Activation of the serine/threonine kinase Akt, also called protein kinase B (PKB), was investigated in human neutrophils. Stimulation of the cells with the chemotactrant fMet-Leu-Phe or the chemokines IL-8 and GROα leads to the rapid and transient activation of PKB. Maximum PKB activation correlates with the well documented kinetics of respiratory burst and exocytosis. Wortmannin, a selective inhibitor of phosphoinositide 3-kinases (PI 3-kinases) in neutrophils, abrogates PKB activation. Similarly homo and heterotypic cross-linking of FcγRIA and FcγRIIB causes a transient activation of PKB that is sensitive to wortmannin treatment. Kinase activity measurements in immunoprecipitates from lysates of the myelocytic GM-1 cells or GM-1/CXCR1 cells, which are transfected with the IL-8 receptor 1, confirmed the transient activation of PKB observed in neutrophils. Stimulation of human monocytes with the CC chemokine RANTES (regulated on activation normal T cell expressed and secreted) also results in the activation of PKB. Preincubation of monocytes and neutrophils with Bordetella pertussis toxin inhibits fMet-Leu-Phe and RANTES-stimulated PKB activation, demonstrating that coupling of the receptors to heterotrimeric G-protein is required. The data show, that activation of PKB by G-protein-coupled receptors is mediated by PI 3-kinase and suggest that PKB is a constituent of neutrophil activating pathways.

Recruitment of neutrophils during inflammation is essential for the defense of the host. Activation of neutrophils is mediated by a variety of chemotactic agonists, such as formylpeptides, C5a and CXC chemokines. The chemotactic agonists bind to distinct heptahedral receptors, which are coupled to Bordetella pertussis toxin-sensitive heterotrimeric G-proteins (1, 2). Stimulation of the receptors in neutrophils triggers chemotaxis, exocytosis, and the respiratory burst (1). The signal transduction induced by the receptors leads to the rapid activation of phospholipase Cβ2 by the βγ subunits of the Gαi-proteins (3, 4) and to the subsequent elevation of cytosolic free calcium and protein kinase C activation (1). Parallel to the activation of phospholipase C, a calcium-independent pathway leads to the activation of phosphatidylinositol 3-kinase (PI 3-kinase)1 (5, 6). Both pathways are required for exocytosis and respiratory burst of neutrophils (5).

Neutrophils can also be activated in vitro by cross-linking Fcy-receptors. The resting cells express the FcγRIIA, a transmembrane protein with a cytoplasmic domain of 76 amino acids, and the FcγRIIB, which is membrane-anchored by glycosyl phosphatidylinositol moeity and does not possess a cytoplasmic tail (7). Cross-linking of the cell-surface receptor in vitro has been used to study distinct Fcγ-receptor-stimulated pathways (8, 9). Fcγ-receptor signaling is not dependent on Gα-proteins and leads to activation of phospholipase Cβ1 by a tyrosine kinase-dependent mechanism (10) and, in the case of FcγRIIA, to the activation of PI 3-kinase (11).

The fungal metabolite wortmannin is a selective and potent inhibitor of PI 3-kinase (6, 12). Treatment of phagocytes with wortmannin revealed that PI 3-kinase activity is required for the stimulation of the respiratory burst, exocytosis, and phagocytosis (5, 6, 13). However, chemotaxis and shape change of neutrophils stimulated by agonists of G-protein-coupled receptors are insensitive to wortmannin, indicating that PI 3-kinase is not involved (14, 15). Several isoforms of PI 3-kinase have been characterized in neutrophils, mainly the classical PI 3-kinaseα, (p85/p110) (6), and the more recently described PI 3-kinaseγ, which is activated by the βγ subunits of G-proteins (16, 17). Little information is available on potential downstream effectors of PI 3-kinase in neutrophils. So far the p21 activated kinase PAK (18) and p47phox, a soluble factor of the NADPH oxidase (19, 20), have been described.

