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Immunology

Association of immunoglobulin G Fc receptor II with Src-like protein-tyrosine kinase Fgr in neutrophils

(tyrosine phosphorylation/*fgr* gene)

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ABSTRACT **The interaction of Fc receptors with antibody–antigen complexes activates multiple biological functions in hematopoietic cells. Recently, protein-tyrosine phosphorylation has been suggested to be involved in Fc receptor-mediated cell signaling. Here we show that the Src-like protein-tyrosine kinase Fgr, which is specifically expressed in mature myelomonocytic cells, coimmunoprecipitates with IgG Fc receptor II ($Fc_{\gamma}RII$), but not with $Fc_{\gamma}RIII$ from detergent lysates of human peripheral neutrophils. Crosslinking of $Fc_{\gamma}RII$ induced a rapid increase in the tyrosine kinase activity and comodulation of Fgr. These results suggest that Fgr is physically and functionally associated with $Fc_{\gamma}RII$ and involved in $Fc_{\gamma}RII$-mediated signal transduction pathways.**

The interaction of immunoglobulin G (IgG) antibody with cells of the immune system induces a wide variety of responses, including phagocytosis, antibody-dependent cellular cytotoxicity, generation of reactive oxygen intermediates, and release of lysosomal enzymes. All these responses are initiated through the binding of the Fc domain of IgG to its specific receptors, IgG Fc receptors ($Fc_{\gamma}Rs$). On the basis of differences in their structures and affinities for IgG, human $Fc_{\gamma}Rs$ are divided into three major classes, I–III. $Fc_{\gamma}RI$ has three immunoglobulin-like extracellular domains that bind monomeric IgG with high affinity, whereas $Fc_{\gamma}RII$ and $Fc_{\gamma}RIII$ have only two immunoglobulin-like extracellular domains and low affinity for monomeric IgG. $Fc_{\gamma}RII$ molecules are encoded by three homologous but distinct genes, termed $Fc_{\gamma}RIIA$, -B, and -C, that generate at least six different transcripts by alternative splicing, whereas $Fc_{\gamma}RIII$ molecules are encoded by two different genes, $Fc_{\gamma}RIIIA$ and -B (1, 2). Although much is known about $Fc_{\gamma}Rs$ at the gene and protein levels, the mechanisms of signal transduction coupled to $Fc_{\gamma}Rs$ are not well understood. However, recent studies have demonstrated that engagement of $Fc_{\gamma}Rs$ with antibodies induces tyrosine phosphorylation of multiple substrates, including phospholipase C-γ1, $Fc_{\gamma}RII$ itself, and CD3 ζ chain, which is one subunit of the multiprotein $Fc_{\gamma}RIIIA$ complex, suggesting that tyrosine phosphorylation may be involved in signal transduction mediated by $Fc_{\gamma}Rs$ (3–8).

Protein-tyrosine kinases have been reported to associate with surface receptors that lack an intracellular catalytic domain: Lck with CD4, CD8, and the β chain of the interleukin 2 receptor on T cells (9–12), Lyn with surface immunoglobulin (sIg) on B cells and the high-affinity IgE receptor ($Fc_{\epsilon}RI$) on mast cells and basophils (13–17), and Fyn with the T-cell antigen receptor (TCR)–CD3 complex and sIg (15, 18, 19). Thus it is highly possible that $Fc_{\gamma}R$ also associates with Src-like kinases. The present study suggests that Fgr, which is specifically expressed in granulocytes, monocytes, and natural killer cells (20, 21), is associated with $Fc_{\gamma}RII$ and involved in $Fc_{\gamma}RII$-mediated signal transduction pathways in neutrophils.

MATERIALS AND METHODS

Isolation of Human Peripheral Neutrophils. Purified human neutrophils from healthy donors were obtained by sedimentation in 3% dextran at room temperature, density centrifugation on Ficoll/Hypaque, and hypotonic lysis of erythrocytes (22). Isolated neutrophils were incubated with 5 mM diisopropyl fluorophosphate (DFP) (Sigma), a potent proteinase inhibitor, before cell lysis (23).

