RAPID COMMUNICATION

Induction of Phagocytosis by a Protein Tyrosine Kinase

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The transmission of extracellular signals to cellular targets by many noncatalytic surface receptors is dependent on interaction between cytoplasmic protein tyrosine kinases (PTKs) and tyrosine-containing sequences in the cytoplasmic domain of the receptor or an associated subunit. Isoforms of each of the three classes of the noncatalytic Fcγ receptors, FcγRI, FcγRII, and FcγRIII, are able to transmit a phagocytic signal in transfected COS-1 cells. Both FcγRI and FcγRIIIA require the γ subunit for this signaling event. The protein tyrosine kinase Syk dramatically enhances phagocytosis mediated by both these receptors and increases the number of cells able to mediate phagocytosis. Two γ chain cytoplasmic YXXL sequences are required for this effect. The action of Syk is less pronounced on the phagocytic FcγRII receptor, FcγRIIA, which does not require the γ chain for phagocytosis. However, Syk allows phagocytosis by the nonphagocytic FcγRII receptor FcγRIIB2, which contains only a single YXXL sequence, when an additional tyrosine-containing sequence, YMTL, is introduced. These studies indicate that the efficiency of phagocytosis is markedly enhanced by the presence of a specific protein tyrosine kinase.

PROTEIN TYROSINE KINASES (PTKs) have been implicated in signaling events initiated by members of the Ig gene superfamily including receptors for the constant region of IgG (FcγRs). Cross-linking Fcγ receptors in hematopoietic cells induces phosphorylation of tyrosine residues of multiple proteins including the receptors themselves and/or their associated subunits.[1-8] Although Fcγ receptors do not possess intrinsic tyrosine kinase activity, their cytoplasmic domains have sequences that facilitate interactions with cellular PTKs. Recently, the cytoplasmic domains of the Fcγ receptors and their associated subunits have received considerable attention because they include conserved tyrosine-containing sequences (YXXL) that have been implicated in signal transduction events.[1,9-23] The cytoplasmic regions of most Fcγ receptors and/or their subunits contain at least one pair of this tyrosine-containing sequence,[1,9,10,12,14,16,19] which is thought to bind to the SH2 (Src homology 2) domain(s) of PTKs.[24,25]

The Fcγ receptors differ from other Ig gene superfamily receptors such as the T-cell antigen receptor and the B-cell antigen receptor in that they mediate the phagocytosis of IgG-coated cells.[26] The mechanism of Fcγ receptor-mediated phagocytosis likely involves elements endogeneous to phagocytic cells; however, it has recently been shown that COS-1 cells, a fibroblast and/or epithelial-like cell line derived from monkey kidney cells, have the capability to mediate a phagocytic signal when transfected with a phagocytic receptor.[11-16,27,28] The transfection of Fcγ receptors into such cells, which do not express endogeneous Fc receptors but have phagocytic potential, has allowed the study of individual Fcγ receptors and the definition of those structures within the receptor molecule important for phagocytosis. For example, FcγRIIA contains the conserved YXXL motif within its cytoplasmic domain[1,11,12,14] and mediates a high level of phagocytosis of IgG-sensitized red blood cells (RBCs) in COS-1 cells and fibroblast transfectants.[11-14] In contrast, the cytoplasmic domains of FcγRIIIA and FcγRI lack this motif and require the cytoplasmic domain of an associated γ chain subunit for phagocytic function[15-17,27] (Table 1).

In these studies, we have further explored factors that influence the efficiency of phagocytosis by Fcγ receptors and have identified a PTK that specifically enhances phagocytosis mediated through the γ chain. The 72-kD PTK Syk, originally cloned from porcine spleen, is associated with B-cell slg and mast cell FcεRI receptors.[29-32] We have observed that Syk can also be isolated in abundance from monocytes and macrophages and that cross-linking of FcγRIIIA stimulates a fourfold increase in Syk kinase activity.[2] Furthermore, after FcγRIIIA cross-linking, Syk was identified by immunoprecipitation and phosphopeptide mapping as a major tyrosine phosphorylation substrate associated with the γ chain.[2] Taken together, these data suggested that Syk may be important for FcγRI and FcγRIIIA signal transduction.

