Akt activation requires phosphorylation of Thr\(^{308}\) and Ser\(^{473}\) by 3-phosphoinositide-dependent kinase-1 and 2 (PDK1 and PDK2), respectively. While PDK1 has been cloned and sequenced, PDK2 has yet to be identified. The present study shows that phosphatidylinositol 3-kinase-dependent p38 kinase activation regulates Akt phosphorylation and activity in human neutrophils. Inhibition of p38 kinase activity with SB203580 inhibited Akt Ser\(^{473}\) phosphorylation following neutrophil stimulation with formyl-methionyl-leucyl-phenylalanine, Fc\(\gamma\)R cross-linking, or phosphatidylinositol 3,4,5-trisphosphate. Concentration inhibition studies showed that Ser\(^{473}\) phosphorylation was inhibited by 0.3 \(\mu\)M SB203580, while inhibition of Thr\(^{308}\) phosphorylation required 10 \(\mu\)M SB203580. Transient transfection of HEK293 cells with adenoviruses containing constitutively active MKK3 or MKK6 resulted in activation of both p38 kinase and Akt. Immunoprecipitation and glutathione S-transferase (GST) pull-down studies showed that Akt was associated with p38 kinase, MK2, and Hsp27 in neutrophils, and Hsp27 dissociated from the complex upon activation. Active recombinant MK2 phosphorylated recombinant Akt and Akt in anti-Akt, anti-MK2, anti-p38, and anti-Hsp27 immunoprecipitates, and this was inhibited by an MK2 inhibitory peptide. We conclude that Akt exists in a signaling complex containing p38 kinase, MK2, and Hsp27 and that p38-dependent MK2 activation functions as PDK2 in human neutrophils.

The serine/threonine kinase protein kinase B, also called Akt, is the cellular homologue of a viral oncogene, v-Akt (1–3). Akt contains a pleckstrin homology domain at its N terminus, a catalytic domain, and a short C-terminal tail, and is closely related to protein kinase A and protein kinase C in its amino- and C-terminal regions. Akt plays a critical role in mediating cell proliferation, differentiation, and survival signals propagated from certain growth factors (4, 5). Akt activation is dependent on phosphatidylinositol 3-kinase (PI-3K), since wortmannin and dominant negative mutants of PI-3K block Akt activation (6) and constitutively active mutants of PI-3K activate Akt (7, 8). Activation of Akt requires that the products of PI-3K, phosphatidylinositol 3,4,5-trisphosphate (PIP\(_3\)) and phosphatidylinositol 3,4-bisphosphate interact with the pleckstrin homology domain of Akt and recruit it to the plasma membrane (4, 9, 10, 11). Subsequently, Akt undergoes phosphorylation at two sites, Thr\(^{308}\) in the kinase domain and Ser\(^{473}\) in the C-terminal domain. Phosphatidylinositol 3,4-bisphosphate and PIP\(_3\) activate 3-phosphoinositide-dependent kinase-1 (PDK1), which phosphorylates Thr\(^{308}\) (4, 12). Phosphorylation of Ser\(^{473}\) is also dependent on products of PI-3K; however, the identity of this kinase, termed PDK2, is unknown (11, 12).

Cellular stresses, such as heat shock and hyperosmolarity, stimulate both p38 kinase and Akt activity (13). p38 kinase, a homologue of the yeast HOG1, is activated by dual phosphorylation on Thr and Tyr within a Thr-Gly-Tyr motif by MAP kinase kinases MKK3 and MKK6 (14, 15). The activation of MKK3 and MKK6 is regulated by phosphorylation on Ser and Thr residues by one of several MAP kinase kinases. Chemotaxtractant stimulation and cross-linking of Fc\(\gamma\)R receptors stimulate PI-3K-dependent transient activation of Akt and p38 kinase in human neutrophils (16–18). MAP kinase-activated protein kinase-2 (MK2), a direct target of p38, has been reported to phosphorylate Ser\(^{473}\) of Akt in vitro (19), and activated Akt associates with a substrate of MK2, heat shock protein 27 (Hsp27) (20). Direct regulation of Akt activity by p38 kinase, however, has not been demonstrated previously. Additionally, Alessi et al. (19) suggested that a role for the p38 kinase module in Akt activation was unlikely in intact cells, since IGF-1 activates Akt, but not MK2, in HEK 293 cells. The

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The present study examined the hypothesis that p38 kinase regulates Akt activation in human neutrophils. We show for the first time that Akt activation is regulated by PI-3K-mediated p38 kinase activity in intact cells. We also report that Akt forms a stable complex with p38 kinase, MK2, and Hsp27 and that, upon stimulation with FMLP, Hsp27 dissociates from this complex.

