JB Review

J. Biochem. 2009;145(3)267–273
doi:10.1093/jb/mvp001

Protein Tyrosine Kinase, Syk: A Key Player in Phagocytic Cells

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Received October 1, 2008; accepted October 30, 2008; published online January 4, 2009

Spleen tyrosine kinase (Syk) is a non-receptor protein tyrosine kinase expressed in a wide range of haematopoietic cells. At the initial stage of investigation, main exploring was toward its functions in platelets and in classical immunoreceptor signalling. However, Syk has now been recognized as a key player in both innate and adaptive immunity. Especially, in phagocytosis, Syk plays essential roles in signalling evoked by various types of receptors such as FcγR, CR3, Dectin-1 and apoptotic cell-recognizing receptor. A variety of upstream immunoreceptor tyrosine-based activation motif-like molecules have been found and are still in the course of new studies. On the contrary, downstream effectors to explain diverse function of Syk are still under exploration. As its novel function, we propose the role of Syk in the regulation of α-tubulin acetylation. Further investigation on the effectors of Syk would give us more information in relation to therapeutic molecular targets.

Key words: ITAM, integrin, macrophage, phagocytosis, Syk.

Abbreviations: BLNK, B-cell linker protein; CHO, Chinese hamster ovary; CR3, complement receptor 3; FcγR, Fcγ receptor; IgG, immunoglobulin; ITAM, immunoreceptor tyrosine-based activation motif; LIBS, ligand-induced binding site; MoAb, monoclonal antibody; NK, natural killer; PSGL-1, P-selectin glycoprotein ligand-1; PTK, protein-tyrosine kinase; SH2, Src homology 2; SLP76, SH2-domain-containing leukocyte protein of 76 kDa; Syk, Spleen tyrosine kinase.

Spleen tyrosine kinase (Syk) is a non-receptor protein tyrosine kinase (PTK), which is present abundantly in a wide range of haematopoietic cells such as B cells, mast cells, neutrophils, macrophages and platelets (*1–6*). There exist over 10 distinct families of non-receptor PTKs including the Src family, and Syk family is composed of two members, Syk and Zap-70. Expression of Zap-70 is restricted to T- and natural killer (NK)-cells, whereas Syk is expressed more widely and also found in various non-immune cells (*7*, *8*). A common structural feature of the Syk family is the presence of two N-terminal Src homology 2 (SH2) domains located in tandem and a C-terminal kinase region (Fig. 1). These three domains are separated by interdomain A (located between the N-SH2 and C-SH2 domains) and interdomain B (located between the C-SH2 and the kinase domain) (Fig. 1). Each domain of Syk appears to function distinctly. The tandem SH2 domains bind the diphosphorylated immunoreceptor tyrosine-based activation motif (ITAM) domain. The interdomain B contains autophosphorylation sites and plays a role in recruiting downstream signalling molecules (*9–12*). The interdomain A plays a role to provide appropriate conformation of the tandem SH2 for the binding to the diphosphorylated ITAM using a helical coiled-coil structure. In addition, C-terminal part of the interdomain A but not tyrosine residue is critical for the regulation of kinase activity at the resting state (*13*). In the early part of this review, we overview the role of Syk in classical immunoreceptor and integrin signalling, and in the later part, together with our data, focus attention on its role in the phagocytic process mediated by diverse function.

THE FUNCTION OF Syk IN CLASSICAL IMMUNORECEPTOR-MEDIATED SIGNALLING

Syk has been reported to play a crucial role in signal transduction through the classical immunoreceptors, including the B-cell receptor (*14*, *15*), Fc receptors (*16*, *17*) and activating receptors of NK cells (*18*). These receptors have diverse mechanisms of ligand recognition, but their intracellular signalling pathways are highly conserved. The ligation of immunoreceptors leads to activation of different members of the Src family kinase depending on the cell type. Src family kinases then phosphorylate ITAMs that are contained within the immunoreceptors themselves or in receptor-associated molecules (*19*). An ITAM consists of two critically spaced tyrosine residues (YXXI/L-$X_{6–12}$-YXXI/L) that form a docking site for the tandem SH2 domains of Syk. Syk translocates to phosphorylated ITAM motifs via the interaction of their SH2 domains and undergoes conformational changes upon autophosphorylation and/or binding to phosphorylated ITAM. Subsequently, recruitment of Syk to ITAM motifs leads to its activation and phosphorylation of a number of molecules such as the adapter protein SH2-domain-containing leukocyte protein of 76 kDa (SLP76), the Vav family of guanine nucleotide exchange factors or B-cell linker

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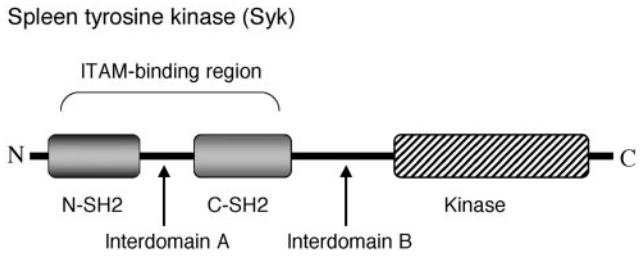

Fig. 1. **Schematic representation of domain structure of Syk.** N-SH2 and C-SH2 indicate Src homology 2 (SH2) domains located in tandem. Interdomain A locates between N-SH2 and C-SH2 domains and interdomain B locates between C-SH2 and the kinase domain.

protein (BLNK) and further leads to downstream pathway (*20–22*). As a consequence, activation of Syk promotes the downstream functional responses following immunoreceptor activation, proliferation, differentiation and effector functions such as degranulation, respiratory burst and cytokine release.

