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Adhesion via CD43 Induces Syk Activation and Cell Proliferation in TF-1 Cells

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The effect of adhesion via CD43 (leukosialin, sialophorin) on cell proliferation and phosphorylation signaling were examined in a growth factor-dependent hematopoietic progenitor cell line, TF-1. TF-1 cells promptly resulted in death after withdrawal of growth factors. However, the viable cell number increased when TF-1 cells were cultured on anti-CD43 monoclonal antibody-coated plates. In this case, sustained activation of protein tyrosine kinase Syk and extracellular signal-regulated kinase (Erk) 1/2 were detected. Overexpression of exogenous Syk on TF-1 cells by the adenovirus vector system induced enhancement of the cell proliferation accompanied with enhancement of the Erk activation by a dominant-positive effect. The signal transducer and activator of transcription (STAT) 5 seemed not to be associated with the CD43-mediated cell proliferation. These results indicated that adhesion via CD43 induces the proliferation of TF-1 cells in the absence of growth factors in part by Syk-dependent Erk 1/2 signaling.

Key Words: **Syk; CD43; cell proliferation; Erk; TF-1.**

Interaction of hematopoietic progenitor cells with stromal cells via adhesion molecules is an important process for the regulation of hematopoiesis. However, the precise functions of individual adhesion molecules in cell proliferation, differentiation and death remain poorly understood.

CD43 is a transmembrane sialoglycoprotein expressed on the surface of almost all hematopoietic cells. It has been regarded as an anti-adhesion molecule due to its structural character. On the other hand, it has been also suggested that CD43 works as an adhesion molecule (1). Several molecules are shown to be ligands for CD43 (2, 3), though it still remains to be elucidated whether they are physiological ligands or not. In addition, engagement of CD43 with a specific antibody mediates the activation of neutrophils (4), monocytes (5), dendritic cells (6), T cells (7) and NK cells (8). Although its roles in mature hematopoietic cells are examined, those of hematopoietic progenitor cells are poorly characterized (9–11). CD43 transduces signaling that leads to the generation of diacylglycerol and inositol phosphates, the mobilization of intracellular Ca^{2+} and the activation of protein kinase C (12). It has also been demonstrated that CD43 mediates the tyrosine phosphorylation signaling (8, 10, 11, 13, 14). Syk is a cytoplasmic protein tyrosine kinase that is widely expressed in hematopoietic cells (15). It has been demonstrated that Syk is involved in coupling of activated immunoreceptors to downstream signaling events that mediate diverse cellular responses including proliferation, differentiation and phagocytosis (16). In addition, recent studies have shown that Syk participates in integrin-mediated signaling and have provided several lines of evidence that Syk plays important roles in cell adhesion (17, 18). In the present study, the effects of adhesion via CD43 on cell proliferation and phosphorylation signaling were assessed in a growth factor-dependent hematopoietic progenitor cell line, TF-1 (19) by the use of anti-CD43 monoclonal antibody-coated plates. In the course of cultivation, we examined cell growth, activation of Syk, and involvement of growth-associated signaling molecules, Erk1/2 and STAT5.

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Abbreviations used: Erk, extracellular signal-regulated kinase; STAT, signal transducer and activator of transcription; mAb, monoclonal antibody; rhGM-CSF, recombinant human granulocyte-macrophage colony-stimulating factor; FCS, fetal calf serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide; PVDF, polyvinylidene difluoride; BSA, bovine serum albumin; (T-)TBS, (Tween 20-)Tris-buffered saline; MOI, multiplicities of infection.

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MATERIALS AND METHODS

Antibodies and reagents. Anti-CD43 mAb DFT1 was obtained from Immunotech (Westbrook, ME). Isotype-matched control mouse IgG1 (MOPC-21) for DFT1 was obtained from Sigma (St. Louis, MO). Anti-phosphotyrosine mAb 4G10 was from Upstate Biotechnology Inc. (Lake Placid, NY). Anti-phospho-Erk mAb and rabbit anti-phospho-STAT5 polyclonal Ab were purchased from Cell Signaling Technology (Beverly, MA). Anti-STAT5 mAb, rabbit anti-Syk Ab and goat anti-Erk Ab were products of Santa Cruz Biotechnology (Santa Cruz, CA). MTT was from Sigma (St. Louis, MO). polyvinylidene difluoride (PVDF) was purchased from Millipore (Bedford, MA). Protein A-sepharose beads were from Amersham Pharmacia Biotech Inc. (Uppsala, Sweden) and rhGM-CSF (specific activity: 5×10^7 U/mg protein) was provided by Hoechst Japan Co. (Tokyo, Japan).

