The signaling pathways activated by the macrophage colony-stimulating factor (M-CSF) to promote survival of monocyte and macrophage lineage cells are not well established. In an effort to elucidate these pathways, we have used two cell types responsive to M-CSF: NIH 3T3 fibroblasts genetically engineered to express human M-CSF receptors (3T3-FMS cells) and human monocytes. M-CSF treatment induced M-CSF receptor tyrosine phosphorylation and recruitment of the p85 subunit of phosphatidylinositol 3-kinase (PI3K) to these receptors. These M-CSF receptor events correlated with activation of the serine/threonine kinase Akt. To clarify that PI3K products activate Akt in response to M-CSF, NIH 3T3 fibroblasts expressing mutant human M-CSF receptors (3T3-FMS(Y809F)) that fail to activate Ras in response to M-CSF also exhibit increased Akt kinase activity in response to M-CSF challenge. Furthermore, Akt appears to be the primary regulator of survival in 3T3-FMS cells, as transfection of genes encoding dominant-negative Akt isoforms into these fibroblasts blocked M-CSF-induced survival. In normal human monocytes, M-CSF increased the levels of tyrosine-phosphorylated proteins and induced Akt activation in a PI3K-dependent manner. The PI3K inhibitor LY294002 blocked M-CSF-mediated monocyte survival, an effect that was partially restored by caspase-9 inhibitors. These data suggest that M-CSF may induce cell survival through Akt-induced suppression of caspase-9 activation.

Monocytes are produced in bone marrow and normally circulate in the bloodstream for 24–48 h (1). In the absence of growth factors, circulating monocytes die of apoptosis (2–4). The growth factor M-CSF appears to be important in monocyte survival (5), as mice lacking M-CSF suffer deficiencies in circulating monocytes and macrophages (6). The phenotype of M-CSF-deficient animals illustrates the role of this growth factor in a variety of human diseases, including osteopetrosis (6), coronary artery disease (7), transplant vascular sclerosis (8), and cancer (9). M-CSF also appears to be important in the survival of human monocytes (4, 5, 10), although the specific intracellular mediators induced by M-CSF to promote cell survival are not well understood.

M-CSF is a growth factor that has its highest biological activity as a disulfide-linked dimer (10, 11). M-CSF dimers cross-link two tyrosine kinase M-CSF receptors (also known as FMS or colony-stimulating factor–1 receptors), inducing auto- and transphosphorylation of tyrosine residues in the cytoplasmic domains of these receptors (11). Phosphotyrosine residues in the cytoplasmic domains of M-CSF receptors induce translocation of intracellular signaling molecules via SH2 interactions and activate signaling cascades, resulting in the activation of both PI3K-dependent and Ras/mitogen-activated protein kinase-dependent pathways (12). Similar to normal human monocytes, NIH 3T3 fibroblasts transfected with the gene encoding the human M-CSF receptor (FMS gene) also activate these intracellular signaling pathways in response to human M-CSF (13). In contrast, NIH 3T3 fibroblasts expressing mutant forms of human M-CSF receptors containing a phenylalanine residue in place of the tyrosine residue at position 809 (3T3-FMS(Y809F)) do not activate Ras or undergo mitogenesis in response to human M-CSF (14, 15).

M-CSF receptors are structurally related to platelet-derived growth factor receptors (16, 17). To promote cellular survival, platelet-derived growth factor has been shown to recruit PI3K to the platelet-derived growth factor receptor and to activate the serine/threonine kinase Akt (also known as protein kinase B) (18, 19). Similarly, PI3K and Akt are activated in the insulin receptor activation pathway (20), and chimeric receptors constructed of the extracellular domains of M-CSF receptors and the cytoplasmic domains of insulin receptors activate Akt in response to M-CSF (21). PI3K appears to activate Akt by generating 3-phosphorylated second messengers, including phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate (22–24). Recent data suggest that phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate are likely important in Akt activation (21, 22), and the inositol 5-phosphatase SHIP and the inositol 3-kinase PTEN both suppress Akt activity (25–27).