THE FUNCTION OF SYK IN OUTSIDE-IN SIGNALLING OF INTEGRINS ON NEUTROPHILS, MACROPHAGES, OSTEOCLASTS AND PLATELETS

Besides these roles in immunoreceptor-mediated signalling, Syk has also been known to be required for integrin signalling in haematopoietic cells (*21*, *23–25*). Integrins are the major cell surface receptors composed of α- and β-chain heterodimers and mediate adhesion of cells to a variety of extracellular matrix proteins and counter receptors expressed on endothelial cells. There are 19 different α- and 8 different β-subunits, which associate in pairs to form distinct αβ-receptors (*26*). Integrin signalling occurs in two phases, firstly inside-out signalling and then outside-in signalling. In response to a variety of intracellular activation signals integrins can undergo changes in their conformation that increase their affinity for ligand and this process is known as 'inside-out signalling'. Integrins also transmit signals into the cell through 'outside-in signalling', which is initiated by the ligand binding to the integrins and bring about a wide variety of cellular processes including actin polymerization and reorganization (*24*, *26*). With regard to Syk, its roles in 'outside-in signalling' have received considerable focus, similar to those in classical immunoreceptor signalling in platelets, neutrophils, monocyte-macrophge and osteoclasts. The platelet-specific integrin αIIbβ3 is expressed on platelets/megakaryocyte-lineage cells and required for normal hemostasis *in vivo*. In *in vitro* assay, integrin αIIbβ3 is required for initiation of a signalling cascade that results in platelet spreading, aggregation and granule secretion when platelets bind to fibrinogen. Many attempts were performed to analyse the function of outside-in signalling of integrin αIIbβ3 distinct from inside-out signalling. Clark *et al*. (*27*) used the anti-ligand-induced binding site (anti-LIBS) monoclonal antibody (MoAb) that alters αIIbβ3 to the activated form and showed that Syk is activated via engagement of αIIbβ3 by fibrinogen only when the cells are pre-treated with anti-LIBS MoAb. In addition, utilizing a megakaryocytic leukaemia cell line CMK, we found that Syk is activated by the single stimulation with soluble fibrinogen (*28*). Gao *et al*. (*29*) also showed that Syk is activated via αIIbβ3 triggered by the binding of soluble fibrinogen in Chinese hamster ovary (CHO) cells by coexpressing Syk and αIIbβ3-integrin subunits. In a similar fashion, platelets lacking Syk fail to spread when plated onto fibrinogen (*30*, *31*). In addition, platelets deficient in Hck, Fgr, Lyn and Src fail to spread on fibrinogen (*30*). These results demonstrate that Syk as well as Src family kinases are essential for the outside-in signalling of integrin αIIbβ3. Additionally, Syk carries an indispensable function in outside-in signalling of β2 integrin-mediated pathway. In human neutrophils, Syk is activated when plated onto fibrinogen in the presence of TNFα (*32*), and similarly in granulocytic-differentiated HL60 cells as a result of 'outside-in' signalling (*33*). Furthermore, neutrophils deficient in Syk fail to spread or undergo respiratory burst or degranulation when plated onto integrin ligands (*23*), although it was confirmed that Syk is not required in the development of neutrophils from generating radiation chimeras using fetal liver from Syk-deficient embryos. As for Src family kinases, neutrophils lacking main Src family members expressed in the myeloid lineage are unable to produce ROS in response to a variety of ligands for β2-integrins and anti-integrin antibodies. Similarly, adhesion-dependent degranulation is also deficient in neutrophils from Syk-deficient mice (*34*). Syk deficiency is also reported to lead to reduction of osteoclast function when cells are differentiated *in vitro*, similar to that seen in Src- or β3-deficient cells (*35*, *36*). Outside-in signalling may also be important for similar pathways to B-cells via β1 integrin (*37*).

In this way, Syk plays an essential role in outside-in signakling in haematopoietic cells as well as Src family kinases. However, these facts bring us to the question how Src and Syk cooperatively transmit signals at the downstream of integrin receptor, because the cytoplasmic region of integrin is too short to have ITAM-like alignment. The recent study that mutation of any one of the ITAM-binding SH2 domains of Syk is sufficient to disrupt known immunoreceptor-mediated signalling may be applicable for αIIbβ3 outside-in signalling (*31*). Furthermore, ITAM-containing DAP-12 and FcγR have been found to associate with Syk and mediate β2-integrin signalling in neutrophils and macrophages (*38*). These facts suggest an importance of the binding of ITAM-containing molecules to SH2 domains of Syk in outside-in signalling (*35*, *39*). The proposed scenario leading to Syk activation in outside-in signalling is as follows; the integrin receptor is ligated, a Src family kinase (which is sometimes associated with β-chain of integrin) is activated, tyrosine residues within the ITAM-containing adapter proteins undergo phosphorylation, and docking sites for tandem SH2 domains of Syk are created. The following recruitment and activation of Syk result in the assembly of a multi-protein signalling complex containing the cytosolic adapter molecules and ultimately cause a multitude of cellular activation responses. Taken together, although cell-specific ligands may regulate the different integrin couple to different ITAM adapters, the

outlined pathway of outside-in signalling reveals an unexpected similarity to the signal transduction mechanisms used by classical immunoreceptors, and ligation of integrins trigger a Syk activation loop that is initiated by Src family kinases and reinforced by binding of Syk to ITAM domains.