MATERIALS AND METHODS

Cell culture and transfection. COS-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing glucose (4.5 mg/mL), glutamine (2 mmol/L), streptomycin (100 U/mL), penicillin (100 μg/mL), and 10% heat-inactivated fetal calf serum. Transient transfection of cells at 70% to 80% confluence was performed in complete media containing 10% Nu-Serum (Collaborative Biomedical Products, Bedford, MA), DEAE-Dextran (1 mg/mL), chloroquine chloride (100 μmol/L), and 2.5 μg plasmid DNA per milliliter of transfection media. After 4 hours at 37°C, the transfection media was replaced with 10% dimethyl sulfoxide (DMSO) in phosphate-buffered saline (PBS) for 2 minutes at room temperature. The cells were then washed, overlaid with fresh media for further incubation, and analyzed after 48 hours.

Flow cytometry. Cell samples incubated with anti-FcγRII monoclonal antibody (MoAb) IV.3 or anti-FcγRI MoAb 32.2 for 30 minutes at 4°C were washed, labeled with fluorescein isothiocyanate (FITC)-conjugated goat-antimouse $F(ab')_2$ IgG (TAGO, Inc, Burlingame, CA) for 30 minutes at 4°C, then washed and fixed with 4% paraformaldehyde. Isotype controls were used for all reactions, and fluorescence was measured on a FACSTAR cytometer (Becton Dickinson, Mountain View, CA).

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Table 1. Effect of the Tyrosine Kinase Syk on Phagocytosis by Fcγ Receptors

Fcγ Receptor	Receptor Alone	+γ Chain	+Syk	+γ Chain + Syk
FcγRIIIA	<50	136†	<50	760
FcγRIIIAΔCYT	<50	151†	<50	680
FcγRI	<50	69†	<50	664
FcγRIΔCYT	<50	62†	<50	434
FcγRIIIA*	<50	60	ND	462
FcγRI*	<50	<50	ND	270
FcγRIIA	500	380	830	525
I-IIA-IIA	540	250	625	450
I-I-IIA	160	117	144	619
FcγRIIB1	<50	<50	<50	<50
FcγRIIB2	<50	<50	<50	<50

Representative experiments are shown for transfection of the γ chain, Syk, or the γ chain + Syk with the indicated Fcγ receptor. Phagocytosis is expressed as phagocytic index (the number of ingested EA per 100 Fcγ receptor expressing cells determined by flow cytometry) in Tables 1 and 2. Syk did not alter Fcγ receptor surface expression. Receptor cell surface expression was equivalent within a single experimental group for all receptors. FcγRIIIA required the γ chain subunit for expression. I-I-IIA and I-IIA-IIA are chimeric receptors containing the extracellular domain of FcγRI, the transmembrane domain of either FcγRI or FcγRIIA and the cytoplasmic domain of FcγRIIA. Abbreviation: ND, not performed.

* Experiments in which the ζ chain was substituted for the γ chain.

† FcγRI and FcγRIIIA with the γ chain induce a low level of phagocytic function in COS-1 cells in the absence of Syk.[15,16,27]

Binding and phagocytosis of IgG-sensitized RBCs. Antibody-sensitized sheep erythrocytes (Rockland, Gilbertsville, PA) (EA) were prepared in magnesium- and calcium-free PBS by incubating 10^9/mL sheep RBCs with an equal volume of the highest subagglutinating concentration of rabbit-antisheep RBC antibody (Cappel Laboratories, West Chester, PA) as previously described.[11,12,14-16,27] COS-1 transfectants were overlayed and incubated with EA at 37°C for 30 minutes, unbound EA were removed by washing with PBS, and the plates were stained with Wright-Giemsa. The percentage of cells binding RBCs was determined by counting in a blinded fashion those cells binding 5 or more sensitized RBCs. To assess phagocytosis, parallel groups of EA incubated COS-1 cells were briefly exposed to hypotonic PBS to remove adherent EA. The cells were then stained with Wright-Giemsa and the number of COS-1 cells with one or more internalized EA determined in a blinded fashion.