Membrane-bound phosphatidylinositol 3,4,5-trisphosphate and phosphatidylinositol 3,4-bisphosphate target Akt via interactions with the pleckstrin homology domain in Akt, leading to the stimulation of its serine and threonine kinase activity (28–30). Activated Akt can then disengage from the membrane and interact with intracellular signaling elements to promote
cellular survival (31, 32). Akt promotes cellular survival by suppressing the activity of pro-apoptotic proteins and by suppressing the activity of the Forkhead DNA transcription factors. In monocytes, activation of the executioner caspase-3 appears to regulate survival (33), and recently, Akt has been shown to phosphorylate and inactivate caspase-9, thereby blocking caspase-3 activation (30).

There are data to suggest that Akt may be important in monocyte survival, as mice deficient in either SHIP or PTEN suffer expansion of tissue macrophage populations (34). As previously outlined, both of these phosphatases normally function to suppress Akt activation (25, 35). Because circulating monocytes do not proliferate and are precursors to tissue macrophages, these data suggest that Akt may also be important in monocyte survival. In light of these findings, this study was directed at identifying the signaling pathway activated by M-CSF to promote cell survival and at determining the role of PI3K and Akt in this signaling pathway.

We find that M-CSF induces tyrosine phosphorylation of human M-CSF receptors and recruitment of the p85 subunit of PI3K to these receptors in 3T3-FMS fibroblasts. Moreover, in these cells, M-CSF induces Akt kinase activity in a PI3K-dependent manner. Akt also appears to be central to M-CSF-induced survival in these fibroblasts, as dominant-negative Akt constructs block M-CSF-induced fibroblast survival. We found a similar M-CSF-induced signaling pathway in normal human monocytes, as M-CSF leads to Akt activation in these cells in a PI3K-dependent manner. In contrast, 3T3-FMS(Y809F) fibroblasts that do not activate Ras in response to M-CSF (14) display Akt kinase activity when stimulated with M-CSF. Moreover, PI3K inhibitors block M-CSF-induced Akt activation and survival in normal human monocytes. We found that monocytes express caspase-9, and consistent with an important role for Akt in monocyte survival, caspase-9 inhibitors reverse apoptosis induced by PI3K inhibitors in M-CSF-treated monocytes. These data suggest that PI3K and Akt likely play an important role in M-CSF-induced monocyte survival.

**EXPERIMENTAL PROCEDURES**

**Materials and Cell Lines—**Recombinant human M-CSF was purchased from R&D Systems (Minneapolis, MN). LY294002 was from Calbiochem. Dulbecco’s modified Eagle's medium (DMEM) and protein G-agarose were purchased from Life Technologies, Inc. RPMI 1640 medium was obtained from BioWhittaker, Inc. (Walkersville, MD). G-agarose were purchased from Life Technologies, Inc. RPMI 1640 medium was obtained from BioWhittaker, Inc. Walkersville, MD). Fetal calf serum was obtained from Hyclone Laboratories (Logan, UT). Anti-Akt, anti-c-FMS, anti-p85, and anti-phosphotyrosine (clone 4G10) antibodies were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). The caspase-9 inhibitor LEHD-fmk and LEHD-AFC fluorescent substrates were obtained from Enzyme Systems Products (Livermore, CA). Anti-caspase-9 polyclonal antibodies were obtained from Transduction Laboratories (Lexington, KY). NIH 3T3 fibroblasts engineered to express the human M-CSF receptor (3T3-FMS) or a mutant version of the M-CSF receptor (3T3-FMS/Y809F) was generated as described previously (17). All other reagents were from Sigma unless otherwise specified.