As mentioned above, data by many investigators have demonstrated that Src-ITAM-Syk pathway is essential for the downstream signalling of both immunoreceptors and integrin receptors. In the classical immunoreceptors, the ITAM is contained in the intracellular region of subunits associated with the receptors, often in pairs, or is part of the cytoplasmic domain of the receptors themselves. On the contrary, at the downstream of integrin signalling, adapter molecules which contain ITAMs act as mediators, because integrin receptors themselves do not contain such motifs in their cytoplasmic domain. Furthermore, a variety of ITAM-like molecules were found to have a similar activity in other pathways, for example, a signalling mediated by P-selectin glycoprotein ligand-1 (PSGL-1) (*40*), and all of them mediate immune cell function through ITAM-like signalling pathways. This convergence of intracellular signalling pathways illuminates Syk-mediated signalling as a key pathway, which is diverse but important in both innate and adaptive immunity. The next chapter describes a central role of Syk in phagocytosis mediated by a number of receptors; Fcγ receptor, complement receptor, lectin receptor and apoptotic cell recognizing receptor together with our data.

THE FUNCTION IN PHAGOCYTOSIS OF MACROPHAGES VIA SEVERAL RECEPTORS SUCH AS FCγR CR3, LECTIN RECEPTOR AND THE RECEPTORS RECOGNIZING APOPTOTIC CELLS

Phagocytosis is the process in which cells recognize and engulf large particles (usually >0.5 μm in size) such as the pathogens and apoptotic cells, and is a central event in both innate and adaptive immune responses. On the surface of the phagocytes there exist a lot of receptors that are able to recognize and decode their cognate ligands expressed on pathogens and apoptotic cells. Macrophages are one of the professional phagocytes and are among the first lines of defense against invading pathogens. Macrophages express a variety of phagocytic receptors: one type is opsonin-mediated phagocytic receptors including the Fcγ receptor (FcγR) and the integrin αMβ2 (also known as the complement receptor 3 or CR3), and another type is non-opsonin-mediated receptors that directly recognize the lectins or apoptotic cells. Target particles are sometimes recognized by the combination of multiple receptors.

In any receptor system, phagocytosis is initiated by the binding of target particles to cell surface receptors which induces the formation of phagocytic cups at the cell membrane. Subsequently, the target particles are engulfed and the plasma membrane fuses to seal off a mature phagosome.

Opsonin-mediated Phagocytic Receptors—Among the phagocytic receptors expressed on macrophages, FcγRs bind to immunoglobulin (IgG)-opsonized particles and the complement receptor 3 binds to the particles opsonized with complement C3bi. FcγR is one of the classical immunoreceptor and a central role of Syk in the phagocytosis mediated by this receptor was shown in the studies of Syk-deficient murine macrophages (*16*, *17*). That is, following Fcγ-receptor engagement, ITAMs in the receptor are phosphorylated by Src-family kinases, leading to the recruitment and activation of Syk. This in turn leads to the formation of a signalling complex at the membrane, in which Syk-mediated phosphorylation of several adapter proteins causes activation of downstream pathways which give rise to the phagocytic effect.

As for the complement-mediated system which is another opsonin-mediated receptor for phagocytosis, Syk also plays an essential role. Complement-mediated phagocytosis is accomplished by specific binding of complement components to the corresponding receptors CR1, CR3 (another name is integrin αMβ2) and CR4 (another name is integrin αXβ2). C3bi binding to CR3 induces the most effective phagocytosis (*41*). The complement system is composed of three distinct pathways through which the complement components are activated on the pathogen surfaces: (i) classical pathway, (ii) mannose-binding lectin pathway and (iii) alternative pathway. Each pathway follows a sequence of reactions to generate a key protease called C3 convertase. In addition, these systems commonly include three types of functions as follows: (i) opsonization of pathogens toward engulfment by phagocytes bearing receptors for complements (C3b, C3bi); (ii) recruitment of inflammatory cells (C3a, C5a); (iii) direct killing of pathogens by creating pores on the surface membrane (C5b, C6, C7, C8, C9). Among these pathways and functions, opsonization in the alternative pathway is exclusively important in innate immunity. Utilizing this physiological reaction, zymosan (*Saccharomyces cerevisiae*) is bound to C3bi effectively in *in vitro* treatment. C3b is spontaneously produced and destroyed, and when non-self microbes such as zymosan are exposed to the serum at 37°C, C3b connects and binds to zymosan covalently and becomes C3-convertase by the action of cofactor proteins. Cleavage of C3 to C3b and finally to C3bi is amplified, and subsequently zymosan is surrounded by C3bi. Such treated zymosan particles are recognized effectively by phagocytes via CR3.

We examined the role of Syk in *in vitro* complement-mediated phagocytosis assay using macrophage-like differentiated human leukaemic HL60 cells (*42*). After the pretreatment with C3bi-zymosan, Syk was tyrosine-phosphorylated and accumulated at the region of forming phagosomes. Next, we isolated Syk-mutant cell clones, Syk-shRNA/HL (stably expressing Syk-shRNA) and rescue-Syk/Syk-shRNA/HL (expressing recovered amount of wild-type Syk on Syk-shRNA/HL), and found that parental macrophage-like HL60 cells incorporate C3bi-zymosan promptly within 30 min but this phagocytosis is suppressed in Syk-shRNA/HL and transfer of rescue-Syk restores the phagocytic activity. Our data indicate that Syk plays an important role in complement-mediated phagocytosis. From the experiment that discriminates fluorescent zymosan particles between inside and outside the cell, Syk was found to be required for

engulfment of C3bi-zymosan but not for attachment of zymosan to CR3. Finally, we examined the molecular mechanism which signalling works at the downstream of Syk to proceed phagocytosis and indicated that Vav-RhoA signalling generates contractile force leading to engulfment of the particles in Syk dependent, complement (C3bi-CR3)-mediated phagocytosis.

