Regulation of Neutrophil Adhesion by Pituitary Growth Hormone Accompanies Tyrosine Phosphorylation of Jak2, p125FAK, and Paxillin

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*J Immunol* 2000; 165:2116-2123; doi: 10.4049/jimmunol.165.4.2116

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Neutrophils are the primary effector cells in acute inflammation (1). They are rapidly recruited in large numbers from the bloodstream by processes of adhesion to vascular endothelium, transendothelial migration, and chemotaxis to a local inflammatory site (2, 3). In the presence of inflammatory mediators such as chemotactic agonists and cytokines (4), neutrophils interacting with plasma and extracellular matrix proteins (3, 5, 6) or endothelial counter-receptors reorganize their cytoskeleton and spread over the adhesive surface (7, 8), diapedese, produce reactive oxygen intermediates, and release granule constituents (9–13).

Pituitary growth hormone (GH) plays diverse roles in the promotion of cell growth and metabolism (14). GH has been shown to influence the development of the immune organ and the function of immune cells (15–19). The binding of GH to its receptor causes dimerization of two growth hormone receptors (GHR), which, in turn, initiates the signal transduction in the cell. Lacking intrinsic tyrosine kinase activity, the GHR recruits and activates a member of the Janus family of cytosolic kinases (JAKs) upon dimerization (20, 21). In addition to the GHR and itself, Jak2 phosphorylates STATs (22, 23). We and others have previously demonstrated that recombinant GH (rGH) primes and enhances respiratory burst function in human neutrophils through intracellular calcium increase (12, 24–30). Although GH-induced priming of neutrophils is accompanied by an increase in adhesion (16), the molecular mechanisms by which GH activates neutrophil adhesion have not been determined. Therefore, an interesting question in the operation of GH in neutrophil adhesion is how the signal is transduced from stimuli to the adhesion.

Focal adhesion kinase (p125FAK) is a cytosolic kinase that is concentrated in focal contacts (31, 32). Because of its localization, p125FAK has been thought to be involved in regulating cell morphology and cell migration in response to cell adhesion to extracellular matrix proteins (31, 33). p125FAK is rapidly phosphorylated following cell attachment to fibronectin-coated surfaces or integrin clustering by Abs. p125FAK is also considered a focal adhesion docking protein capable of facilitating the recruitment and activation of other tyrosine-phosphorylated signaling molecules, such as pp60csrc and paxillin (31, 34, 35). Paxillin is another signaling molecule that localizes to focal adhesions and becomes tyrosine phosphorylated either during integrin-mediated or growth factor-induced adhesion (34, 35). Paxillin contains a domain that interacts with the C-terminus of p125FAK (36), and p125FAK recruitment to focal contacts appears to require paxillin binding (36, 37). Paxillin has been demonstrated to be a substrate for p125FAK phosphorylation in both in vitro (38) and in vivo systems (35, 39). Recently, treatment with several peptide hormones such as prolactin, insulin-like growth factor I (IGF-I), hepatocyte growth factor,
vacular endothelial growth factor, and platelet-derived growth factor (PDGF) have been shown to augment the tyrosine phosphorylation of p125^FAK_ (24–30, 40).

The aim of the present study was to determine how neutrophil adhesive functions are modulated by human pituitary GH. Herein we provide data that pituitary GH treatment potentiates neutrophil adhesion and triggers the tyrosine phosphorylation of Jak2, p125^FAK_ and paxillin and F-actin formation that may involve in the adhesion signaling of human neutrophils.