Immunoprecipitation and analysis of phosphoproteins. After stimulation of Fcγ receptor transfected COS-1 cells by incubation with EA at 37°C for 20 minutes, externally bound RBCs were removed by brief hypotonic shock. Transfected COS-1 cells were lysed directly on plates with RIPA buffer (1% Triton X-100 [Sigma, St Louis, MO], 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 158 mmol/L NaCl, 10 mmol/L Tris pH 7.2, 5 mmol/L NaEDTA, 1 mmol/L phenylmethylsulphonyl fluoride, 1 mmol/L Na_3VO_4) at 4°C for 30 minutes. Clarified cell lysates were immunoprecipitated with anti-Syk antibody[33] (Upstate Biotechnology Inc, Lake Placid, NY) and immune complexes were bound to Pansorbin (Calbiochem, La Jolla, CA) in lysis buffer. Pellets were washed three times in lysis buffer and adsorbed proteins eluted into reducing sample buffer were resolved on 7.5% SDS-polyacrylamide gels. After electrophoretic transfer to nitrocellulose, immunoblotting was performed with antiphosphotyrosine MoAb 4G10 (UBI). Blots were developed with horseradish peroxidase-conjugated goat-antimouse IgG (Bio-Rad, Richmond, CA) and bound proteins were detected using enhanced chemiluminescence (ECL) (Amersham Corp, Arlington Heights, IL) and Kodak XAR-5 film (Eastman Kodak, Rochester, NY).[1,12,14,27,33] 2×10^6 COS-1 cells were analyzed per lane.

Construction of mutant receptor molecules. Two-step overlap extension polymerase chain reaction (PCR) was used to construct mutant cDNAs.[14,16,27] γY1F and γY2F indicate γ chain mutants in which either cytoplasmic tyrosine 1 (Y1F) or tyrosine 2 (Y2F) have been replaced with phenylalanine. γΔ80 and γΔ65 are γ chain mutants truncated at amino acid 80 or 65, respectively. γΔ71-73 is a γ chain mutant in which amino acid residues 71-73 have been deleted. In the γ-FcγRIIA mutant, the 7-amino acid sequence NTRSQET between the two YXXLs of the γ chain has been replaced by the 12-amino acid sequence NPRAPTDDDKNI between the two YXXLs of FcγRIIA. I-I-IIA and I-IIA-IIA are chimeric receptors containing the extracellular domain of FcγRI, the transmembrane domain of either FcγRI or FcγRIIA and the cytoplasmic domain of FcγRIIA. B2/YMTL represents FcγRIIB2 in which a YMTL sequence is inserted after amino acid 221. B2/YQNRI represents FcγRIIB2 in which amino acid 243 is replaced by tyrosine to create a YXXXI sequence. B2/YMTL/YQNRI is a FcγRIIB2 mutant containing both YMTL and YQNRI (see Table 2).

RESULTS AND DISCUSSION

We have observed that even with comparable cell surface expression in COS-1 cells both FcγRIIIA/γ and FcγRI/γ mediate phagocytosis at a considerably lower level than does FcγRIIA (Table 1). Because FcγRIIIA and FcγRI efficiently induce phagocytosis in human cultured monocytes and macrophages,[27,34,35] we hypothesized that COS-1 cells lack an element(s) present in cells of monocyte/macrophage lineage that optimizes γ chain–mediated phagocytosis.

