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Forum Review

Role of Protein-Tyrosine Kinase Syk in Oxidative Stress Signaling in B Cells

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ABSTRACT

Oxidative stress induces the activation of multiple signaling pathways related to various cellular responses. In B cells, Syk has a crucial role in intracellular signal transduction induced by oxidative stress as well as antigen receptor engagement. Treatment of B cells with hydrogen peroxide (H_2O_2) induces enzymatic activation of Syk. Syk is essential for Ca^{2+} release from intracellular pools through phospholipase C-γ2 and the activation of c-Jun N-terminal kinase, p38 mitogen-activated protein kinase, and phosphatidylinositol 3-kinase–Akt survival pathway following H_2O_2 stimulation. Oxidative stress-induced cellular responses in B cells follow different patterns, such as necrosis, apoptosis, and mitotic arrest, according to the intensity of H_2O_2 stimulation. Syk is involved in the protection of cells from apoptosis and induction of G2/M arrest. Syk leads to the activation of the phosphatidylinositol 3-kinase–Akt survival pathway, thereby enhancing cellular resistance to oxidative stress-induced apoptosis. On the other hand, Syk-dependent phospholipase C-γ2 activation is required for acceleration toward apoptosis following oxidative stress. These findings suggest that oxidative stress-induced Syk activation triggers the activation of several pathways, such as proapoptotic and survival pathways, and the balance among these various pathways is a key factor in determining the fate of a cell exposed to oxidative stress. *Antioxid. Redox Signal.* 4, 533–541.

INTRODUCTION

REACTIVE OXYGEN SPECIES (ROS), such as the superoxide radical, the hydroxyl radical, and hydrogen peroxide (H_2O_2), are continuously being generated *in vivo* in normal metabolic processes (18). At physiological concentrations, ROS may function as mediators of cellular responses, including activation in lymphocytes and proliferation in fibroblasts, and mimic effects of insulin on adipocytes (4, 13, 39, 41, 48, 75). However, oxidative stress occurs when the concentrations of ROS are increased above the physiological levels. Oxidative stress is involved in the pathological development of various diseases, such as diabetes mellitus, atherosclerosis, and neurological disorders (18, 19). The pathology associated with ROS is derived from their ability to modify cellular components such as proteins, lipids, and nucleic acids, to disrupt cellular function, and to cause cellular death (19). The generation of ROS has been reported for a variety of cells stimulated with cytokines (38, 42, 47, 67), growth factors (1, 29, 60), agonists of heptahelical receptors (6, 16), or phorbol esters (53). Intracellular ROS can act as second messengers in the transduction of signals that are involved in many cellular functions, such as oxidant-induced stress, apoptosis, and proliferation.

H_2O_2 is a small, diffusible, and ubiquitous molecule that can be synthesized as well as destroyed rapidly in response to external stimuli. The addition of exogenous H_2O_2 to cells has been used extensively to investigate oxidative stress-induced cellular signaling pathways. H_2O_2 has been shown to stimulate multiple signaling pathways related to cell activation, proliferation, and apoptosis (4, 39, 45, 66). One of the earliest events in response to H_2O_2 stimulus is the activation of protein-tyrosine kinases (PTKs). This is followed by Ca^{2+} mobilization, the activation of mitogen-activated protein kinases (MAP kinases), and transcription factors (26, 44, 45, 56–58, 61).

Syk belongs to Syk/ZAP-70 family of PTKs and contains two Src homology 2 (SH2) domains, but no myristoylation

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site, and is distributed in cytosol (55, 65, 78). Syk is expressed in a wide range of hematopoietic and non-hematopoietic cells, such as epithelial cells, fibroblasts, hepatocytes, neuronal cells, and breast tissue (33, 68, 69, 72, 77, 79). It has been clear that Syk plays a crucial role in B-cell antigen receptor (BCR) and Fc receptor-mediated signaling (55, 78). Recent findings reveal that expression of Syk in nonhematopoietic cells may be involved in a wide variety of cellular functions and pathogenesis of malignant tumors (33, 68, 69, 72, 77, 79). We have demonstrated that Syk also plays an important role in H_2O_2-induced signal transduction in B cells.