**EXPERIMENTAL PROCEDURES**

**Materials**—PD98059, SB203580, Wortmannin, and LY294002 were obtained from Calbiochem. Final concentrations used were: PD98059, 10 μM; SB203580, 10 μM Wortmannin, and 100 μM LY294002, except where otherwise indicated. FMLP and histone H2B were obtained from Sigma. GST-Akt agarose beads, active recombinant MK2, anti-Akt2, and anti-Akt3 were obtained from Upstate Biotechnology, Inc. (Lake Placid, NY). Anti-phospho-p38, anti-phospho, and anti-phospho-Ser185,-Thr189,-Akt, anti-phospho-Thr308,-Akt, and anti-Akt antibodies were obtained from New England Biolabs, Inc. (Beverly, MA). Recombinant Hsp27 and anti-mouse Hsp27 were obtained from Stressgen Biotechnologies Corp. (Victoria, Canada). Anti-MK2 was obtained from Research Biochemicals International (Natick, MA). Anti-FcγRIIIa Fab monoclonal antibody (IV.3) and FcγRIIB F(ab’2) monoclonal antibody (3G8) were obtained from Medarex (Annandale, NJ). Goat anti-mouse IgG conjugated to horseradish peroxidase IgG were obtained from Bethyl Laboratories, Inc. (Montgomery, CA). Goat anti-mouse IgG specific for F(ab’)2 was obtained from Biocytogen, Inc. (Lexington, KY). The synthetic EGFR peptide (NH2-RRELVE-QTVDRSLNGVAEIR-amine) was obtained from the Macromolecular Structure Analysis Facility at the University of Kentucky (Lexington, KY). The synthetic EGF peptide (NH2-RELEVE-PLTFSGEPNQALLR-COOH) was obtained from Macromolecular Resources, Colorado State University (Fort Collins, CO).

**Neutrophil Isolation**—Neutrophils were isolated from healthy donors using plastic Percoll gradients, as described previously (21). After isolation, neutrophils were washed and resuspended with lipopolysaccharide-free Krebs-Ringer phosphate buffer (pH 7.2) containing 0.2% dextrose (Kreb’s). Microscopic evaluation of isolated cells treated by trypan blue exclusion indicated that 95% of cells were neutrophils and those were >98% viable.

**p38 MAP Kinase Activity**—For cross-linking, for p38 MAP kinase activity was measured by as-saying the ability of immunoprecipitated enzyme to phosphorylate glutamine aptamer for 30 min and the reaction was terminated by the addition of 6 μl of 6× Laemmli buffer. Samples were boiled for 5 min, the products were resolved by 10% SDS-PAGE, and products were visualized by autoradiography.

**Measurement of p38 Kinase Activity**—p38 MAP kinase activity was assayed by using the ability of immunoprecipitated enzyme to phosphorylate glutamine aptamer for 30 min and the reaction was terminated by the addition of 6 μl of 6× Laemmli buffer. Samples were boiled for 5 min, the products were resolved by 10% SDS-PAGE, and products were visualized by autoradiography.

**Preparation of GST and GST-MK2-Sepharose Beads**—GST, pGEX-2T and MK2-GST-pGEX2T DNA were transformed into E. coliDH5α and E. coli BL21(DE3)pLysS, and the expression and purification of GST and GST-MK2 fusion protein was performed as described previously (25).

**Western Blot Analysis of Phospho-p38 and Phospho-Akt**—Tyrosine phosphorylation of ERK and p38 kinase and phosphorylation of Ser473 or Thr308 on Akt was determined by Western blotting with phosphospecific antibody. Following lysis, proteins were separated by electrophoresis using 1% SDS-PAGE and transferred to polyvinylidene difluoride membranes. Membranes were blocked with 5% milk/TBST and then incubated with anti-phospho-ERK, or anti-phospho-Akt (1:1000), anti-Hsp27 (1:1000), anti-MK2 (1:2000), and anti-insulin receptor substrate (IRS) antibodies. Membranes were washed three times with TBST and then incubated with horseradish peroxidase-conjugated, secondary antibody in 5% milk/TBST. Membranes were washed and processed for autoradiography.

