RAPID COMMUNICATION

Physical and Functional Association of FcαR With Protein Tyrosine Kinase Lyn

By Heinz Gulle, Aysen Samstag, Martha M. Eibl, and Hermann M. Wolf

In this report, we show that the Src family nonreceptor protein tyrosine kinase (PTK) Lyn associates with aggregated IgA Fc receptor (FcαR) in the monocyte cell line THP-1. Receptor aggregation and subsequent immunoprecipitation of receptor complexes with hulA adsorbed to nitrocellulose particles shows that Lyn associates with FcαR by a mechanism sensitive to short treatment with the Src family-selective inhibitor PPI. However, interaction of Lyn with IgG Fc receptor (FcγR) in THP-1 cells was unaffected by short treatment with the PTK inhibitor. Cross-linking of FcαR induced tyrosine phosphorylation of several cellular proteins, including p72<sup>Syk</sup>, which appears to be a major target of early PTK activity. Unexpectedly, in vitro kinase assays showed that FcαR aggregation-induced tyrosine phosphorylation of Syk did not result in upregulation of Syk activity. Despite the lack of enhanced Syk kinase activity, downstream signaling after FcαR cross-linking was functional and induced the release of significant amounts of interleukin-1 receptor antagonist and interleukin-8. The induction of cytokine release was completely blocked by PPI, thus confirming the biological significance of the association of Lyn with aggregated FcαR. Our data show that early signal transduction after FcαR cross-linking as well as FcαR-mediated activation of cellular effector functions depends on Src family kinase activity. The Src-family PTK involved in FcαR-mediated tyrosine phosphorylation appears to be Lyn, which coprecipitated with aggregated FcαR complexes. © 1998 by The American Society of Hematology.

Igδ of the IgA Isotype prevail over other Ig isoforms in the mucosal compartment, where they play a critical role in protection against environmental challenges. In addition to functions dependent on specific antibody activity, such as neutralization of bacterial toxins and inhibition of attachment of pathogenic microorganisms to the mucosal epithelium, IgA appears to be a regulatory molecule capable of modulating immune and inflammatory responses. The immunoregulatory functions of IgA seem to be confined to interaction of IgA with receptors for the Fc portion of IgA expressed on the surface of cells of the immune system, such as monocytes, macrophages, neutrophils, or T cells, leading to upregulation or downregulation of receptor complexes with human IgA adsorbed to nitrocellulose. Receptor aggregation and subsequent immunoprecipitation of receptor complexes with hulA adsorbed to nitrocellulose particles shows that Lyn associates with FcαR by a mechanism sensitive to short treatment with the Src family-selective inhibitor PPI. However, interaction of Lyn with IgG Fc receptor (FcγR) in THP-1 cells was unaffected by short treatment with the PTK inhibitor. Cross-linking of FcαR induced tyrosine phosphorylation of several cellular proteins, including p72<sup>Syk</sup>, which appears to be a major target of early PTK activity. Unexpectedly, in vitro kinase assays showed that FcαR aggregation-induced tyrosine phosphorylation of Syk did not result in upregulation of Syk activity. Despite the lack of enhanced Syk kinase activity, downstream signaling after FcαR cross-linking was functional and induced the release of significant amounts of interleukin-1 receptor antagonist and interleukin-8. The induction of cytokine release was completely blocked by PPI, thus confirming the biological significance of the association of Lyn with aggregated FcαR. Our data show that early signal transduction after FcαR cross-linking as well as FcαR-mediated activation of cellular effector functions depends on Src family kinase activity. The Src-family PTK involved in FcαR-mediated tyrosine phosphorylation appears to be Lyn, which coprecipitated with aggregated FcαR complexes. © 1998 by The American Society of Hematology.

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has been described in U937 monocytic cells and this interaction was necessary for functional transmembrane signal transduction in a transfected B-cell line. However, little is known about the PTKs responsible for FcγR-mediated tyrosine phosphorylation of cellular proteins. A preliminary report described interaction of Syk with this receptor in U937 monocytic cells, but the Src family PTKs involved in FcγR signaling have not been identified so far.