The serine/threonine protein kinase B (PKB, also called Akt or RAC-PK), first identified as an oncogene, was subsequently shown to be a major target of PI 3-kinase-dependent signaling in growth factor stimulation (21–27). In line with this observation, expression of constitutively active forms of PI 3-kinase (20, 28) induce stimulus-independent activation of PKB in COS-7 cells (28) and in stably transfected myelocytic GM-1 cells (20). Furthermore, regulation of neuronal survival and suppression of c-Myc-induced apoptosis implicate that PKB is involved in the transduction of survival signals (29, 30). However, the mitogen lysophosphatidic acid (LPA) which binds to G-protein-coupled receptors was reported to have no effect on the activation of PKB in fibroblasts (25). Recent data, by contrast, suggest that PKB becomes transiently activated upon stimulation of Gα-protein-coupled receptors in platelets (31) and by Gα-protein-coupled receptors in adipocytes (32).

Chemokine receptors were recently shown to function as co-receptors during HIV-infection of CD4+ cells (33). A homozy-
gous defect in the CCR5 gene, that encodes for the receptor of the CC chemokines RANTES, MIP1α, and MIP1β, was shown to provide resistance to HIV infection of multiply exposed individuals (33). It was further reported that RANTES and RANTES antagonists prevent infection of lymphocytes with macrophage tropic HIV isolates (33), indicating that binding of the HIV envelope protein gp120 the co-receptor is essential for virus uptake. However, the contribution of signal transduction stimulated by gp120 binding to CD4 and the G1-protein coupled co-receptors for HIV infection is still controversial (34, 35).

We show here for the first time that in phagocytes activation of PKB can be stimulated by a variety of G1-protein-coupled receptor-agonists and by cross-linking of Fcγ-receptors. The transient PKB activation observed in neutrophils correlates with other typical responses of the cells, such as the respiratory burst and exocytosis. The findings implicate that PKB is part of the signal cascade involved in neutrophil activation.

**EXPERIMENTAL PROCEDURES**

**Materials**

RPMI 1640 media, Hank’s balanced salt solution, antibiotics, and additional cell culture supplements were obtained from Life Technologies, Inc. Protein A-Sepharose CL-4B was purchased from Pharmacia Biotech. Immunoblotting-P (PVDF) was from Millipore Corp, histone 2B was from Fluka, [γ-32P]ATP was from Amersham Life Science, Inc. and *B. pertussis* toxin was from List Biochemicals. Alkaline phosphatase-conjugated goat anti-rabbit IgG was obtained from Bio-Rad, and the goat anti-mouse (GaM) IgG Fab(α)2 fragments were from Dianova. All other reagents were of molecular biology grade. The anti-RACα2–β50 antibodies were prepared as described previously (23). The monoclonal antibodies against Fcγ receptors IIA (mAb IV.3) and IIIB (mAb SD2) were kindly provided by Dr. A. J. Verhoeven. Chemokines IL-8, GROα, and RANTES were chemically synthesized by Dr. I. Clark-Lewis (Vancouver, Canada). The CXCR1 (IL-8R1) gene was kindly supplied by Dr. B. Moser, and 17-hydroxywortmannin was a gift from Dr. T. Payne.

**Cells**

GM-1 and GM-1/CXCR1—The CXCR1 gene was inserted into pPUR (pPUR/CXCR1) under the control of the CMV-1 promoter. GM-1 cells (36) were transfected with the pPUR/CXCR1 as described (20). Resistant clones (GM-1/CXCR1) were selected in RPMI 1640 media supplemented with 10% FCS and 0.2 μg/ml puromycin. Parental GM-1 cells were cultured in RPMI 1640 supplemented with 10% FCS and where indicated, differentiated with IFNy as described previously (36).

Neutrophil and Monocyte Cell Preparation—Neutrophils were isolated from freshly drawn blood of healthy volunteers (37) or fromuffy coats of citrated donor blood stored up to 20 h at 4–10 °C provided from the Swiss Red Cross (Bern, Switzerland) (38). Monocytes were prepared fromuffy coats as described (39).