**Phosphorylation with Active Recombinant MK2**—Neutrophils (2 x 10^6) were lysed with 200 μl of IP lysis buffer. GST-Akt agarose, GST-MK2 Sepharose, protein A-Sepharose, or GST-Sepharose beads were added to the precleared neutrophil lysate and incubated overnight at 4 °C with shaking. Protein A-Sepharose beads (15 μl) were then added, and samples were rotated for an additional 2 h at 4 °C. Beads were washed once by centrifugation in Krebs buffer and then resuspended in 50 μl of 2× Laemmli buffer and boiled for 3 min. Proteins were separated by electrophoresis using 10% SDS-PAGE. Proteins were transferred onto nitrocellulose membrane and blocked with 5% milk/TBST for 1 h. Blots were probed with anti-p38 (1:1000), anti-Hsp27 (1:1000), anti-MK2 (1:2000), or anti-Akt (1:1000) antibody in 5% BSA/TBST. Products were visualized by chemiluminescence. To verify equal loading of protein in each lane, the blots were stripped and reprobed for total p38, ERK, or Akt.

**Immunoprecipitation of p38, MK2, Hsp27, and Akt**—Neutrophils (2 x 10^6) were prewarmed for 37 °C for 5 min prior to stimulation with or without 0.5 μM FMLP. The reactions were stopped by centrifugation followed immediately by the addition of 200 μl of immunoprecipitation (IP) lysis buffer containing 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% (v/v) Triton X-100, 0.5% (v/v) Nonidet P-40, 1 mM EDTA, 1 mM EGTA, 20 mM sodium orthovanadate, 10 μM p-nitrophenol phosphate, 20 μM NaF, 5 mM phenylmethylsulfonyl fluoride, 21 μg/ml aprotinin, and 5 μg/ml leupeptin. Following centrifugation at 15,000 × g for 15 min at 4 °C, cleared lysates were incubated with 5 μl of anti-Akt antiseraum, 3 μl of anti-p38, 2 μl of anti-Hsp27, or 5 μl of anti-MK2 overnight with continuous rotation at 4 °C. Protein A-Sepharose beads (15 μl) were then added and samples were rotated for an additional 2 h at 4 °C. Beads were washed once by centrifugation in Krebs buffer and then resuspended in 50 μl of 2× Laemmli buffer and boiled for 3 min. Proteins were separated by electrophoresis using 10% SDS-PAGE transferred to nitrocellulose membrane and blocked with 5% milk/TBST for 1 h. Blots were probed with anti-p38 (1:1000), anti-Hsp27 (1:1000), anti-MK2 (1:2000), or anti-Akt (1:1000) antibody. Membranes were visualized by chemiluminescence.
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**RESULTS**

**p38 kinase but Not ERK Regulates Akt Phosphorylation**—Both formyl peptide receptors and Fcy receptors stimulate Akt phosphorylation in human neutrophils (16). To determine the optimal time of stimulation, a time course of Akt Ser\(^{473}\) phosphorylation following the addition of 3 \times 10^{-7} \text{M} fMLP or Fc\(\gamma\)R cross-linking was performed. Both agonists stimulated optimal Akt phosphorylation at 2 min (data not shown).

To investigate the involvement of ERK and p38 in PI-3K-dependent Akt activation in neutrophils, we measured fMLP-stimulated Akt Ser\(^{473}\) phosphorylation in the presence or absence of LY294002, 100 \text{nm} Wortmannin, PD98059, or SB203580. Fig. 1a shows that wild-type, LY294002, and SB203580 inhibited fMLP-stimulated Akt Ser\(^{473}\) phosphorylation, while PD98059 had no effect. To determine whether p38 regulation of Akt Ser\(^{473}\) phosphorylation was unique to chemotaxtractant receptors, we examined the effect of SB203580 on Fc\(\gamma\)R-stimulated Akt Ser\(^{473}\) phosphorylation (Fig. 1b). Pretreatment with SB203580 blocked Akt Ser\(^{473}\) phosphorylation stimulated by Fc\(\gamma\)IIa and Fc\(\gamma\)IIb receptor cross-linking. Thus, p38 kinase inhibition attenuates both formyl peptide receptor and Fc\(\gamma\)R-stimulated Akt phosphorylation.