Antibodies. $F(ab')_2$ fragments of goat anti-mouse IgG were from Organon Teknika-Cappel. The anti-human $Fc_{\gamma}RII$ (2E1), $Fc_{\gamma}RIII$ (3G8), and complement receptor III (BEAR 1) antibodies were purchased from Cosmo Bio (Tokyo). Monoclonal antibodies (mAbs) against Src-like protein-tyrosine kinases were raised against synthetic peptides corresponding to the unique amino-terminal regions of the individual Src-like kinases. For anti-Fgr the mAb was raised against a peptide corresponding to Ala-48 to Asp-67; for anti-Fyn, Ser-25 to Val-141 (24); and for anti-Lyn, Arg-25 to Ala-119 (14). These mAbs were able to bind to SDS-denatured Fgr, Fyn, and Lyn proteins as well as native ones (14, 24). Heat-aggregated human IgG was prepared by incubation at 5 mg/ml in phosphate-buffered saline (PBS: 137 mM NaCl/2.7 mM KCl/1.5 mM KH_2PO_4/8.1 mM Na_2HPO_4, pH 7.3) at 68°C for 30 min and washing with PBS to remove soluble IgG.

Coimmunoprecipitation, *in Vitro* Phosphorylation, and Reimmunoprecipitation. Freshly isolated human peripheral neutrophils, pretreated with 5 mM DFP, were lysed at 2×10^7 cells per ml in lysis buffer [1% (vol/vol) Nonidet P-40/50 mM Tris·HCl, pH 8.0/150 mM NaCl/2 mM EDTA/5 mM NaF/ 250 μM Na_3VO_4/5 mM DFP containing aprotinin, leupeptin, and *p*-amidinophenylmethanesulfonyl fluoride hydrochloride at 10 μg/ml] for 30 min at 4°C. The lysate was centrifuged and cleared with protein G-Sepharose (Pharmacia LKB). Aliquots of the cleared lysate were incubated with various mAbs and immune complexes were precipitated with protein G-Sepharose. The immunoprecipitates were washed five times with lysis buffer and twice with kinase buffer [40 mM Hepes, pH 7.5/10 mM $MgCl_2$/3 mM $MnCl_2$/10% (vol/vol) glycerol], suspended in 30 μl of reaction buffer (40 mM Hepes, pH 7.5/10 mM $MgCl_2$/3 mM $MnCl_2$/10% glycerol/1 mM dithiothreitol with aprotinin, leupeptin, and *p*-amidinophenylmethanesulfonyl fluoride hydrochloride at 10 μg/ml) containing 10 μCi of [γ-^{32}P]ATP (5000 Ci/mmol; 1 Ci = 37 GBq), and incubated for 20 min at 25°C. The reaction was stopped by two washes with lysis buffer containing 20 mM EDTA. For reprecipitation, the phosphoproteins were boiled in 1% SDS, to dissociate the multiple components in the

Abbreviations: DFP, diisopropyl fluorophosphate; $Fc_{\gamma}R$, IgG Fc receptor; mAb, monoclonal antibody; sIg, surface immunoglobulin; TCR, T-cell antigen receptor.

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immune complexes, and diluted 10-fold with lysis buffer. Fgr and other Src-family kinases were immunoprecipitated with specific mAbs. The samples were suspended in sample buffer, denatured by boiling, and then subjected to SDS/PAGE and autoradiography.

Partial Proteolytic Peptide Mapping. ^{32}P-labeled bands of the directly immunoprecipitated Fgr and Fc$_{\gamma}$RII-associated Fgr (Fig. 1 *A* and *B*) were excised from the gel, partially digested with various amounts of *Staphylococcus aureus* V8 protease according to Cleveland *et al.* (25), and electrophoresed in an SDS/16% polyacrylamide gel. Gels were analyzed on a Fuji image analyzer, model BAS2000.