The PTK Syk, which is present in hematopoietic cells,[2,3,6,29-32,36-38] coimmunoprecipitates with the γ chain associated with FcγRIIIA in macrophages and FcεRI in mast cells.[2,31,32] Syk is also phosphorylated on tyrosine after cross-linking of FcγRI and/or FcγRIIIA on cells of the monocyte/macrophage lineage.[2,3,6,37,38] Therefore, to define the signal transduction requirements for phagocytosis, we cotransfected Syk, the γ chain, and either FcγRIIIA or FcγRI into COS-1 cells and examined the ability of these cells to ingest IgG-sensitized RBCs (EA). Syk dramatically enhanced

Table 2. Effect of Syk on Phagocytosis Mediated by Mutants of the Nonphagocytic Receptor FcγRIIB2

		Phagocytosis	
Fcγ Receptor		−Syk	+Syk
FcγRIIB2		<50	<50
B2/YMTL		60	410
B2/YQNRI		<50	<50
B2/YMTL/YQNRI		90	820
	221		243
FcγRIIB2 WT	NPTNPDEADKV		GAENTITYSLLMHPDALEEPDDQNRI
FcγRIIB2/YMTL	NPTNPDEADKVYMTL		GAENTITYSLLMHPDALEEPDDQNRI
FcγRIIB2/YQNRI	NPTNPDEADKV		GAENTITYSLLMHPDALEEPDYQNRI
FcγRIIB2/YMTL/YQNRI	NPTNPDEADKVYMTL		GAENTITYSLLMHPDALEEPDYQNRI

Representative experiments are shown for FcγRIIB2 and its mutants. B2/YMTL represents FcγRIIB2 in which a YMTL sequence is inserted after amino acid 221. B2/YQNRI represents FcγRIIB2 in which amino acid 243 is replaced by tyrosine to create a YXXXI sequence. B2/YMTL/YQNRI is a FcγRIIB2 mutant containing both YMTL and YQNRI. Mutations were constructed using two-step overlap PCR.[14]