**Ser\(^{473}\) is More Sensitive to SB203580 Inhibition than Thr\(^{308}\)—**A previous study found that SB203580 inhibited PDK1 phosphorylation of Akt Thr\(^{308}\) at concentrations greater than 3 \muM (27). To separate the effects of SB203580 on PDK1 and p38 kinase, we performed concentration inhibition experiments on phosphorylation of Thr\(^{308}\) and Ser\(^{473}\). Neutrophils were pretreated with varying concentrations of SB203580 for 1 h prior to stimulation with fMLP. Concentration inhibition studies showed that SB203580 reduced fMLP-stimulated phosphorylation of Ser\(^{473}\) at 0.3 \muM, while at least 10 \muM SB203580 was required to see a diminution of Thr\(^{308}\) phosphorylation (Fig. 2). The concentrations of SB203580 required to inhibit Akt Thr\(^{308}\) phosphorylation (10 \muM) are \(\sim\)20-fold higher than the IC\(_{50}\) for p38 kinase inhibition (0.3–0.5 \muM) (27), while Akt Ser\(^{473}\) phosphorylation is inhibited by SB203580 at the IC\(_{50}\) for p38 kinase. These results suggest that inhibition of p38 kinase attenuates Ser\(^{473}\) phosphorylation, while Thr\(^{308}\) phosphorylation is independent of p38 kinase.

**p38 Kinase Mediates PIP\(_3\)-dependent Akt Phosphorylation**—To determine whether p38 kinase is upstream or downstream of PI-3K in the pathway leading to Akt activation, we examined the ability of PIP\(_3\) to stimulate p38 kinase and Akt Ser\(^{473}\) phosphorylation in neutrophils. PIP\(_3\) stimulated both p38 kinase (Fig. 3a) and Akt Ser\(^{473}\) phosphorylation (Fig. 3b) in a time-dependent manner with optimal stimulation at 1 min. To determine whether PIP\(_3\)-stimulated Akt phosphorylation was mediated by p38 kinase, neutrophils were pretreated with 10 \muM SB203580 prior to the addition of PIP\(_3\). Fig. 3b shows that SB203580 inhibited PIP\(_3\)-mediated Akt Ser\(^{473}\) phosphorylation, indicating that p38 kinase activation is necessary for PI-3K-mediated activation of Akt in human neutrophils. PIP\(_3\) also stimulated ERK activation with a time course similar to p38 activation (data not shown).

**Constitutively Active MKK3/6 Stimulates Akt Activation in HEK 293 Cells**—Since neutrophil half-life is not long enough to allow genetic manipulation, HEK 293 cells were transiently transfected with adenoviruses containing empty vector, MKK3\(\beta\)E (constitutively active MKK3), MKK3A (dominant negative MKK3), MKK6\(\beta\)E (constitutively active MKK6), or MKK6A (dominant negative MKK6) to directly examine the ability of p38 kinase to stimulate Akt activation. Fig. 4a shows that MKK3\(\beta\)E and MKK6\(\beta\)E stimulated increased Akt activity, while the dominant negative mutants had no effect. Both MKK3\(\beta\)E and MKK6\(\beta\)E stimulated increased p38 kinase activity (Fig. 4b), as measured by an in vitro kinase assay using ATP\(_{\gamma}\)S as substrate.

**Akt Is Physically Associated with Components of the p38 Kinase Pathway**—Previous studies reported that MK2 phosphorylates Akt Ser\(^{473}\) in vitro and that Hsp27 associates only with active Akt (19, 20). Coupled with our data showing that phosphorylation of Ser\(^{473}\) is dependent on p38 kinase, we postulated that Akt exists in a signaling complex with MK2 and p38 kinase. Therefore, the association of Akt with p38 kinase, MK2, and Hsp27 was examined in unstimulated and stimulated neutrophils. Lysates prepared from unstimulated and fMLP-stimulated cells were immunoprecipitated with anti-Akt antibody. Proteins in the immunoprecipitate were separated by SDS-PAGE and immunoblotted with anti-Akt, anti-p38, anti-MK2, and anti-Hsp27. Fig. 5a shows that p38 kinase, MK2, and Hsp27 were all present in Akt immunoprecipitates from unstimulated cells. Stimulation with fMLP resulted in a time-dependent dissociation of Hsp27 from the complex. Additionally,
neutrophil lysates immunoprecipitated with anti-Hsp27, anti-MK2, or anti-p38 were immobiloblotted for Akt. Akt was detected in all three immunoprecipitates (data not shown).