Crosslinking of Fc$_{\gamma}$RII. Neutrophils were incubated in the presence of a saturating concentration of anti-Fc$_{\gamma}$RII mAb (2E1) for 30 min at 4°C and then washed with cold PBS. Crosslinking of Fc$_{\gamma}$RII was initiated by the addition of bridging antibodies, F(ab')$_2$ of goat anti-mouse IgG (10 μg/ml), in serum-free Dulbecco's modified Eagle's medium (DMEM). In some experiments Fc$_{\gamma}$Rs were crosslinked by heat-aggregated IgG. After crosslinking, cells were incubated at 37°C for various periods and then lysed directly in DMEM by addition of 2× lysis buffer.

Immunoblotting. The lysates were immunoprecipitated with anti-Fgr mAb covalently conjugated to CNBr-activated Sepharose (in order to avoid the interference from the heavy-chain band of the mAb). The immunoprecipitates were washed with lysis buffer, suspended in sample buffer, denatured by boiling, and subjected to SDS/10% PAGE. The proteins were transferred to a poly(vinylidene difluoride) membrane filter (Immobilon-P, Millipore) and immunoblotted with anti-Fgr mAb. The Fgr proteins were detected by using horseradish peroxidase-conjugated protein A and an enhanced chemiluminescence detection system (Amersham).

Immune-Complex Kinase Assay. Fgr was immunoprecipitated with anti-Fgr mAb and protein G-Sepharose. The immunoprecipitates were incubated in 30 μl of reaction buffer (40 mM Hepes, pH 7.5/10 mM $MgCl_2$/3 mM $MnCl_2$/10% glycerol/1 mM dithiothreitol/10 μM ATP) containing 10 μCi of [γ-^{32}P]ATP (5000 Ci/mmol) for 5 min at 25°C with or without rabbit muscle enolase (Sigma), an exogenous substrate, and the reactions were stopped by the addition of sample buffer. The samples were denatured by boiling, subjected to SDS/10% PAGE, and analyzed on a Fuji BAS2000 image analyzer.