Preparation of antibody-coated plates. Culture plates or 96-well microplates were pre-coated for 3 h at room temperature with 20 μg/mL antibody in adhesion buffer (20 mmol/L Tris/HCl [pH 8.2], 150 mmol/L NaCl) (8). The plates were blocked with 1% BSA in adhesion buffer for 30 min at room temperature, washed once with PBS and then applied to cell proliferation and stimulation assays.

Cell proliferation and stimulation assays. TF-1 cells were kindly provided by Dr. T. Kitamura (Tokyo University, Tokyo, Japan). The cells were incubated in growth factor-free culture medium (RPMI 1640 supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin) for 16 h prior to cell proliferation and stimulation assays. For cell proliferation assays, TF-1 cells were cultured on the antibody-coated 96-well microplates for the indicated period. Cell proliferation was assessed by MTT assay (20). Cell viability was also confirmed by trypan blue dye exclusion assay. For cell stimulation assays, TF-1 cells were cultured on the antibody-coated culture plates for the indicated period and then lysed in nonionic lysis buffer (1% Triton X-100, 50 mM Tris/HCl [pH 7.4], 150 mM NaCl, 5 mM EDTA, 1 mM sodium vanadate and 1 mM phenylmethyl sulfonyl fluoride).

Preparation of whole cell lysates and immunoprecipitates. Cell lysates were kept on ice for 10 min followed by micro-centrifugation at 15,000rpm for 10 min at 4°C. Supernatants were directly solubilized with SDS sample buffer (2% SDS, 62.5 mM Tris/HCl [pH 6.8], 10% glycerol, 5% 2-mercaptoethanol and 0.001% bromophenol blue and) and boiled for 3 min. Immunoprecipitates were prepared by incubation of the cell lysates with indicated Abs for 1 h at 4°C, and subsequently with protein A-sepharose for another 1 h at 4°C. The immunoprecipitates were washed three times with lysis buffer. For immunoblotting analyses, the immunoprecipitates were boiled with SDS sample buffer for 3 min.

In vitro kinase assays. Immunoprecipitates were incubated in a reaction mixture (50 mM Hepes/NaOH [pH 8.0], 10 μM sodium vanadate, 50 mM Mg acetate, 150 mM NaCl and 1 μM ATP) for 10 min at 30°C. The reaction was terminated by adding SDS sample buffer and boiling for 3 min. The samples were separated by SDS–PAGE, transferred to PVDF membranes and subjected to immunoblotting analysis with anti-phosphotyrosine mAb.

Immunoblotting analyses. Whole cell lysates or immunoprecipitates were separated by SDS–PAGE and transferred to PVDF membranes. The membranes were blocked with 1% BSA in low-salt T-TBS (10 mM Tris/HCl [pH 7.4], 100 mM NaCl containing 0.1% Tween 20) for 30 min at 37°C. This was followed by incubation with primary Abs in low-salt T-TBS for 1 h at room temperature. The membranes were washed and incubated with horseradish peroxidase-conjugated secondary Abs in low-salt T-TBS for 30 min at room temperature. After washing with low-salt T-TBS and subsequently with low-salt TBS (10 mM Tris/HCl [pH 7.4], 100 mM NaCl), enhanced chemiluminescence (ECL) assays were performed to visualize positive bands on X-ray films.

Gene transfection studies. Gene transfection was performed by the adenovirus vector system. Adenovirus vectors were constructed by the use of an adenovirus expression kit (Takara, Tokyo, Japan). The cDNA fragment coding full-length wild-type porcine syk, kinase-negative porcine syk or lacZ was inserted into the Swa1 site of the pAxCAwt cosmid vector. This vector contains the CAG promoter, but in which the E1A, E1B and E3 genes are deleted. A virus clone which strongly expresses wild-type Syk (pAxCA-Syk/wild), kinase-negative Syk (pAxCA-Syk/kn) or LacZ (pAxCA-LacZ) was prepared by homologous recombination of the expression cosmid cassette and parental viral genome, and amplified to achieve a stock with a titer of approximately 10^9 plaque-forming units/ml (21). Aliquots of TF-1 cells were mixed with the recombinant adenovirus solution at the indicated MOI and incubated for 1 h at 37°C. The cells were then subjected to cell proliferation and stimulation assays.