Materials and Methods

Reagents

Human pituitary GH, genistein, and phallolidin-FITC were purchased from Sigma (St. Louis, MO). Protein A-Sepharose, dextran T-500, and Ficol-Paque were purchased from Pharmacia Biotechn (Piscataway, NJ). Anti-phosphotyrosine mAb 4G10 (Upstate Biotechnology, Lake Placid, NY) was used for Western blotting, and anti-phosphorysine Ab PB-20-agarose conjugate (Santa Cruz Biotechnology, Santa Cruz, CA) was used for immunoprecipitation studies. Rabbit polyclonal anti-Jak2, anti-p125^FAK_ (H-7), and anti-STAT3 Ab were purchased from Santa Cruz Biotechnology. Anti-p125^FAK_ mAb (clone 77) and anti-paxillin mAb was obtained from Upstate Biotechnology and Transduction Laboratories (Lexington, KY), respectively. The enhanced chemiluminescence (ECL) Western blotting system was obtained from Amersham (Arlington Heights, IL). DuPont-New England Nuclear (Boston, MA) was the source of [gamma-32P]ATP. Four- and 96-well tissue culture plates and 100- and 35-mm dishes were purchased from Nunc (North Aurora Road, IL). RPMI containing l-arginine (200 mg/L), HBSS, and FBS and other tissue culture reagents were purchased from Nunc (North Aurora Road, IL). RPMI containing l-arginine (200 mg/L), HBSS, and FBS and other tissue culture reagents were purchased from Life Technologies (Gaithersburg, MD).

PMN isolation

Human venous blood (10 ml) was collected from healthy adult volunteers into heparinized tubes (12). To obtain neutrophils from peripheral blood, the heparinized blood was centrifuged for 10 min at 1000 rpm to remove platelet-rich plasma. After 2% dextran sedimentation of erythrocytes for 30 min, neutrophils were isolated under sterile conditions by density gradient centrifugation on Ficol-Paque cushions in cornical tubes. The tubes were centrifuged at 2500 rpm for 30 min in swing-out buckets at room temperature. Contaminating erythrocytes were lysed with hypotonic solution containing NH4Cl/EDTA and then washed twice. The cells were gently resuspended in magnesium-free HBSS containing 200 mM NaCl, 5 mM MgCl2, 5 mM MnCl2, 0.1 mM Na3VO4, and 10 mM HEPES (pH 7.4) containing [gamma-32P]ATP (5 mM) and immediately used for immunoprecipitation or Western blot.

Immunoprecipitation and Western blot analysis

Lysates (1 ml) obtained as described above were precleared by the addition of 30 l of protein A-Sepharose (50% (v/v) slurry) for 1 h at 4°C. Then the precleared lysates were incubated with 5 l of free Abs for 4–6 h at 4°C on an oscillating platform. Twenty-five microliters of protein A-Sepharose was then added into lysates and left for 1 h at 4°C. In the presence of immunoprecipitates of phosphotyrosine (pY), this was followed by the addition of 10 l of protein A-agarose-conjugated anti-pY Ab (Santa Cruz Biotechnology) for 4–6 h at 4°C. All beads were collected by centrifugation and were washed twice with modified extraction buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 1 mM PMSF, 10 l/mL leupeptin, 1 l/mL pepstatin, 1 mM N-ethylmaleimide, 2 mM Na3VO4, 20 mM sodium pyrophosphate, and 50 mM NaF. Lysates were centrifuged at 15,000 rpm at 4°C for 30 min. The clear cytosol was separated from the insoluble pellet fractions and immediately used for immunoprecipitation or Western blot.