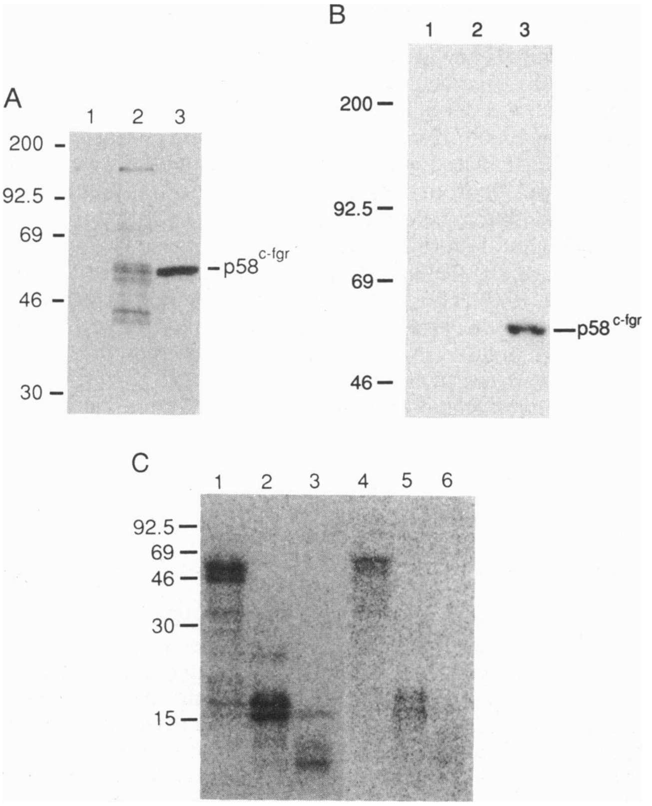

FIG. 1. Coimmunoprecipitation of Fgr with Fc$_{\gamma}$RII. (*A*) Coimmunoprecipitation of kinase activity with Fc$_{\gamma}$RII. Freshly isolated human peripheral neutrophils (2 × 10^7 cells per lane) were lysed and subjected to immunoprecipitation with mAbs to Fc$_{\gamma}$RIII (3G8) (lane 1) and Fc$_{\gamma}$RII (2E1) (lane 2). A lysate of 2 × 10^6 neutrophils was immunoprecipitated with anti-Fgr mAb (lane 3). Following *in vitro* kinase reaction, precipitates were analyzed by SDS/10% PAGE followed by autoradiography. Position of Fgr (p58$^{c\text{-}fgr}$) is indicated at right. Size markers (kDa) are at left. (*B*) Fgr in anti-Fc$_{\gamma}$RII immunoprecipitates. Neutrophils (2 × 10^7 cells per lane) were lysed and subjected to immunoprecipitation with mAbs to complement receptor III (BEAR 1) (lane 1), Fc$_{\gamma}$RIII (lane 2), and Fc$_{\gamma}$RII (lane 3). Following *in vitro* kinase reaction, immune complexes were dissociated in 1% SDS and diluted for reimmunoprecipitation with anti-Fgr mAb. The precipitates were analyzed by SDS/7.5% PAGE and autoradiography. (*C*) Peptide mapping of directly precipitated Fgr and Fc$_{\gamma}$RII-associated Fgr. ^{32}P-labeled bands of the directly immunoprecipitated Fgr and Fc$_{\gamma}$RII-associated Fgr (*A* and *B*) were subjected to partial proteolytic peptide analysis using *S. aureus* V8 protease. Directly precipitated Fgr (lanes 1–3) and Fc$_{\gamma}$RII-associated Fgr (lanes 4–6) were digested with various amounts of V8 protease (1 μg/ml, lanes 1 and 4; 20 μg/ml, lanes 2 and 5; 400 μg/ml, lanes 3 and 6). After SDS/16% PAGE, the gel was analyzed on a Fuji BAS2000 image analyzer.

RESULTS

Coimmunoprecipitation of Fgr with Fc$_{\gamma}$RII. Physical association between Fc$_{\gamma}$R and Fgr was examined by sequential immunoprecipitation experiments using neutrophils in which Fc$_{\gamma}$RIIA, -IIC, and -IIIB are preferentially expressed. Freshly isolated human peripheral neutrophils were lysed in buffers containing 1% Nonidet P-40, and Fc$_{\gamma}$RII and Fc$_{\gamma}$RIII were immunoprecipitated. Incubation of the anti-Fc$_{\gamma}$RII immunoprecipitates with [γ-^{32}P]ATP resulted in phosphorylation of several proteins, including 58- and 40-kDa proteins which were suspected to be autophosphorylated Fgr and Fc$_{\gamma}$RII itself. However, no corresponding phosphoproteins were detected in anti-Fc$_{\gamma}$RIII immunoprecipitates (Fig. 1*A*). To verify that the 58-kDa phosphoprotein was phosphorylated Fgr, we solubilized the Fc$_{\gamma}$R immunoprecipitate with 1% SDS, diluted it 10-fold with buffer containing 1% Nonidet P-40, and subjected the solution to reimmunoprecipitation with anti-Fgr mAb. The phosphorylated Fgr was precipitated from the anti-Fc$_{\gamma}$RII immune complexes, but not from anti-Fc$_{\gamma}$RIII or anti-complement receptor III immune complexes (Fig. 1*B*). Reprecipitation of Fgr from the anti-Fc$_{\gamma}$RII immune complexes was blocked by preincubation of the antibody with the peptide used for immunization (data not shown). To further confirm the identity of the 58-kDa phosphoprotein, we performed peptide mapping analysis. The 58-kDa phosphoprotein reimmunoprecipitated from the anti-Fc$_{\gamma}$RII immune complexes was partially digested with *S. aureus* V8 protease and then analyzed by SDS/PAGE. The digestion pattern of the 58-kDa phosphoprotein was very similar to that of Fgr directly precipitated from neutrophils with anti-Fgr mAb and subjected to autophosphorylation (Fig. 1*C*). These data suggest that Fgr is physically associated with Fc$_{\gamma}$RII in neutrophils.