RESULTS

Engagement of CD43 induces tyrosine phosphorylation of proteins including Syk in TF-1 cells. We first examined effects of engagement of CD43 on protein tyrosine phosphorylation in TF-1 cells by immunoblotting analyses. TF-1 cells were allowed to adhere to the plates coated with anti-CD43 mAb or control mouse IgG1. The increase in tyrosine phosphorylation of several proteins was detected in the cells adherent to anti-CD43 mAb-coated plates, while such phosphorylation was not detected in the cells cultured on control IgG1-coated plates (Fig. 1A). We next assessed whether Syk is involved in CD43-mdiated signaling in the same time-course study. As shown in Fig. 1B, tyrosine phosphorylation of Syk was detected from 15 min in the cells adherent to anti-CD43 mAb-coated plates, while it was not detected in the cells cultured on control IgG1-coated plates. These results indicated that engagement of CD43 induces tyrosine phosphorylation of proteins including Syk in TF-1 cells.

Engagement of CD43 induces the proliferation of TF-1 cells. Our observation that engagement of CD43 generated tyrosine phosphorylation signaling (Fig. 1) led us to postulate that engagement of CD43 could affect the proliferation of TF-1 cells. Thus, we examined cell proliferation by MTT assay under the conditions that TF-1 cells were allowed to adhere to anti-CD43 mAb-coated microplates in the absence of growth factors. TF-1 cells proliferate only in the presence of growth factors such as GM-CSF (19). In our experiment, TF-1 cells cultured on control IgG1-coated microplates resulted in death immediately after withdrawal of growth factor (Fig. 2, closed circles). However, the number of viable cells cultured on anti-CD43 mAb-coated microplates increased by 1.5 times the initial cell number on day 7 (Fig. 2, open circles). These results indicated that engagement of CD43 induces the proliferation of TF-1 cells in the absence of growth factors.

Syk gene transfer to TF-1 cells by the adenovirus vector system. Recent reports suggested that Syk is involved in cell growth (26, 27). To examine whether

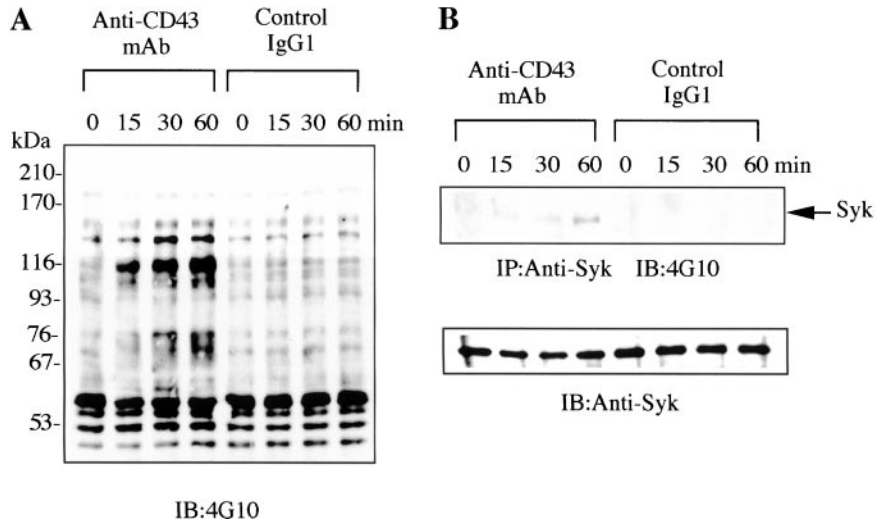

FIG. 1. Time-course study of tyrosine phosphorylation of proteins including Syk by engagement of CD43 with anti-CD43 mAb in TF-1 cells. TF-1 cells were allowed to adhere to culture plates coated with anti-CD43 mAb or control IgG1 for the indicated times. (A) Whole cell lysates were subjected to immunoblotting analysis with anti-phosphotyrosine mAb 4G10. In each lane, the equivalent amount of protein (10 μg) from whole cell lysates was loaded. (B) Lysates from the equivalent number of the cells (1×10^6 cells) were immunoprecipitated with 2 μg anti-Syk Ab. Immunoblotting analysis was performed with 4G10 (upper panel). The membrane was reprobed with anti-Syk Ab (lower panel).