Non-opsonin-mediated Phagocytic Receptors—Recently, some reports showed that Syk is also important in Dectin-1-mediated phagocytosis (*43*). Dectin-1 is an NK-cell receptor-like C-type lectin and thought to be involved in innate immune responses to fungal pathogens (*44*). Dectin-1 has the cytoplasmic ITAM-like motif which resembles sequences found in other activation molecules such as DAP12 and FcγR. Classical ITAMs consist of a tandem repeat of YXXI/L sequences which become tyrosine-phosphorylated by Src kinases. This leads to docking and activation of the tandem SH2-domain-containing Syk, and the initiation of downstream signalling events such as phagocytosis or cellular activation. Although, the sequence of the first amino-terminal membrane-distal repeat is different from the classical ITAM (this alignment of Dectin-1 contains one more X in the repeat; that is, YXXXI for mouse; YXXXL for human). In fact, this repeat is unnecessary and only the membrane-proximal YXXL seems to be required for all of the Dectin-1 functions studied (*43*, *45*). However, unexpectedly, phosphorylation of this single ITAM sequence is sufficient to mediate an interaction with Syk through another mechanism (possibly by bridging two Dectin-1 molecules in 44 and our scheme in Fig. 2), and reveals a new signalling pathway for innate immune responses that might be used with similar single cytoplasmic repeat sequences as found in many C-type lectins (*43*, *45*). In addition, these reports also described that requirement of Syk for Dectin-1 function is cell-type specific and Syk can directly induce cellular responses such as the respiratory burst and interleukin-10 production, and that Syk contributes to phagocytosis only in dendritic cells but not in macrophages through an unidentified mechanism (*45*). Further investigation about the roles of Syk in Dectin-1signalling is needed.

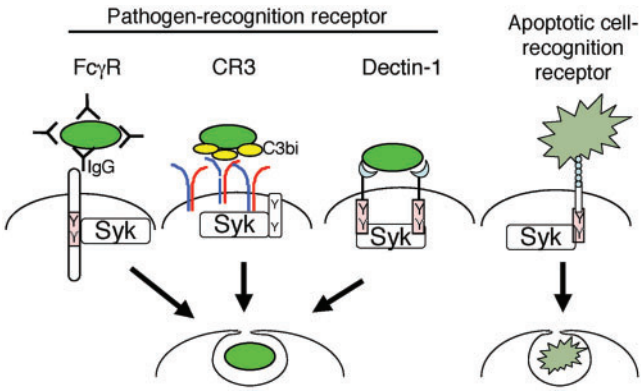

Fig. 2. **Syk plays essential roles in signalling evoked by various types of phagocytic receptors as Fcγ receptor (FcγR), complement receptor (CR3), Dectin-1 (NK-cell receptor-like C-type lectin) and apoptotic cell-recognizing receptor.** Syk transmits signals from the ITAM or ITAM-like molecules to the downstream events, which result in engulfment of target particles and phagosome formation. Pathogen such as fungus or bacterium, Apoptotic cell, Y Y ITAM-like motif.

During morphogenesis and embryogenesis, millions of cells undergo programmed cell death, and a large number of cells are generated and die during physiological or pathological processes. It is apparent that for a multicellular organism the majority of phagocytosed materials is derived from self-cells and not invading pathogens. Unlike pathogens, apoptotic cells are anti-inflammatory and production of proinflammatory cytokines from macrophages is limited (*46*).

The mechanism of phagocytosis of apoptotic cells is well conserved between species. The *Caenorhabditis elegans* engulfment receptor CED-1 (*47*) and its *Drosophila* orthologue, Draper are important components. The CED-1/Draper signalling pathway is also essential for phagocytosis of necrotic cells (*48*). Recently, Ziegenfuss *et al*. (*49*) showed that *Drosophila* Shark, a non-receptor tyrosine kinase (similar to vertebrate Syk) binds Draper through an ITAM motif in the Draper intracellular domain. They showed that Shark activity is essential for Draper-mediated signalling pathways including phagocytosis of dead neuronal cells by glial cells. They also showed that the Src family kinase Src42A increases Draper phosphorylation which is essential for phagocytic activity of glial cells. Draper-Src42A-Shark interactions probably resemble immunoreceptor-Src family kinase–Syk signalling events in mammalian immune system (*19*). These results suggest that Syk also plays a crucial role in phagocytic signalling of dead cells mediated by Src-ITAM-Syk pathway.

In summary, Syk plays an essential role in phagocytosis at the downstream of various types of receptors by transmitting the signals from the ITAM or ITAM-like molecules to the downstream events including engulfment of target particles and phagosome formation (Fig. 2).

THE ROLE OF Syk IN THE OSTEOCLAST FUNCTION

Osteoclasts are multinucleated cells generated from cell fusion among mononuclear macrophages and degrade the bone by releasing the acidic granules containing degradative enzymes into the delimited space on the bone matrix. Although the targets of osteoclasts are too large to engulf intracellularly, some signalling pathways may transmit in a similar fashion as to phagocytosis (Fig. 3). As for Syk, both its roles in osteoclast differentiation (*50–52*) and osteoclast function have been intensively studied through integrin αvβ3 activation (*35*) and M-CSF-DAP12-pathway (*36*). These studies demonstrated that the ITAMs in DAP12 or FcγR is tyrosine-phosphorylated by ligation of αvβ3 integrin or M-CSF/c-fms signalling dependent on c-Src and that DAP12 phosphorylation recruits Syk via its SH2 domain, resulting in its autophosphorylation. Phosphorylated Syk ultimately mediates the cytoskeletal organization and actin-ring formation.

