Minireview

Tyrosine phosphorylation and Fcγ receptor-mediated phagocytosis

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Abstract Phagocytosis of IgG-opsonized particulate material in hematopoietic cells is mediated by Fcγ receptors (FcγRs). Interaction of the receptors with Fc domains of IgG triggers transduction of phagocytic signal in which a key role is played by phosphorylation of tyrosine residues of the receptors. These residues are arranged into a specific motif (immunoreceptor tyrosine-based activation motif; ITAM) which is located either in the cytoplasmic part of FcγRIIA or in γ chains associated with FcγRI and FcγRIIIA. The conserved tyrosine residues are phosphorylated by, and associate with, tyrosine kinases of Src and Syk families. Coordinated action of these components initiates numerous intracellular events leading finally to local rearrangement of the actin-based cytoskeleton and internalization of the particles.

Key words: Fcγ receptor; Tyrosine phosphorylation; Protein tyrosine kinase; Phagocytosis

1. Introduction

Several receptors that mediate binding and ingestion of particulate material have been identified in hematopoietic cells. These receptors may interact either directly with the particles recognizing determinants present on their surface or may bind to opsonins coating the particles. FcγRs are the opsonin-dependent membrane constituents which recognize the Fc domain of opsonin IgG. This interaction triggers a variety of responses, among them actin-dependent internalization of the particles. Being engaged in phagocytosis of IgG-coated targets (e.g. microorganisms) FcγRs play a crucial role in the immune defence system. Various aspects of FcγR structure and function have been considered previously [1–3]. In this review, we focus on the role of tyrosine phosphorylation in regulation of FcγR-mediated phagocytosis.

2. Fcγ receptors

Three distinct classes of FcγRs have been identified: FcγRI, FcγRII and FcγRIII. They are members of the Ig gene superfamily and share a highly homologous extracellular portion which contains the IgG binding domain; however, there are some differences in the receptors' structure. FcγRII is a monomer while FcγRI and FcγRIII exist as oligomeric complexes in which the α chain, bearing the IgG binding domain, associates with dimers composed of homologous, disulphide-linked, γ and ζ chains [1]. The α chain of FcγRIII interacts with γ and ζ homo- and heterodimers [4,5]. In the case of FcγRI only the γ accessory chain has been identified [6].

FcγRI (α chain; a 72 kDa sialoglycoprotein) binds monomeric IgG with high affinity and is expressed on monocytes, macrophages and interferon-γ stimulated neutrophils. FcγRII (a 40 kDa sialoglycoprotein) is a low-affinity receptor which preferentially binds to multivalent ligands. Its several isoforms originate from the expression of three genes: FcγRIIA, B, C and alternative splicing of FcγRIIB (FcγRIIB1 and FcγRIIB2) and have different distribution in hematopoietic cells [7,8]. FcγRIIA occurs mainly in phagocytic cells (monocytes, macrophages and granulocytes) and is the only FcγR identified in platelets. FcγRIIB is preferentially expressed in B- and T-cells. FcγRIIC is the result of unequal cross-over between FcγRIIB and FcγRIIA that contains the extracellular and transmembrane portion of FcγRIIB and the FcγRIIA tail [9]. It is present in neutrophils, monocytes and B-cells. FcγRIII is also a low-affinity receptor with the molecular mass of the α chain ranging from 51 to 73 kDa. It is encoded by two genes: FcγRIIIA and B. FcγRIIIA is expressed as a transmembrane form on NK cells and macrophages. FcγRIIIB is a glycosylphosphatidylinositol-linked receptor lacking transmembrane and cytoplasmic domains, and may mediate the phagocytic signal in cooperation with other receptors. It is expressed solely in neutrophils [10,11].

During activation of FcγRs, upon onset of phagocytosis or by cross-linking of the receptors with specific antibodies, tyrosine residues in the cytoplasmic parts of the receptors become phosphorylated [12–15]. The tyrosines are located within a common motif forming a minimal functional segment of the receptors [16]. The motif consists of paired tyrosines and leucines in the consensus sequence $YxxL(x)_{7/12}YxxL$, and is referred to as the immunoreceptor tyrosine-based activation motif (ITAM). The role of ITAM in transduction of phagocytic signal was elucidated using several model systems, among which COS cells, lacking endogenous FcγRs and transfected with various constructs of human receptors, were especially useful. COS cells transfected with FcγRIIA ingest IgG-sensitized particles [17]. Transfection of a human FcγRIIA cDNA induces also T-cells to become phagocytic [18]. The cytoplasmic domain of FcγRIIA contains two YxxL sequences (Y282 and Y298) arranged into an ITAM motif plus an additional tyrosine residue at position 275. Further studies showed that deletion of Y282 or Y298 from ITAM inhibited phagocytosis by 50 to 65%, but replacement of Y275 did not affect phagocytosis. These data indicate that at least two cytoplasmic tyrosines, including the typical single YxxL motif, are required for phagocytosis mediated by this receptor. It was proposed that Y275 plays a 'back-up' function when one tyrosine residue of ITAM is missing [19]. FcγRIIB has only a single YxxL sequence. In the human FcγRIIB1