CD43-mediated cell proliferation was affected by the modulation of Syk kinase activity, the exogenous syk gene and its mutant were introduced to TF-1 cells by the adenovirus vector system. First, we tested the eligibility of the adenovirus vector system. To examine gene transfection efficiency, TF-1 cells were infected with pAxCA-LacZ at 50 MOI. After cultivation for 4 days, the cells were stained with 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) and more than 90% of the total cells proved to be positive for LacZ (data not shown). To examine whether syk gene is successfully transfected, the expression of exogenous (porcine) Syk was assessed by immunoblotting analysis with anti-Syk Ab. TF-1 cells were infected with pAxCA-Syk/wild at 50 MOI and then cultured for 14 days. It was confirmed in a dose-escalating study that the expression of exogenous Syk on day 4 reached plateau at 50 MOI (data not shown). As shown in Fig. 3, anti-Syk Ab reacted on both human and porcine Syk, and they could be distinguishable each other from a little difference of mobilities under SDS–PAGE. The expression of the exogenous Syk was abundant from day 1 to day 7 and decreased thereafter. The expression of mutant Syk in pAxCA-Syk/kn-infected cells showed the similar pattern as that of pAxCA-Syk/wild-infected cells (data not shown). These results indicated that the adenovirus vector system brings about sufficient expression of exogenous genes transiently in most of TF-1 cells at 50 MOI.

CD43-mediated cell proliferation is affected by the modulation of Syk kinase activity. We next assessed the proliferation of exogenous Syk-overexpressing cells. TF-1 cells were infected with pAxCA-Syk/wild or pAxCA-Syk/kn at 50 MOI and then cultured on anti-CD43 mAb-coated microplates for the indicated period in the absence pf growth factors. The number of viable cells was increased by 2.5 times the initial cell number in wild-type Syk-overexpressing cells (Fig. 2, open squares). On the other hand, the viable cell number remained unaltered over time in kinase-negative Syk-overexpressing cells (Fig. 2, open diamonds). It was almost the same in LacZ-overexpressing cells as that of untransfected cells (Fig. 2, open triangles versus open circles). From these data, Syk proved to have supportive effect on the proliferation of TF-1 cells initiated by engagement of CD43 in the absence of growth factors.

Syk was tyrosine-phosphorylated from day 1 to day 7 in untransfected cells adherent to anti-CD43 mAb-coated plates (Fig. 4A, lanes 1–4). To test whether Syk was activated when it was tyrosine-phosphorylated, we performed *in vitro* kinase assays of anti-Syk immunoprecipitates. In the presence of ATP, the increase in tyrosine phosphorylation of Syk was detected in anti-Syk immunoprecipitates of untransfected cells cultured for 4 days (Fig. 4B). As shown in Fig. 4A, the tyrosine phosphorylation of Syk was enhanced in wild-type Syk-overexpressing cells (lanes 5–7), whereas it was suppressed in kinase-negative Syk-overexpressing cells (lanes 8–10). *In vitro* kinase assays showed that the increase in tyrosine phosphorylation of Syk was detected in anti-Syk immunoprecipitates of wild-type Syk-overexpressing cells (Fig. 4C, lanes 1 and 2), while it was only slightly detected in that of kinase-negative Syk-overexpressing cells (Fig. 4C, lanes 3 and 4). It is a matter of interest whether or not overexpression of