**Isolation of Peripheral Blood Monocytes and Cell Culturing—**Monocytes (66 ± 2.1% CD14+) were isolated from the heparinized blood of normal volunteers as described previously (4). For DNA fragmentation analysis, monocytes were cultured under the indicated conditions immediately after isolation from blood. For signaling experiments, monocytes were subsequently grown in RPMI 1640 medium + 10% fetal calf serum + 100 ng/ml recombinant human M-CSF for 16 h at 37°C. Samples were then serum-starved on ice in RPMI 1640 medium alone for 2 h before being subjected to stimulation with M-CSF as indicated in the figure legends. For signaling experiments using 3T3-FMS and 3T3-FMS/Y809F cells, samples were serum-starved in DMEM alone for 18 h and then subjected to stimulation as indicated in the figure legends with 100 ng/ml M-CSF.

**Immunoprecipitation and Immunoblotting—**Monocytes (10 x 10^6) in 100 µl of Hanks’ balanced salt solution (Life Technologies, Inc.) per sample were stimulated with 100 ng/ml M-CSF for the indicated times and then lysed by the addition of 900 µl of ice-cold lysis buffer (50 mM Tris-HCl (pH 7.2), 150 mM NaCl, 10 mM NaF, 0.5% deoxycholate, 10 mM EDTA, 0.1% SDS, and 1% Nonidet P-40) and incubated on ice for 15 min. Nuclei were removed by centrifugation, and samples were subjected to immunoprecipitation with anti-c-FMS (M-CSF receptor) antibodies overnight at 4°C. Immunoprecipitates were recovered by the addition of 0.5 mg/ml protein G-agarose for 1 h at 4°C. Laemmli sample buffer containing 2-mercaptoethanol was then added to the samples, and they were incubated at 95°C for 5 min. Samples were resolved by 10% SDS-PAGE, transferred to nitrocellulose, and probed with primary antibodies as indicated.

**Akt in Vitro Kinase Assays—**Akt in vitro kinase assays were performed as described previously (4). Briefly, samples were aliquoted at 5–10 x 10^6 cells/100 µl in RPMI 1640 medium and preincubated with the PI3K inhibitor LY294002 or an equal concentration of dimethyl sulfoxide carrier for 20 min at 37°C. Samples were then stimulated as indicated with 100 ng/ml recombinant human M-CSF. Samples were subsequently lysed by the addition of 900 µl of ice-cold buffer A (50 mM Tris-HCl (pH 7.5), 0.1% (w/v) Triton X-100, 1 mM EGTA, 50 mM NaF, 10 mM sodium glycerophosphate, 5 mM sodium pyrophosphate, 1 mM sodium orthovanadate, and 0.1% 2-mercaptoethanol) and incubated on ice for 30 min. Nuclei were then removed by centrifugation, and lysates were subjected to immunoprecipitation with 2 µg/sample anti-Akt antibodies (previously bound to protein G-agarose) for 90 min at 4°C. Samples were washed three times with buffer A, two times with buffer B (50 mM Tris-HCl (pH 7.5), 0.02% (w/v) Brij-35, 0.1 mM EGTA, and 0.1% 2-mercaptoethanol), and one time with assay dilution buffer (20 mM MOPS (pH 7.2), 2.5 mM sodium glycerophosphate (pH 7.0), 1 mM sodium orthovanadate, and 1 mM diithiothreitol). Immune complexes were then resuspended in 30 µl of assay dilution buffer containing 10 µg of histone 2B, 25 mM MgCl2, 200 µM ATP, and 10 µCi [gamma-32P]ATP (3000 Ci/mmol) and incubated for 20 min at 30°C. Reactions were stopped by the addition of the appropriate volume of 5 x Laemmli sample buffer and heated to 95°C for 5 min. Samples were resolved by 15% SDS-PAGE, transferred to nitrocellulose, and evaluated by autoradiography. Nitrocellulose filters were subsequently probed with antibodies to Akt and evaluated by enhanced chemiluminescence (ECL, Pierce).