We describe here PTKs engaged in transmembrane signaling of FcγR in the monocytic cell line THP-1. To investigate receptor-associated molecules, we cross-linked and subsequently immunoprecipitated FcγR complexes with the natural ligand, hulgA, adsorbed to nitrocellulose (NC) particles. Furthermore, these particles were used to study the effect of FcγR cross-linking on downstream cellular functions, such as the release of cytokines. Our results show that, comparable to other FcRs, aggregation of FcγR recruits PTK Lyn. However, the mechanism of kinase/receptor interaction appears to be different for FcγR and FcγR.

**MATERIALS AND METHODS**

**Cells.** The THP-1 monocytic cell line was obtained from the American Type Culture Collection (ATCC; Rockville, MD) and cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FCS; Hyclone Lab, Logan, UK), 2 mmol/L L-glutamine, 100 IU/mL penicillin, and 100 µg/mL streptomycin (GIBCO BRL, Paisley, UK; complete medium). Cell density was 1 × 10^5 and 2 × 10^6/mL. Aliquots of 5 × 10^6 cells were transferred into 1.5-mL reaction tubes, incubated for 30 minutes at 37°C, and stimulated with antibodies for 5 minutes at 37°C. NC particles adsorbed with human Ig preparations were used at a concentration of 1 mg of coated particles per tube (dry weight). FcγR-specific MoAb My43 (cell culture supernatant) was added 1:2 to the cell suspensions, and the anti-CD14 MoAb Mo2 was used at a concentration of 5 µg/mL. The activation reaction was stopped by addition of 500 µL of ice-cold PBS containing 1 mmol/L sodium orthovanadate (Na_2VO_4), followed by brief centrifugation and immediate lysis of cells for 20 minutes on ice in Nonidet P-40 lysis buffer (20 mmol/L Tris/HCl, pH 7.5, 150 mmol/L NaCl, 1% Nonidet P-40, 0.1% sodium azide, 1 mmol/L Na_2VO_4, 1 mmol/L sodium molybdate, Na_2MoO_4, 2 mmol/L phenylmethanesulfonyl fluoride (PMSF), 5 mmol/L EDTA, and 10 µg/mL of proteinase inhibitors aprotinin and leupeptin). Nuclei and cellular debris were removed by centrifugation for 10 minutes at 14,000g and soluble proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 8% polyacrylamide gels under reducing conditions.

**Immunoprecipitation and in vitro kinase assays.** THP-1 cells were washed and resuspended in RPMI 1640 medium to a density of 1.5 × 10^6/mL. Aliquots of 7.5 × 10^5 or 1 × 10^6 cells were transferred into 1.5-mL reaction tubes and cells were activated for 5 minutes with human Ig-adsorbed NC particles (concentrations of NC particles are given in the respective figure legends) as described above. Cell activation was stopped by addition of 0.9 mL of Brij 96 (polyoxyethyl-ene 10 oleyl ether) lysis buffer (20 mmol/L Tris/HCl, pH 7.5, 150 mmol/L NaCl, 1% Brij 96, 0.1% sodium azide, 1 mmol/L Na_2VO_4, 1 mmol/L Na_2MoO_4, 2 mmol/L PMSF, 1 mmol/L MgCl_2, and 10 µg/mL of proteinase inhibitors aprotinin and leupeptin) and 20 µL/mL of Dnase I (Boehringer Mannheim GmbH, Mannheim, Germany). The lysed cells were incubated for 20 minutes on ice to precipitate the NC particles. Supernatants were discarded, and the particles were afterwards washed five times with Brij 96 lysis buffer and precipitated on ice as described. Subsequently, immunocomplexes were eluted with sample buffer and separated by SDS-PAGE on 8% to 12% polyacrylamide gradient gels under nonreducing conditions.