*B. pertussis* Toxin Treatment of Neutrophils and Monocytes—Pre-treatment of neutrophils and monocytes with *B. pertussis* toxin was as described previously (40). Briefly, neutrophils or monocytes (1.35 × 10⁶/ml) were incubated for 90 min at 37 °C in the absence or presence of 1 μg/ml *B. pertussis* toxin in a balanced salt solution (110 mM NaCl, 10 mM KCl, 1 mM MgCl₂, 10 mM glucose, 30 mM HEPES, and 0.1% w/v bovine serum albumin).

**Cell Stimulation**

**Chemotaxtractant Stimulation**—The cells were washed twice with phosphate-buffered saline, and resuspended in Hank’s balanced salt solution containing 20 mM HEPES, pH 7.4 (6 × 10⁶ cells/ml for neutrophils or monocytes). Aliquots (500 μl) were incubated for 10–15 min at 37 °C in the absence or presence of 100 mM wortmannin prior to stimulation with the indicated stimuli. The reactions were stopped by the addition of trichloroacetic acid to a final concentration of 10%. Precipitation with the indicated stimuli or as described above for Fcγ-receptor cross-linking. The reactions were stopped by the addition of concentrated lysate buffer giving final concentrations of 50 mM Tris-HCl, pH 7.5, 1% Nonidet P-40, 120 mM NaCl, 1 mM EDTA, 50 mM NaF, 40 mM β-glycerophosphate, 0.1 mM sodium vanadate, 0.5 mM phenylmethylsulfonyl fluoride, and 1 mM benzamidine. In vitro kinase assays were performed as described previously (20), with the exception that histone 2B was used as a substrate. The incorporation of 32P into histone 2B was determined from 13.5% SDS-PAGE using a PhosphorImager (Molecular Dynamics). The PKB content of the immunoprecipitates were measured on Western blots (see below).

**Western Blot Analysis of PKB**

**RESULTS**

**Activation of PKB by Chemoattractants**—The determination of PKB activity by substrate phosphorylation from chemoattractant-stimulated neutrophils gave inconsistent results. The high proteolytic and phosphatase activities found in detergent lysates of neutrophils cause the rapid degradation and dephosphorylation of PKB, even in the presence of high concentrations of protease and phosphatase inhibitors. However, activation of PKB was demonstrated to coincide with the phosphorylation on Ser473 and Thr308 resulting in an altered electrophoretic mobility (42, 43) which is detected on Western blots using an antibody directed against the carboxy terminal of the kinase (23). Fig. 1 shows the transient activation of PKB in human neutrophils stimulated by fMet-Leu-Phe, IL-8, and GROα. Phosphorylated PKB with reduced electrophoretic mobility is detectable as early as 10 s after stimulation of the neutrophils.

**Fig. 1.** Transient activation of PKB in human neutrophils stimulated with fMet-Leu-Phe, IL-8, or GROα. Neutrophils were preincubated in the absence (left panels) or presence (right panels) of 100 mM wortmannin and then stimulated with 100 μM fMet-Leu-Phe, IL-8, or GROα for the indicated times. Activation of PKB was detected on Western blots as retarded mobility during electrophoresis (closed arrow heads).

HEPES, pH 7.4 (1.2 × 10⁶ cells/ml for immunoprecipitations or 6 × 10⁶ cells/ml for total protein precipitations). Aliquots (500 or 250 μl) were incubated for 10–15 min at 37 °C in the absence or presence of 100 mM wortmannin prior to stimulation with cross-linking GaM IgG Fab(α)2 fragments. The reactions were terminated either with trichloroacetic acid, as above, or ice-cold lysis buffer (described below).