**Transient Transfections—**In six-well plates, 3T3-FMS cells were incubated with DMEM supplemented with 5% fetal calf serum at 37°C and 5% CO2 until 70–80% confluent. Dr. Thomas Franke (Columbia University, New York, NY) provided Akt constructs encoding wild-type Akt (hemagglutinin-Akt) or catalytically inactive Akt (K179M) inserted into pCMV6 vectors and pCMV6 vectors alone. Akt constructs or the pCMV6 vector alone was transfected into cells using LipofectAMINE (Life Technologies, Inc.) according to the manufacturer’s protocol for transient transfections. Briefly, samples were incubated with a LipofectAMINE DNA mixture for 6 h at 37°C. DMEM containing 20% fetal calf serum was then added 1:1 to the transfection mixture, and samples were incubated for an additional 24 h. Samples were then washed and incubated in serum-free DMEM containing 100 ng/ml M-CSF as the only growth factor.

**Cytosolic DNA Fragmentation Analysis—**Monocytes were incubated in RPMI 1640 medium supplemented with 5% fetal calf serum and 10 µg/ml polymixin B at 37°C and 5% CO2 as indicated for 18 h before oligonucleosomal DNA fragmentation analysis. Apoptotic DNA fragments were purified from 2 x 10^6 monocytes/sample using a DNA isolation kit (Suicide-Track DNA Isolation kit, Oncogene Research Products, Cambridge, MA). DNA fragments were resolved by 1.2% agarose gel electrophoresis. DNA bands were visualized by staining with ethidium bromide (Molecular Probes, Inc., Eugene, OR), and the DNA fragments were analyzed on a digital gel documentation system (GelDoc 1000, Bio-Rad).

**Detection of Caspase-9-like Activity by Fluorogenic LEHD substrate—**Cell lysates were assayed for LEHD activity using a fluorosubstrat. Extracts were incubated with LEHD-AFC in a cyto-buffer (10% glycerol, 50 mM PIPES (pH 7), 1 mM EDTA) containing 1 mM diithiothreitol and 20 µM LEHD-AFC. Release of free AFC was determined using a CytoFluor 4000 (Perspective Co.).

**Statistical Analysis—**Densitometry of small molecular weight cytosolic DNA fragments was carried out on DNA gels using the GelDoc 1000 imaging system. These densitometric measurements under different conditions were compared using analysis of variance testing with Tukey’s post-hoc analysis (Minitab Software, State Park, PA). Statistical significance was defined as p < 0.05.

**RESULTS**

M-CSF Treatment Induces M-CSF Receptor Tyrosine Phosphorylation and Association with p85 PI3K in 3T3-FMS Fibro-
M-CSF Induces Cell Survival through Akt Activation

FIG. 1. M-CSF induces receptor phosphorylation and p85 recruitment in M-CSF receptor-expressing NIH 3T3 fibroblasts (3T3-FMS). 3T3-FMS fibroblasts (5 × 10^6/sample) were serum-starved and stimulated for the indicated times (in minutes) with 100 ng/ml M-CSF or were not stimulated (NS). Samples were then lysed and subjected to immunoprecipitation with anti-M-CSF receptor antibodies or control IgG (denoted by C). Immunoprecipitates were resolved by 10% SDS-PAGE, transferred to nitrocellulose, and blotted with antibodies to phosphotyrosine (A). The filter shown in A was subsequently stripped and reprobed with antibodies to the p85 subunit of PI3K (B). These data are representative of two independent experiments.

M-CSF Mediates Fibroblast Survival in Response to M-CSF Stimulation—To explore the role of Akt in cell survival, we next transfected genes encoding wild-type Akt isoforms, dominant-negative Akt isoforms, or control pCMV6 vector constructs into 3T3-FMS fibroblasts. Cells transfected with dominant-negative Akt constructs (K179M) demonstrated reduced numbers of surviving cells in the presence of M-CSF as the only growth factor, whereas survival was maintained in cells transfected with genes encoding wild-type Akt or vector constructs (Fig. 3A). To statistically compare the effects of the various constructs on cell survival, we arbitrarily defined the cells transfected with genes encoding wild-type Akt as the control condition. In comparison with wild-type Akt-transfected cells, 3T3-FMS fibroblasts transfected with genes encoding dominant-negative K179M Akt isoforms demonstrated significantly reduced numbers of surviving cells at 18 h, whereas those transfected with vector constructs did not (p = 0.016 for numbers of cells transfected with K179M Akt isoforms versus other conditions) (Fig. 3B).