Another method for detecting protein-protein interactions is a GST pull-down assay. GST-fused Akt or MK2 proteins were expressed in E. coli and were immobiloblotted on glutathione-agarose or glutathione-Sepharose beads. Neutrophil lysates were incubated with the protein-immobilized beads or GST-Sepharose beads. The proteins attached to the beads were separated by SDS-PAGE and immunoblotted for Akt, p38 kinase, MK2, and Hsp27. GST-MK2 associated with p38 kinase, MK2, and Hsp27. GST-Sepharose beads alone did not bind to Akt, p38 kinase, MK2, or Hsp27 from neutrophil lysates (data not shown).

**MK2 Phosphorylation of Akt**—A direct target of p38 kinase, MK2, phosphorylates Akt in vitro (19). A previous report suggested, however, that MK2 is unlikely to mediate Akt activation because agonists that activate Akt in HEK 293 cells failed to activate MK2. We examined the ability of recombinant active MK2 to phosphorylate recombinant Akt (Fig. 6a) and Akt present in anti-Akt, anti-MK2, anti-p38, and anti-Hsp27 immunoprecipitates from human neutrophils. Fig. 6a, a and b, shows that MK2 stimulated phosphorylation of a 66-kDa protein in all conditions. The phosphorylated protein was trypsin-digested, and resulting peptides were subjected to matrix-assisted laser desorption mass spectroscopic analysis and identified by peptide mass fingerprinting to be Akt. Recombinant MK2 also stimulated phosphorylation of Ser473 Akt in anti-Akt, anti-MK2, and anti-Hsp27 immunoprecipitates from neutrophils (Fig. 6c). To determine whether the differences in the role of MK2 as PKD2 could be due to cell-specific differences in the Akt isoforms, we immobiloblotted neutrophil lysates with anti-Akt1, anti-Akt2, and anti-Akt3 antibodies. All three isoforms of Akt were present in human neutrophils (data not shown).

**Inhibition of Akt Phosphorylation and Activation by MK2 Inhibitory Peptide**—Zu et al. (22) reported that a peptide representing the phosphorylation site of Hsp27 inhibited MK2 phosphorylation of substrates. A concentration inhibition study showed that a 160 μM concentration of the MK2 inhibitory peptide was required to reduce fMLP-stimulated MK2 phosphorylation of Hsp27 (Fig. 7a). A nonrelated peptide at the same concentration did not reduce MK2-mediated Hsp27 phosphorylation. The concentration of the MK2 peptide required was significantly greater than the 30 μM concentration reported by Zu et al. (22). A similar concentration of the MK2 peptide was required to inhibit the ability of active recombinant MK2 to phosphorylate Akt from neutrophil anti-Akt immunoprecipitates (Fig. 7b). To determine the role of MK2 in Akt phosphorylation and activation, we preincubated neutrophils with the MK2 inhibitory peptide prior to stimulation with fMLP. Intracellular delivery of the inhibitory peptide reduced both fMLP-stimulated Akt Ser473 phosphorylation (Fig. 7c) and Akt activity (Fig. 7d), while a nonrelated peptide had no effect. Taken together, our results indicate that MK2 phosphorylates Ser473, which leads to activation of Akt in human neutrophils.

**ERK Activation Is Independent of Akt**—We examined the effect of pretreatment with 50 μM PD98059 on PIP3-stimulated Akt 473 phosphorylation and found that PD98059 did not alter PIP3-stimulated Akt phosphorylation (data not shown), suggesting that ERK is not upstream of Akt. To determine whether ERK is downstream of Akt, we pretreated neutrophils with SB203580 prior to stimulation with fMLP and measured ERK activity by an in vitro kinase assay and by immunoblot for phospho-ERK. Inhibition of p38 kinase did not alter fMLP-stimulated ERK activity by either of the two methods (data not shown). These data suggest that ERK does not participate in the Akt signaling pathway, despite the requirement of PI-3K for ERK activation.