**Immunoprecipitation and in Vitro Kinase Assay**

GM-1/CXCR1 and IFNy differentiated GM-1 cells were serum-starved overnight or for 3 h at 37 °C in RPMI 1640. The cells were washed twice with phosphate-buffered saline and resuspended in Hank’s balanced salt solution containing 20 mM HEPES, pH 7.4 (1.2 × 10⁶ cells/ml). Aliquots (500 μl) were incubated for 10–15 min at 37 °C in the absence or presence of 100 mM wortmannin prior to stimulation with the indicated stimuli or as described above for Fcγ-receptor cross-linking. The reactions were stopped by the addition of concentrated lysate buffer giving final concentrations of 50 mM Tris-HCl, pH 7.5, 1% Nonidet P-40, 120 mM NaCl, 1 mM EDTA, 50 mM NaF, 40 mM β-glycerophosphate, 0.1 mM sodium vanadate, 0.5 mM phenylmethylsulfonyl fluoride, and 1 mM benzamidine. In vitro kinase assays were performed as described previously (20), with the exception that histone 2B was used as a substrate. The incorporation of 32P into histone 2B was determined from 13.5% SDS-PAGE using a PhosphorImager (Molecular Dynamics). The PKB content of the immunoprecipitates were measured on Western blots (see below).

**Western Blot Analysis of PKB**

Immunoprecipitated or total protein samples were resolved by 8.0% SDS-PAGE, transferred to PVDF membranes, and incubated with the anti-RACα2–β50 antibody. Detection was performed using alkaline phosphatase-conjugated anti-rabbit antibody.

**Activation of PKB by Chemoattractants**—The determination of PKB activity by substrate phosphorylation from chemoattractant-stimulated neutrophils gave inconsistent results. The high proteolytic and phosphatase activities found in detergent lysates of neutrophils cause the rapid degradation and dephosphorylation of PKB, even in the presence of high concentrations of protease and phosphatase inhibitors. However, activation of PKB was demonstrated to coincide with the phosphorylation on Ser473 and Thr308 resulting in an altered electrophoretic mobility (42, 43) which is detected on Western blots using an antibody directed against the carboxy terminal of the kinase (23). Fig. 1 shows the transient activation of PKB in human neutrophils stimulated by fMet-Leu-Phe, IL-8, and GROα. Phosphorylated PKB with reduced electrophoretic mobility is detectable as early as 10 s after stimulation of the neutrophils.
and shows a maximum of activation at ~40–80 s. Stimulation with fMet-Leu-Phe leads to a marked phosphorylation of PKB that is still apparent after 5 min. IL-8, which binds with high affinity to both IL-8 receptors CXCR1 and CXCR2, elicits a more transient activation of PKB. Stimulation with GROα, which binds only to CXCR2 with high affinity (2), causes an even less pronounced response. Pretreatment of neutrophils with wortmannin abolishes the phosphorylation of PKB in all cases, confirming previous observations that PKB is a downstream effector of PI 3-kinase (20, 28).

To measure PKB activity we used promyelocytic GM-1 cells that can be differentiated with IFNγ to respond to stimulation with fMet-Leu-Phe (36). Endogenous PKB was immunoprecipitated with affinity purified antibodies from detergent lysates of GM-1 cells and subjected to Western blot analysis. Like in neutrophils, stimulation with fMet-Leu-Phe results in the wortmannin-sensitive transient phosphorylation of PKB (Fig. 2, left). The electrophoretic mobility shift of endogenous PKB in serum-starved GM-1 cells can be detected as early as 10 s, shows a maximum at ~40–80 s, and is still apparent 5 min after stimulation. We assessed protein kinase activity from the same anti-PKB immunoprecipitates from lysates of IFNγ-differentiated cells using histone 2B as substrate. Exposure of serum-starved GM-1 cells to fMet-Leu-Phe stimulates ~3-fold the activity of endogenous PKB. The increase in PKB activity shows a maximum at 40–80 s and is still measurable after 5 min. Incubation of the anti-PKB immunoprecipitates with wortmannin did not inhibit the activity of the kinase, confirming that PKB is not a target. However, pretreatment of the cells with wortmannin blocks agonist-dependent PKB activity, indicating that PI 3-kinase is an upstream regulator. In agreement with previous results (42), incubation of the immunoprecipitates with protein phosphatase 2A (PP2A) abolished PKB activity and the electrophoretic mobility shift (data not shown).