Syk kinase was precipitated from cell lysates of 1.5 × 10^6 THP-1 cells, which have been stimulated for 5 minutes with My43 or Mo2 as described and lysed in Nonidet P-40 lysis buffer without EDTA. To reduce unspecific binding of proteins to Syk-specific antibody, lysates were incubated on ice for 1 hour with 5 µg/mL of rabbit polyclonal IgG followed by two successive treatments for 30 minutes at 4°C with 30 µL of a 10% Staph aureus slurry. Lysates were then incubated on ice for 1 hour with 5 µg/mL anti-Syk rabbit polyclonal antibody or the same amount of control rabbit IgG. Antibody-bound molecules were precipitated by addition of Staph aureus cells, and immunocomplexes were washed three times with lysis buffer and once with 25 mmol/L HEPES, pH 7.2, containing 150 mmol/L NaCl, 0.1% Nonidet P-40, and 5 mmol/L MnCl_2. Pelleted Staph aureus cells were resuspended in 90 µL kinase buffer (25 mmol/L HEPES, pH 7.2, 5 mmol/L MnCl_2) and one third of the slurry was used for in vitro kinase assay. The rest of each sample was pelleted and bound proteins were eluted with reducing sample buffer followed by separation on 8% to 12% polyacrylamide gradient gels. To determine the PTK activity of Syk, immunocomplexes were incubated for 15 minutes at 30°C in the presence of 10 µC [γ-32P] ATP. Bound proteins were then eluted with reducing sample buffer and resolved on 8% to 12% gradient gels. Syk kinase activity was visualized by autoradiography of dried gels.
**Immunoblot analysis.** Electrophoretically separated proteins were transferred onto NC sheets and the transfer efficiency was examined by staining with 0.5% Ponceau S. Free protein binding sites were blocked by incubation of immunoblots in Tris-buffered saline, pH 7.5, containing 0.1% Tween-20 and 1% nonfat dry milk (Bio-Rad Lab, Hercules, CA). Tyrosine phosphorylation of cellular proteins was probed with MoAb 4G10 followed by HRP-labeled sheep antimouse antibody. After removal of excess antibody by washing with TBST, specific binding was visualized by ECL. For reprobing of immunoblots, phosphotyrosine-specific antibody was removed by treatment with 100 mmol/L glycine/HCl, pH 2.5 (3 times for 20 minutes), on a shaker. Reprobing with rabbit polyclonal antibodies specific for Syk, Lyn, or Hck was performed after blocking of glycine-treated immunoblots with TBST containing 1% nonfat dry milk. Specific binding was detected by ECL.

**Induction of cytokine release.** THP-1 cells were cultured for 40 hours in complete medium in 6-well tissue culture plates (Macro Tray nonfat dry milk. Specific binding was detected by ECL. For reprobing of immunoblots, phosphotyrosine-specific antibody was removed by treatment with 100 mmol/L glycine/HCl, pH 2.5 (3 times for 20 minutes), on a shaker. Reprobing with rabbit polyclonal antibodies specific for Syk, Lyn, or Hck was performed after blocking of glycine-treated immunoblots with TBST containing 1% nonfat dry milk. Specific binding was detected by ECL.

**RESULTS**

**Cross-linking of FcR induces tyrosine phosphorylation of cellular proteins in THP-1 cells.** To study transmembrane signaling after FcR triggering, we cross-linked the receptor with the MoAb My43, or hulgA adsorbed to NC particles, on the surface of the human monocytic cell line THP-1. Both stimuli induced tyrosine phosphorylation of an identical set of cellular proteins, with the exception of an unidentified 115-kD protein, which was phosphorylated exclusively by cross-linking of FcR with NC particles adsorbed with the natural ligand, hulgA (Fig 1A, lanes 2 and 6 through 10). In contrast, treatment of cells with the CD14-specific MoAb Mo2, with monomeric hulgA, or with NC particles adsorbed with BSA failed to induce tyrosine phosphorylation, indicating that induction of signaling was specific for FcR ligation and that signal transduction required FcR cross-linking (Fig 1A, lanes 1, 3, and 11). Unexpectedly, cross-linking of monomeric hulgA with F(ab')2 fragments of a specific antibody failed to induce tyrosine phosphorylation of cellular proteins, suggesting the need for extensive FcR aggregation by multivalent ligand (Fig 1A, lane 4).