In addition to these data, we have demonstrated a novel role of Syk in the process of osteolysis.

By developing a traceable and reproducible *in vitro* analysing system for osteoclast function, we found that ATP-signalling via $P2X_7$ receptor gives rise to the two essential events for osteolysis; one is the formation of sealing-zone, ring-like adhesion structures and the other is the delivery and secretion of lytic granules towards the bone matrix. We further found that deacetylation of α-tubulin is a critical reaction for these functions. Pharmacological inhibition of α-tubulin deacetylation resulted in: (i) failure of the sealing-zone like structure formation and (ii) ceased secretion of lytic granules. Additionally, kinetics of deacetylation was found to be regulated by Syk. These data suggest a novel $P2X_7$-Syk-microtubular regulation pathway for therapeutic targets in osteolytic diseases (Hazama, R. and Tohyama, Y., manuscript submitted for publication). We recently reported that Syk acts at the upstream of RhoA signalling in integrin αMβ2-dependent phagocytosis (*42*), and as for osteoclasts previous studies (*53*) showed that RhoA is involved in the control of α-tubulin acetylation (*53*). Furthermore, integrin αvβ3 activation, dependent on c-Src-ITAM-Syk is a critical step for sealing-zone formation (*35*). Together with the current data, our hypothesis is presented in Fig. 4. (i) ATP-signalling mediated by the nucleotide receptors, mainly by $P2X_7$ (the ionotropic receptor) and in part by the G-protein-coupled P2Y, induces an inside-out signalling of integrin αvβ3, and to form a sealing-zone, Syk is recruited and bound to the tyrosine-phosphorylated ITAM proteins in an SH2 domain-dependent manner; (ii) activated Syk regulates a deacetylase such as HDAC6 via RhoA activation by forming molecular complexes including integrin αvβ3, Syk, RhoA and deacetylase such as HDAC6 in adherent site; (iii) subsequent regulation of α-tubulin acetylation links the sealing-zone formation and the delivery of lytic granules. Finally, we propose the importance of ATP/$P2X_7$-Syk-microtubule deacetylation pathway as potential therapeutic targets in osteolytic diseases.

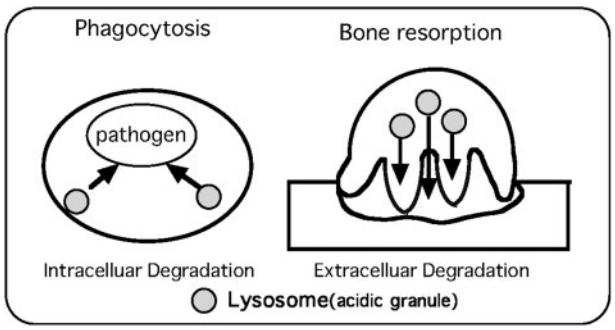

Fig. 3. **Osteoclasts are giant macrophages but the targets of osteoclasts are too large to engulf intracellularly.** Some signalling pathways may transmit in a similar fashion as phagocytosis.

CONCLUSION AND PERSPECTIVE

The roles of Syk were explored mainly in platelets and in classical immunoreceptor-signalling in adaptive immunity at the early stage of its discovery. However, Syk has now been recognized to be a key player in innate immunity as well as in adaptive immunity. A variety of upstream ITAM molecules and ITAM-like molecules have been found and are still in the course of discovery as the mediators between Syk and the upstream receptors.

On another front, considering diverse physiological effects of Syk in a variety of cells, downstream effector

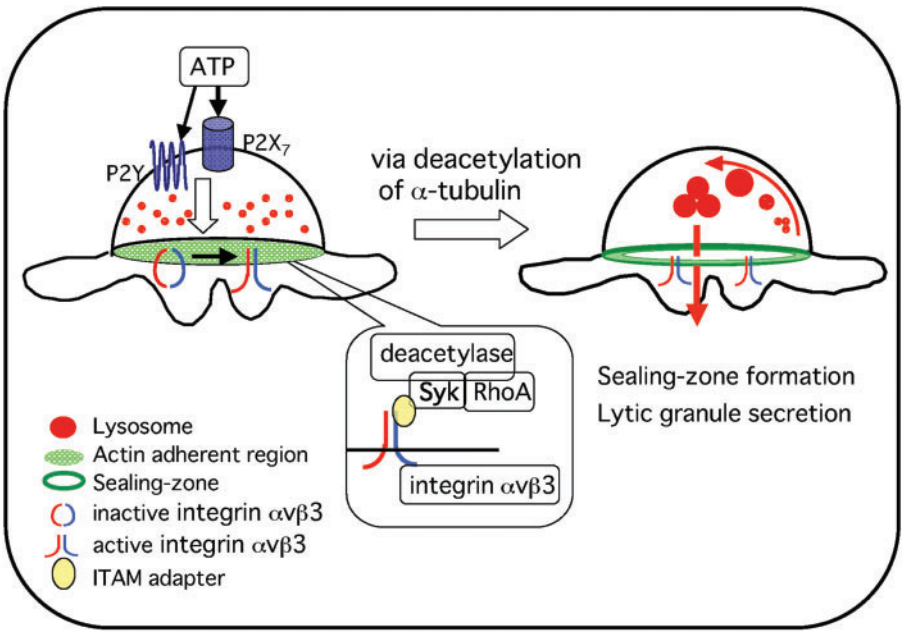

Fig. 4. **The hypothesis of osteoclast function induced by ATP-mediated signalling via integrin αvβ3-Syk-α-tubulin deacetylation.** ATP-signalling transmitted by P2X7 and P2Y receptors evokes osteoclast function. Inside-out signalling of integrin αvβ3 is induced and then Syk recruits to the activated integrin and forms a complex with a deacetylase which deacetylates α-tubulin such as HDAC6. As a result, sealing-zones are created, and lytic granules are delivered and secreted.