Differentiation induced with IFNγ may alter signaling pathways which could regulate PKB activation. To exclude this possibility, we introduced the IL-8 receptor CXCR1 into GM-1 cells and cloned stable transfectants. GM-1 cells expressing CXCR1 (GM-1/CXCR1) showed a typical rise in cytosolic free calcium upon stimulation with IL-8 (data not shown). The cells were then used for testing IL-8-dependent activation of PKB. Lysates of control and IL-8-stimulated cells were treated with anti-PKB antibodies, and the immunoprecipitates were resolved on SDS-PAGE. Like in neutrophils, stimulation of serum-starved GM-1/CXCR1 cells results in the transient phosphorylation of PKB (Fig. 2, right). The effect is fully prevented by pretreatment of the cells with 100 nM wortmannin. Measurements of kinase activity associated with the immunoprecipitates from GM-1/CXCR1 cells confirm the transient IL-8-stimulated activation of PKB. The kinase activity shows a maximum at 40 s after stimulation. As in the case of neutrophils, stimulation with IL-8 results in a less pronounced and more transient activation of PKB than stimulation with fMet-Leu-Phe. Measurements of PKB activity stimulated by different concentrations of IL-8 displays a bell-shaped dose-response curve (Fig. 3). Maximum stimulation, about 3-fold, is obtained with 100 nM IL-8. At higher IL-8 concentrations, a slightly weaker activation of PKB is observed (Fig. 3). This effect could be mediated by an increase of phosphatase activities, which are more potently stimulated at high agonist concentrations. However, stimulation of kinase activity can be detected in anti-PKB immunoprecipitates from cells exposed to concentrations as low as 1 nM IL-8, suggesting a close coupling of PKB activation to the chemokine receptor.

Activation of PKB by Fcγ-receptor Cross-linking—Cross-linking of Fcγ-receptors has been reported to induce the activation of PI 3-kinase (44). We have therefore tested if homo or heterotypic cross-linking of Fcγ-receptors IIa and/or IIb (41) leads to activation of endogenous PKB. Fig. 4 shows the effect of goat anti-mouse F(ab′)2-stimulated cross-linking of Fcγ-receptors in neutrophils that were preincubated with receptor-specific monoclonal antibodies, mAb IV.3 (anti-FcγRIIA) and/or mAb SD2 (anti-FcγRIIB). The phosphorylation-induced electrophoretic mobility shift of PKB was absent if the cells were pretreated with 100 nM wortmannin or if the cells were incubated only with receptor-specific antibodies or cross-linking F(ab′)2. In contrast, following cross-linking, FcγRII induces the transient activation of PKB which shows a maximum at ~90–120 s and was nearly absent after 5 min. Similarly, heterotypic
FIG. 3. IL-8 concentration dependence of PKB activity. PKB activity was measured in immunoprecipitates of GM-1/CXCR1 cells stimulated with the indicated concentrations of IL-8 for 60 s (for details see Fig. 2). Samples are representative of two experiments performed in duplicate.

FIG. 4. Activation of PKB by Fcγ-receptor cross-linking in neutrophils. Homotypic (FcγRII and FcγRIII) or heterotypic (FcγRII + FcγRIII) cross-linking of Fcγ-receptors induces the transient activation of PKB in neutrophils (closed arrowhead). Preincubation with 100 nM wortmannin (WT) abolishes activation. Neither the addition of the primary monoclonal antibody (mAb) or the cross-linking GaM IgG F(ab')2 fragments (IgG), alone, induced PKB activation. PKB activation was detected as in Fig. 1. The Western blots are each representative of at least two similar experiments.

cross-linking of both receptors causes the transient phosphorylation of PKB.