Here, we describe the role of Syk in oxidative stress signaling in B cells stimulated with H_2O_2. B cells are relatively sensitive to H_2O_2 stimulation (15, 34, 35), and high concentrations of H_2O_2 (1 m*M*) induce cell necrosis, whereas cell death induced by low concentrations of H_2O_2 (10–100 μM) seems to reflect cellular apoptosis in B cells (11). Treatment of B cells with both high and low concentrations of H_2O_2 can induce Syk activation. However, the responses of signaling pathways following Syk activation are different, depending upon the intensity of H_2O_2 stimulation. To examine the role of Syk in H_2O_2-mediated cellular signaling, we have performed biochemical and genetic studies by using Syk-deficient DT40 chicken B cells in which *syk* gene is disrupted by gene targeting (62). We report H_2O_2-mediated Syk activation and downstream signal transduction such as Ca^{2+} mobilization and the activation of MAP kinases and Akt. Furthermore, we discuss the significance of our findings in light of the roles of Syk in oxidative stress-induced cellular responses.

SYK ACTIVATION

Oxidative stress induces the activation of Syk

It has been shown that oxidative stress induces the activation of Src- and Syk/ZAP-70-family PTKs in T and B lymphocytes identical to that observed following antigen receptor engagement (56–58). Syk plays an essential role in BCR- and Fc receptor-mediated signaling and regulates the biological outcomes of function and development in immune cells (7, 70). In B cells exposed to H_2O_2, Syk is rapidly activated in a dose-dependent manner (49, 50). One of the earliest events upon H_2O_2 stimulation is tyrosine phosphorylation of cellular proteins. In Syk-deficient B cells, tyrosine phosphorylation of cellular proteins was completely abolished (10). Syk has a central role in the increased tyrosine phosphorylation of cellular proteins although it remains unknown whether Syk phosphorylates all the downstream molecules directly or via other PTKs subsequently activated by Syk after H_2O_2 stimulation.

Syk activation is dependent on Lyn

BCR stimulation leads to the phosphorylation of immunoreceptor tyrosine-based activating motif (ITAM) in Igα and Igβ by Src-family PTK, Lyn. Subsequent association of Syk SH2 domains with phosphorylated ITAM results in the activation of Syk through both autophosphorylation and phosphorylation by Lyn (32, 62). In B cells exposed to H_2O_2, Syk activation is also dependent on Lyn. Lyn-deficient cells largely abrogated tyrosine phosphorylation of Syk and other cellular proteins after H_2O_2 stimulation (49). However, the N-terminal SH2 domain mutant of Syk [mSH2(N)Syk] demonstrates a stronger tyrosine phosphorylation than wild-type Syk, and the C-terminal SH2 domain mutant of Syk [mSH2(C)Syk] shows the same degree of tyrosine phosphorylation as wild-type Syk following H_2O_2 stimulation (10, 51). mSH2(N)Syk and mSH2(C)Syk contain point mutation in each of the phosphotyrosine-dependent binding motifs of SH2 domains and can hardly bind to phosphorylated Igα and Igβ in BCR (32). These findings suggest that recruitment of Syk to BCR is not necessary for Syk activation after H_2O_2 stimulation. Recently, it has been reported that the rafts can be the direct targets of oxidative stress (46). Rafts are sphingolipid- and cholesterol-rich membrane microdomains and contain a variety of signaling molecules, such as glycosylphosphatidylinositol-linked proteins, Src-family PTKs, and growth factor receptors (2). Therefore, the suspected mechanism of Lyn-dependent Syk activation seems as follows: Oxidative stress triggers crosslinking of cell-surface proteins on cysteine SH groups in rafts, inducing the clustering and subsequent activation of raft-associated Lyn (46) that results in Syk activation. However, the molecular mechanism of Lyn-dependent Syk activation remains unknown.

SIGNAL TRANSDUCTION FOLLOWING SYK ACTIVATION

Syk is required for Ca^{2+} release from intracellular pools

It has been shown that oxidative stress induces a rapid increase in cytoplasmic free Ca^{2+} ($[Ca^{2+}]_i$). B cells exposed to H_2O_2 stimulation also exhibit a rapid increase in $[Ca^{2+}]_i$ (Fig. 1A; 30, 57). The presence of EGTA, a chelator for extracellular Ca^{2+}, partially inhibits H_2O_2-induced Ca^{2+} mobilization (49, 50). Thus, oxidative stress-induced Ca^{2+} responses seem to be due to both an extracellular Ca^{2+} influx and a Ca^{2+} release from intracellular pools. Compared with the wild-type cells, Syk-deficient cells show a slow and small increase in $[Ca^{2+}]_i$ after H_2O_2 treatment (Fig. 1A). Interestingly, the increase in $[Ca^{2+}]_i$ in Syk-deficient cells after H_2O_2 stimulation is largely abrogated under the complete absence of extracellular Ca^{2+} (49, 50).