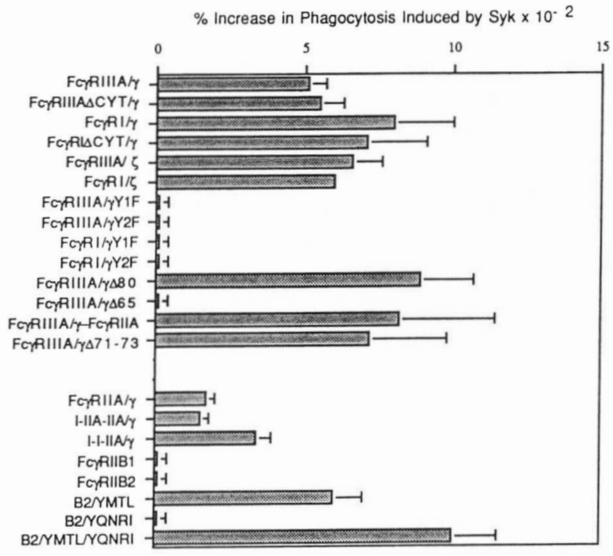

Fig 1. Effect of Syk on phagocytosis mediated by Fcγ receptors. The increase in efficiency of phagocytosis by transfected wild-type and mutant Fcγ receptors in the presence of the PTK Syk is expressed as percent increase in phagocytic index (number of ingested EA per 100 Fcγ receptor expressing cells determined by flow cytometry). Error bars indicate ±SEM. The number of cells that ingest at least one RBC is also increased in the presence of Syk. For example, the fold increase in percent phagocytic cells is 3.0 ± 0.2 for FcγRIIIA/γ, 3.6 ± 0.5 for FcγRI/γ, and 6.3 ± 1.7 for FcγRIΔCYT/γ. Transfectants of FcγRI and FcγRIIIA with wild-type and mutant γ chains are grouped on the upper section of the graph and wild-type and mutant FcγRII transfectants are presented on the lower section of the graph. FcγRIΔCYT and FcγRIIIAΔCYT indicate mutants in which the cytoplasmic domain has been deleted. γY1F and γY2F indicate γ chain mutants in which either cytoplasmic tyrosine 1 (Y1F) or tyrosine 2 (Y2F) have been replaced with phenylalanine. γΔ80 and γΔ65 are γ-chain mutants truncated at amino acid 80 or 65, respectively. γΔ71-73 is a γ-chain mutant in which amino acid residues 71-73 have been deleted. In the γ-FcγRIIA mutant, the sequence between the two YXXLs of the γ chain has been replaced by the sequence between the two YXXLs of FcγRIIA. The chimeras I-I-IIA and I-IIA-IIA represent receptor molecules containing the EC of FcγRI, the CYT of FcγRIIA and the TM of either FcγRI or FcγRIIA, respectively. The definition of the FcγRIIB2 mutants is given in Table 2. FcγRI and FcγRIIIA were transfected with the γ-chain subunit (eg, FcγRI/γ and FcγRIIIA/γ) or the ζ chain as indicated. Data are shown with the γ chain for FcγRIIA and the two FcγRIIA chimeras to illustrate the enhancement by Syk of I-I-IIA–mediated phagocytosis in the presence of the γ chain (Table 1). For most receptors, data are derived from at least four experiments.

(threefold to sevenfold) the phagocytosis of EA by FcγRIIIA and γ cotransfectants (Fig 1, Table 1). The low level of phagocytosis noted for FcγRI and γ chain cotransfectants was also greatly enhanced (3-fold to 10-fold) by Syk (Fig 1, Table 1). Syk also increased the percent of cells able to phagocytose EA (3.0 ± 0.2-fold for FcγRIIIA/γ, 3.6 ± 0.5-fold for FcγRI/γ). Thus, the PTK Syk dramatically enhances phagocytosis mediated by these receptors and also allows some nonphagocytic Fcγ receptor expressing cells to acquire phagocytic capability.

In the absence of the γ chain, Syk did not induce phagocytosis by either FcγRIIIA or FcγRI, and consistent with the concept that the effect of Syk requires sequences in the γ chain, the cytoplasmic domain of neither FcγRIIIA nor FcγRI was required for the stimulation of phagocytosis by Syk (Fig 1, Table 1). There was no effect when either FcγRI or FcγRIIIA and the γ chain were cotransfected with nonsense Syk or vector alone. Flow cytometry showed that the Syk effect was not a consequence of increased Fcγ receptor expression (Fig 2). Furthermore, cross-linking of FcγRI in COS-1 transfectants expressing FcγRI, the γ chain, and Syk induced tyrosine phosphorylation of Syk, suggesting that Syk is activated under these conditions (Fig 3). This result is consistent with the observations in monocytes and macrophages which show that Fcγ receptor cross-linking enhances tyrosine phosphorylation of Syk kinase.[2,3,6,37,38] These data indicate that Syk markedly enhances the phagocytic signal in two Fcγ receptors associated with the γ chain and demonstrates that introduction of a specific tyrosine kinase can induce a physiologically important cellular function.

The ζ chain of the T-cell receptor is homologous to the γ chain in that it contains conserved cytoplasmic YXXL sequences. It functions as a subunit of FcγRIIIA in some cells, eg, natural killer cells[39] and is also able to transmit a phagocytic signal.[15] Coexpression of Syk with the ζ chain and either FcγRIIIA or FcγRI also enhanced ζ chain–mediated phagocytosis but did not increase the level of phagocytosis to that of the γ chain (Table 1). This result is consistent with our previous observations which indicate that the ζ chain is less efficient than the γ chain in inducing phagocytosis in transfected COS-1 cells.[15]

Signaling pathway(s) used for phagocytosis by FcγRI and FcγRIIIA appear to differ from that used by the other phagocytic Fcγ receptor, FcγRIIA. For example, in contrast to FcγRI and FcγRIIIA, FcγRIIA-induced phagocytosis occurs efficiently in COS-1 transfectants in the absence of the

