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Stimulation of human monocytes with macrophage colony-stimulating factor induces a Grb2-mediated association of the focal adhesion kinase pp125^{FAK} and dynamin

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ABSTRACT **Macrophage colony-stimulating factor (M-CSF) is required for the growth and differentiation of mononuclear phagocytes. In the present studies using human monocytes, we show that M-CSF induces interaction of the Grb2 adaptor protein with the focal adhesion kinase pp125^{FAK}. The results demonstrate that tyrosine-phosphorylated pp125^{FAK} directly interacts with the SH2 domain of Grb2. The findings indicate that a pYENV site at Tyr-925 in pp125^{FAK} is responsible for this interaction. We also demonstrate that the Grb2–FAK complex associates with the GTPase dynamin. Dynamin interacts with the SH3 domains of Grb2 and exhibits M-CSF-dependent tyrosine phosphorylation in association with pp125^{FAK}. These findings suggest that M-CSF-induced signaling involves independent Grb2-mediated pathways, one leading to Ras activation and another involving pp125^{FAK} and a GTPase implicated in receptor internalization.**

Macrophage colony-stimulating factor (M-CSF) regulates the growth, differentiation, and survival of mononuclear phagocytes (1). M-CSF receptor (M-CSF-R) is a transmembrane glycoprotein with intrinsic protein tyrosine kinase (PTK) activity (2). This receptor, encoded by the c-*fms* gene, is related to the platelet-derived growth factor (α and β) receptors (3). M-CSF stimulates receptor dimerization and autophosphorylation at Tyr-697, Tyr-706, and Tyr-721 in the kinase domain (4). The autophosphorylated M-CSF-R associates with signal-transducing proteins such as phosphatidylinositol 3-kinase (4, 5), GTPase-activating protein (GAP) (6), and GAP-associated protein p62 (6). Each of these proteins contains Src homology 2 (SH2) domains that bind specific phosphotyrosine [Tyr(*P*)]-containing sequences within activated growth factor receptors and other cellular PTKs (7, 8). The specificity of these protein–protein interactions is dictated by the SH2 domain and the sequence surrounding the Tyr(*P*) (4, 9).

Recent studies in Rat-2 fibroblasts, which express murine M-CSF-Rs, have shown that ligand-induced autophosphorylation on Tyr-697 mediates binding to the Grb2 adaptor protein (10). Grb2 binds to activated growth factor receptors and other tyrosine-phosphorylated proteins through its SH2 domain. Grb2 also binds to the guanine nucleotide-releasing factor Sos through its SH3 domains (11–14). The interaction with Grb2 translocates Sos to the plasma membrane where it increases the exchange of GDP for GTP on membrane-bound Ras (12, 13). Binding of proteins to SH3 domains occurs through short proline-rich sequences (2, 13), such as the PXXPPPXXP motifs of 3BP-1 and 3BP-2 (15–17). Dynamin, a 100-kDa GTPase protein involved in synaptic transmission, endocytosis, and receptor internalization, also contains proline-rich sequence motifs and interacts with the SH3 domains of Grb2 (17, 18).

Oncogenic nonreceptor PTKs, such as pp60$^{c\text{-}src}$, transduce signals involved in growth-factor-stimulated proliferation and alterations of cell morphology and adhesion (19). Ligand-induced autophosphorylation of the M-CSF-R has been shown to induce an association with pp60$^{c\text{-}src}$ (20). pp60$^{c\text{-}src}$ induces tyrosine phosphorylation of specific cellular proteins that are associated with elements of the cytoskeleton network and focal adhesions (19, 21–24). For example, tyrosine phosphorylation of pp125^{FAK} is increased in cells transformed by oncogenic variants of pp60$^{c\text{-}src}$ (22, 25). pp125^{FAK} is a unique PTK that contains a highly conserved tyrosine kinase catalytic domain flanked by amino and carboxyl domains. Tyrosine phosphorylation of pp125^{FAK} is increased as a consequence of the engagement of integrins with the extracellular matrix or the cross-linking of integrins with integrin-specific antibodies (26).

In this report, we present evidence for the direct association of Grb2 with pp125^{FAK} in M-CSF-stimulated normal human peripheral blood monocytes. Our results indicate that the SH2 domain of Grb2 and a pYENV site at Tyr-925 in pp125^{FAK} are responsible for this interaction. We also demonstrate that the Grb2–FAK complex associates with the GTPase dynamin.