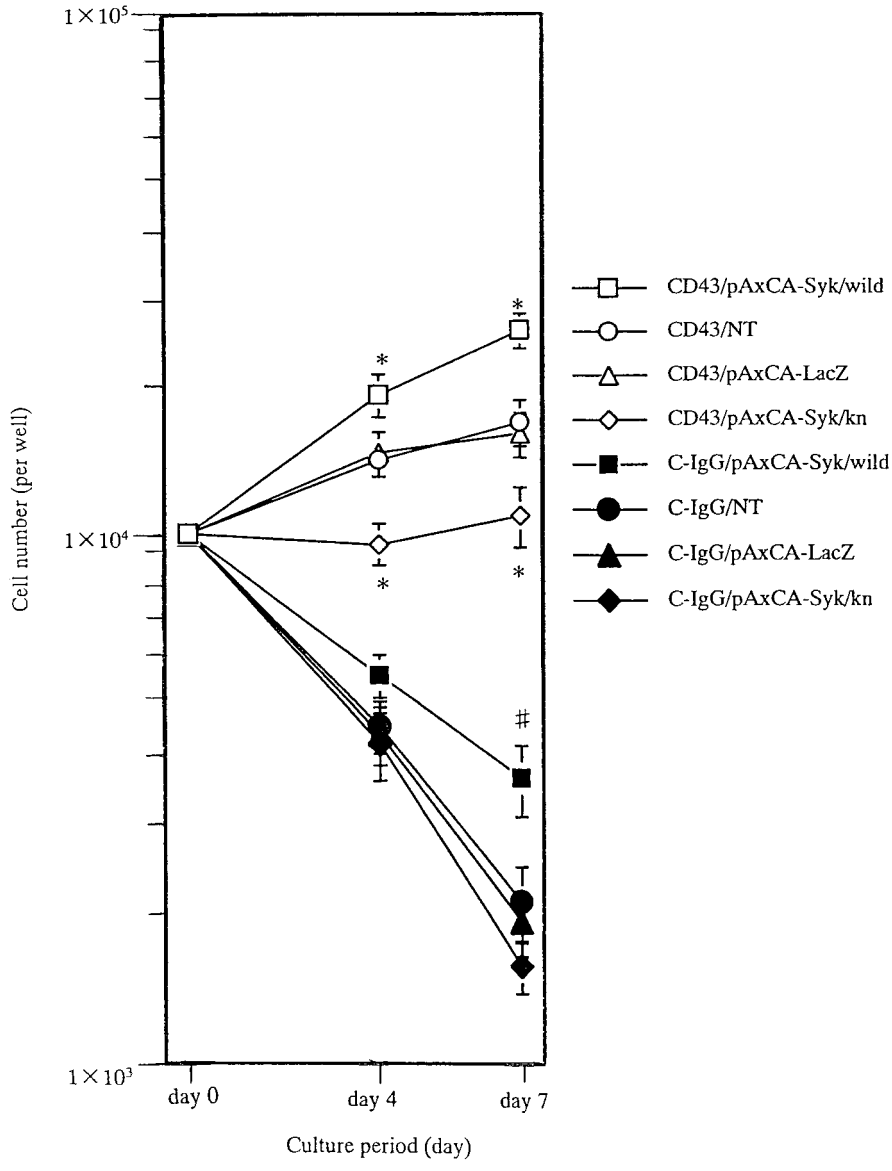

FIG. 2. Growth of TF-1 cells cultured on antibody-coated microplates in the absence of growth factors. TF-1 cells (1×10^4 cells/well) were cultured for 7 days on 96-well microplates coated with anti-CD43 mAb (open circles) or control IgG1 (closed circles). Some aliquots of the cells were infected with the following adenovirus vectors at 50 MOI on day 0: pAxCA-Syk/wild (open squares for anti-CD43 mAb-coated microplates, closed squares for control IgG1-coated microplates); pAxCA-Syk/kn (open diamonds for anti-CD43 mAb-coated microplates, closed diamonds for control IgG1-coated microplates); pAxCA-LacZ (open triangles for anti-CD43 mAb-coated microplates, closed triangles for control IgG1-coated microplates). Cell proliferation was evaluated by MTT assays on day 4 and day 7. Results are the means ± SD of more than 3 independent experiments. $P < 0.001$ (*), wild-type Syk-overexpressing cells or kinase-negative Syk-overexpressing cells versus untransfected cells cultured for corresponding period on anti-CD43 mAb-coated microplates; $P < 0.01$ (#), wild-type Syk-overexpressing cells versus untransfected cells cultured for 7 days on control IgG1-coated microplates. NT, untransfected cells. C-IgG, control IgG1.

Syk affects the expression of CD43 molecules. Hence we performed flow cytometric analysis and confirmed that the expression of CD43 molecule on exogenous Syk-overexpressing TF-1 cells is almost the same level as that of untransfected cells (data not shown). These results indicated that CD43-mediated cell proliferation is affected by the modulation of Syk kinase activity.

To test the direct effect of Syk on the cell proliferation, exogenous Syk-overexpressing TF-1 cells were cultured on control IgG1-coated microplates for the indicated period in the absence of growth factors. The loss of viability was observed, but it was rescued to some extent in wild-type Syk-overexpressing cells (Fig. 2, closed squares) compared with that of untransfected cells (Fig. 2, closed circles) on day 7. It was not affected in LacZ-overexpressing (Fig. 2, closed triangles) or in kinase-negative Syk-overexpressing cells (Fig. 2, closed diamonds).