Src-Like Tyrosine Kinases in anti-Fc$_{\gamma}$RII Immunoprecipitates. We also examined the association of Fc$_{\gamma}$RII with other Src-like tyrosine kinases. In neutrophils, while Fyn was not detectably expressed, Lyn was expressed as a doublet generated by alternative splicing (Fig. 2*A*). However, this kinase was scarcely detectable in the anti-Fc$_{\gamma}$RII immune complexes (Fig. 2*B*).

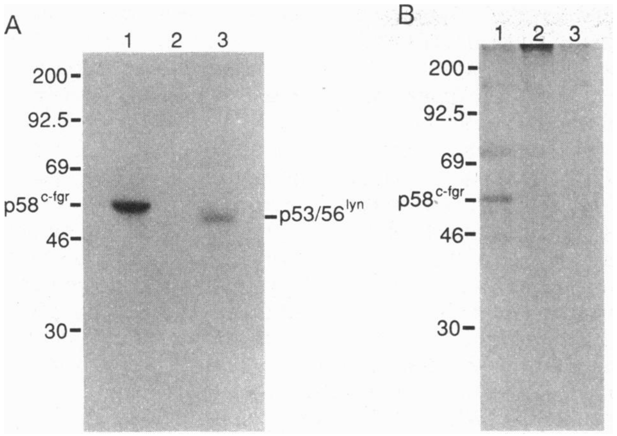

FIG. 2. Src-like tyrosine kinases in anti-Fc$_\gamma$RII immunoprecipitates. (A) Expression of Src-like tyrosine kinases in neutrophils. Lysates of neutrophils (5×10^5 cells per lane) were immunoprecipitated with mAbs to Src-like tyrosine kinases. After kinase reactions, the immune complexes were subjected to SDS/10% PAGE. Lane 1, anti-Fgr; lane 2, anti-Fyn; lane 3, anti-Lyn. Positions of Fgr (p58$^{c\text{-}fgr}$) and Lyn (p53/56^{lyn}) are indicated. (B) Src-like tyrosine kinases in anti-Fc$_\gamma$RII immunoprecipitates. Lysates of 5×10^6 (lane 1) and 2.5×10^7 (lanes 2 and 3) neutrophils were subjected to immunoprecipitation with anti-Fc$_\gamma$RII mAb. After kinase reactions, the immune complexes were reimmunoprecipitated with mAbs to Src-like tyrosine kinases and subjected to SDS/10% PAGE. Lane 1, anti-Fgr; lane 2, anti-Fyn; lane 3, anti-Lyn.

Modulation of Fgr by Fc$_\gamma$RII Crosslinking. Lck, which is associated with CD4 and CD8 in T cells, has been shown to be comodulated with CD4 and CD8 molecules following antibody-mediated crosslinking (9). We therefore examined the effects of crosslinking of Fc$_\gamma$RII molecules on the level of the Fgr protein. Crosslinking of Fc$_\gamma$RII with anti-Fc$_\gamma$RII antibody and F(ab')$_2$ of goat anti-mouse IgG resulted in a significant loss (≈50%) of detergent-soluble Fgr within 15 min, as measured by immunoblotting with anti-Fgr mAb (Fig. 3 *A* and *C*). Treatment of neutrophils with F(ab')$_2$ fragments of goat anti-mouse IgG alone did not induce significant loss of Fgr (Fig. 3*B*). Since the abundance of Fgr in SDS lysates was not affected by crosslinking of Fc$_\gamma$RII for at least 15 min, it seems that Fgr changed into a detergent-insoluble form after the crosslinking (data not shown). These observations are consistent with reports that crosslinking of Fc$_\gamma$Rs with specific antibodies or immune complexes induces their rapid internalization within 15 min (26–29). Indeed, our experiments showed that the time course of internalization of Fc$_\gamma$RII from the cell surface was similar to that of the reduction in the level of detergent-soluble Fgr (data not shown). These results further suggest that the Fgr protein is physically associated with Fc$_\gamma$RII in neutrophils.