MATERIALS AND METHODS

Monocyte Isolation and Culture. Human monocytes were isolated from the peripheral blood of healthy volunteers by Ficoll/Paque separation followed by adherence for 1 h and removal of the nonadherent cells. Monocytes were isolated as described (27, 28) and treated with recombinant human M-CSF at 1000 units/ml (specific activity, 1.90×10^6 units/ml; Genetics Institute, Cambridge, MA) for 1 min at 37°C.

Reagents and Antibodies. Anti-Grb2 and anti-Sos antibodies were purchased from Santa Cruz Biotechnology (San Diego); anti-Shc, anti-M-CSF-R, and anti-Tyr(*P*) were obtained from Upstate Biotechnology. Anti-FAK (clone 2A7) and anti-dynamin antibodies were from J. Thomas Parsons (University of Virginia) and Mark McNiven (Mayo Clinic, Rochester, MN), respectively.

Immunoprecipitation and Immunoblot Analysis. Cell lysates were prepared by resuspending monocytes in lysis buffer for 30 min on ice and immunoprecipitations were as described (29). Proteins were transferred to nitrocellulose and probed with the indicated antibodies. The blots were developed by ECL chemiluminescence (Amersham).

Fusion Protein Binding Assays. The glutathionine *S*-transferase (GST)–Grb2 (full length), GST–Grb2-SH2, GST–Grb2-N-SH3, GST–Grb2-C-SH3, and GST–Lyn-SH2 fusion proteins were purchased from Santa Cruz Biotechnology (San Diego). Peptides were synthesized by using Fmoc-Tyr(PO_3Me_2)-OH for incorporation of Tyr(*P*) as follows: P1, SNDKVpYEN-

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Abbreviations: M-CSF, macrophage colony-stimulating factor; PTK, protein tyrosine kinase; M-CSF-R, M-CSF receptor; Tyr(*P*), phosphotyrosine; GST, glutathionine *S*-transferase.

†S.K. and A.S. made equal contributions.

VTGL; P2, VQTIQpYSNSEDK; and P3, SETDDpYAEIIDE. Cell lysates were incubated with 2.0 μg of immobilized GST or GST fusion proteins in the presence or absence of 50 μM peptides for 2 h at 4°C. The protein complexes were washed three times with lysis buffer and boiled for 5 min in SDS sample buffer. The complexes were then separated by SDS/PAGE on 7.5% gels and subjected to silver staining or immunoblot analysis with anti-FAK or anti-Tyr(*P*) antibodies.

pp125^{FAK} Autophosphorylation, Reprecipitation, and Binding Assays. Cell lysates were immunoprecipitated with anti-FAK. After three washes with lysis buffer containing 0.1% Brij 96 and once with kinase buffer (20 mM Hepes, pH 7.6/10 mM $MgCl_2$/10 mM $MnCl_2$/1 mM dithiothreitol), *in vitro* kinase assays were performed by adding 10 μCi of [γ-^{32}P]ATP (1 Ci = 37 GBq) for 15 min at 30°C. The proteins were analyzed by SDS/PAGE on 7.5% gels and autoradiography. FAK protein was excised from the gel and eluted at 37°C in elution buffer (50 mM NH_4HCO_3/1% SDS/1 mM dithiothreitol). The eluate was diluted 1:10 with lysis buffer and reprecipitated with anti-FAK, GST, or GST–Grb2-SH2. The precipitates were analyzed by SDS/PAGE on 7.5% gels and autoradiography.

RESULTS

Recent studies in Rat-2 fibroblasts that express murine M-CSF-Rs have shown that ligand-induced autophosphorylation on Tyr-697 mediates binding to the SH2/SH3-containing Grb2 adaptor protein (10). To determine whether similar events occur in a physiologic model, we stimulated human peripheral blood monocytes with M-CSF and assayed anti-Grb2 immunoprecipitates for substrates containing Tyr(*P*). Proteins ranging from 150 to 46 kDa exhibited increased reactivity with anti-Tyr(*P*) in the M-CSF-treated monocytes (Fig. 1*A* and data not shown). Analysis of the anti-Grb2 immunoprecipitates with an anti-M-CSF-R antibody confirmed an increase in binding of Grb2 to M-CSF-Rs upon ligand stimulation (Fig. 1*A*). The M-CSF-induced interaction between these two proteins was associated with phosphorylation of M-CSF-Rs on tyrosine.