Ca^{2+} release from intracellular pools is achieved through the activation of phospholipase C (PLC) followed by the generation of inositol 1,4,5-trisphosphate (IP_3). Although the sources of released Ca^{2+} remain controversial (30), Syk-dependent Ca^{2+} release seems to be derived from IP_3-sensitive stores, because Ca^{2+} mobilization upon H_2O_2 stimulation in Syk-deficient cells is essentially similar to that in PLC-γ2-deficient cells (Fig. 1A). In Syk-deficient cells, tyrosine phosphorylation of PLC-γ2 and IP_3 production were completely abolished following H_2O_2 stimulation (Fig. 1B, 10, 20). Moreover, BLNK (B-cell linker protein), one of the adaptor proteins in B cells (14), is required for coupling Syk to PLC-γ2 activation following H_2O_2 stimulation as well as BCR engagement, because H_2O_2-induced Ca^{2+} mobilization in BLNK-deficient cells is identical to that observed in Syk-

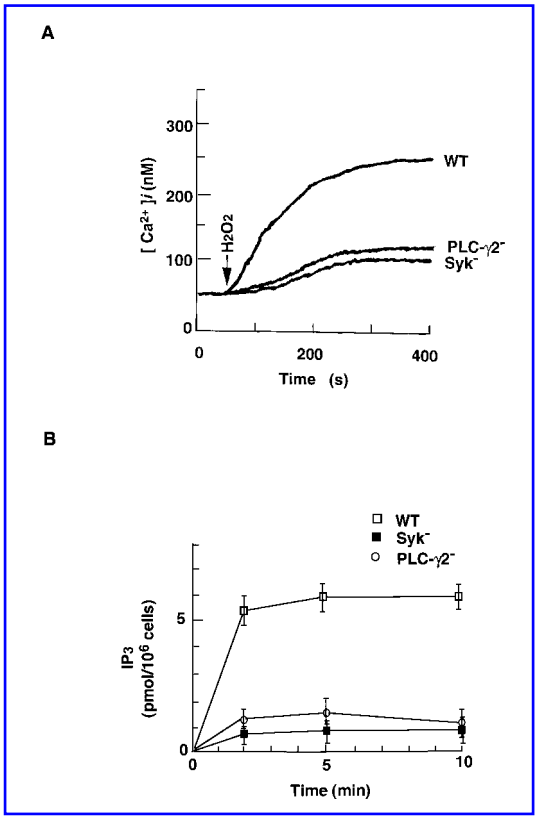

FIG. 1. Ca^{2+} mobilization following oxidative stress. (**A**) Ca^{2+} mobilization analysis. Intracellular free Ca^{2+} levels in Fura-2-loaded wild-type (WT), Syk-deficient (Syk$^-$), and PLC-γ2-deficient (PLC-γ2$^-$) B cells were monitored by a spectrophotometer after stimulation with 100 μ*M* H_2O_2 (20). (**B**) IP_3 generation. B cells were stimulated with 100 μ*M* H_2O_2 for the indicated times, and IP_3 production was measured (20).

deficient and PLC-γ2-deficient cells (20, 25). The following mechanism for the induction of Ca^{2+} release is suggested: H_2O_2 activates Syk, which in turn phosphorylates BLNK and induces an increased association of BLNK with cell membrane. BLNK in combination with Syk draws PLC-γ2 from cytosol to plasma membrane, and Syk subsequently phosphorylates PLC-γ2. PLC-γ2 catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to diacylglycerol and IP_3, which induce activation of protein kinase C (PKC) and Ca^{2+} release from intracellular pools via IP_3 receptors, respectively (20). Thus, Syk activation induces Ca^{2+} release from intracellular pools through tyrosine phosphorylation of PLC-γ2 following oxidative stress.