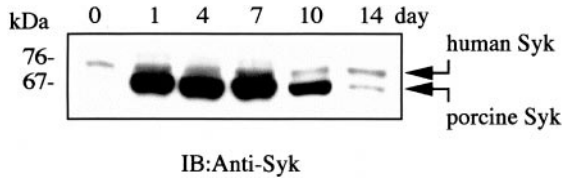

FIG. 3. Expression of exogenous (porcine) Syk protein in TF-1 cells by the adenovirus vector system. TF-1 cells were infected with pAxCA-Syk/wild at 50 MOI on day 0 and cultured for the indicated days. Whole cell lysates were subjected to immunoblotting analysis with anti-Syk Ab. In each lane, the equivalent amount of protein (4 μg) from whole cell lysates was loaded.

Engagement of CD43 induces sustained activation of Erk1/2 in TF-1 cells. GM-CSF activates Janus kinase (JAK) 2-STAT5 and Ras-mitogen-activated protein kinase (MAPK) signalings to induce the proliferation of TF-1 cells (22, 23). We examined whether engagement of CD43 gives rise to the growth factor-mediated signaling in TF-1 cells. On stimulation with 10ng/mL rhGM-CSF, the tyrosine phosphorylation of STAT5 occurred in a time-dependent manner (Fig. 5A). On the other hand, its phosphorylation was neither detected in a time-course (Fig. 5A) nor in a day-course study (data not shown) by engagement of CD43. As for Erk, engagement of CD43 induced sustained phosphorylation of Erk1/2 from day 1 to day 7 (Fig. 5B). Next, we assessed the effects of overexpression of exogenous Syk on the phosphorylation of Erk1/2. As shown in Fig. 5C, the phosphorylation of Erk1/2 was enhanced in wild-type Syk-overexpressing cells, while it was suppressed in kinase-negative Syk-overexpressing cells. These results indicated that engagement of CD43 induces sustained activation of Erk1/2, which is affected by the modulation of the kinase activity of Syk.

DISCUSSION

TF-1 cells are strictly growth factor-dependent and have the ability to differentiate toward both mature erythroid and myeloid lineage cells (19). Such cells are considered to have some properties close to primary hematopoietic progenitor cells (24). In the present study, we showed that adhesion to anti-CD43 mAb-coated plates induced the proliferation of TF-1 cells in the absence of growth factors (Fig. 2). The result provides a possibility that interaction with stromal cells via CD43 is involved in the proliferation of hematopoietic progenitor cells in the bone marrow environment. It was reported that CD34-positive primitive quiescent cells cultured on anti-CD43 mAb-coated plates showed resistance to apoptosis (9). Taken together, CD43-mediated signaling seems to have a supportive effect on the growth of hematopoietic progenitor cells in quiescent status.

Constitutive activation of Syk by engagement of CD43 (Fig. 4) led us to postulate that Syk is involved in CD43-mediated proliferation of TF-1 cells. Piceatannol has been used as an inhibitor of Syk, but it was demonstrated to inhibit not only Syk but also other protein tyrosine kinases (25). We therefore conducted gene transfection studies to examine the effects of modulation of Syk kinase activity on the cell proliferation. The Syk activity was enhanced in wild-type Syk-overexpressing cells, while it was suppressed in kinase-negative Syk-