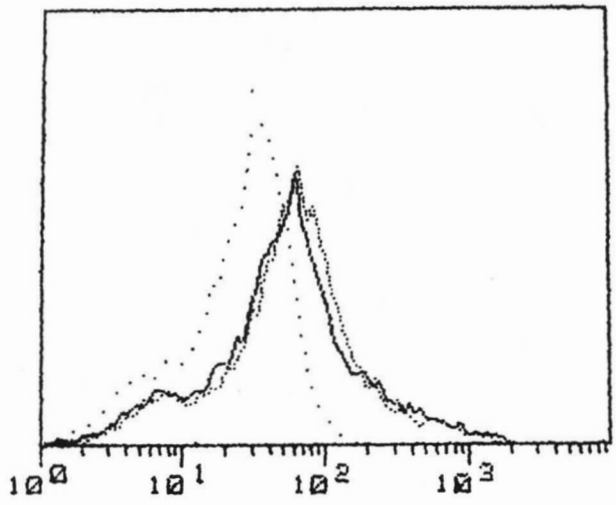

Fig 2. Flow cytometric analysis of COS-1 cells cotransfected with FcγRI and the γ chain with (· · ·) or without (—) Syk. The fluorescence of an isotype control is indicated by (· · ·). COS-1 cells transfected with FcγRI were incubated with anti-FcγRI MoAb 32.2 and labeled with FITC-conjugated goat F(ab′)$_2$ antimouse IgG (TAGO, Inc, Burlingame, CA).[11,27] Expression of FcγRI was similar in the presence and absence of Syk.

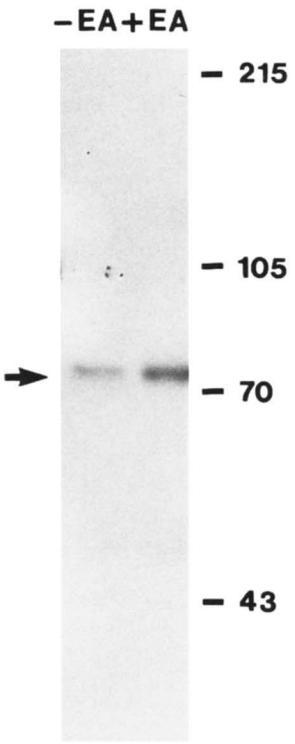

Fig 3. Tyrosine phosphorylation of Syk in FcγRI, γ chain, and Syk COS-1 cell cotransfectants. Transfectants were left unstimulated (−EA), or stimulated (+EA) with IgG-sensitized RBCs for 20 minutes at 37°C. After immunoprecipitation of cell lysates with anti-Syk antibody,[33] nitrocellulose blots were probed with antiphosphotyrosine antibody (see Materials and Methods). The arrow indicates the position of phosphorylated Syk (72 kD).

γ chain or Syk.[11,12,14] Nevertheless, the induction of Syk phosphorylation after cross-linking of FcγRII in monocytes/macrophages[3,6,38] suggested that Syk may play a role in phagocytosis by FcγRIIA. Syk modestly increased the efficiency of phagocytosis by FcγRIIA (Fig 1, Table 1) and, similarly, modestly increased phagocytosis by the chimeric receptor I-IIA-IIA which contains the cytoplasmic domain (CYT) and transmembrane domain (TM) of FcγRIIA and the extracellular domain (EC) of FcγRI (Fig 1, Table 1). Consistent with the thesis that association of FcγRI with the γ chain occurs through the TM of FcγRI,[27,40-42] greater enhancement of phagocytosis by Syk in the presence of the γ chain was observed for the chimera I-I-IIA (EC-TM-CYT) than for either FcγRIIA or the chimeric receptor I-IIA-IIA (Fig 1, Table 1). It is likely that association with the γ chain occurs through the FcγRI-derived TM of I-I-IIA and that recruitment of the γ chain allows a larger Syk phagocytic response by this chimera. It is also noteworthy that in these experiments, the γ chain decreased phagocytosis mediated by FcγRIIA in the absence of Syk (Table 1). Although the reason for this apparent inhibitory effect is unknown, one possible explanation is that the γ chain competes for a substrate(s) important for phagocytosis by FcγRIIA.