Indirect labeling methods were undertaken to detect the localization of p125^FAK_ , paxillin, and phosphotyrosine in neutrophils. The cellular distribution of F-actin was directly stained using FITC-labeled phallloidin. Adherent cells (4 105 cells/ml) were seeded onto two-well chamber slides and incubated for 30 min with serum-free RPMI 1640 medium. Cells were then stimulated with GH (100 ng/ml) for the indicated period of time, and the cell layers were washed twice with PBS and fixed for 15 min with 4% paraformaldehyde (PFA) at room temperature. After washing with PBS, fixed cells were blocked for 1 h with blocking solution containing 0.3% Triton X-100, 5% BSA, and 3% goat serum and then treated with a 1/200 dilution of rabbit polyclonal anti-Laminu’s b (1/2) by 100 mM Tris-HCl (pH 6.8), 4% SDS, 200 mM DTT, 20% glycerol, 2% SDS, 0.2% bromophenol blue, 10 l/mL aprotinin, and 10 l/mL leupeptin) was then added. The samples were boiled for 10 min at 100°C and then spun at 15,000 rpm for 10 s. Immunoprecipitates were divided into equal aliquots before separation on the SDS-PAGE as described below. The denaturated proteins were electrophoresed on 8% SDS-polycrylamide gel and transferred to cellulose membrane. Membranes were blocked in 5% skim milk in Tris (pH 7.4), 150 mM NaCl, and 0.05% Tween-20 for 30 min at room temperature. Blots were probed with primary Abs overnight at 4°C. This was followed by incubation with anti-rabbit or anti-mouse IgG conjugated with HRP (Bio-Rad, Richmond, CA) for 2 h. Signals were detected by using the ECL system (Amersham).

In vitro kinase assay

Anti-p125^FAK_ immunoprecipitates were isolated from neutrophil lysates treated with GH for 0–60 min. Immunoprecipitates were washed three times with a lysis buffer and then divided into two equal parts. Each part was resuspended in an equal volume (30 l) of kinase assay buffer (50 mM NaCl, 5 mM MgCl2, 5 mM MnCl2, 0.1 mM Na3VO4, and 10 mM HEPES (pH 7.4) containing [gamma-32P]ATP (5 lC/min) for 30 min at 37°C. The kinase reaction was stopped by the addition of 5 l of sample buffer for SDS-PAGE, separated on 8% gel, and visualized by autoradiography. The other part of immunoprecipitates was separated on 8% gel and probed with an anti-p125^FAK_ Ab.

Flow cytometry

For the effect of GH on F-actin formation, we used the method described by Lee et al. (42). Briefly, neutrophils were incubated for 1 h at 37°C with GH (100 ng/mL). Reactions were terminated, and cells were fixed by the
addition of 4% PFA for 15 min. Cells were washed twice with PBS, and phalloidin-FITC (100 nM) was added for 5 min. The changes in fluorescence activity were measured using FACStar (Becton Dickinson, Mountain View, CA).

Data analysis
Data are expressed as the mean and SEM. Differences were compared using ANOVA (repeated measures) and the Mann-Whitney test. A significant level was designated at the 95% level (p < 0.05). Statistical calculations were completed using the StatView 512 software package (Abacus Concepts, Berkeley, CA).

Results
Pituitary GH increases neutrophil adhesion
In the first series of experiments the time- and dose-dependent effects of pituitary GH on neutrophil adhesion were determined. The time course for the direct adherence of neutrophils to the plastic substratum of cell cultureware was assessed with or without GH. Maximal adherence of neutrophils was observed at 30 min for both control and GH-treated samples (Table I). GH augmented the neutrophil adhesion in a bell-shaped dose-response fashion, with the peak effect observed at 100 ng/ml (Fig. 1A). The effect of GH on neutrophil adhesion was modest, and maximally a 32% increase was observed 30 min after GH stimulation. Then we examined the effect of genistein, a typical tyrosine kinase inhibitor, on GH-stimulated neutrophil adhesion. Fig. 1B shows that 10 μM genistein decreased the adherence of neutrophils by itself and blocked the effect of pituitary GH. Concentrations ranging from 1 to 10 μM genistein significantly blocked the effect of GH on neutrophil adhesion (data not shown). Photomicrographs of Fig. 1C showed that neutrophils in response to GH (100 ng/ml) for 30 min adopted more elongated shapes (Fig. 1C, b and d) than control cells, which show a round shape (Fig. 1C, a and c). Interestingly, GH-treated neutrophils showed membrane ruffles and exhibited more spreading compared with control neutrophils. The bipolar shape and uropod formation were also observed (Fig. 4, C, F, and I). The sustained effect on the shape change by GH was most evident in the distribution of cell adherence by 30 min. These data indicated that the signaling by GH is an effective stimulus for shape change of neutrophils.