The PTK Syk is a major target in FcR-mediated tyrosine phosphorylation. Among the targets that became phosphorylated after cross-linking of FcR was a predominant protein band of 75 kD. Because involvement of Syk, a PTK of similar specificity, was examined using commercially available enzyme-linked immunosorbent assay (ELISA) kits for tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-8 (IL-1β-EASIA, TNF-alpha-EASIA, and IL-8-EASIA; Medgenix Diagnostics, Fleurus, Belgium) or IL-1ra (Quantikine Human Interleukin 1 Receptor Antagonist Immunoassay; R&D Systems, Minneapolis, MN). Results are expressed as nanograms per milliliter or as a percentage of control relative to the cytokine release observed in THP-1 cells stimulated in the absence of PTK inhibitor.

**Statistical analysis.** Results of the determination of cytokine release are expressed as the mean ± SEM of repeated experiments performed on different days. Statistical evaluation of the observed hulgA-mediated induction of cytokine release by calculating the differences between more than two study groups was performed with the nonparametric Kruskal-Wallis one-way ANOVA by ranks or the Newman-Keuls multiple comparisons test. For statistical evaluation of the differences between two study groups, the Mann-Whitney U-test or the Student’s t-test for paired samples was used, as appropriate. P < .05 was considered a statistically significant difference.
molecular mass, has been shown for various FcRs, we wanted to clarify whether the 75-kD band corresponded to p72Syk. As shown in Fig 1B, rabbit polyclonal IgG specific for human Syk recognized a single protein band of identical molecular mass. To further characterize the effect of FcαR-mediated signaling on this PTK, we immunoprecipitated Syk kinase from resting and stimulated THP-1 cells and performed anti-PY immunoblot analysis and in vitro kinase assay. FcαR cross-linking with MoAb My43 induced extensive tyrosine phosphorylation of Syk protein (6-fold increase in phosphotyrosine; Fig 2A, lane 3), whereas treatment of cells with the MoAb Mo2 (also of the IgM isotype) had no effect (Fig 2A, lane 2). Although FcαR aggregation after stimulation with My43 induced Syk tyrosine phosphorylation, the enzymatic activity of precipitated Syk was unchanged (Fig 2C). Anti-Syk immunoblot analysis shows that an equal amount of Syk was immunoprecipitated from resting and FcαR-activated cells (Fig 2B).

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The Src family kinase Lyn coperipitates with FcαR. The results shown above prompted us to investigate whether PTKs associate with FcαR. Therefore, we adsorbed huIgA to NC particles and used these complexes for cross-linking and subsequent immunoprecipitation of aggregated FcαR. Several tyrosine-phosphorylated proteins were present in the FcαR-specific immunoprecipitate, whereas no proteins were found with NC particles coated with BSA or two different preparations of human myeloma IgM (Fig 3A). Among the proteins that coprecipitated with the receptor was a notable double band of 56 and 58 kD. Similar migration patterns have been described for the Src family kinases Hck and Lyn. To further characterize the double band, we stripped the immunoblot in low pH buffer and reprobed it with antibodies specific for human Hck or Lyn. Our results show that the anti-Lyn antibody reacted with protein bands of identical molecular mass, whereas no proteins were detected with the Hck-specific antibody (Fig 3B, and data not shown). Additionally, Lyn kinase was also present in receptor complexes immunoprecipitated with NC particles adsorbed with the MoAb My43, but not with MoAb Mo2 (data not shown).

To exclude that precipitation of Lyn kinase was due to specific antibody activities present in the huIgA preparation, a chimaeric huIgA antibody specific for the hapten NP (4-hydroxy-3-nitrophenacetyl) was used to immunoprecipitate FcαR. Interaction of this hapten-specific antibody with THP-1 cells depends entirely on its huIgAFc domain and is, therefore, restricted to ligation of FcαR. Anti-PY analysis shows that proteins of 55, 56, and 58 kD were present in the receptor-specific immunoprecipitates using either huIgA or chimaeric huIgA adsorbed to NC particles (Fig 4A, lanes 1 and 2, respectively). Again, protein bands of identical molecular mass were recognized in both immunoprecipitates by reprobing of the immunoblot with the Lyn-specific antibody (Fig 4B).