molecules of Syk that are reported could not be enough to explain. Cell-lineage specific molecules or regulation mechanisms will surely exist. As one candidate, we propose a novel system in which Syk controls α-tubulin acetylation and finally the ratio of intracellular transport of acidic granules along microtubules in osteoclasts (*54*, Hazama, R. and Tohyama, Y., manuscript submitted for publication). Further investigation on the function-specific effectors of Syk would give us more extended information in relation to therapeutic molecular targets for anti-inflamation, anti-osteolysis and anti-cancer therapies.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Taniguchi, T., Kobayashi, T., Kondo, J., Takahashi, K., Nakamura, H., Suzuki, J., Nagai, K., Yamada, T., Nakamura, S., and Yamamura, H. (1991) Molecular cloning of a porcine gene syk that encodes a 72-kDa protein-tyrosine kinase showing high susceptibility to proteolysis. *J. Biol. Chem.* **266**, 15790–15796
2. Kurosaki, T., Takata, M., Yamanashi, Y., Inazu, T., Taniguchi, T., Yamamoto, T., and Yamamura, H. (1994) Syk activation by the Src-family tyrosine kinase in the B cell receptor signaling. *J. Exp. Med.* **179**, 1725–1729
3. Yanagi, S., Kurosaki, T., and Yamamura, H. (1995) The structure and function of nonreceptor tyrosine kinase $p72^{SYK}$ expressed in hematopoietic cells. *Cell Signal.* **3**, 185–193
4. Cheng, A.M., Rowley, B., Pao, W., Hayday, A., Bolen, J.B., and Pawson, T. (1995) Syk tyrosine kinase required for mouse viability and B-cell development. *Nature* **378**, 303–306
5. Turner, M., Mee, P.J., Costello, P.S., Williams, O., Price, A.A., Duddy, L.P., Furlong, M.T., Geahlen, R.L., and Tybulewicz, V.L. (1995) Perinatal lethality and blocked B-cell development in mice lacking the tyrosine kinase Syk. *Nature* **378**, 298–302
6. Sada, K., Takano, T., Yanagi, S., and Yamamura, H. (2001) Structure and function of Syk protein-tyrosine kinase. *J. Biochem.* **130**, 177–186
7. Coopman, P.J.P., Do, M.T.H., Barth, M., Bowden, E.T., Hayes, A.J., Basyuk, E., Blancato, J.K., Vezza, P.R., McLeskey, S.W., Mangeat, P.H., and Mueller, S.C. (2000) The Syk tyrosine kinase suppresses malignant growth of human breast cancer cells. *Nature* **406**, 742–747
8. Yanagi, S., Inatome, R., Ding, J., Kitaguchi, H., Tybulewicz, V.L., and Yamamura, H. (2001) Syk expression in endothelial cells and their morphological defects in embryonic Syk-deficient mice. *Blood* **98**, 2869–2871
9. Law, C.L., Chandran, K.A., Sidorenko, S.P., and Clark, E.A. (1996) Phospholipase C-gamma1 interacts with conserved phosphotyrosyl residues in the linker region of Syk and is a substrate for Syk. *Mol. Cell Biol.* **16**, 1305–1315
10. Hong, J.J., Yankee, T.M., Harrison, M.L., and Geahlen, R.L. (2002) Regulation of signaling in B cells through the phosphorylation of Syk on linker region tyrosines. A mechanism for negative signaling by the Lyn tyrosine kinase. *J. Biol. Chem.* **277**, 31703–31714
11. Zhang, J., Berenstein, E., and Siraganian, R.P. (2002) Phosphorylation of Tyr342 in the linker region of Syk is critical for FcγRI signaling in mast cells. *Mol. Cell Biol.* **22**, 8144–8154
12. Simon, M., Vanes, L., Geahlen, R.L., and Tybulewicz, V.L. (2005) Distinct roles for the linker region tyrosines of Syk in FcγRI signaling in primary mast cells. *J. Biol. Chem.* **280**, 4510–4517