Analysis of the anti-Grb2 immunoprecipitates also revealed proteins of 125, 100–95, 52, and 46 kDa that exhibit M-CSF-induced increases in tyrosine phosphorylation (Fig. 1). Since Shc is known to associate with Grb2 as a result of growth factor receptor stimulation (30), we analyzed anti-Shc immunoprecipitates for reactivity with anti-Tyr(*P*). While increased tyrosine phosphorylation of p52/p46 Shc was found in M-CSF-treated monocytes, there was no evidence for tyrosine-phosphorylated M-CSF-Rs in the anti-Shc immunoprecipitates (Fig. 1*B* and data not shown). Immunoblot analysis of the anti-Grb2 and anti-Shc immunoprecipitates, however, demonstrated M-CSF-induced increases in the association between Grb2 and Shc (Fig. 1*B*). These findings suggested that Grb2 molecules interact independently with M-CSF-Rs and Shc as a consequence of M-CSF treatment.

The anti-Grb2 immunoprecipitates also contained a 125-kDa protein that exhibited increased Tyr(*P*) in the M-CSF-stimulated monocytes (Fig. 1*A*). The focal adhesion kinase pp125^{FAK} is a tyrosine kinase that colocalizes with components of cellular focal adhesions, such as tensin and vinculin (31). To determine whether the 125-kDa Grb2-associated protein is pp125^{FAK}, we assayed the anti-Grb2 immunoprecipitates with an anti-FAK antibody (Fig. 2*A*). The results demonstrate that anti-FAK reacts with a 125-kDa protein in the anti-Grb2 precipitate from M-CSF-treated monocytes. Incubation of the anti-Grb2 antibody with the Grb2 peptide (aa 195–217 used as immunogen) prior to immunoprecipitation completely blocked detection of the anti-FAK signals. Conversely, lysates from control and M-CSF-treated monocytes were subjected to immunoprecipitation with anti-FAK and immunoblot analysis with anti-Grb2 revealed increased reactivity with a 26-kDa protein after M-CSF stimulation. Although these findings indicated that Grb2 associates with pp125^{FAK} and that this interaction is increased by M-CSF-induced phosphorylation of FAK on tyrosine, other studies (12, 13) have shown that Grb2 links PTK receptors to the guanine nucleotide exchange protein Sos and, thus, to the Ras activation pathway. Increased binding of Sos was observed in anti-Grb2 immunoprecipitates from M-CSF-treated monocytes (Fig. 2*B* and data not shown). However, anti-Sos reactivity in anti-FAK immunoprecipitates was not detectable under the conditions used in this study. These results support the existence of independent pools of Grb2, one bound to M-CSF-Rs and the other bound to pp125^{FAK}.

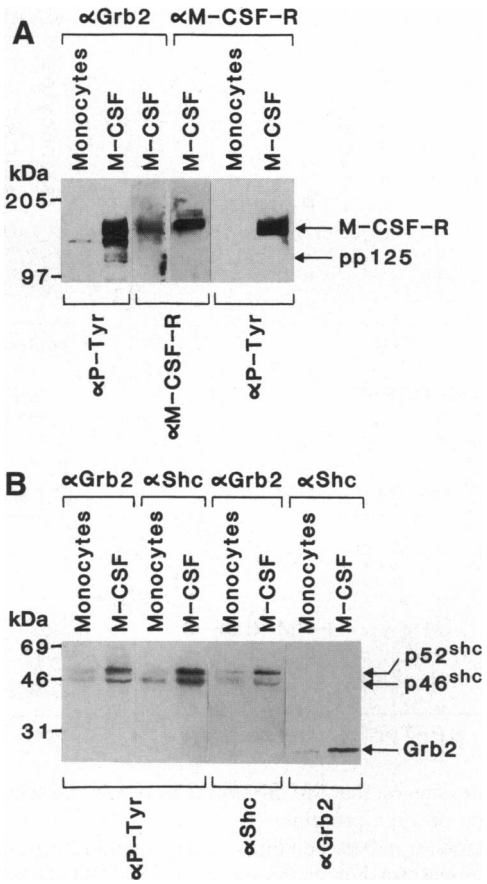

FIG. 1. M-CSF stimulation of human monocytes induces binding of Grb2 to M-CSF-Rs and p52/p46 Shc. Human peripheral blood monocytes were stimulated with M-CSF for 1 min at 37°C. (*A*) Cell lysates were immunoprecipitated with anti-Grb2 (αGrb2) or anti-M-CSF-R (αM-CSF-R) antibodies (indicated above lanes). The immunoprecipitated proteins were resolved by SDS/PAGE and subjected to immunoblot analysis with anti-Tyr(*P*) (αP-Tyr) or αM-CSF-R (indicated below lanes). (*B*) Lysates were subjected to immunoprecipitation with αGrb2 or anti-Shc (αShc) (indicated above lanes). The immunoprecipitates were assayed for reactivity with αP-Tyr, αShc, or αGrb2 (indicated below lanes).