Because the association of Syk with phosphorylated γ[2,31,32] and the enhancement by Syk of Fcγ receptor phagocytosis suggest involvement of Syk in γ chain mediated Fcγ receptor function, we examined whether particular sequences in the γ chain are important for induction of phagocytosis by Syk (Fig 1). Replacement of either tyrosine by phenylalanine in the conserved YXXL motifs of the cytoplasmic domain of the γ chain eliminates both FcγRI- and FcγRIIIA-mediated phagocytosis[15] (Fig 1). Syk was unable to induce phagocytosis in these γ-chain mutants lacking one YXXL tyrosine (FcγRI/γY1F, FcγRI/γY2F, FcγRIIIA/γY1F, and FcγRIIIA/γY2F, Fig 1), suggesting that Syk engages two functional SH2 binding domains for its interaction with γ in phagocytosis. In addition, there was no induction of phagocytosis by Syk in the γ-chain truncation mutation which removed YXXL sequences (γΔ65) whereas enhancement of phagocytosis by Syk was observed with the γ-chain mutants that lack residues downstream of the YXXL sequences (γΔ80). These results confirm the importance of the region containing the two YXXL sequences for phagocytosis and suggest that Syk associates with the γ chain through this conserved sequence.

We examined whether the spacing between the two conserved YXXL sequences of the γ chain affects Syk function. Seven amino acids separate the two YXXL sequences of the γ chain whereas 12 amino acids separate the two YXXLs in FcγRIIA. In the γ chain mutant γ-FcγRIIA, the sequence separating the two cytoplasmic YXXLs of the γ chain was replaced with the intervening sequence from FcγRIIA. This lengthened the sequence between the two YXXLs by five amino acids. Syk stimulated the phagocytic efficiency of this mutant as well as that of a γ chain mutant in which four amino acids were deleted from the conserved sequence separating the two YXXLs (γΔ71-73). Thus, a sequence of between 3 and 12 amino acids between the two conserved YXXL sequences allows Syk to function in Fcγ receptor/γ chain–mediated phagocytosis.

To further define the Fcγ receptor sequences important for the induction of phagocytosis by Syk, we also examined FcγRII isoforms and mutants of these receptors. In contrast to the phagocytic receptor FcγRIIA, FcγRIIB1 and FcγRIIB2 contain a single cytoplasmic YXXL sequence and do not mediate phagocytosis in COS-1 transfectants.[14] Cotransfection with Syk did not induce phagocytosis by FcγRIIB1 or FcγRIIB2 (Fig 1, Table 1). To establish an additional YXXL sequence in FcγRIIB2, YMTL (the first YXXL from FcγRIIA) was inserted after Val^{221}, seven amino acids upstream of the existing YSLL (B2/YMTL) (Table 2). The insertion of YMTL into FcγRIIB2 enabled Syk to enhance the phagocytic efficiency of this receptor more than sixfold (Table 2, Fig 1). In contrast, Syk did not affect phagocytosis by B2/YQNRI, in which Asp^{243} was changed to Tyr, creating a YXXXI motif at position 243, 11 amino acids downstream of the existing YSSL. However, Syk increased by ninefold the phagocytic efficiency of B2/YMTL/YQNRI, a mutant with two additional tyrosine-containing motifs. These data suggest that Syk requires at least two YXXL/YXXXI motifs to induce phagocytosis and that YMTL may be one of the permissible sites for the effect of Syk.