Pituitary GH stimulates tyrosine phosphorylation of p125FAK and paxillin
Next, the effect of GH stimulation on tyrosine phosphorylation of Jak2, STAT3, p125FAK, and paxillin was examined. Neutrophils were incubated at 37°C with different concentrations of GH (10–500 ng/ml) for 30 min and lysed with detergent. GH induced the tyrosine phosphorylation of Jak2 (Fig. 2A), STAT3 (Fig. 2B), p125FAK (Fig. 2C), and paxillin (Fig. 2D) in a bell-shaped, concentration-dependent manner. The half-maximal and maximal increases in the level of tyrosine phosphorylation of Jak2 and

Table I. Effect of pituitary GH on the neutrophils adhesion

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>GH (100 ng/ml)</th>
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<tbody>
<tr>
<td>30</td>
<td>0.414 ± 0.023</td>
<td>0.480 ± 0.017**</td>
</tr>
<tr>
<td>60</td>
<td>0.406 ± 0.025</td>
<td>0.449 ± 0.031*</td>
</tr>
<tr>
<td>90</td>
<td>0.326 ± 0.046</td>
<td>0.349 ± 0.049</td>
</tr>
</tbody>
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* Values are the absorbency at 570 nm. Neutrophils (5 × 10⁶ cells/ml) were treated with pituitary GH (100 ng/ml) according to the indicated time interval at 37°C. After washing, a total of 200 μl of 0.5% crystal violet in 12% neutral formaldehyde solution and 10% ethanol were added to each well for 1 h to fix and stain cells. The samples were then thoroughly washed with distilled water and air dried for 30 min. Crystal violet was extracted by the addition of 1% SDS and absorbency was measured at 570 nm. Results are presented as the mean ± SEM of five separate experiments.

* p < 0.05; ** p < 0.01; significantly different from the control at 30 min; †p < 0.05; significantly different from each time control (no treatment of GH).

FIGURE 1. Pituitary GH increases the adhesion of human neutrophils. A. Dose-dependent effect of pituitary GH on the adhesion of human neutrophils (n = 8). The neutrophils (4 × 10⁶) were incubated with medium alone or with the indicated concentration of GH for 30 min in a 96-well plate. B. Inhibitory effect of genistein on the GH (100 ng/ml)-stimulated adhesion of neutrophils (n = 6). The cells were preincubated with genistein (10 μM) for 30 min. Values show the change in absorbency at 570 nm. *, Significantly different from each time control of GH, p < 0.01; †, Significantly different from the value of GH-stimulated cells, p < 0.05. C. Morphological changes in neutrophils induced by pituitary GH. Cells were incubated in chamber slides with GH (100 ng/ml) for 30 min and examined by phase contrast light microscopy (a and b). Cells were fixed with 4% paraformaldehyde and stained with Wright-Giemsa solution (c and d). a and c, Medium control; b and d, pituitary GH treatment.
STAT3 occurred at 50 and 100 ng/ml, respectively. Furthermore, GH caused a striking increase in the tyrosine phosphorylation of p125FAK and paxillin. The maximal tyrosine phosphorylation of p125 FAK and paxillin was observed at 100 ng/ml of GH. Subsequent blotting with respective Abs demonstrated that equal amounts of the protein were loaded in each pair of lanes.

To examine the kinetics of phosphorylation of Jak2, STAT3, p125 FAK, and paxillin, cells were stimulated with GH (100 ng/ml) for 5, 15, 30, and 60 min before lysis. As illustrated in Fig. 3A, increased tyrosine phosphorylation of Jak2 was detected 5 min after stimulation, reached its maximum within 15 min, and declined thereafter. The tyrosine phosphorylation of STAT3 (Fig. 3B) was also evident after 5 min of stimulation and reached its maximum at 15 min of treatment with GH. After 30 min, the tyrosine phosphorylation of STAT3 was almost reduced. It was noted that GH-stimulated tyrosine phosphorylation of p125 FAK and paxillin reached a maximum at 30 min and declined at 60 min. Pretreatment with genistein (10 mM) inhibited the tyrosine phosphorylation of p125 FAK (Fig. 3C) and paxillin (Fig. 3D).