**DISCUSSION**

Both Akt and p38 kinase are rapidly activated in neutrophils by a number of inflammatory mediators, and one or both kinases participate in respiratory burst activity, chemotaxis, priming, and apoptosis (17, 18, 29, 31, 32). Previous studies indicated that activation of both kinases is mediated by products of PI-3K (18, 30). Our study provides evidence for the first time that p38 kinase participates in the signal transduction pathway leading to Akt activation. Akt activation requires its translocation from a cytosolic location to the plasma membrane, phosphorylation of Thr308 by PDK1, and phosphorylation of Ser473 by an unknown kinase heretofore called PDK2 (12, 33). All three of these activation steps are dependent on products of PI-3K (30, 33). Previous reports suggested that phosphorylation of Ser473 was the result of autophosphorylation following PDK1-dependent phosphorylation of Akt Thr308 or was due to sequential phosphorylation of Thr308 and then Ser473 by PDK1 (34). Our results indicate that p38 kinase is
required for PIP₃-stimulated activation of p38 kinase and Akt in human neutrophils, and PIP₃-dependent phosphorylation of Ser473 on Akt is inhibited by SB203580. The pyridinyl imidazole SB203580 is a relatively specific inhibitor of the α and β isoforms of p38 kinase (35). Recently, Lali et al. (27) reported that SB203580 inhibited PDK1 at concentrations significantly greater than the IC₅₀ for p38 kinase. We excluded this explanation for our results by demonstrating different SB203580 concentration-inhibition curves for Thr308 and Ser473 phosphorylation. SB203580 at the IC₅₀ for p38 kinase (0.3 μM) inhibited Ser473 phosphorylation, while concentrations of SB203580 below 10 μM failed to inhibit Thr308 phosphorylation. Further evidence that p38 kinase participates in Akt activation was provided by the ability of constitutively active MKK3 and MKK6 to stimulate Akt activation following transfection into HEK 293 cells. These studies also suggest that p38 kinase participates in Akt activation independent of PI-3K. Neutrophils were inadequate for these genetic studies, since they undergo constitutive apoptosis in culture resulting in survival of less than 40% of cells at 48 h (36).

Alessi et al. (19) previously showed that MK2 phosphorylates recombinant Akt on Ser⁴⁷³; however, they discounted a role for MK2 in intact cells, since fibroblasts showed IGF-1-dependent Akt activation in the absence of MK2 activation. The present study demonstrates that p38 kinase-stimulated Akt Ser⁴⁷³ phosphorylation is mediated by MK2. Not only did active recombinant MK2 phosphorylate recombinant Akt in vitro, but Akt immunoprecipitated from neutrophil lysates was phosphorylated by active recombinant MK2 as well. Direct evidence for MK2-mediated phosphorylation of Akt Ser⁴⁷³ in intact neutrophils was obtained using an MK2 inhibitory peptide described by Zu et al. (22). Introduction of the inhibitory peptide into freshly isolated neutrophils inhibited phosphorylation of Hsp27 following immunoprecipitation of MK2 from fMLP-stimulated cells. Similarly, the MK2 inhibitory peptide reduced fMLP-stimulated Akt activation and Ser⁴⁷³ phosphorylation, while the control EGFR peptide had no effect. Taken together, these data indicate that MK2 acts as PDK2 in human neutrophils.

The concept that signal transduction pathway components form multimeric complexes held together by scaffolding pro-
teins, rather than existing free in the cytosol, has received significant experimental support recently. Scaffolding proteins have been described for two other MAP kinase modules, ERK and JNK (37, 38). Therefore, the possibility that p38 kinase, MK2, and Akt form a signaling complex was examined. Using two separate methods, immunoprecipitation and GST pull-down, the present study shows for the first time that p38 kinase, MK2, and Akt exist as a complex that does not dissociate upon activation. Hsp27 was previously reported to associate with activated Akt (20). Therefore, we examined the association of Hsp27 with Akt, MK2, and p38 kinase following Akt, MK2, or p38 immunoprecipitation and following GST pull-down with MK2 or Akt. Hsp27 was present in these complexes in unstimulated neutrophils. As opposed to MK2 and p38 kinase, Hsp27 dissociated from Akt immunoprecipitates following neutrophil stimulation with fMLP. Taken together, our data indicate that three components of the p38 kinase module, p38 kinase, MK2, and Akt, form a signaling complex with Akt. MK2, which has been shown to phosphorylate Hsp27 (39, 40), directly phosphorylates Ser473 of Akt in all of the immunoprecipitates.
from the complex during stimulation, suggesting that Hsp27 performs a regulatory function. Our data do not indicate whether Hsp27 dissociates from the complex before or after translocation or phosphorylation of Akt; therefore, no conclusion as to whether Hsp27 acts as a positive or negative regulator is possible. The presence of other components in the signaling complex and the scaffolding protein that binds the complex together remain to be determined. Identity of scaffolding protein is unknown.

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