Qin *et al.* have recently shown that activation of phosphatidylinositol 3-kinase (PI 3-kinase) regulates H_2O_2-induced IP_3 production through Bruton's tyrosine kinase (Btk) activation (52). We have demonstrated that Syk is required for PI 3-kinase activation following H_2O_2 stimulation (11). Therefore, there may exist an alternative pathway leading to H_2O_2-induced PLC-γ2 activation through PI 3-kinase and Btk.

Syk is required for activation of JNK and p38

It is now clear that oxidative stress triggers certain intracellular pathways that lead to the activation of various transcription factors and expression of specific genes (40). Signal transduction via MAP kinases is involved in this process. MAP kinases include the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAP kinase (p38). In general, ERKs are normally associated with growth factors to induce proliferation, whereas JNK and p38 are stimulated by stress stimuli and cytokines to mediate cell apoptosis and differentiation (23, 37, 54, 71, 74).

We have demonstrated that H_2O_2 stimulation induces activation of ERK, JNK, and p38 in chicken B cells (Fig. 2; 20, 22, 50). Interestingly, the patterns of activation are different according to the intensity of H_2O_2 stimulation. The activation of ERK is induced in B cells treated with either low (10–100 μ*M*) or high (1 m*M*) concentrations of H_2O_2 and the activation in Syk-deficient cells is identical to that in wild-type cells (Fig. 2A, 22, 50), suggesting that Syk is not required for ERK activation following H_2O_2 stimulation. JNK activation in B cells is observed only following treatment with the highest concentrations of H_2O_2 and is abolished in Syk-deficient cells for all H_2O_2 levels (Fig. 2B; 20). On the other hand, p38 activation is reduced in Syk-deficient cells treated with low concentrations of H_2O_2 (Fig. 2C; 22). Thus, Syk is essential for the activation of JNK and p38, depending upon the intensity of oxidative stress. Although tyrosine phosphorylation of Syk is detectable in B cells treated with 100 μ*M* H_2O_2 (11), Syk activation is associated with distinct pathways for MAP kinase activation according to the intensity of H_2O_2 stimula-

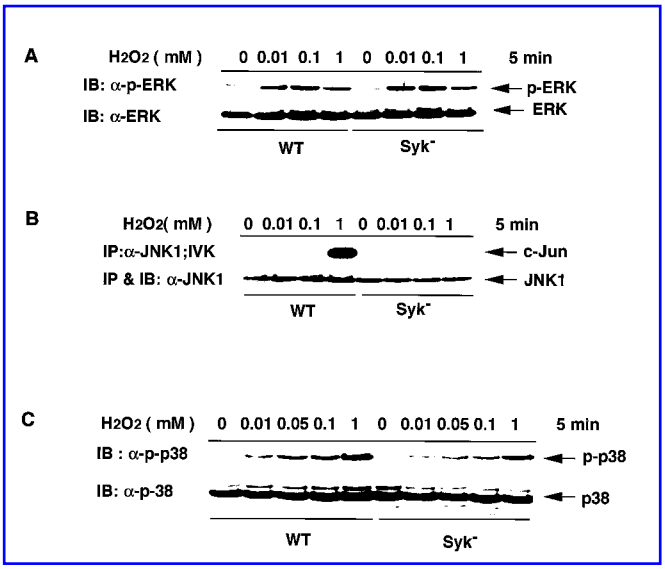

FIG. 2. Oxidative stress-induced MAP kinase phosphorylation. (**A**) ERK phosphorylation. After wild-type (WT) and Syk-deficient (Syk$^-$) B cells were stimulated with the indicated concentrations of H_2O_2 for 5 min, cell lysates were subjected to immunoblot analysis using anti-phospho-ERK or anti-ERK antibodies (22). (**B**) JNK activation. JNK activity in the immunoprecipitates was determined using a specific *in vitro* kinase assay (IVK) (20). (**C**) p38 phosphorylation. Lysates of stimulated B cells were subjected to immunoblot analysis using anti-phospho-p38 or anti-p38 antibodies (22).

tion. It remains unknown how Syk mediates selective activation of MAP kinases after oxidative stress.