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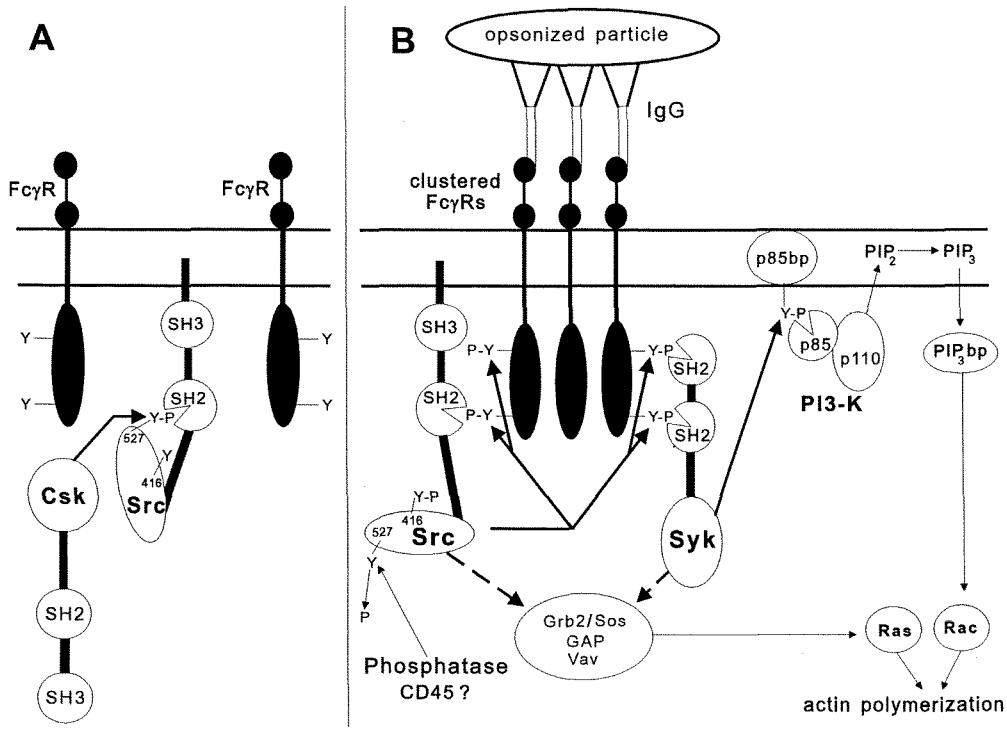

Fig. 1. Scheme of initial events induced after Fcγ receptors stimulation in phagocytic cells. (A) Resting cell; (B) cell with activated Fcγ receptors. For detailed description, see text.

isoform the YxxL sequence comprises Y292 while in the FcγRIIB2 isoform this sequence starts at Y273 [20]. COS cells transfectants expressing either FcγRIIB1 or FcγRIIB2 or mutated FcγRIIA lacking the cytoplasmic tail, efficiently bind particles but do not ingest them. The phagocytic incompetence of these cells was attributed to a lack of phosphorylation of the expressed receptor isoforms. The phagocytic activity of the non-phagocytic FcγRIIB2 was partially restored by insertion of the YxxL sequence upstream of the original one, which resulted in formation of a sequence resembling ITAM of FcγRIIA. The activity was additionally improved by introduction of the tyrosine residue downstream of the motif giving a final combination of three tyrosine residues arranged into two ITAM-related configurations [21]. The α chains of FcγRI and FcγRIIIA do not possess tyrosine residues and these receptors transmit a phagocytic signal through associated γ chains bearing one intact ITAM motif [22–24]. The ζ chain which contains multiple copies of ITAM is less efficient in induction of phagocytosis than the γ chain [25,26]. Interestingly, ζ-associated isoforms of FcγRIIIA are confined to NK-cells and seem to be specialized to accompany cytotoxic activity of these cells [4].

These studies point to the importance of the ITAM motif for FcγR-mediated phagocytosis. It is believed that the conserved tyrosine residues of FcγRs serve as substrates for intracellular tyrosine kinases and subsequently as docking sites for SH2 domain(s) of the kinases.

3. Fcγ-associated tyrosine kinases

Two classes of protein tyrosine kinases, members of the Src and Syk families, have been implicated in the function of the FcγRs.