Because the protein kinases of the Src family Lyn, Fyn, Fgr, Lck, and Src, like Syk, are expressed in phagocytic cells such as monocytes/macrophages,[1-3,6,37,38,43] we examined the effect of these protein kinases on γ chain–mediated phagocytosis. In contrast to the effect of Syk, phagocytosis by FcγRIIIA/γ and by FcγRI/γ was not increased by introduction of any of these tyrosine kinases. The observation that

of these protein kinases only Syk kinase enhanced phagocytosis by FcγRI or FcγRIIIA in the presence of the γ chain suggests a specificity of Syk for γ chain sequences. The low levels of FcγRI/γ- and FcγRIIIA/γ-mediated phagocytosis in COS-1 cells in the absence of transfected Syk may be caused by endogeneous tyrosine kinases,[1,12] less able than Syk to function with γ-chain sequences, or to low levels of Syk that may be present in some COS-1 cells. In addition, it is not yet known whether endogeneous COS-1 cell Src family kinases (SRTKs) are necessary for Syk's effect or whether Syk-induced phagocytosis in the presence of the γ chain is independent of SRTKs. Variability in the surface density of the Fcγ receptors may also influence phagocytic function; however, the adherent properties of COS-1 cells make it difficult to use cell sorting to determine whether cells expressing high densities of Fcγ receptors are able to mediate phagocytosis in the absence of Syk.

Our studies show that introduction of a PTK can induce an important cellular function, phagocytosis. Previous studies have demonstrated an association between the γ chain and Syk kinase[2,31,32] in macrophages and rat basophilic leukemia cells. However, although activation of FcγRIIIA on monocytes and macrophages induces the phosphorylation of the γ chain and association with Syk,[2] the mechanism by which Fcγ receptors interact with distinct PTKs and the role(s) of Syk in Fcγ receptor function are unknown. We have observed that Syk dramatically increases phagocytosis by FcγRI and FcγRIIIA, mediated through the γ chain. The effect of Syk requires the conserved tyrosines within the γ chain cytoplasmic domain and two intact YXXL sequences, providing two potential SH2 binding sites. Syk is also able to induce phagocytosis in a previously nonphagocytic Fcγ receptor, FcγRIIB2, after the insertion of an additional YXXL sequence(s) (Table 2).

Although isoforms of each class of Fcγ receptor are able to induce the phagocytosis of IgG-coated cells,[11-15,27,34,35] their mechanisms for phagocytosis differ. FcγRIIA induces phagocytosis through the phosphorylation of tyrosines within the conserved motif of its own cytoplasmic domain[11,12,14] whereas both FcγRI and FcγRIIIA require the tyrosines within the conserved cytoplasmic region of the γ subunit for phagocytosis[16] (Fig 1). Efficient phagocytosis by transfected FcγRIIA is mediated by endogeneous kinases in COS-1 cells (presumably Src family kinases)[1,12] because high levels of phagocytosis are observed in the absence of cotransfected Syk. Enhancement by Syk of FcγRIIA-mediated phagocytosis was minimal compared with FcγRI/γ or FcγRIIIA/γ, further supporting the concept that the pathway for phagocytosis mediated through FcγRIIA is distinct from the pathway(s) used by FcγRI and FcγRIIIA.[11,12,15] Furthermore, in contrast to γ chain–mediated phagocytosis, phagocytosis by FcγRIIA is detectable in the presence of a single intact YXXL sequence.[12,14,16,17] It is of note that Syk does not enhance phagocytosis by these FcγRIIA mutants lacking one YXXL sequence (unpublished observation, March 1994).

These studies begin to define the structural features for the induction of phagocytosis by Syk. The observation that cotransfection of Syk alters the efficiency of an important Fcγ receptor–mediated function provides an approach for examining the specificity and requirements for tyrosine kinases involved in signaling by Fcγ receptors. A similar model has been helpful for reconstructing the association of T-cell receptor molecules after their introduction into COS cells.[44,45] Our experiments show that receptor function is modified by introduction of a kinase and constitute, to our knowledge, the first direct demonstration of an in vivo functional consequence of the action of a PTK.

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