Tyrosine kinase inhibitor PP1 differentially affects association of Lyn with FcαR and FcγR. Treatment of RBL-2H3 cells with the Src family inhibitor piceatannol has been shown to partially inhibit FcβR-mediated protein tyrosine phosphorylation, but to completely block downstream cellular responses. The molecule responsible for tyrosine phosphorylation of the remaining phosphorylated proteins appeared to be the Src family kinase Lyn, which is constitutively associated with the FcαR β-chain and, hence, may function upstream of Lyn. We used the Src family-selective inhibitor PP1 to study the role of Src kinases in FcαR-mediated protein tyrosine phosphorylation. Activation of THP-1 cells in the presence of 100 μmol/L PP1 abolished FcαR-mediated protein tyrosine phosphorylation of whole lysate proteins, including Lyn and Syk (data not shown). PP1 blocked FcαR- and FcγR-mediated tyrosine phosphorylation of Lyn kinase in a dose-dependent way and phosphorylation of Lyn was completely abolished at a concentration of 100 μmol/L (Fig 5A, lanes 3 and 7). Interestingly, association of Lyn with FcγR was sensitive to short-term treatment with PP1 and completely blocked at 100 μmol/L. However, interaction of Lyn with FcγR was not affected by PP1 (Fig 5B, lanes 1 through 3 and 5 through 7, respectively). These results support the hypothesis that association of Lyn with FcαR or FcγR differs in the need of Src kinase activity before and/or after receptor aggregation.
Cross-linking of FcαR with huIgA adsorbed to NC particles induces IL-1ra and IL-8 release in THP-1 cells.

In a previous study, we showed that stimulation of human mononuclear cells or isolated human monocytes with huIgA induces the production of IL-1ra, a naturally occurring inhibitor of IL-1 activity previously shown to be produced by monocytes/macrophages in response to FcγR stimulation or LPS, 37,38 The present results confirm and extend these previous findings by showing that cross-linking of FcαR by huIgA adsorbed onto nitrocellulose particles induces IL-1ra release in THP-1 cells (Fig 6A). In addition, stimulation of THP-1 cells by FcαR cross-linking induces the production of IL-8 (Fig 6B), an 8-kD chemokine and angiogenic factor previously shown to be produced by cells of the monocyte/macrophage lineage, epithelial cells, or endothelial cells in response to lipopolysaccharide (LPS), FcγR cross-linking, or cytokines such as TNF-α. 39,40

Induction of cytokine release by particle-bound huIgA was dose-dependent and became statistically significant even at a relatively low concentration of particle-adsorbed huIgA (ie, 100 µg/mL). In contrast, levels of IL-1ra and IL-8 produced by cells cultured in the presence of the same amount of BSA-adsorbed nitrocellulose particles were only slightly elevated as compared with background cytokine release, indicating that induction of cytokine release by particle-bound huIgA was specific and mediated through triggering of FcαR. Cross-linking of FcαR by particle-bound ligand was required for induction of IL-1ra and IL-8 release, because stimulation of THP-1 cells with soluble monomeric huIgA at the same concentration had no effect on cytokine production, although flow cytometric analysis confirmed strong binding of soluble huIgA to the cells under these conditions (data not shown). In contrast to phorbol 12-myristate 13-acetate (PMA), which stimulates production of IL-8, IL-1ra, TNF-α, and IL-1β by directly activating protein kinase C independent of surface receptor triggering, particle-adsorbed huIgA specifically induced IL-1ra and IL-8 release accompanied by only very low levels of TNF-α or IL-1β release (TNF-α

Fig 3. Lyn coimmunoprecipitates with aggregated FcαR. THP-1 cells (7.5 × 10⁶/sample) were activated at 37°C for 5 minutes with 2 mg of NC particles adsorbed with BSA only (lane 1) or huIgA (lane 2). Immunoprecipitation, separation of precipitated proteins by 8% to 12% SDS-PAGE, and antiphosphotyrosine immunoblot analysis of separated proteins were performed as described before (A). The immunoblot was then stripped and reprobed with a rabbit polyclonal anti-Lyn antibody (B).