13. Adachi, T., Wienands, J., Tsubata, T., and Kurosaki, T. (2007) Interdomain A is crucial for ITAM-dependent and -independent regulation of Syk. *Biochem. Biophys. Res. Commun.* **364**, 111–117
14. Kurosaki, T., Johnson, S.A., Pao, L., Sada, K., Yamamura, H., and Cambier, J.C. (1995) Role of the Syk autophosphorylation site and SH2 domains in B cell antigen receptor signaling. *J. Exp. Med.* **182**, 1815–1823
15. Takata, M., Sabe, H., Hata, A., Inazu, T., Homma, Y., Nukada, T., Yamamura, H., and Kurosaki, T. (1994) Tyrosine kinases Lyn and Syk regulate B cell receptor-coupled Ca^{2+} mobilization through distinct pathways. *EMBO J.* **13**, 1341–1349
16. Kiefer, F., Brumell, J., Al-Alawi, N., Latour, S., Cheng, A., Veillette, A., Grinstein, S., and Pawson, T. (1998) The Syk protein tyrosine kinase is essential for Fcgamma receptor signaling in macrophages and neutrophils. *Mol. Cell Biol.* **18**, 4209–4220
17. Crowley, M.T., Costello, P.S., Fitzer-Attas, C.J., Turner, M., Meng, F., Lowell, C.A., Tybulewicz, V.L., and DeFranco, A.L. (1997) A critical role for Syk in signal transduction and phagocytosis mediated by Fcgamma receptors on macrophages. *J. Exp. Med.* **186**, 1027–1039
18. Lanier, L.L. (2005) NK cell recognition. *Annu. Rev. Immunol.* **23**, 225–274
19. Underhill, D.M. and Goodridge, H.S. (2007) The many faces of ITAMs. *Trends Immunol.* **28**, 66–73
20. Koretzky, G.A., Abtahian, F., and Silverman, M.A. (2006) SLP76 and SLP65: complex regulation of signalling in lymphocytes and beyond. *Nat. Rev. Immunol.* **6**, 67–78
21. Schymeinsky, J., Sindrilaru, A., Frommhold, D., Sperandio, M., Gerstl, R., Then, C., Mócsai, A., Scharffetter-Kochanek, K., and Walzog, B. (2006) The Vav binding site of the non-receptor tyrosine kinase Syk at Tyr 348 is critical for beta2 integrin (CD11/CD18)-mediated neutrophil migration. *Blood* **108**, 3919–3927
22. Johmura, S., Oh-hora, M., Inabe, K., Nishikawa, Y., Hayashi, K., Vigorito, E., Kitamura, D., Turner, M., Shingu, K., Hikida, M., and Kurosaki, T. (2003) Regulation of Vav localization in membrane rafts by adapter molecules Grb2 and BLNK. *Immunity* **18**, 777–787
23. Mocsai, A., Zhou, M., Meng, F., Tybulewicz, V.L., and Lowell, C.A. (2002) Syk is required for integrin signaling in neutrophils. *Immunity* **16**, 547–558
24. Kasirer-Friede, A., Kahn, M.L., and Shattil, S.J. (2007) Platelet integrins and immunoreceptors. *Immunol. Rev.* **218**, 247–264
25. Jakus, Z., Fodor, S., Abram, C.L., Lowell, C.A., and Mócsai, A. (2007) Immunoreceptor-like signaling by beta 2 and beta 3 integrins. *Trends Cell Biol.* **17**, 493–501
26. Clare, L., Abram, C.L., and Lowell, C.A. (2007) Convergence of immunoreceptor and integrin signaling. *Immunol. Rev.* **218**, 29–44
27. Clark, E.A., Shattil, S.J., Ginsberg, M.H., Bolen, J., and Brugge, J.S. (1994) Regulation of the protein tyrosine kinase pp72syk by platelet agonists and the integrin alpha IIb beta 3. *J. Biol. Chem.* **269**, 28859–28864
28. Tohyama, Y., Tohyama, K., Tsubokawa, M., Asahi, M., Yoshida, Y., and Yamamura, H. (1998) Outside-In signaling of soluble and solid-phase fibrinogen through integrin αIIbβ3 is different and cooperative with each other in a megakaryoblastic leukemia cell line, CMK. *Blood* **92**, 1277–1286
29. Gao, J., Zoller, K.E., Ginsberg, M.H., Brugge, J.S., and Shattil, S.J. (1997) Regulation of the pp72syk protein tyrosine kinase by platelet integrin αIIbβ3. *EMBO J.* **16**, 6414–6425

