
retinal pigment epithelium (11). Phagocytosis was decreased in MonoMac-1 cells or undifferentiated THP-1 cells that express less FAK. FAK expression during macrophage differentiation may simply correlate with the expression of other phenotypic traits that promote phagocytosis. Alternatively, it is possible that some degree of FAK expression facilitates receptor clustering or cytoskeletal assembly required for effective phagocytosis. FAK expression may also activate downstream kinases required for a phagocytic phenotype. This awaits further study. However, inhibiting FAK in differentiated THP-1 cells stimulated phagocytosis.

Activation of FAK by mechanical forces such as pressure has been reported in other types of cells, including colon cancer cells (31, 53), NIH/3T3 fibroblasts (59), and cardiomyocytes (46, 64). In our system, pressure inhibited activation of FAK-Y397 in PMA-differentiated THP-1 macrophages, leading to enhanced latex bead uptake. Inhibition of FAK by transfection with siRNA targeted to FAK also increased THP-1 macrophage phagocytosis. These results are internally consistent, although they differ from the promotion of retinal epithelial phagocytosis by FAK activation (11). Although both inhibition of FAK-Y397 phosphorylation by extracellular pressure and transfection with FAK siRNA augmented THP-1 macrophage phagocytosis, pressure was markedly less effective in cells in which FAK expression had already been inhibited. Pressure may therefore alter macrophage phagocytosis by modulating FAK activation.

ERK is activated by cross-linking of integrins and phagocytosis of particle antigens in macrophages, and this ERK activation subsequently influences cytokine expression, cell survival, and control of intracellular pathogen growth (22, 28, 35, 44, 45). However, a role for ERK in the regulation of phagocytosis is controversial.

ERK is not required for phagocytosis in monocytic cells not differentiated to a macrophage phenotype (16, 24), but ERK promotes Fc receptor-mediated phagocytosis in polymorphonuclear leukocytes, a different cell lineage (32, 42). Garcia-Garcia et al. (15) have reported that inhibition of ERK by PD-98059 inhibition did not alter the nonspecific phagocytosis of erythrocytes by differentiated THP-1 cells, but inhibited IgG-opsonized erythrocyte phagocytosis in THP-1 cells stimulated with IFN-γ for 24 h. Our results under ambient pressure conditions differ from those of Garcia-Garcia et al. in that PD-98059 treatment stimulated serum-opsonized latex bead phagocytosis in our hands. This difference may be explained by the different pathways required for Fc receptor-mediated phagocytosis of IgG-opsonized erythrocytes and the complement- or integrin-mediated phagocytosis that is required for serum-opsonized latex bead uptake (1, 35). In addition, relatively shorter and weaker differentiation with IFN-γ employed by Garcia-Garcia et al. (15) may yield a different and less differentiated phenotype from the 3-day PMA treatment that we used to stimulate macrophage differentiation.

In addition, we observed enhancement of serum-opsonized latex bead phagocytosis in PMA-differentiated THP-1 macrophages in the setting of increased pressure, when ERK was also inhibited, and the inactivation of ERK by pressure appeared to contribute to the pressure effect on phagocytosis, because the pressure effect was substantially attenuated in the presence of ERK blockade. These data suggest that ERK inhibition may promote at least complement receptor- or integrin-mediated phagocytosis macrophage phagocytosis in some settings.

The inhibition of ERK by pressure in THP-1 cells is interesting because ERK is typically activated by mechanical forces in various other types of cells, such as osteoblasts (23), smooth muscle cells (57), intestinal epithelial cells (31, 58), cardiac fibroblasts (62), and cardiac myocytes (33). However, ERK is not activated by pressure in malignant colonocytes in suspension (53) or in rat neonatal cardiac fibroblasts expressing prominent stress fibers and high basal levels of smooth muscle actin (62). Interestingly, although cell stretching of mouse fibroblast L929 cells or human embryonic kidney-derived cells activated ERK in one report, the same authors found that the opposite deformation, contraction of a tonically stretched substrate on which these cells had been maintained, resulted in inactivation of ERK (48). Thus two different physical force deformations had different effects on ERK in these cells.

ERK is activated by a complex intracellular cascade converging on MEK, but an upstream role for FAK in ERK activation in response to some (although not all) stimuli has been postulated. For instance, overexpressing FAK in hepatocytes leads to ERK activation (8). Repetitive strain activates ERK in intestinal epithelial cells by a FAK-dependent pathway (31). On the other hand, ERK activation in SH-SY5Y human neuroblastoma cells by insulin-like growth factor-1 is independent of FAK (26). Our results suggest that the inhibition of FAK activation by pressure may subsequently inhibit ERK activation in differentiated THP-1 cells because the inhibitory effect on ERK activation by pressure did not appear in PMA-differentiated THP-1 macrophages when FAK protein expression was inhibited by transfection with FAK siRNA. Thus these results are consistent with a pathway in which inhibition of FAK-Y397 phosphorylation by pressure decreases ERK activation, leading in turn to the stimulation of macrophage phagocytosis.

In summary, these results demonstrate the previously unappreciated contribution of FAK and ERK activation to the regulation of particle phagocytosis in THP-1 macrophages. Furthermore, constant 20 mmHg pressure increased PMA-differentiated THP-1 cell phagocytosis through inhibition of FAK and ERK activation. The regulation of macrophage phagocytosis is likely to be a complex process and may involve other intracellular signals, such as protein kinase C, Syk, phosphatidylinositol 3-kinase, and the GTPases. The potential role of such other kinases in mediating the effect of pressure awaits further study. Furthermore, these are obvious differences between bead phagocytosis by THP-1 cells in culture and phagocytosis in human tissue. However, these observations raise the possibility that changes in tissue or capillary pressure in inflamed, infected, or edematous tissue may modulate macrophage phagocytosis by a novel pathway involving the inhibition of FAK and ERK.

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