Activation of Fgr by Fc$_\gamma$RII Crosslinking. We next examined the effect of crosslinking of Fc$_\gamma$RII on the tyrosine kinase activity of Fgr. To crosslink Fc$_\gamma$RII, we incubated neutrophils with heat-aggregated human IgG or specific antibody to Fc$_\gamma$RII and F(ab')$_2$ of goat anti-mouse IgG. Within 0.5–2 min, both treatments resulted in 3- to 5-fold increases in the *in vitro* kinase activity of Fgr, measured as autophosphorylation and phosphorylation of enolase, the model substrate (Fig. 4 *A* and *B*). On antibody-mediated stimulation, alteration in the Fgr kinase activity was detected only after addition of the bridging antibody (goat anti-mouse), suggesting that physical crosslinking is required for activation of the kinase activity (Fig. 4*C*). The bridging antibody alone did not induce any activation of the Fgr kinase activity. The change in the kinase activity observed here seems to be due to an alteration in the specific activity, since the amount of Fgr detected by immunoblotting analysis in neutrophils remained unchanged until at least 5 min after the initiation of crosslinking (Fig. 3*A*).

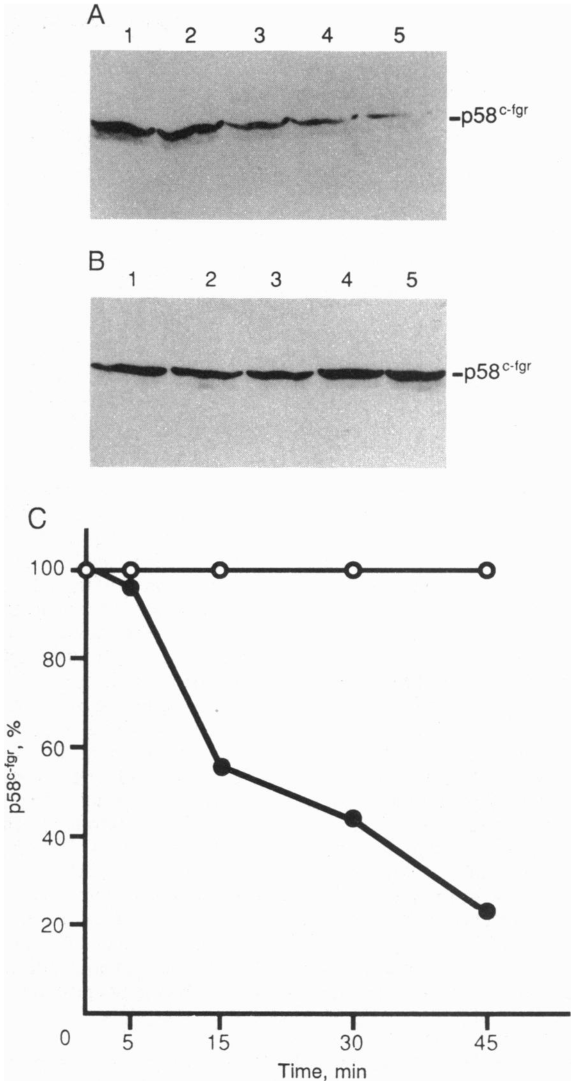

FIG. 3. Modulation of Fgr by Fc$_\gamma$RII crosslinking. (A) Time course of alteration in the amount of detergent-soluble Fgr induced by anti-Fc$_\gamma$RII mAb and F(ab')$_2$ of goat anti-mouse IgG. Neutrophils were incubated in the presence of anti-Fc$_\gamma$RII mAb (2E1) for 30 min at 4°C, and then crosslinking of Fc$_\gamma$RII was initiated by the addition of bridging antibodies, F(ab')$_2$ of goat anti-mouse IgG. After incubation for various periods, the cell lysates were prepared and subjected to immunoprecipitation with anti-Fgr mAb covalently conjugated to Sepharose. The Fgr immunoprecipitated was detected by immunoblotting with anti-Fgr mAb. Lane 1, untreated control; lane 2, 5 min; lane 3, 15 min; lane 4, 30 min; lane 5, 45 min. Position of Fgr (p58$^{c\text{-}fgr}$) is indicated. (B) Time course of alteration in the amount of detergent-soluble Fgr induced by F(ab')$_2$ of goat anti-mouse IgG alone. An experiment similar to that shown in A was performed with neutrophils incubated in the absence of