Fig 4. Immunoprecipitation of FcαR depends on the Fc domain of huIgA. THP-1 cells (1 × 10⁷/sample) were activated at 37°C for 5 minutes with 2.5 mg of NC particles adsorbed with huIgA (lane 1), chimeric huIgA (lane 2), or BSA only (lane 3). Immunoprecipitation, separation of precipitated proteins by 8% to 12% SDS-PAGE, and antiphosphotyrosine immunoblot analysis of isolated proteins were performed as described. Immunoblot analysis of tyrosine phosphorylated proteins (A). Reprobing of the same immunoblot with Lyn-specific antibody (B).
release, in nanograms per milliliter, mean ± SEM [n]: BSA-adsorbed particles, 0.29 ± 0.04 [9]; hulgA-coated particles, 0.76 ± 0.14 [9]; PMA [100 ng/mL], 3.98 ± 1.07 [5]; IL-1β, in nanograms per milliliter, mean ± SEM [n]: BSA-adsorbed particles, 0.12 ± 0.02 [9]; hulgA [100 µg/mL]-coated particles, 0.82 ± 0.22 [9]; PMA [100 ng/mL], 8.45 ± 1.66 [5]; ratio IL-1ra/IL-1β: hulgA coated onto NC particles, 28 ± 7; PMA: 5 ± 1; P = .00135, Mann-Whitney U-test). Furthermore, particle-adsorbed hulgA triggered cytokine release even under conditions in which endotoxin-induced cytokine release was blocked by the addition of polymyxin B (data not shown), thus indicating that FcαR cross-linking and LPS stimulate cytokine release in THP-1 cells through different mechanisms (Fig 6A and B).

**Induction of IL-1ra and IL-8 release after cross-linking of FcαR by multivalent ligand requires Src family protein tyrosine kinase activity.** In the present study, we show that signaling events after FcαR cross-linking involve association of the PTK Lyn with FcαR and tyrosine phosphorylation of proteins involved in signal transduction such as Syk. The results presented in Fig 6B and C show that Src family PTK activity is essential for FcαR-mediated activation of cellular functions, because the Src family-specific inhibitor PP1 abolished the

**Fig 5.** Association of Lyn with FcαR is sensitive to treatment with PP1. THP-1 cells (1 x 10⁷/sample) were incubated at 37°C for 30 minutes in RPMI 1640 cell culture medium only (lanes 1, 4, and 5) and cell culture medium containing 10 µmol/L of PP1 (lanes 2 and 6) or 100 µmol/L of PP1 (lanes 3 and 7). Subsequently, cells were activated at 37°C for 5 minutes with 2.5 mg of NC particles adsorbed with hulgA (lanes 1 through 3), hulgG (lanes 5 through 7), or BSA only (lane 4). Immunoprecipitation, separation of precipitated proteins by 8% to 12% SDS-PAGE, and antiphosphotyrosine immunoblot analysis of isolated proteins were performed as described in Materials and Methods (A). Reprobing of the same immunoblot with Lyn-specific antibody (B).