M-CSF Induces Akt Kinase Activity in a PI3K-dependent Manner—We next investigated the effects of M-CSF treatment on the activation of the survival factor Akt in these 3T3-FMS fibroblasts. M-CSF treatment led to transient but significant increases in Akt kinase activity in these cells, which were suppressed by preincubation with the PI3K inhibitor LY294002 (Fig. 2A). Akt immunoblots of the membranes used for the Akt in vitro kinase assays demonstrated equal loading of Akt under each condition (Fig. 2B). Of note, the PI3K inhibitor wortmannin (10 nM) also suppressed Akt activation (data not shown).

As proof that the induction of Akt by M-CSF does not require Ras activation, we found that M-CSF also stimulated increases in Akt kinase activation in 3T3-FMS(Y809F) cells (Fig. 2C), even though this mutant receptor cannot activate Ras in response to M-CSF (15, 37). Akt immunoblots of these samples demonstrated that equal amounts of Akt were assayed under the various conditions (Fig. 2D).

Akt Mediates Fibroblast Survival in Response to M-CSF Stimulation—To explore the role of Akt in cell survival, we next transfected genes encoding wild-type Akt isoforms, dominant-negative Akt isoforms, or control pCMV6 vector constructs into 3T3-FMS fibroblasts. Cells transfected with dominant-negative Akt constructs (K179M) demonstrated reduced numbers of surviving cells in the presence of M-CSF as the only growth factor, whereas survival was maintained in cells transfected with genes encoding wild-type Akt or vector constructs (Fig. 3A). To statistically compare the effects of the various constructs on cell survival, we arbitrarily defined the cells transfected with genes encoding wild-type Akt as the control condition. In comparison with wild-type Akt-transfected cells, 3T3-FMS fibroblasts transfected with genes encoding dominant-negative K179M Akt isoforms demonstrated significantly reduced numbers of surviving cells at 18 h, whereas those transfected with vector constructs did not (p = 0.016 for numbers of cells transfected with K179M Akt isoforms versus other conditions) (Fig. 3B).

M-CSF Induces Akt Kinase Activity in Normal Human Monocytes—M-CSF stimulated significant increases in total cellular tyrosine phosphorylation in normal human monocytes, which peaked at 2–5 min (Fig. 4A). Similar to the M-CSF-activated survival pathway defined in fibroblasts, M-CSF also stimulated Akt kinase activity in these monocytes (Fig. 4B). Moreover, the PI3K inhibitor LY294002 suppressed M-CSF-activated Akt kinase activity in these cells, whereas the mitogen-activated protein kinase kinase inhibitor PD098059 did not (Fig. 4C). We hypothesized that if Akt is important in monocyte survival in response to M-CSF, then PI3K inhibitors would suppress monocyte survival. Consistent with an important role of Akt in monocyte survival, the PI3K inhibitor LY294002 blocked M-CSF-induced survival in a dose-dependent manner (Fig. 5). Of note, wortmannin at doses of 10 and 1 nM also suppressed M-CSF-induced monocyte survival (data not shown).