To further understand the kinetics of M-CSF-R autophosphorylation and tyrosine phosphorylation of pp125^{FAK}, we treated monocytes with M-CSF at 25°C and assayed anti-M-CSF-R and anti-FAK immunoprecipitates with anti-Tyr(*P*). Phosphorylation of M-CSF-Rs was detectable 1 min after M-CSF stimulation and returned to near baseline levels at 10 min (Fig. 2*C*). In contrast, while there was little phosphorylation of pp125^{FAK} at 1 min, increases in anti-Tyr(*P*) reactivity were detected at 5 and 10 min. Tyrosine phosphorylation of pp125^{FAK} is thus delayed compared to increases in kinase activity of the stimulated M-CSF-R. Moreover, the absence of detectable pp125^{FAK} in the anti-M-CSF-R precipitates and the absence of M-CSF-Rs in the anti-FAK

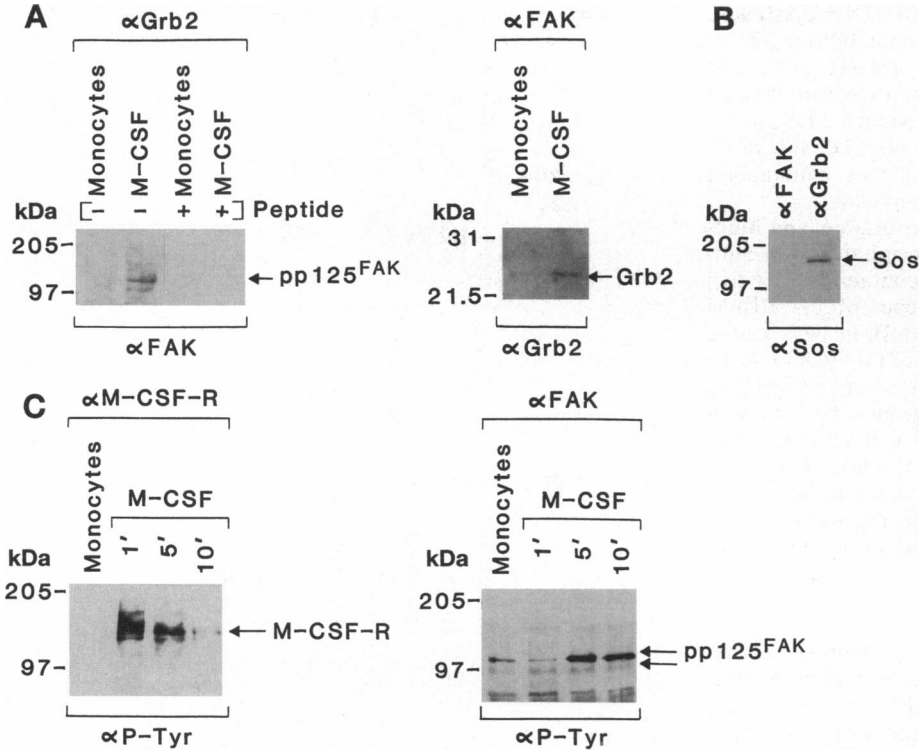

FIG. 2. M-CSF induces binding of Grb2 to tyrosine-phosphorylated pp125^{FAK}. (*A*) Lysates from M-CSF-treated cells were immunoprecipitated with anti-Grb2 (αGrb2) (lanes −) or αGrb2 preincubated in the presence of a 30-fold excess of Grb2 peptide (lanes +) used as immunogen. The αGrb2 immunoprecipitates were subjected to immunoblot analysis with anti-FAK (αFAK) (*Right*). Anti-FAK immunoprecipitates were also subjected to immunoblot analysis with αGrb2 (*Left*). (*B*) M-CSF-treated lysates were subjected to immunoprecipitation with αFAK or αGrb2. The immunoprecipitates were subjected to immunoblot analysis with anti-Sos (αSos) antibodies. (*C*) Monocytes were incubated with M-CSF at 25°C for the indicated times in min. Lysates were immunoprecipitated with the anti-M-CSF-R (αM-CSF-R) (*Left*) or αFAK antibodies (*Right*). The immunoprecipitates were subjected to immunoblot analysis with anti-Tyr(*P*) (αP-Tyr).

precipitates suggest that M-CSF stimulation is not associated with interaction of these proteins.