**Fig 6.** Src family PTK activity is required for induction of IL-1ra and IL-8 in THP-1 cells after cross-linking of FcαR by hulgA-adsorbed NC particles. THP-1 cells (3 x 10⁶ cells/mL/well) were stimulated for 24 hours with hulgA adsorbed to nitrocellulose particles (IgA-NC); parallel cultures were incubated in the presence of corresponding concentrations of soluble hulgA or BSA-adsorbed nitrocellulose particles (NC). In (A) and (B), cells were cultured in complete medium (Med) or in complete medium containing PMB (final concentration, 10 µg/mL) or PP1 (final concentration, 100 µmol/L). In (C), THP-1 cells were stimulated for 24 hours with hulgA adsorbed to NC particles (final concentration of hulgA, 100 µg/mL) in the presence of PP1 at the indicated concentrations. Release of IL-1ra and IL-8 was examined in cell-free supernatants as described in Materials and Methods. Results are expressed as nanograms per milliliter (mean ± SEM of 3 experiments [A] or 6 experiments [B]) or (C) as a percentage of control (mean ± SEM of 3 experiments) relative to the cytokine release observed in cells stimulated with hulgA-particles in the absence of PP1 (cytokine release, in nanograms per milliliter, mean ± SEM: IL-1ra, 7.99 ± 0.84; IL-8, 1.41 ± 0.04). (*) Statistically significant difference between cultures stimulated with hulgA-NC and cells cultured in the presence of hulgA or NC alone (both in the presence or absence of polymyxin B, 10 µg/mL; P < 0.05, Newman-Keuls multiple comparisons test; P = .004, analysis of variance). (#) Statistically significant difference as compared with cultures containing soluble hulgA or NC particles alone (P < .1, Kruskal-Wallis test for comparison of more than two samples). (x) Statistically significant difference as compared with cells stimulated with hulgA adsorbed to NC particles in the absence of PP1 (P = .01, Mann-Whitney U test). (+) Statistically significant inhibition of cytokine release after stimulation with hulgA adsorbed to NC particles (P < .025, Student's t-test for paired samples).
huIgA-mediated induction of IL-1ra and IL-8 release in THP-1 cells. PP1-induced inhibition of cytokine production was dose-dependent over a 3-log range, with almost complete inhibition of cytokine release at a concentration of 100 µmol/L of PP1 (Fig 6C).

**DISCUSSION**

In the present report, we show that, in the monocytic cell line THP-1, the Src family kinase Lyn physically associates with FcγR after receptor aggregation by multivalent ligand. The association of Lyn with FcγR was found in immunoprecipitates of the native ligand of the receptor, huIgA, or an MoAb of the IgM isotype directed against the ligand-binding site of FcγR, thus excluding precipitation of FcγR-bound Lyn. Furthermore, precipitation of Lyn kinase due to specific antibody activities was excluded by the use of a chimeric huIgA consisting of a hapten-specific murine F(ab')2 component and the Fc part of human IgA.

Our approach, to cross-link and immunoprecipitate FcγR with huIgA-adsorbed NC particles, does not allow us to study whether Lyn is constitutively associated with FcγR in unstimulated cells. However, the sensitivity of this interaction to short treatment with the PTK inhibitor PP1 argues in favor of an aggregation-induced association dependent on immediate kinase activity. PP1 has been shown to selectively inhibit Src family PTKs, including Lck, Fyn, Src, and Hck, at nanomolar concentrations, whereas no effect on the kinase activity of ZAP-70, a member of the Syk-family of PTKs, was observed even in the presence of 100 µmol/L of the inhibitor. Furthermore, Amoui et al recently showed that Lyn in vitro kinase activity was highly sensitive to PP1, whereas Syk activity was not influenced by the inhibitor. The selectivity of PP1 provides, therefore, strong evidence that the PTK regulating FcγR/Lyn association is a Src family kinase, probably Lyn itself.

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which has been shown to associate with FcγRs in THP-1 cells, additionally emphasizes the crucial role of Lyn for FcγR-induced signaling processes. Although no other Src family kinases have been described to be involved in signal transduction via FcRs in THP-1 cells, involvement of Src kinases apart from Lyn cannot be completely ruled out. Aggregation of FcγR induced the release of considerable amounts of IL-1α, a cytokine with potent anti-inflammatory properties in vitro and in vivo, as well as IL-8. Although IL-8 was initially described as a proinflammatory neutrophil chemotactic factor secreted by lipopolysaccharide-stimulated mononuclear cells, IL-8 has recently been shown to play a more complex role in the regulation of the inflammatory response, eg, by exerting a wide range of modulatory effects on neutrophil-endothelial adhesive interactions. Our finding that cross-linking of FcγR by particle-bound hIgA induces the release of IL-1α- and IL-8- confirms and extends previous studies suggesting that hIgA, in addition to the protective functions mediated by specific antibody responses by interacting with the FcγR on cells of the monocyte lineage.

ACKNOWLEDGMENT

The authors thank Eleonore Gschaider and Astrid Lehner for expert technical assistance and Paul Brent for excellent photographic assistance.

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Physical and Functional Association of FcαR With Protein Tyrosine Kinase Lyn

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