Monocytes Express Caspase-9, and Caspase-9 Inhibitors Rescue Monocytes from Apoptosis—We next sought to analyze caspase-9 expression in human monocytes. Immunoprecipitation experiments with anti-caspase-9 or control antibodies revealed a band the size of caspase-9 in the immune antibody precipitates (Fig. 6A), but not immunoprecipitation experiments performed with control antibodies. Consistent with functional caspase-9 in human monocytes, fluorogenic assays with a specific caspase-9 substrate (LEHD) revealed caspase-9-like activity in nonstimulated apoptotic cells, but this was reduced in cells incubated with M-CSF (Fig. 6B). Since Akt presumably suppresses caspase-9 activity, we reasoned that if Akt is the PI3K-dependent mediator of monocyte survival induced by M-CSF, then caspase-9 inhibitors may rescue monocytes from apoptosis. Caspase-9 inhibitors reversed monocyte apoptosis.
found in growth factor-deprived cells (Fig. 6C). Moreover, these inhibitors also partially reversed apoptosis induced by PI3K inhibitors in M-CSF-treated monocytes (Fig. 6C). As determined by densitometry of four separate experiments, caspase-9 inhibitors significantly suppressed monocyte apoptosis induced by either growth factor deprivation or PI3K inhibitors in the presence of M-CSF (p = 0.016). The data represent two independent experiments.

DISCUSSION

This study begins to identify the signaling pathway activated by M-CSF to induce cell survival. Initially using 3T3 fibroblasts that express human M-CSF receptors (3T3-FMS), we have shown that human M-CSF induces tyrosine phosphorylation in normal human monocytes. Monocytes were isolated from the peripheral blood of normal human donors as outlined under “Experimental Procedures.” Cells (3 × 10^6/sample) were serum-starved and then stimulated for the indicated times (in minutes) with 100 ng/ml M-CSF or were not stimulated (NS). Some samples were preincubated with 50 μM LY294002 or 10 μM PD098059 (both in Me2SO (DMSO)) or an equal concentration of Me2SO alone for 15 min before M-CSF stimulation. Samples not receiving LY294002 received an equal concentration of Me2SO alone. Apoptotic DNA was isolated according to procedures outlined under “Experimental Procedures” and resolved by agarose gel electrophoresis. These data are representative of three independent studies. bp, base pair.

FIG. 5. The PI3K inhibitor LY294002 inhibits M-CSF-stimulated monocyte survival. Monocytes were isolated from the peripheral blood of normal human donors as outlined under “Experimental Procedures.” Cells (3 × 10^6/sample) were incubated for 18 h in 100 ng/ml M-CSF (except where indicated) and the indicated amounts of the PI3K inhibitor LY294002. Samples not receiving LY294002 received an equal concentration of Me2SO alone. Apoptotic DNA was isolated according to procedures outlined under “Experimental Procedures” and resolved by agarose gel electrophoresis. These data are representative of three independent studies. bp, base pair.

FIG. 4. M-CSF activates tyrosine phosphorylation and Akt activation in normal human monocytes. Monocytes were isolated from the peripheral blood of normal human donors as outlined under “Experimental Procedures.” Cells (10 × 10^6/sample) were serum-starved and then stimulated for the indicated times (in minutes) with 100 ng/ml M-CSF or were not stimulated (NS). Some samples were preincubated with 50 μM LY294002 or 10 μM PD098059 (both in Me2SO (DMSO)) or an equal concentration of Me2SO alone for 15 min before M-CSF stimulation. Samples were lysed and immunoprecipitated with anti-phosphotyrosine antibodies (A), anti-Akt antibodies (B and C), or control antibodies (denoted by C above the first lanes in B and C). A, immunoprecipitates were resolved by SDS-PAGE, transferred to nitrocellulose, and blotted with anti-phosphotyrosine antibodies. B, Akt immunoprecipitates were subjected to an in vitro kinase assay using histone 2B (H2B) as a substrate and [γ-32P]ATP as the phosphate donor. Kinase reaction products were resolved by SDS-PAGE, transferred to nitrocellulose, and exposed to film. C, the filter shown in B was subsequently reprobed with antibodies to Akt. Note that the PI3K inhibitor LY294002 suppressed Akt kinase activity in response to M-CSF. These data are representative of two independent studies. IgG H.C., IgG heavy chain.