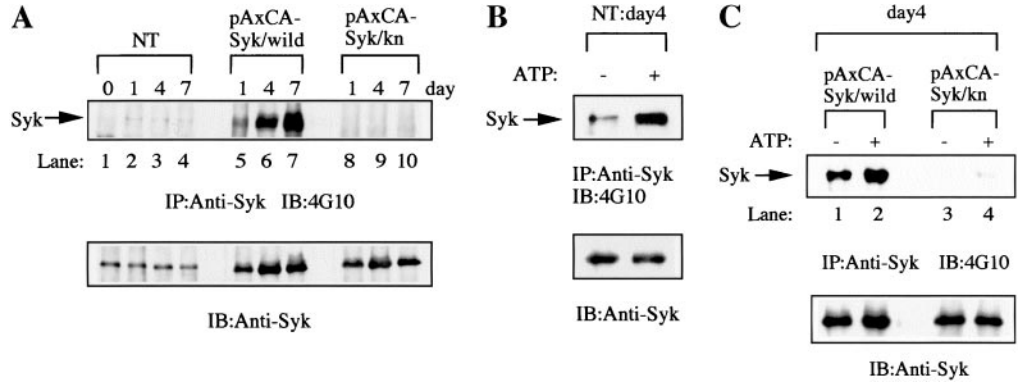

FIG. 4. (A) Day-course study of tyrosine phosphorylation of Syk by engagement of CD43 with anti-CD43 mAb in TF-1 cells. TF-1 cells were allowed to adhere to anti-CD43 mAb-coated culture plates for the indicated days (lanes 1–4). Some aliquots of the cells were infected with pAxCA-Syk/wild (lanes 5–7) or pAxCA-Syk/kn (lanes 8–10) at 50 MOI on day 0. Lysates from the equivalent amount of protein (400 μg) were immunoprecipitated with 1 μg anti-Syk Ab. Immunoblotting analysis was performed with anti-phosphotyrosine mAb 4G10 (upper panel). The membrane was reprobed with anti-Syk Ab (lower panel). NT: untransfected cells. (B, C) *In vitro* kinase assays of anti-Syk immunoprecipitates in TF-1 cells adherent to anti-CD43 mAb-coated plates. TF-1 cells were allowed to adhere to anti-CD43 mAb-coated culture plates for 4 days. Some aliquots of the cells were infected with pAxCA-Syk/wild (C, lanes 1 and 2) or pAxCA-Syk/kn (C, lanes 3 and 4) at 50 MOI on day 0. Lysates from the equivalent amount of protein (800 μg) were immunoprecipitated with 2 μg anti-Syk Ab. The anti-Syk immunoprecipitates were subjected to *in vitro* kinase assays in the presence or absence of ATP and immunoblotting with anti-phosphotyrosine mAb 4G10 (upper panel). Each membrane was reprobed with anti-Syk Ab (lower panels). NT, untransfected cells.