The association between Grb2 and tyrosine-phosphorylated pp125^{FAK} was further examined by using GST–Grb2 fusion proteins. Lysates from control and M-CSF-treated monocytes were first incubated with the GST–Grb2 (full length) fusion protein. Analysis of the adsorbates analyzed on immunoblots with anti-Tyr(*P*) revealed increased phosphorylation of 150-, 125-, and 100- to 95-kDa proteins in M-CSF-treated monocytes (Fig. 3*A*, lanes 2 and 3). Reprobing with anti-FAK confirmed the presence of pp125^{FAK} in the GST–Grb2 adsorbate. Moreover, the increased anti-FAK signal when using M-CSF-treated compared to control monocytes supported an increased association between Grb2 and pp125^{FAK}. Proteins were also released from the GST–Grb2 beads, immunoprecipitated with anti-FAK, and then subjected to immunoblot analysis with anti-Tyr(*P*). The finding that the anti-FAK immunoprecipitate contains a 125-kDa protein that undergoes tyrosine phosphorylation in M-CSF-treated monocytes further confirmed the increased interaction between Grb2 and tyrosine-phosphorylated pp125^{FAK} (Fig. 3*B*). A protein of 100–95 kDa that coimmunoprecipitated with pp125^{FAK} and Grb2 was also phosphorylated on tyrosine in M-CSF-induced monocytes (Fig. 3*B*, lane 3).

Lysates from control and M-CSF-treated monocytes were incubated with GST fusion proteins prepared from the SH3 (C and N terminal) and SH2 domains of Grb2. Adsorbates obtained with the GST–Grb2-C–SH3 fusion protein contained a 125-kDa protein that exhibited increased levels of Tyr(*P*) after M-CSF treatment (Fig. 4*A*). A similar finding was obtained with adsorbates from the GST–Grb2-N-SH3 fusion protein. In contrast, adsorbates from GST–Grb2-SH2 but not a GST–Lyn-SH2 (data not shown) revealed more pronounced M-CSF-induced increases in binding of tyrosine-phosphorylated pp125^{FAK} and the interaction with tyrosine-phosphorylated M-CSF-Rs. The identity of the adsorbed proteins was confirmed by reprobing the filters with anti-M-CSF-R and anti-FAK (data not shown). These findings indicated that