It has been reported that PLC-γ2-dependent signals (PKC activation or Ca^{2+} mobilization) and GTP-binding proteins (Ras or Rac1) are required for BCR-induced MAP kinase responses (21). In oxidative stress signaling, it has been reported that Ras, which is an upstream activator of ERK, is a more general target for ROS and senses cellular redox status (33). ERK activation following H_2O_2 stimulation may be associated with the Raf-MEK (MAPK/ERK kinase) pathway via Ras, which is directly activated by H_2O_2 but not with the response of PLC-γ2-mediated pathway because ERK activation is not dependent on Syk after H_2O_2 stimulation. On the other hand, JNK and p38 activation are abrogated in PLC-γ2-deficient as well as Syk-deficient cells treated with H_2O_2 (20, 22). These findings indicate that upon oxidative stress Syk induces JNK and p38 responses through PLC-γ2-mediated pathways. Moreover, the PLC-γ2 dependence of p38 activation is most likely due to PKC activation rather than Ca^{2+} mobilization. An absence of intracellular and extracellular Ca^{2+} using chelators dooes not affect p38 activation after H_2O_2 stimulation, and phorbol 12-myristate 13-acetate, a PKC-specific activator, induces p38 activation (22). On the other hand, it has been reported that an increase in $[Ca^{2+}]_i$ is required for JNK activation following oxidative stress (24). Involvement of Rac1 in H_2O_2-induced JNK and p38 activation remains unknown.

Syk is required for activation of PI 3-kinase–Akt survival pathway

Oxidative stress can trigger the activation of those signaling pathways that are involved in cell survival, including the phosphorylation cascades leading to the activation of ERK, nuclear factor-κB (NF-κB), and serine-threonine kinase Akt (17, 28, 43). Treatment of B cells with H_2O_2 induces the activation of Akt in a PI 3-kinase-dependent manner (11). Activation of Akt and PI 3-kinase was considerably reduced in Syk-deficient cells treated with low concentrations of H_2O_2 (20–100 μ*M*), but not in Syk-deficient cells treated with high concentrations of H_2O_2 (1 m*M*) (Fig. 3A; 11, 20). Thus, Syk is required for the activation of Akt via PI 3-kinase. It has been reported that activation of Akt is necessary for the prevention of apoptosis, which occurs by phosphorylation of the Bcl-x inhibitor BAD or caspase-9 and by regulation of signaling via transcription factors such as NF-κB (5, 9, 27). Interestingly, during stimulation with H_2O_2, caspase-9 activity in Syk-deficient cells is increased greatly compared with that of wild-type cells (Fig. 3B; 11). These findings have suggested that Akt, which is activated in a Syk-dependent manner following oxidative stress, phosphorylates caspase-9 and inhibits its activity.

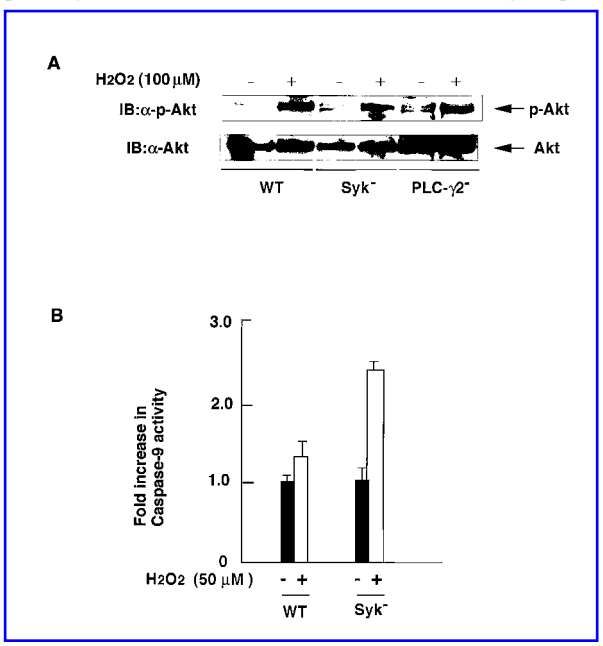

FIG. 3. Activation of Akt survival pathway following oxidative stress. (**A**) Akt activation. Wild-type (WT), Syk-deficient (Syk⁻), and PLC-γ2-deficient (PLC-γ2⁻) B cells were treated with 100 μ*M* H_2O_2 for 10 min, and cell lysates were subjected to immunoblot analysis using anti-phospho-Akt or anti-Akt antibodies (20). (**B**) Caspase-9 activity. B cells were treated with 50 μ*M* H_2O_2 for 12 h at 37°C, and cell lysates were assayed for caspase-9 activity (11).