Localization of p125 FAK, paxillin, and phosphotyrosine in pituitary GH-stimulated neutrophils

Previous studies have shown that the initial effect of growth factors such as IGF-I and PDGF is to promote the formation of ruffles and the extension of filopodia or lamellipodia (29, 43, 44). We also tested whether GH could modulate the morphological changes in neutrophils. Our data obtained from confocal microscopy showed that pituitary GH stimulates typical membrane ruffling and uropod formation in neutrophils (Fig. 4, C, F, and I). Interestingly, p125 FAK and paxillin were localized and concentrated at these focal adhesion sites, such as peripheral ruffling (Fig. 4, B and E). These peripheral complexes were clearly distinguished from control cells both morphologically and in the intensity of staining (Fig. 4, A and D). Otherwise, pY staining showed the punctate structures in control and GH-stimulated neutrophils (Fig. 4, G and H). Although the pY was densely stained in membrane ruffling in the presence of GH (Fig. 4H), the distribution of pY showed different patterns from p125 FAK and paxillin in neutrophils (Fig. 4, B and E). In part, these data showed that several proteins other than p125 FAK and paxillin are tyrosine phosphorylated upon GH stimulation in neutrophils.

Pituitary GH stimulates the association of p125 FAK with Jak2 via STAT3 and in vitro phosphorylation of p125 FAK

Although the association between Jak2 and p125 FAK has been reported, the possible association mechanism has not been confirmed (45). To investigate the association mechanism of p125 FAK with Jak2, neutrophils were treated for 0–60 min with 100 ng/ml pituitary GH. Cells were then lysed, and equally aliquoted lysates were separately immunoprecipitated using anti-Jak2, STAT3, and
p125FAK. Western blot analysis, as assessed by coimmunoprecipitation, showed a time-dependent association of p125FAK with Jak2 (Fig. 5A). Association between p125FAK and Jak2 was apparent at 15–30 min and diminished 60 min after pituitary GH stimulation. Subsequent immunoblotting with the immunoprecipitating Ab showed the presence of equal amounts of protein. Conversely, we observed the Jak2 and p125FAK association by immunoprecipitation with Jak2 and immunoblotting with p125FAK (Fig. 5B). The physical association of Jak2 and p125FAK was enhanced after stimulation with pituitary GH, but there was little constitutive association between these molecules in the controls not treated with pituitary GH. Because it is possible that Jak2 and p125FAK are indirectly linked by another protein and because STAT3 was also shown to be coimmunoprecipitated with Jak2 in neutrophils (Fig. 5B), we examined the possibility that STAT3 may mediate the association between Jak2 and p125FAK. Interestingly, as illustrated in Fig. 5, C and D, p125FAK was present in the STAT3 immunoprecipitates, and STAT3 was present in the p125FAK immunoprecipitates. The association between STAT3 and p125FAK was also increased after stimulation with pituitary GH.

To determine whether GH signal is involved in the phosphorylation of p125FAK, an in vitro kinase assay was performed using immunoprecipitated p125FAK obtained from the GH-stimulated neutrophils. Fig. 6A shows that the autoprophosphorylation of p125FAK is time dependent. It was shown that the autophosphorylation activity of p125FAK started to increase at 5 min and was maximal at 30 min. Then, the autophosphorylation activity of p125FAK was decreased, but persisted for 60 min (Fig. 6A). We further observed that genistein blocked the stimulatory effect of GH on the autophosphorylation of p125FAK (Fig. 6B).