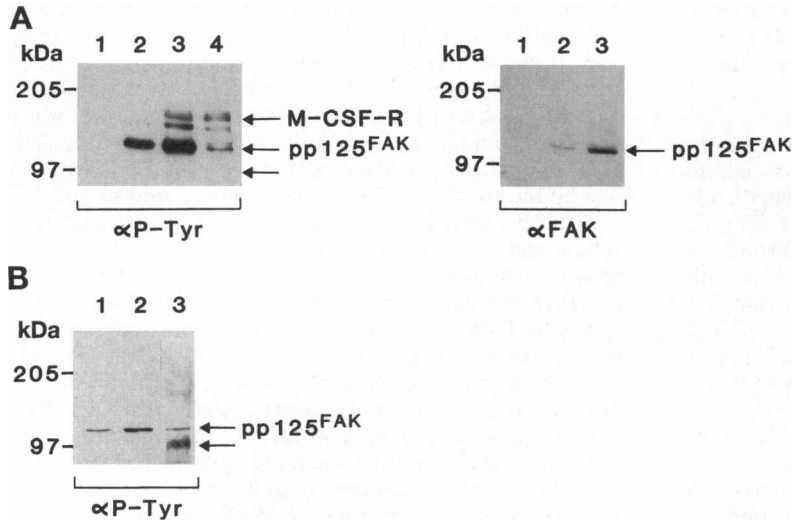

FIG. 3. Binding of pp125^{FAK} to Grb2 *in vitro*. (*A*) Lysate from M-CSF-treated cells was incubated with GST immobilized on glutathione-Sepharose (lane 1). Lysates from control (lane 2) and M-CSF-treated (lane 3) monocytes were incubated with immobilized GST–Grb2 (full length) fusion protein. The adsorbed proteins were separated by SDS/PAGE and subjected to immunoblot analysis with anti-Tyr(*P*) (αP-Tyr) (*Left*) or anti-FAK (αFAK) (*Right*). M-CSF-treated cell lysates were incubated with anti-Grb2 and the immunoprecipitated proteins were subjected to immunoblot analysis with αP-Tyr (lane 4). (*B*) Lysates from control (lane 1) and M-CSF-treated (lane 2) cells were incubated with GST–Grb2. Proteins adsorbed to the GST–Grb2 fusion protein were released by boiling in 50 mM Tris·HCl, pH 8.0/0.5% SDS/1 mM dithiothreitol and then immunoprecipitated with αFAK. Lysates from M-CSF-treated cells were also immunoprecipitated with αFAK (lane 3). Immunoprecipitates were subjected to immunoblot analysis with αP-Tyr.

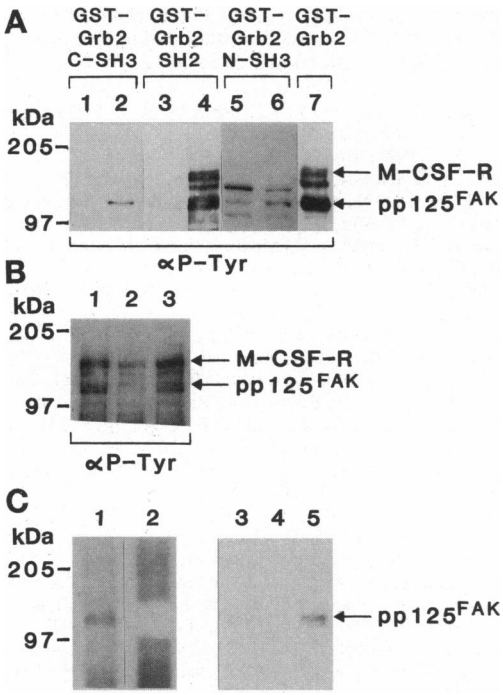

FIG. 4. SH2 domain of Grb2 binds to the pYENV (Tyr-925) motif of pp125^{FAK}. (*A*) Lysates from control (lanes 1, 3, and 5) and M-CSF-treated (lanes 2, 4, 6, and 7) monocytes were incubated with the indicated GST fusion proteins. The adsorbates were subjected to immunoblot analysis with anti-Tyr(*P*) (αP-Tyr). The blot for GST–Grb2-N-SH3 was overexposed to demonstrate protein binding. (*B*) GST–Grb2-SH2 protein was incubated in the absence (lane 1) or presence of 50 μM tyrosine-phosphorylated synthetic peptides P1 (lane 2), P2 (lane 3), and P3 (data not shown). The fusion proteins were incubated with lysate from M-CSF-treated monocytes and the adsorbate was subjected to immunoblot analysis with αP-Tyr. (*C*) M-CSF-treated cell lysates were immunoprecipitated with anti-FAK antibodies. After *in vitro* autophosphorylation, the proteins were analyzed by SDS/PAGE and autoradiography (lane 1). FAK protein was excised from the gel (lane 2), eluted, and subjected to reimmunoprecipitation with anti-FAK (lane 3), GST (lane 4), or GST–Grb2-SH2 (lane 5). The precipitates were analyzed by SDS/PAGE and autoradiography.

there is some constitutive non-tyrosine-phosphorylation-dependent association of Grb2 and pp125^{FAK} through Grb2-SH3 domains and that this interaction is increased to some extent by M-CSF treatment. However, the findings also indicate that the M-CSF-induced association between pp125^{FAK} and Grb2 is mediated primarily through a tyrosine-phosphorylation-dependent interaction with the SH2 domain of Grb2.