OXIDATIVE STRESS-INDUCED CELLULAR RESPONSES IN B CELLS

Distinct patterns of cellular response according to the intensity of H_2O_2 stimulation

Different cell lines exhibit different susceptibilities to oxidative stress, and lymphocytes appear to be more sensitive to oxidative stress than other cells (15, 34, 35). It has been reported that exogenously added H_2O_2 can stimulate growth responses in a variety of cultured mammalian cell types, such as fibroblasts (4). However, in T cells mitogenic stimuli induce H_2O_2 generation, which interferes with cell proliferation (75). The exposure of T cells to H_2O_2 is shown to result in the suppression of mitogen-induced lymphocyte proliferation and interleukin-2 expression (39, 48). Also, we have not observed any enhancement of proliferation in B cells exposed to H_2O_2. Thus, lymphocytes seem to exhibit no stimulation of growth response after oxidative stress.

We have demonstrated that distinct patterns of H_2O_2-induced cellular response occur according to the magnitude of H_2O_2 stimulation in B cells (11, 22). The high concentrations of H_2O_2 (1 m*M*) induce a necrotic form of cell death characterized by smeared patterns of DNA digestion and morphological evidence of primary cytoplasm and plasma membrane damage. In contrast, stimulation with 20–100 μ*M* H_2O_2 mainly induces apoptosis characterized by DNA ladder formation (11). In addition, B cells treated with 10–20 μ*M* H_2O_2 are observed to exhibit suppression of cell growth, and flow cytometric analysis with propidium iodide staining reveals inhibition of cell division in M phase rather than the induction of apoptosis (22). The mechanistic basis for the exertion of a given cellular response following oxidative stress remains unknown.

Syk is involved in the protection of cells from apoptosis and induction of G2/M arrest

We have examined the role of Syk in oxidative stress-induced cellular responses. Treatment with millimolar concentrations of H_2O_2 rapidly leads to cellular necrosis in Syk-

deficient as well as wild-type cells. On the other hand, the viability of Syk-deficient cells after exposure to 20–100 μM H_2O_2 declines dramatically compared with that of wild-type cells (11, 20), and a deficiency of Syk produces a significantly enhanced, dose-dependent apoptotic response as assessed by DNA ladder formation (Fig. 4B; 11, 20). Moreover, when cell-cycle profiles were determined by flow cytometric analyses, significantly more G2/M-phase cells than G1-phase cells were present in wild-type population treated with 10–20 μM H_2O_2. Surprisingly, Syk-deficient cells exhibit no induction of G2/M delay after H_2O_2 stimulation (22). These results indicate that in B cells exposed to low concentrations of H_2O_2, Syk plays important roles in the protection of cells from apoptosis and induction of G2/M arrest.

Syk triggers the activation of both pro-apoptotic and survival pathways

One prominent feature of the role of H_2O_2 in cell apoptosis is to activate mitochondrial permeability transition pore and release the mitochondrial protein cytochrome *c* into the cytoplasm (59). In the cytosol, cytochrome *c* in combination with Apaf-1 binds to procaspase-9, resulting in the proteolytic activation of caspase-9. Active caspase-9 then directly activates caspase-3, which leads to the activation of caspase cascade and the proteolytic activation of pivotal proteins for apoptosis, such as caspase-activated DNase (12, 36). Although the viability of Syk-deficient cells following H_2O_2 stimulation is relatively lower than that of wild-type cells, H_2O_2-induced cytochrome *c* release in Syk-deficient cells is not different from that in wild-type cells. Syk is not involved in initiating a cascade of caspase activation following oxidative stress.