Because of the rapid degradation of PKB in neutrophil lysates, we used GM-1 cells to assess Fcγ-receptor-stimulated kinase activity in anti-PKB immunoprecipitates. Cross-linking of FcγRII results in 2.5–3-fold stimulation of PKB activity. Maximum activity (100%), from three separate experiments, was determined at 60 s after addition of cross-linking IgG. Controls: (white bars, left to right) (i) mock immunoprecipitates lacking PKB antibody, (ii) cells treated with secondary Ab alone (primary FcγRIIA mAbs were omitted), and (iii) non-treated cells in which both the primary anti-FcγRIIA and GaM IgG F(ab')2 fragments (IgG) were omitted. Phosphorylated histone 2B was resolved on SDS-PAGE, and the 32P content was determined using a PhosphorImager.

FIG. 5. PKB activity upon Fcγ-receptor cross-linking in GM-1 cells. Cross-linking of Fcγ-receptors (IIA) stimulates PKB activity in GM-1 cells. PKB activity was determined in immunoprecipitates prepared from lysates of cells that were preincubated with primary anti-FcγRIIA mAb followed by cross-linking for 60 s with GaM IgG F(ab')2 fragments (IgG). Preincubation with 100 nM wortmannin (WT; black bars), as compared with nontreated cells (gray bars), inhibits PKB activity. Maximum activity (100%), from three separate experiments, was determined at 60 s after addition of cross-linking IgG. Controls: (white bars, left to right) (i) mock immunoprecipitates lacking PKB antibody, (ii) cells treated with secondary Ab alone (primary FcγRIIA mAbs were omitted), and (iii) non-treated cells in which both the primary anti-FcγRIIA and GaM IgG F(ab')2 fragments (IgG) were omitted. Phosphorylated histone 2B was resolved on SDS-PAGE, and the 32P content was determined using a PhosphorImager.

DISCUSSION

We have investigated the activation of PKB in phagocytes upon stimulation with chemoattractants that bind to G-protein-coupled receptors and following Fcγ-receptor cross-linking. In response to stimulation with chemoattractants, the kinetics of PKB activation complies with other well-characterized neutrophil responses, such as respiratory burst and granule exocytosis. The findings suggest that activation of PKB is involved in the regulation of neutrophil responses. The more pronounced activation of PKB, upon stimulation with fMet-Leu-Phe, is in line with the previously reported stronger potency of the formyl-peptide to induce the respiratory burst and exocytosis of neutrophils (40, 46). Chemokines, which induce a weaker superoxide production than fMet-Leu-Phe, stimulate equally potent the chemotaxis of neutrophils, a response, how-
ever, that is independent of PI 3-kinase activity and thus may not involve PKB (14).

Our experiments reveal that PI 3-kinase activity is necessary for chemoattractant and Fc-receptor-mediated activation of PKB. Recent reports, however, suggest that wortmannin-insensitive pathway(s) may exist that stimulate PI 3-kinase-independent activation of PKB. The reports include activation by βγ-adrenoreceptors (32), which couple to Gs- and Gi-type heterotrimeric G-proteins (47), by elevation of intracellular cAMP levels (48) or by a stress-induced pathway (49). It is conceivable that the alternative pathways are not linked to the phagocyte responses, which are activated upon stimulation with agonists of Gα-linked receptors or during Fe-mediated phagocytosis.

Until now only glycogen synthase kinase 3 (GSK3) has been characterized as substrate, which is phosphorylated by PKB upon stimulation of cells with insulin (27). The present data suggest that PKB is involved in rapid signal transduction pathways that lead to transient secretory responses. Stimulation of platelets with thrombin-receptor activating peptide resulted in comparable kinetics of PKB activation as we describe here for platelets with thrombin-receptor activating peptide. Stimulation of platelets with thrombin-receptor activating peptide resulted in comparable kinetics of PKB activation as we describe here for platelets with thrombin-receptor activating peptide.