So far, five out of nine members of the Src gene family kinases have been identified in phagocytic cells: Src, Fyn, Fgr, Hck, Lyn [27]. The conserved region of the Src family kinases can be divided into five domains: the extreme N-terminal domain contains myristoylated glycine which is responsible for membrane association; the src homology SH3 and SH2 regions; the catalytic domain and the C-terminal non-catalytic tail. Within the tail a tyrosine residue is located (e.g. Y527 in Src) which, when phosphorylated, inhibits catalytic activity of the kinases. It is commonly accepted that this phosphorylated tyrosine interacts intramolecularly with the SH2 domain of its own kinase, which results in the formation of an inactive enzyme (for review see [28,29]) (Fig. 1A). The negative regulation of Src family kinase activity is probably the result of phosphorylation by Csk which is capable of phosphorylating the C-terminal tyrosine residue of Src, Lyn and Fyn [30,31]. Dephosphorylation of this tyrosine residue exposes the SH2 domain which can then bind to the phosphotyrosine residue in the activated receptor (Fig. 1B). However, activation of members of the Src family may also be induced by (auto)phosphorylation of another tyrosine residue (Y416) identified within the catalytic domain of Src [28]. Src, and to a lesser extent Lyn and Fyn, phosphorylate FcγRIIA in vitro suggesting a possible function of the kinases in vivo [32,33]. In accordance with this supposition, Src-deficient cells are less capable of FcγRIIA-mediated phagocytosis and receptor phosphorylation [33]. In neutrophils FcγRII is associated with Fgr and the receptor cross-linking is accompanied by Fgr activation [34]. FcγRI and FcγRII in monocytic cells are physically and functionally associated with two other Src family kinases – Hck and Lyn. Furthermore, activation of the receptors is followed by tyrosine phosphorylation of Hck and Lyn and an increase in these kinases' activity, thus resembling

the FcγRII-Fgr interaction [35,36]. Members of the Src family kinases are implicated in the very early stages of phagocytic signal transduction although the details of the FcγR-kinase interaction, regulated probably in a cell-specific manner, are unknown [32,35,37].

Syk and closely related ZAP-70 kinases are not myristoylated and hence are not constitutively bound to the plasma membrane. They bear two N-terminal SH2 domains and a C-terminal catalytic domain. Syk (72 kDa) is expressed in all hematopoietic cells, whereas ZAP-70 (70 kDa) occurs exclusively in T- and NK-cells [38,39]. Several lines of evidence point to interaction of Syk with FcγRI and FcγRIIIA which is mediated by the γ chain of the receptors: (i) in macrophages, the enzymatic activity of Syk is enhanced and tyrosine residues of the kinase are phosphorylated upon activation of FcγRIIIA [15,37]; (ii) Syk coimmunoprecipitates with the γ chain of FcγRIIIA in these cells [37]; (iii) based on similar data: kinase hyperphosphorylation and activation after FcγRI cross-linking as well as coimmunoprecipitation with γ chain of the receptor, Syk is thought to be involved in signal transduction through the FcγRI in monocytes [14,40]; (iv) cotransfection of Syk with the γ chain and FcγRI or FcγRIIIA markedly enhanced the phagocytic capability of COS cells compared to that of FcγRI/γ or FcγRIIA/γ transfectants. Due to requirement of the presence of both tyrosine residues in the ITAM motif of the γ chain for the action of Syk, it is proposed that Syk binds to, via its two SH2 domains, and is activated by tyrosine phosphorylated ITAMs [24] (Fig. 1B); (v) confirming the last suggestion, Syk was identified as a necessary component acting downstream of γ chains in the pathway leading from ITAM activation to actin assembly [41]. In contrast to Syk, ZAP-70 kinase required additionally participation of Fyn kinase to enhance phagocytosis in COS cells transfected with FcγRIIIA and the γ (or ζ) chain [26]. These data suggest possible cooperation between the Syk and Src tyrosine kinase families. The function of Syk is less clear in the case of FcγRIIA which does not associate with the γ chain and is able to retain partial phagocytic ability with one tyrosine residue of ITAM missing [19]. However, since cross-linking of FcγRII induces phosphorylation and activation of Syk, it seems possible that Syk is also involved in phagocytosis mediated by this receptor [12,40,42]. Recent studies on chimeric proteins composed of the extracellular domain of FcγRIII connected to various tyrosine kinases indicated the importance of Syk kinases for Fcγ-mediated phagocytosis when the chimeric proteins were transfected into COS cells. Only Syk, and to a lesser extent ZAP-70 transfectants gained phagocytic abilities, provided the enzymatic activity of the kinase was preserved [43].