As described above, Syk is essential for the activation of the PI 3-kinase–Akt survival pathway following oxidative stress. Syk inhibits the activation of caspase-9 through Akt after oxidative stress, thereby protecting cells from oxidative stress-induced apoptosis. On the other hand, although PLC-γ2 is one of the important molecules downstream of Syk, PLC-γ2-deficient cells treated with 20–100 μM H_2O_2 show a higher percentage of cell viability than wild-type cells (Fig. 4A) and exhibit a reduction in apoptotic response as evidenced by DNA fragmentation (Fig. 4B). It is believed that PLC-γ2 plays the role of an accelerator for apoptosis following oxidative stress as well as BCR engagement (20, 63). In PLC-γ2-deficient cells, an increase in $[Ca^{2+}]_i$ is attenuated to an extent equal to that in Syk-deficient cells, whereas ~60% of Akt phosphorylation remains following H_2O_2 stimulation (Fig. 3A). These findings have led to the suggestion that oxidative stress-induced Syk activation triggers the responses of both proapoptotic and survival pathways, and the balance among these various pathways is a key factor in determining the fate of a cell exposed to oxidative stress.

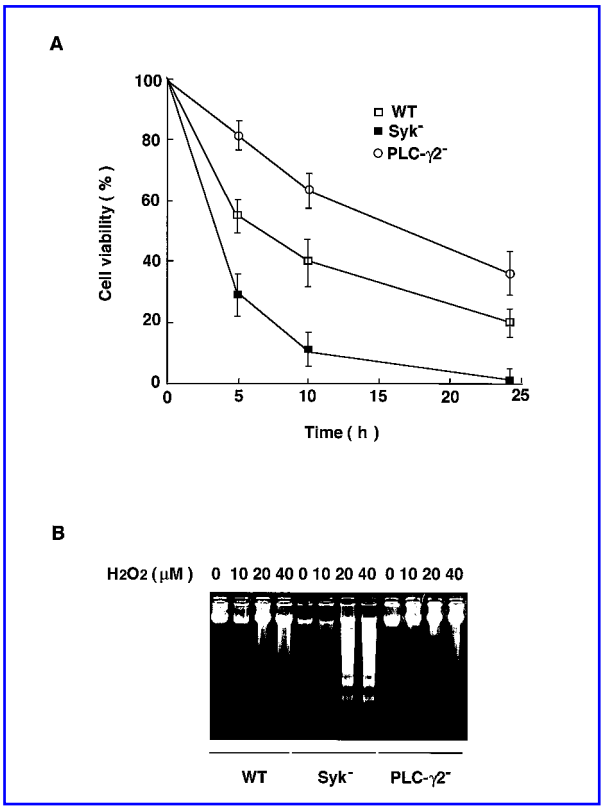

FIG. 4. Cell viability and DNA ladder formation after oxidative stress. (**A**) Cell viability. Wild-type (WT), Syk-deficient (Syk$^-$), and PLC-γ2-deficient (PLC-γ2$^-$) cells were treated with 50 μM H_2O_2 for the indicated times, and cell viability was determined by the trypan blue dye exclusion method (20). (**B**) Oxidative stress-induced apoptosis. Cells were treated with the indicated concentrations of H_2O_2 for 12 h, and cell lysates were subjected to DNA fragmentation analysis (20).

Role of p38 activation

It has been reported that the dynamic balance between ERK and JNK pathways is important in determining whether a cell survives or undergoes apoptosis following growth factor withdrawal (76). Treatment of HeLa cells with H_2O_2 results in the induction of apoptosis accompanied by the sustained activation of all three MAP kinases. This apoptosis is potentiated by inhibition of ERK and decreased by inhibition of JNK, whereas p38 has no influence on the outcome (73). In B cells exposed to 100 μM H_2O_2, the concentration that induces cell apoptosis, ERK and p38 are activated, whereas JNK activation is not observed. Activation of ERK and p38 is thought to be involved in oxidative stress-induced apoptosis. However, inhibition of p38 activity with SB203580, a p38-specific inhibitor, has no effect on cell viability after treatment with H_2O_2 (22), indicating that p38 activation upon H_2O_2 stimulation has no influence on cell apoptosis in B cells.