**Discussion**

It is well established that GH is a physiological mediator of immune cell functions (15–19), and many of the actions of this stimulant are likely to be transduced through the Jak2 pathway (20, 21, 23). Although it has been reported that GH increases neutrophil adhesion, the possible mechanisms have not been examined (16). The present study demonstrates that pituitary GH stimulates neutrophil adhesion through the modulation of focal adhesion kinase (p125FAK), paxillin, and actin polymerization and further suggests...
that the neutrophil adhesion pathway by pituitary GH is under Jak2-p125 FAK -paxillin tyrosine phosphorylation. In the present study pituitary GH increased neutrophil adhesion to the plastic substratum. GH also triggered the tyrosine phosphorylation of Jak2 and STAT3 in neutrophils; this is consistent with previous studies of GH-stimulated tyrosine phosphorylation of Jak2 and STATs in the other cell types (20 –23). Our results revealed that the tyrosine phosphorylation of Jak2 occurs about 10 –15 min before p125 FAK and paxillin tyrosine phosphorylation in neutrophils. There was a 10-min lag between the tyrosine phosphorylation of Jak2 and the p125 FAK and paxillin, which indicates those p125 FAK and paxillin phosphorylations occur down-stream of Jak2. It suggests that GH-stimulated tyrosine phosphorylation of p125 FAK and paxillin may occur through the Jak2 signaling event from the GH receptor and subsequently modulate actin polymerization in neutrophils. It is noteworthy to add that a small amount of constitutive tyrosine phosphorylation of Jak2 and p125 FAK was observed in neutrophils without any stimulation. In comparison with other experiments, the constitutive tyrosine phosphorylation of Jak2 and p125 FAK in our system might be due to the difference in cell types (23, 27, 45). Inhibition of neutrophil adhesion and tyrosine phosphorylation of Jak2, p125 FAK , and paxillin by genistein implies that GH-induced neutrophil adhesion is related to the pattern of tyrosine phosphorylation of these signaling molecules. This observation is consistent with a previous report that hepatocyte growth factor-mediated tyrosine phosphorylation of p125 FAK is inhibited by the tyrosine kinase inhibitor, herbimycin A, which also blocks spreading and the migratory response of oral squamous carcinoma cells (40).

The earliest and most important events in acute inflammation are the adhesion and emigration of neutrophils from the blood into the tissue. It has been noted that adherent inflammatory cells extended the pseudopodia-like process into underlying endothelial cells (2). To target cells to specific sites, neutrophils should become activated and adhere. Following adhesion, neutrophils undergo cell shape change before they transmigrate across the vessel wall and migrate along a chemotactic gradient (2, 6). The precise mechanism(s) for the adhesion signaling from neutrophil migration to firm adhesion remains largely unknown. Recent results have demonstrated that rGH and prolactin stimulate p125 FAK and paxillin tyrosine phosphorylation through the Jak2 pathway (37, 45). In accordance with previous studies, our findings demonstrates that a low dose of pituitary GH (50 ng/ml) induces a striking increase in tyrosine phosphorylation of p125 FAK. Also, GH stimulated the tyrosine phosphorylation of paxillin, which is the focal adhesion component and putative p125 FAK substrate. Several
growth factors have also been reported to increase the tyrosine phosphorylation of paxillin, presumably through the regulation of p125FAK in various kinds of cells (25, 26, 29, 46, 47). Our data showed that the tyrosine phosphorylation of p125FAK and paxillin exhibited a typical bell-shaped dose-response curve, in agreement with the pattern of adhesion and the tyrosine phosphorylation of Jak2. The disruptive effect of pituitary GH at high concentration on the tyrosine phosphorylation of p125FAK and paxillin in neutrophils is similar to the prolactin- or PDGF-induced tyrosine phosphorylation of p125FAK and paxillin in a breast carcinoma cell line or in Swiss 3T3 cells (25, 27).