To define the site in pp125^{FAK} responsible for association with Grb2, we identified three potential candidate sequences. Tyr-925 in the C-terminal domain of FAK is followed by Glu-Asn-Val, and Tyr-304 is followed by Ser-Asn-Ser. The SH2 domain of the pp60$^{c\text{-}src}$ binds to Tyr-397 of pp125^{FAK} and this site is followed by Ala-Gly-Ile. Chemically phosphorylated synthetic peptides corresponding to aa 920–931 (P1), 299–310 (P2), and 392–402 (P3) of pp125^{FAK} were used in competition assays. Preincubation of GST–Grb2-SH2 with P1 inhibited binding of pp125^{FAK} from lysates of M-CSF-treated monocytes (Fig. 4*B*), but there was no detectable inhibition of pp125^{FAK} binding when P2 or P3 was competitor (Fig. 4*B* and data not shown). Binding of GST–Grb2-SH2 to tyrosine-phosphorylated M-CSF-R was also selectively blocked by P1. To determine whether the interaction between Grb2 and pp125^{FAK} is direct, we subjected FAK immunoprecipitates from M-CSF-treated monocytes to autophosphorylation. The ^{32}P-labeled pp125^{FAK} was isolated from SDS/polyacrylamide gels (Fig. 4*C*, lanes 1, 2) and then incubated with anti-FAK, GST, or GST–Grb2-SH2. Analysis of the second precipitates demonstrated direct binding of the SH2 domain of Grb2 to pp125^{FAK} (Fig. 4*C*, lanes 3–5). These findings and selective inhibition of the Grb2–FAK interaction by peptide P1 supported involvement of Tyr-925 in pp125^{FAK}.

In addition to interactions with Sos, the SH3 domains of Grb2 have been shown (15, 32) to bind dynamin. Since the Grb2–FAK complexes from M-CSF-treated monocytes also contain a 100- to 95-kDa tyrosine-phosphorylated species (Fig. 3*B*), we asked whether this protein is dynamin. The results demonstrate that dynamin associates with the SH3 domains of Grb2 (Fig. 5*A*) and that dynamin is detectable in anti-FAK immunoprecipitates from M-CSF-treated monocytes (Fig. 5*B*). Since pp125^{FAK} may phosphorylate dynamin on tyrosine, we assayed anti-dynamin immunoprecipitates for reactivity with anti-Tyr(*P*) and found that dynamin is phosphorylated on tyrosine in response to M-CSF stimulation and associates with a 125-kDa protein that reacts with anti-FAK (Fig. 5*C* and data not shown).

DISCUSSION

Several autophosphorylation sites have been identified in the ligand-activated M-CSF-R. Tyr-697, Tyr-706, and Tyr-721 have been mapped in the murine M-CSF-R kinase insert domain (4, 10, 33). One of these sites, Tyr-697, has been shown to function as a binding site for Grb2. Other studies have shown that Grb2 also interacts with tyrosine-phosphorylated Shc and, in turn, binds to the guanine nucleotide exchange factor Sos (13, 34). The present studies show that p46/52 Shc is tyrosine-phosphorylated and associates with Grb2 in M-CSF-treated

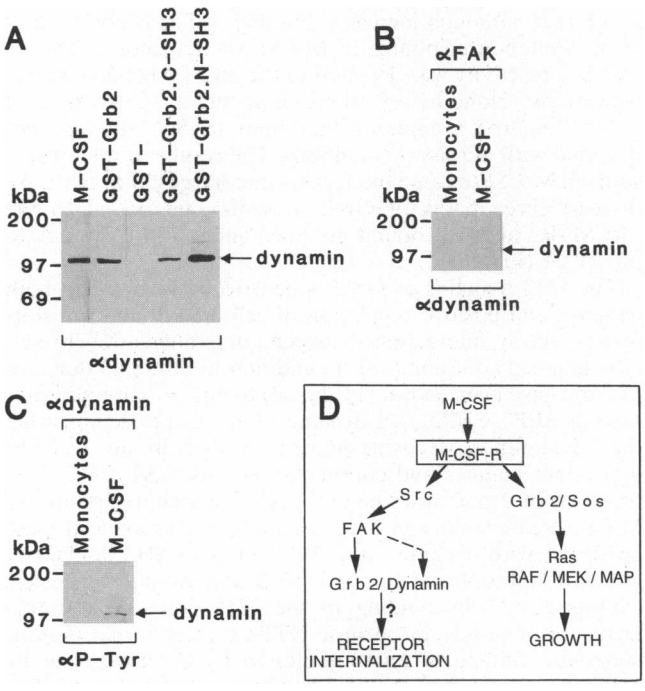

FIG. 5. Coimmunoprecipitation of pp125^{FAK} with tyrosine-phosphorylated dynamin. (*A*) M-CSF-treated cell lysates were subjected to immunoblot analysis with anti-dynamin (αdynamin) (lane 1). Lysates were also incubated with GST (lane 3) or the indicated GST fusion proteins. The adsorbed proteins were separated by SDS/PAGE and subjected to immunoblot analysis with αdynamin. (*B*) Lysates from control and M-CSF-treated monocytes were immunoprecipitated with anti-FAK (αFAK). The precipitated proteins were separated by SDS/PAGE and subjected to immunoblot analysis with αdynamin. (*C*) Lysates from control and M-CSF-treated monocytes were immunoprecipitated with αdynamin and analyzed for reactivity with anti-Tyr(*P*) (αP-Tyr). (*D*) Proposed schema for M-CSF-induced signaling pathways.