FIG. 3. Akt mediates M-CSF-induced survival in 3T3-FMS fibroblasts. 3T3-FMS fibroblasts were grown to 70% confluence and then transfected with genes encoding wild-type Akt in the pCMV6 vector (Wt-Akt), catalytically inactive Akt (K179M), or pCMV6 vector alone (Vector) as outlined under “Experimental Procedures” (A). Samples were subsequently grown in serum-free medium supplemented with M-CSF as the only growth factor for 24 h, upon which time cells were quantified by averaging the numbers of surviving cells in three high-powered fields (HPF) (B). 3T3-FMS fibroblasts transfected with genes encoding the catalytically inactive isoform of Akt reduced survival versus cells transfected with genes encoding wild-type Akt or vector transfections (p = 0.016).
M-CSF Induces Cell Survival through Akt Activation

Monocytes contain functional caspase-9, and caspase-9 inhibitors block monocyte apoptosis. A, monocytes (10 × 10⁶/condition) were lysed, and immunoprecipitations were performed using a control IgG antibody or anti-caspase-9 IgG. The samples were separated by SDS-PAGE and immunoblotted with anti-caspase-9 IgG. B, monocytes (3 × 10⁶/condition) were left untreated or treated with M-CSF (100 ng/ml) alone or with the caspase-9 inhibitor LEHD-fmk (5 or 50 μM) for 18 h. The cells were lysed, and caspase-9 activity was measured using a fluorescent-labeled LEHD substrate. C, monocytes (3 × 10⁶/ml) were incubated as indicated with the cell-permeable caspase-9 inhibitor LEHD-fmk (100 μM) or the PI3K inhibitor LY294002 (25 μM) for 30 min. M-CSF (100 ng/ml) was then added to the cells (except where indicated) for 18 h. D, apoptosis was defined as cytosolic oligonucleosomal DNA fragmentation and by comparing the percent of densitometric units (D.U.) from cytosolic low molecular weight DNA bands from cells incubated in the absence of M-CSF or inhibitors (control) with those under other experimental conditions (p < 0.01 for M-CSF + LY294002 + LEHD-fmk or cells treated with LEHD-fmk alone from control cells). These data are representative of two independent studies. bp, base pair.
first time that Akt is activated by M-CSF and that the Akt kinase pathway is potentially important in normal human monocytes, presumably by suppressing caspase-9 activation. We also demonstrate that PI3K and Akt are important mediators of survival induced by M-CSF.

This study suggests that PI3K and Akt mediate monocyte survival in response to M-CSF. Findings in mutant and transgenic animals support an important role for PI3K and Akt in the monocyte survival pathway. Transgenic animals deficient in the inositol 3-phosphatase PTEN or the inositol 5-phosphatase SHIP suffer macrophage accumulation and organ dysfunction (34, 47). Interestingly, both PTEN and SHIP can suppress Akt kinase activity (23–25, 48). Based on our findings that M-CSF induces Akt kinase activity and that Akt is required for 3T3-FMS fibroblast survival in response to M-CSF as the lone growth factor, we speculate that Akt may also play an important role in monocyte survival. Consistent with this, we found evidence for an important role for Akt in monocyte survival via M-CSF. PI3K inhibitors block M-CSF-induced monocyte survival and Akt activation, which ultimately results in caspase-9-dependent apoptosis.

In summary, this study shows that M-CSF induces Akt kinase activity in a PI3K-dependent manner in NIH 3T3 fibroblasts expressing human M-CSF receptors and in normal human monocytes. M-CSF-induced recruitment of PI3K to the M-CSF receptor and subsequent PI3K activity are critical in mediating the protective effects of M-CSF on monocyte survival. Transfection studies also demonstrated that Akt is a critical determinant of survival in response to M-CSF in these M-CSF receptor-bearing fibroblasts. Together, these data suggest that PI3K and Akt are important regulators of survival in cell types responsive to M-CSF, at least in part, by suppressing caspase-9 activation. Given these data, these enzymes may represent potential targets for therapies aimed at limiting inappropriate monocyte survival in inflammatory human diseases.

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