anti-Fc$_\gamma$RII mAb (2E1). Lane 1, untreated control; lane 2, 5 min; lane 3, 15 min; lane 4, 30 min; lane 5, 45 min. (C) Alteration in the amount of detergent-soluble Fgr induced by anti-Fc$_\gamma$RII mAb. The intensity of the Fgr bands was quantitated by densitometry. Open circles, F(ab')$_2$ of goat anti-mouse IgG alone; filled circles, anti-Fc$_\gamma$RII and F(ab')$_2$ of goat anti-mouse IgG.

DISCUSSION

Src-like protein-tyrosine kinase Fgr is specifically expressed in peripheral monocytes, granulocytes, and natural killer cells and accumulates during myelomonocytic differentiation

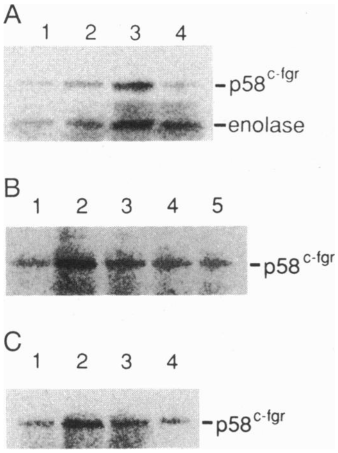

FIG. 4. Activation of Fgr kinase activity by Fc$_\gamma$RII crosslinking. (*A*) Time course of activation of Fgr kinase activity after stimulation at 37°C with heat-aggregated human IgG. The cell lysates were subjected to immunoprecipitation with anti-Fgr mAb, and then immune-complex kinase reactions were performed with rabbit muscle enolase, an exogenous substrate. The samples were analyzed by SDS/PAGE. Lane 1, untreated control; lane 2, PBS alone for 2 min; lane 3, heat-aggregated IgG for 2 min; lane 4, heat-aggregated IgG for 10 min. Positions of Fgr (p58$^{c\text{-}fgr}$) and enolase are indicated. (*B*) Time course of activation of Fgr kinase activity after stimulation with anti-Fc$_\gamma$RII mAb. Neutrophils were incubated with a saturating concentration of anti-Fc$_\gamma$RII mAb (2E1) for 30 min at 4°C. Crosslinking of Fc$_\gamma$RII was initiated by adding F(ab')$_2$ of goat anti-mouse IgG at 37°C for various periods. Fgr was then immunoprecipitated and subjected to *in vitro* kinase reaction. Lane 1, untreated control; lane 2, 30 sec; lane 3, 1 min; lane 4, 5 min; lane 5, 10 min. (*C*) Activation of Fgr kinase activity after various treatments with anti-Fc$_\gamma$RII mAb. Neutrophils were incubated in the presence or absence of anti-Fc$_\gamma$RII mAb for 30 min at 4°C and then treated with or without F(ab')$_2$ of goat anti-mouse IgG for 1 min at 37°C. Lane 1, untreated control; lane 2, anti-Fc$_\gamma$RII plus F(ab')$_2$ of goat anti-mouse IgG; lane 3, anti-Fc$_\gamma$RII alone; lane 4, F(ab')$_2$ of goat anti-mouse IgG alone.