30. Obergfell, A., Eto, K., Mocsai, A., Buensuceso, C., Moores, S.L., Brugge, J.S., Lowell, C.A., and Shattil, S.J. (2002) Coordinate interactions of Csk, Src, and Syk kinases with αIIbβ3 initiate integrin signaling to the cytoskeleton. *J. Cell Biol.* **157**, 265–275
31. Abtahian, F., Bezman, N., Clemens, R., Sebzda, E., Cheng, L., Shattil, S.J., Kahn, M.L., and Koretzky, G.A. (2006) Evidence for the requirement of ITAM domains but not SLP-76/Gads interaction for integrin signaling in hematopoietic cells. *Mol. Cell Biol.* **26**, 6936–6949
32. Yan, S.R., Huang, M., and Berton, G. (1997) Signaling by adhesion in human neutrophils: activation of the p72syk tyrosine kinase and formation of protein complexes containing p72syk and Src family kinases in neutrophils spreading over fibrinogen. *J. Immunol.* **158**, 1902–1910
33. Miura, Y., Tohyama, Y., Hishita, T., Lala, A., De Nardin, E., Yoshida, Y., Yamamura, H., Uchiyama, T., and Tohyama, K. (2000) Pyk2 and Syk participate in functional activation of granulocytic HL-60 cells in a different manner. *Blood* **96**, 1733–1739
34. Lowell, C.A., Fumagalli, L., and Berton, G. (1996) Deficiency of Src family kinases p59/61hck and p58c-fgr results in defective adhesion-dependent neutrophil functions. *J. Cell Biol.* **133**, 895–910
35. Zou, W., Kitaura, H., Reeve, J., Long, F., Tybulewicz, V.L., Shattil, S.J., Ginsberg, M.H., Ross, F.P., and Teitelbaum, S.L. (2007) Syk, c-Src, the αvβ3 integrin, and ITAM immunoreceptors, in concert, regulate osteoclastic bone resorption. *J. Cell Biol.* **176**, 877–888
36. Zou, W., Reeve, J.L., Liu, Y., Teitelbaum, S.L., and Ross, F.P. (2008) DAP12 couples c-Fms activation to the osteoclast cytoskeleton by recruitment of Syk. *Mol. Cell* **31**, 422–431
37. Matsusaka, M., Tohyama, Y., He, J., Shi, Y., Hazama, R., Kadono, T., Kurihara, R., Tohyama, K., and Yamamura, H. (2005) Protein-tyrosine kinase, Syk is required for CXCL12-induced polarization of B cells. *Biochem. Biophys. Res. Commun.* **328**, 1163–1169
38. Mocsai, A., Abram, C.L., Jakus, Z., Hu, Y., Lanier, L.L., and Lowell, C.A. (2006) Integrin signaling in neutrophilsand macrophages uses adapters containing immunoreceptor tyrosine-based activation motifs. *Nat. Immunol.* **7**, 1326–1333
39. Boylan, B., Gao, C., Rathore, V., Gill, J.C., Newman, D.K., and Newman, P.J. (2008) Identification of FcgammaRIIa as the ITAM-bearing receptor mediating alphaIIbbeta3 outside-in integrin signaling in human platelets. *Blood* **112**, 2780–2786
40. Zarbock, A., Abram, C.L., Hundt, M., Altman, A., Lowell, C.A., and Ley, K. (2008) PSGL-1 engagement by E-selectin signals through Src kinase Fgr and ITAM adapters DAP12 and FcR{gamma} to induce slow leukocyte rolling. *J. Exp. Med.* **205**, 2339–2347
41. Tohyama, Y. and Yamamura, H. (2006) Complement-mediated phagocytosis—the role of Syk. *IUBMB Life* **58**, 304–308
42. Shi, Y., Tohyama, Y., Kadono, T., He, J., Miah, S.M., Hazama, R., Tanaka, C., Tohyama, K., and Yamamura, H. (2006) Protein-tyrosine kinase, Syk is required for pathogen engulfment in complement-mediated phagocytosis. *Blood* **107**, 4554–4562
43. Rogers, N.C., Slack, E.C., Edwards, A.D., Nolte, M.A., Schulz, O., Schweighoffer, E., Williams, D.L., Gordon, S., Tybulewicz, V.L., Brown, G.D., and Reis e Sousa, C. (2005) Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. *Immunity* **22**, 507–517
44. Brown, G.D. (2006) Dectin-1: a signalling non-TLR pattern-recognition receptor. *Nat. Rev. Immunol.* **6**, 33–43
45. Underhill, D.M., Rossnagle, E., Lowell, C.A., and Simmons, R.M. (2005) Dectin-1 activates SYK tyrosine kinase in a dynamic subset of macrophages for reactive oxygen production. *Blood* **106**, 2543–2550
46. Fadok, V.A., Bratton, D.L., Konowal, A., Freed, P.W., Westcott, J.Y., and Henson, P.M. (1998) Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J. Clin. Invest.* **101**, 890–898
47. Zhou, Z., Hartwieg, E., and Horvitz, H.R. (2001) CED-1 is a transmembrane receptor that mediates cell corpse engulfment in C. elegans. *Cell* **104**, 43–56
48. Chung, S., Gumienny, T.L., Hengartner, M.O., and Driscoll, M. (2000) A common set of engulfment genes mediates removal of both apoptotic and necrotic cell corpses in C. elegans. *Nat. Cell Biol.* **2**, 931–937
49. Ziegenfuss, J.S., Biswas, R., Avery, M.A., Hong, K., Sheehan, A.E., Yeung, Y.G., Stanley, E.R., and Freeman, M.R. (2008) Draper-dependent glial phagocytic activity is mediated by Src and Syk family kinase signalling. *Nature* **453**, 935–939
50. Mócsai, A., Humphrey, M.B., Van Ziffle, J.A., Hu, Y., Burghardt, A., Spusta, S.C., Majumdar, S., Lanier, L.L., Lowell, C.A., and Nakamura, M.C. (2004) The immunomodulatory adapter proteins DAP12 and Fc receptor gamma-chain (FcRgamma) regulate development of functional osteoclasts through the Syk tyrosine kinase. *Proc. Natl Acad. Sci. USA* **101**, 6158–6163
51. Faccio, R., Teitelbaum, S.L., Fujikawa, K., Chappel, J., Zallone, A., Tybulewicz, V. L., Ross, F.P., and Swat, W. (2005) Vav3 regulates osteoclast function and bone mass. *Nat. Med.* **11**, 284–290
52. Shinohara, M., Koga, T., Okamoto, K., Sakaguchi, S., Arai, K., Yasuda, H., Takai, T., Kodama, T., Morio, T., Geha, R. S., Kitamura, D., Kurosaki, T., Ellmeier, W., and Takayanagi, H. (2008) Tyrosine kinases Btk and Tec regulate osteoclast differentiation by linking RANK and ITAM signals. *Cell* **132**, 794–806
53. Destaing, O., Saltel, F., Gilquin, B., Chabadel, A., Khochbin, S., Ory, S., and Jurdic, P. (2005) A novel Rho-mDia2-HDAC6 pathway controls podosome patterning through microtubule acetylation in osteoclasts. *J. Cell Sci.* **118**, 2901–2911
54. He, J., Tohyama, Y., Yamamoto, K., Kobayashi, M., Shi, Y., Takano, T., Noda, C., Tohyama, K., and Yamamura, H. (2005) Lysosome is a primary organelle in B cell receptor-mediated apoptosis: an indispensable role of Syk in lysosomal function. *Genes Cells* **10**, 23–35