FIG. 6. Activation of PKB in human neutrophils and monocytes, effect of B. pertussis toxin. Neutrophils (top panel) or monocytes (bottom panel) were incubated in the absence (control) or presence of B. pertussis toxin (PTX). Neutrophils were stimulated for 60 s with 100 nM fMet-Leu-Phe (fMLP, top panel) or by cross-linking FcγRII and FcγRIII (FcγRII + FcγRIII, middle panel). Monocytes were stimulated for 60 s with RANTES (bottom panel). Pertussis toxin inhibits PKB activation (closed arrow heads) in response to fMet-Leu-Phe (top panel) or RANTES (bottom panel) but has no effect on Fcγ cross-linking (middle panel).

formed upon dephosphorylation of PI(3,4,5)P3, the main product of PI 3-kinase (52). The previously reported kinetics of the formation D3 polyphosphoinositides in platelets and neutrophils stimulated with G-protein receptor-coupled agonists (52–54) is in excellent agreement with the present data on the activation of PKB.

The two receptors CXCR1 and CXCR2 bind IL-8 with high affinity, whereas GROα is only recognized by CXCR2 with high affinity (55). Both receptors mediate chemotaxis and exocytosis of neutrophils, but the respiratory burst is activated solely by CXCR1 (56, 57). The signaling pathways of CXCR1 and CXCR2 lead to the same degree of calcium mobilization from intracellular stores via phospholipase C (58, 59) and activation of MAP kinase (60). However, only CXCR1 stimulates activation of phospholipase D (56, 61). We show here that both chemokine receptors stimulate the activation of PKB via PI 3-kinase.

A role of PI 3-kinase in promoting cell survival under conditions that would mediate apoptosis has been implicated (62). Recently this effect was shown to be mediated by PKB (29, 30, 50). The observation that chemokines activate PKB may suggest that chemokine receptors could promote the survival of immune cells. However, resistance to apoptosis would also result in improved conditions for the replication of intracellular pathogens. Presently it is not known if binding of HIV to the chemokine receptors expressed on CD4+ cells results in the activation of PKB.

Treatment of L6 myotubules with insulin results in a marked (~12-fold) activation of PKB (43). In CHO cells, which express low levels of endogenous insulin receptors, insulin mediates only a moderate 3-fold activation of PKB activity (26). Thus the 2.5–4-fold stimulation of endogenous PKB in GM-1 and GM1/CXCR1 cells may be limited by the levels of endogenous proteins involved in activation. G-protein-coupled receptor-induced activation of PKB may involve a distinct isoform of PI 3-kinase that is regulated by the βγ-subunits of heterotrimeric G-proteins (16, 17, 63). Activation of PKB by Fcγ-receptor cross-linking is mediated by signal transduction mechanisms, which resemble growth factor receptor signaling, and involves tyrosine phosphorylations, coupling to adaptor proteins and the activation of the classical PI 3-kinase p85/p110 (11, 64, 65).

Signal transduction initiated by the glycosyl phosphatidylinositol-anchored FcγRIIB of neutrophils (7) leads to calcium mobilization (66, 67), translocation of Src-related tyrosine kinases (68), actin polymerization, and enhanced phagocytosis (69), but does not activate MAP-kinase as shown for the transmembrane splice variant FcγRIIA that is expressed on mononuclear cells (70). The coupling of the GPI-anchored FcγRIIB to cytoplasmic signaling molecules is not established (71). We report here that cross-linking of the FcγRIIB triggers the rapid activation of PKB by a PI 3-kinase-dependent pathway.

The rapid activation of PKB by Fcγ-receptor cross-linking suggests that PKB may be involved in early signaling events associated with antibody-mediated phagocytosis.

The precise role of PKB in neutrophil signal transduction remains to be established. Stimulation via Fcγ-receptors and the more pronounced activation of PKB upon stimulation with fMet-Leu-Phe as compared with the chemokines IL-8 and GROα, may indicate that PKB is involved in secretory responses, such as the respiratory burst and exocytosis.

Acknowledgments—We thank Drs. I. Clark-Lewis, B. Moser, T. Payne, and A. J. Verhoeven for providing valuable reagents.

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