Recently, it has been reported that p38 has a potential role in the regulation of cell-cycle progression (3, 31, 64). Treatment of U937 promyelocytic cells with 20 μM H_2O_2 causes a selective activation of p38 and mitotic arrest, which is abolished by treatment with SB203580 (31). Furthermore, Bulavin *et al.* have reported that ultraviolet radiation leads to Cdc25B phosphorylation by p38, resulting in the initiation of the G2/M checkpoint after ultraviolet radiation (3). We have observed that a cell-cycle profile of G2/M arrest appears in B cells exposed to 10–20 μM H_2O_2, and SB203580 partially inhibits this arrest, whereas Syk-deficient cells exhibit no induction of G2/M arrest (22). Although p38 activation may have a partial influence on the initiation of a G2/M checkpoint following oxidative stress, there exist alternative pathways via Syk, independent of p38, to regulate cell-cycle pro-

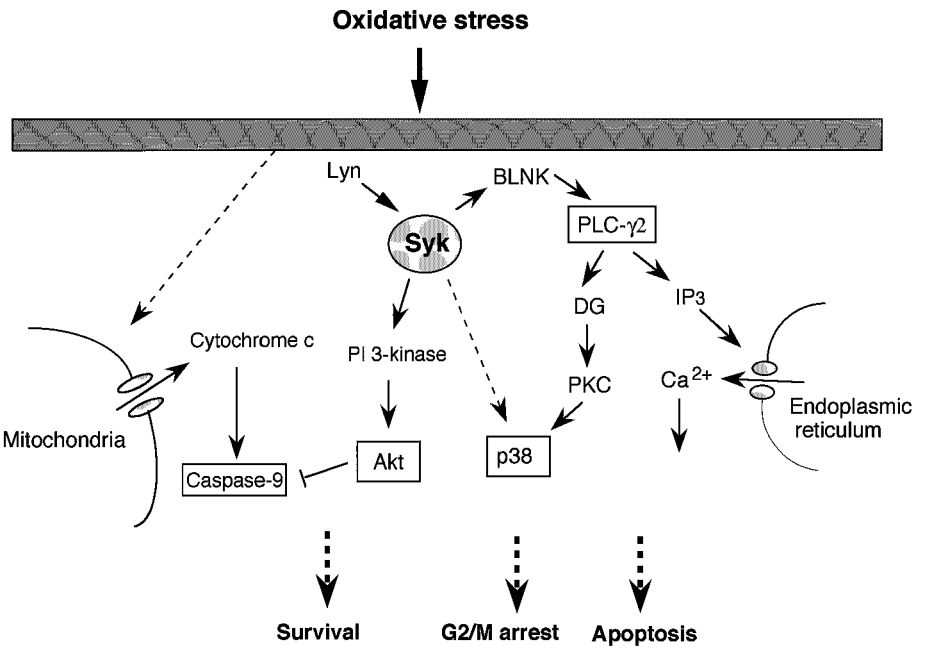

FIG. 5. Role of Syk in oxidative stress signaling in B cells. DG, diacylglycerol.

gression after oxidative stress. Further investigations are required for clarifying the regulatory mechanisms of cell-cycle progression after oxidative stress.

CONCLUSION

The suggested pathways of signal transduction in B cells exposed to H_2O_2 that induce apoptosis or G2/M arrest are illustrated in Fig. 5. Oxidative stress activates Syk through Lyn. Syk activation turns on several pathways, such as the Akt survival pathway and PLC-γ2-mediated pathway. A deficiency of Syk in B cells produces an enhanced cell apoptosis or an abolished G2/M arrest in response to H_2O_2 stimulation. Syk may modulate the balance among a variety of signal transduction pathways in B cells following oxidative stress, thereby determining the fate of a cell exposed to oxidative stress. Further investigation is warranted to clarify how Syk maintains a good balance between activation of downstream pathways following oxidative stress.

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ABBREVIATIONS

BCR, B-cell antigen receptor; BLNK, B cell linker protein; Btk, Bruton's tyrosine kinase; $[Ca^{2+}]_i$, cytoplasmic free calcium; ERK, extracellular signal-regulated kinase; H_2O_2, hydrogen peroxide; IP_3, inositol 1,4,5-trisphosphate; ITAM, immunoreceptor tyrosine-based activating motif; JNK, c-Jun N-terminal kinase; MAP kinase, mitogen-activated protein kinase; mSH2(C)Syk, C-terminal SH2 domain mutant of Syk; mSH2(N)Syk, N-terminal SH2 domain mutant of Syk; NF-κB, nuclear factor-κB; p38, p38 MAP kinase; PI 3-kinase, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLC, phospholipase C; PTK, protein-tyrosine kinase; ROS, reactive oxygen species; SH2, Src homology 2.

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