monocytes. Tyrosine-phosphorylated M-CSF-Rs were not detected in the anti-Shc immunoprecipitates from M-CSF-stimulated monocytes (data not shown). Thus, these results and findings from other studies (10) suggest that the M-CSF-R and Shc independently associate with Grb2 in an M-CSF-dependent manner.

Our results further demonstrate that M-CSF induces interaction of Grb2 with the focal adhesion tyrosine kinase pp125^{FAK}. While pp125^{FAK} contains a catalytic domain characteristic of other PTKs, it lacks domains involved in binding to membranes and other proteins (35). The findings indicate that the M-CSF-induced association between pp125^{FAK} and Grb2 is mediated primarily through the SH2 domain of Grb2. To define the site in pp125^{FAK} responsible for association with Grb2, we identified three potential candidate sequences: (*i*) Tyr-925 in the C-terminal domain of FAK, (*ii*) Tyr-304, and (*iii*) Tyr-397 (which is also an autophosphorylation site). Peptide competition studies demonstrate that the SH2 domain of Grb2 binds to a highly conserved pYENV (Tyr-925) sequence in the C-terminal domain of pp125^{FAK}. The results also demonstrate that Grb2 binds directly to pp125^{FAK}. While further studies are needed to identify events responsible for phosphorylation of pp125^{FAK} at Tyr-925, autophosphorylation of Tyr-397 in pp125^{FAK} contributes to binding of Src-like kinases such as pp60$^{c\text{-}src}$ or pp59^{fyn} (13, 21, 36). Since pp60$^{c\text{-}src}$ is activated in M-CSF-treated cells (20) and the Tyr-925 site in pp125^{FAK} (DXXYENV) is similar to the autophosphorylation site of Src (37), pp60$^{c\text{-}src}$ or Src-like kinases may be responsible for transducing signals from the activated M-CSF-Rs by phosphorylating pp125^{FAK}.

Grb2 has been shown to link the PTK receptor to Sos and, thus, to the Ras activation pathway (33). Our results demonstrate that, although increased binding of Sos is observed in Grb2 immunoprecipitates in M-CSF-treated monocytes, no anti-Sos reactivity was detected in the anti-FAK immunoprecipitations. Nonetheless, there may be a subfraction of pp125^{FAK}–Grb2 complexes that bind to Sos and are not detected with our assay conditions. The results of the present study in M-CSF-treated monocytes thus suggest that there are at least three pools of Grb2 present—one bound to the M-CSF-R, another bound to Shc, and a third bound to pp125^{FAK} (Fig. 5*D*).

The SH3 domains of Grb2 appear to be involved in both negative and positive regulation of cell growth and transformation and in interactions between components of cytoskeleton and cell adhesion (38). In addition to Sos, SH3 domains of Grb2 have been shown (15, 16, 30) to bind to other proteins such as 3BP1, 3BP2, and dynamin. Our results demonstrate that dynamin is tyrosine-phosphorylated in an M-CSF-dependent manner and coprecipitates with GST–Grb2. The presence of dynamin in the anti-FAK immunoprecipitates of M-CSF-treated monocytes further confirmed an association of pp125^{FAK} with dynamin. pp125^{FAK} binds to SH2 domain of Grb2 after phosphorylation at Tyr-925 and dynamin associates with pp125^{FAK} by binding to the SH3 domains of Grb2. Dynamin possesses an intrinsic GTPase activity that is stimulated by binding of microtubules to its C terminus or by binding of dynamin to the SH3 domains of Grb2 (17, 18). The binding of dynamin to SH3-domain-containing proteins also appears to be important for formation of the protein complex required for the endocytic processing of activated receptor PTKs (17). Since dynamin is involved in endocytosis mechanisms and receptor internalization, the interaction of dynamin with Grb2–FAK in M-CSF-treated monocytes may contribute to internalization of M-CSF-Rs and thus may negatively regulate M-CSF-R-mediated signaling.

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