(20, 21). This fact suggests that the physiological role of Fgr is associated with functions in differentiated cells. Indeed, we previously found that Fgr induced a monocyte-specific enzyme, NaF-sensitive α-naphthyl butyrate esterase, in NIH 3T3 mouse cells (30). In the present study, we have found that Fgr is associated with and comodulated with Fc$_\gamma$RII and is activated after receptor engagement with specific mAbs or natural ligands such as heat-aggregated human IgG, suggesting that Fgr is physically and functionally associated with Fc$_\gamma$RII in neutrophils.

Crosslinking of Fc$_\gamma$Rs leads to breakdown of phosphatidylinositol bisphosphate, resulting in activation of protein kinase C and an increase in intracellular Ca^{2+} (31–33). Interestingly, recent studies revealed that activation of Fc$_\gamma$R induces tyrosine phosphorylation of phospholipase C-γ1. Further, the protein-tyrosine kinase inhibitor herbimycin A was shown to strongly inhibit the induction of intracellular Ca^{2+} flux and tumor necrosis factor α mRNA accumulation following the Fc$_\gamma$R crosslinking. These findings suggest that protein-tyrosine phosphorylation may play an important role in the Fc$_\gamma$R-mediated signal transduction (3, 7). Our finding that Fgr is activated after Fc$_\gamma$RII crosslinking suggests that Fgr acts as a critical molecule in the signal-transduction cascade elicited by Fc$_\gamma$RII.

The Fgr protein that was coimmunoprecipitated with Fc$_\gamma$RII was 1–2% of the total Fgr protein based on the densitometric analysis (Fig. 1 *A* and *B*). However, it is unlikely that only 1–2% of Fgr is associated with Fc$_\gamma$RII *in vivo*, because at least 60–80% of Fgr was comodulated with Fc$_\gamma$RII following crosslinking with specific mAbs (Fig. 3 *A* and *C*). Possibly, the Fgr–Fc$_\gamma$RII complexes dissociate during cell lysis under our extraction conditions. Similarly, only small amounts of Lck and Lyn have been reported to coimmunoprecipitate with sIg and interleukin 2 receptor β chain, respectively (12, 13).

Although the molecular basis of the interaction between Fc$_\gamma$RII and Fgr is not clear at the present, it is interesting that Lyn and Fyn were recently reported to associate with the antigen-receptor homology 1 motif (ARH1) in the cytoplasmic portion of the B-cell antigen-receptor signal-transduction molecules Ig-α and Ig-β (34). Fyn has also been demonstrated to associate with the region containing the ARH1 motif of the TCR subunit ζ through the first 10 amino acids in its unique amino-terminal domain (35). This motif is known to be present in a number of other signal-transducer chains, including the TCR ζ, η, γ, δ, and ε polypeptides, the Fc$_\varepsilon$RI β and γ polypeptides, and the Fc$_\gamma$RIIIA γ polypeptide, and has been implicated in receptor-mediated cell activation (36–39). Of particular interest is the fact that the cytoplasmic tail of human Fc$_\gamma$RIIA and -IIC also contains the ARH1 motif (37, 40), and a truncated form of Fc$_\gamma$RIIA lacking a part of the ARH1 motif cannot initiate calcium mobilization and phagocytosis of large particles (29). Moreover, the first 10 amino acid residues of Fgr are very similar to those of Fyn, with amino acid differences at only three positions. Thus, it is possible that Fgr associates with the ARH1 motif of Fc$_\gamma$RIIA and -IIC through its unique amino-terminal domain. This idea is consistent with our result that Fgr does not associate with Fc$_\gamma$RIIIB which has no ARH1 motif in its cytoplasmic tail.

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