The involvement of protein tyrosine kinases in FcγR-mediated signalling events was confirmed by using inhibitors of the kinases. Genistein, tyrphostin 23, erbstatin, or herbimycin-A block both phagocytosis and phosphorylation of numerous proteins involved in these events, as has been demonstrated in monocytes, macrophages and COS cell transfectants [5,13,23,25,44,45]. In neutrophils, however, neither genistein nor erbstatin reduced FcγR-mediated phagocytosis of particulate material although they almost completely inhibited tyrosine phosphorylation of FcγRII. It has been suggested that, in neutrophils, the phosphorylation of FcγRII is a process not directly involved in phagocytosis but rather in other cell responses associated with phagocytosis [46].

4. Substrates of tyrosine kinases

Activation of FcγRs induces rapid and transient tyrosine phosphorylation of various cytoplasmic and membrane proteins including FcγRII and γ subunits of FcγRI and FcγRIIIA [12,13,15,37,40,44,45]. Some of the other phosphotyrosine-containing proteins have been identified, e.g. paxilin (68 kDa), a cytoskeleton-associated, Src tyrosine kinase substrate which colocalizes with F-actin beneath nascent phagosomes [15,31]. The tyrosine phosphorylated proteins of 50–60 kDa are likely to belong to the Src family kinases [34–36]. The polypeptide of 72 kDa has been suggested to be Syk kinase [12,15,37,40,42].

Another phosphorylated polypeptide (115 kDa) is known to be associated with PI3-kinase (PI3-K), presumably by binding to the SH2 domain of p85, a regulatory subunit of the enzyme. The activity of PI3-K increases after FcγR stimulation [47]. The importance of PI3-K for FcγR-mediated phagocytosis was confirmed by studies using wortmanin, a specific PI3-K inhibitor, which blocks phagocytosis in monocytes, neutrophils as well as in COS cells transfected with either of the three classes of FcγRs [2,47]. In COS transfectants PI3-K coimmunoprecipitated with Syk kinase after FcγRI cross-linking which indicates that the kinases may form a complex [2].

Phospholipase C (PLC) γ1 and PLCγ2 also belong to the proteins phosphorylated on tyrosine upon FcγR activation. Being phosphorylated PLCγ1 causes hydrolysis of PIP_2 and intracellular calcium mobilization [12,48]. Nevertheless, several data contradict any involvement of PLCγ and calcium ions in FcγR-mediated phagocytosis suggesting, however, their participation in some phagocytosis-related events ([3] and references therein).

Proteins of the Ras signalling pathway form another group of polypeptides found to be phosphorylated during FcγR activation. This group includes p95Vav, p62/GAP-associated protein and p21Ras-GAP [12,37].

Many of the cellular responses discussed above are similar to the cascade of events generated by B- and T-cell receptors, the cytoplasmic domains of which also contain a homologous signalling motif ITAM (for review see [49]).

5. Signal generation

On the basis of the data discussed above the following scheme of initial events of the FcγR-mediated phagocytosis can be proposed (Fig. 1). In non-stimulated cells Csk phosphorylates the C-terminal tyrosine residue of Src kinases (e.g. Y527 in Src). The phosphorylated tyrosine binds intramolecularly its own SH2 domain, making the enzyme inactive (Fig. 1A). In stimulated cells, binding of particulate ligands to the extracellular domains of FcγRs causes clustering of the receptors (Fig. 1B). Under these conditions Src kinase is activated by dephosphorylation of the C-terminal tyrosine residue. In this process CD45, a tyrosine phosphatase of hematopoietic cells, may be engaged, as it was found to dephosphorylate and activate the Src family kinases in T-cells [49]. Co-cross-linking of CD45 and FcγRs inhibits overall tyrosine phosphorylation, which follows the receptor activation, and blocks some of the downstream tyrosine kinase-dependent events, supporting this possibility [12,44]. Activation of the Src family kinases is additionally enhanced by phosphorylation of another tyrosine residue located within the catalytic domain (e.g. Y416 in

Src). The activated Src phosphorylates tyrosine residues within the ITAM motif of FcγRs. Phosphorylated tyrosines in ITAM serve as high-affinity binding sites for SH2 domains of Src and Syk kinases. Syk phosphorylates a putative p85-binding protein (p85bp), which may serve as a specific docking molecule for the SH2 domain of p85, a regulatory subunit of PI3-K. Reciprocal interactions between phosphorylated tyrosine residues and SH2 domains of Syk and PI3-K kinases are also possible [50]. Additionally, PI3-K may bind to the SH3 domain of Src family kinases [51]. The p110 subunit of PI3-K catalyses formation of phosphorylated phosphoinositides such as PIP_3 which binds to a putative PIP_3-binding protein (PIP_3bp). PIP_3 as well as GAP, Grb2/Sos and Vav directly or indirectly, by Rac or Ras may promote actin polymerization [37]. The local actin filament assembly is believed to be an essential step in internalization of particulate material.

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