The diverse signal transduction pathways of GH stimulation have also been studied in various cell types (15, 16, 45, 48). It was previously been reported that GH promotes association of the p85 subunit of phosphatidylinositol 3-kinase (PI-3 kinase) with insulin receptor substrate-1 and Jak2, and increases enzymatic activity of PI-3 kinase (48, 49). Zhu et al. have recently confirmed that p125FAK is associated with Jak2 after the stimulation of rGH in the CHO cell line (45). Further, it has been shown that the GH signal can be transmitted to the actin cytoskeleton via Jak, p125FAK, and tensin, and the actin polymerization produced by GH stimulation occurs through the PI-3 kinase activation (41). Moreover, it has been suggested that the indirect association of Jak2 with p125FAK occurs via Tec and PI-3 kinase (41, 50, 51). In the present study we found that the physical association between Jak2 and p125FAK could be mediated through STAT3. This association was significantly increased by the disruption of the physical association between Jak2 and p125FAK could be mediated through STAT3. Thus, the STAT3 molecule can play an adapter function, which couples another signaling molecule to PI-3 kinase through STAT3. The integrity of the actin cytoskeleton is essential for the maintenance of p125FAK tyrosine phosphorylation, and may serve to anchor and compartmentalize kinases and other signaling molecules as part of the signal transduction complex (29, 59 – 61). The diversity of signal transduction pathways of GH stimulation is an alteration in cell morphology and cytoskeletal architecture through their respective receptors (29, 30, 41, 45). Recombinant GH, IGF-I, and PDGF have shown the activation of PI-3 kinase, which modulates direct membrane ruffling (24, 30, 43, 46). Similar to IGF-I and PDGF, our results strongly suggest that p125FAK and paxillin are involved in mediating the biological effects of pituitary GH, which stimulates neutrophil adhesion at least in terms of inducing membrane ruffling and uropod formation.

After the stimulation of cells by agonists, globular actin molecules recruit and polymerize to F-actin, which appears to be the prerequisite for the formation of new pseudopodia and subsequent directed movement (57, 58). According to previous reports, we also proposed that pituitary GH may modulate F-actin formation in the focal adhesion sites in neutrophils. We observed an apparent increase in F-actin level and localization in focal adhesion foci such as membrane ruffles and uropods in neutrophils after GH treatments. The cytoskeleton also plays a key role in growth factor-dependent tyrosine phosphorylation, and may serve to anchor and compartmentalize kinases in other signaling molecules as part of the signal transduction complex (29, 59 – 61). The integrity of the actin cytoskeleton is essential for the maintenance of p125FAK tyrosine phosphorylation in many cell types (29, 34, 37, 57, 61, 62). Although we have not shown an association of p125FAK and paxillin with the actin cytoskeleton, our results demonstrate that part of the cytoskeleton and adhesion kinases are mainly colocalized in focal adhesion-like sites such as membrane ruffles and uropods when neutrophils are stimulated by pituitary GH.

The present study shows that the relationship between neutrophil adhesion and Jak2, p125FAK, and paxillin tyrosine phosphorylation may be a key aspect of the neutrophil adhesion signaling by pituitary GH. The findings that GH stimulated tyrosine phosphorylation of p125FAK and paxillin and that GH promoted their recruitment to focal adhesion is consistent with these components in GH stimulation of neutrophil adhesion. Our findings have implications for an understanding of the molecular mechanisms that underlie GH-induced neutrophil adhesion. GH, as a classical physiological hormone, could function by an endocrine mechanism to promote the neutrophil adhesion and to regulate the inflammatory process.

Acknowledgments
We thank Dr. Rajiv R. Ratan for help and comments on this study. Drs. Benjamin A. Kruskal and Brian Wilson are greatly appreciated for critical reading of the manuscript, and Vera Zachariassen is appreciated for preparation of the manuscript. Hwa-Jung Huh, Byung-Ki Kim, and Myung-Soo Lee are greatly acknowledged for their fine technical assistance.

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