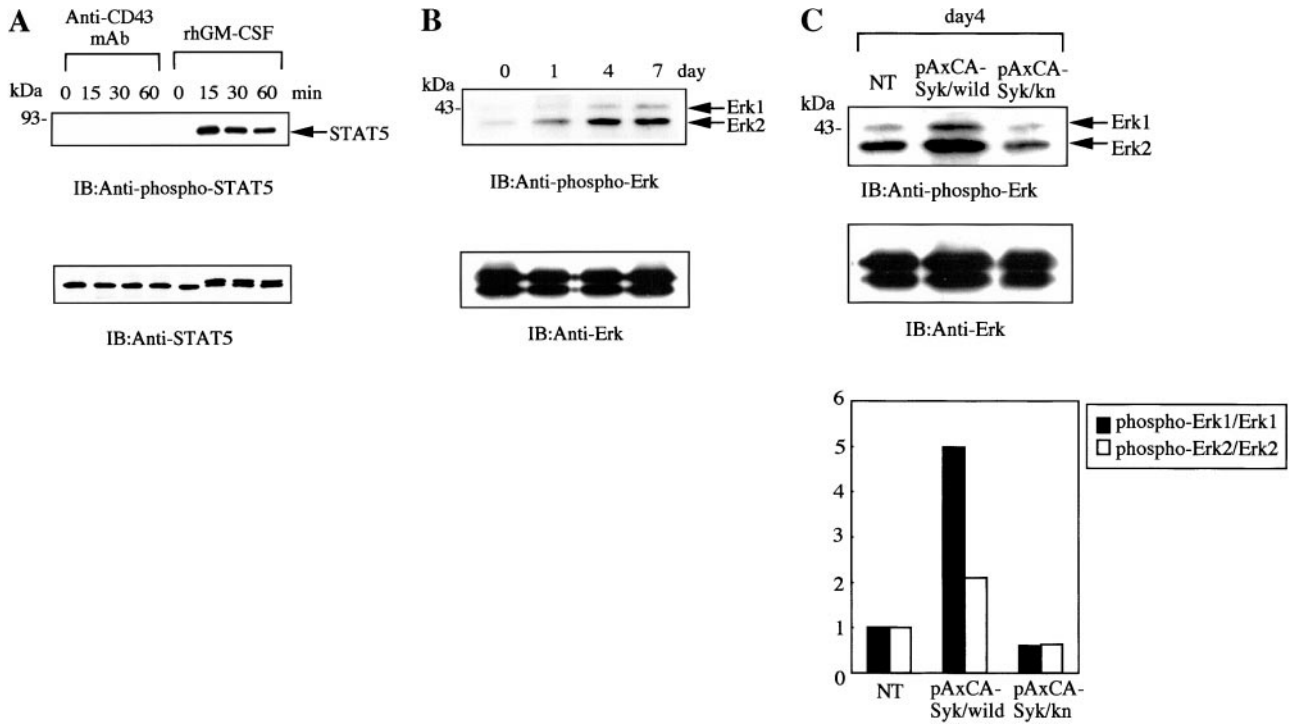

FIG. 5. (A) Time-course study of phosphorylation of STAT5 by engagement of CD43 with anti-CD43 mAb in TF-1 cells. (A) TF-1 cells were either allowed to adhere to anti-CD43 mAb-coated culture plates or incubated in suspension in polypropylene microtubes with 10 ng/ml rhGM-CSF for the indicated times. Whole cell lysates were subjected to immunoblotting analysis with anti-phospho-STAT5 Ab (upper panel). In each lane, the equivalent amount of protein (10 μg) from whole cell lysates was loaded. The membrane was reprobed with anti-STAT5 mAb (lower panel). (B, C) Day-course study of phosphorylation of Erk1/2 by engagement of CD43 with anti-CD43 mAb in TF-1 cells. TF-1 cells were allowed to adhere to anti-CD43 mAb-coated culture plates for the indicated days (B) or for 4 days (C). Whole cell lysates were subjected to immunoblotting analysis with anti-phospho-Erk mAb (B and C, upper panels). In each lane, the equivalent amount of protein (10 μg) from whole cell lysates was loaded. In (C), some aliquots of the cells were infected with pAxCA-Syk/wild or pAxCA-Syk/kn at 50 MOI on day 0. Each membrane was reprobed with anti-Erk Ab (B, lower panel, and C, middle panel). The phosphorylation level of Erk 1/2 proteins was normalized to the expression level of corresponding Erk1/2 proteins (C, lower panel). The density of each protein band was measured by ATTO Densitograph version 4.1 software (ATTO, Tokyo, Japan). NT, untransfected cells.

overexpressing cells (Fig. 4). Cell proliferation was affected in proportion to Syk kinase activity (Fig. 2). These results indicate that Syk has supportive effect on the proliferation of TF-1 cells initiated by engagement of CD43. Recently several studies have shown the involvement of Syk on cell growth, but effects of Syk on cell growth are controversial (26, 27). One study demonstrated that transfection of wild-type Syk inhibited the growth of breast cancer cells (26). The other suggested that Syk had supportive effects on the growth of human umbilical vein endothelial cells (HUVECs) (27). In hematopoietic cells, it was demonstrated that TEL-Syk fusion protein contributed to the advance of myelodysplastic syndrome and to the growth factor-independent cell proliferation of murine leukemia BaF3 cells (28), but the direct effect of Syk on cell proliferation has not been documented. In our observation, the loss of viability after growth factor withdrawal was significantly rescued to some extent in wild-type Syk-overexpressing TF-1 cells (Fig. 2, closed squares). Consistent with the results in HUVECs, Syk seemed to have some supportive effects on cell growth in human growth factor-dependent hematopoietic progenitor TF-1 cells.

A close relationship between Syk and Erk has been reported (29). Enhancement of the Syk activity induced enhancement of the cell proliferation (Fig. 2) and the Erk1/2 activity (Fig. 5C) in TF-1 cells adherent to anti-CD43 mAb-coated plates. On the other hand, suppression of the Syk activity induced suppression of the cell proliferation (Fig. 2) and the Erk1/2 activity (Fig. 5C). These results suggested that Erk1/2 participates in the cell proliferation initiated by engagement of CD43 as a downstream molecule of Syk in TF-1 cells.

In summary, our results suggest that adhesion via CD43 induces the proliferation of TF-1 cells in the absence of growth factors in part by means of Syk-dependent Erk1/2 signaling pathway. STAT5 seems not to be associated with CD43-mediated cell proliferation. Though detailed functions of CD43 and Syk in cell proliferation and intracellular signaling with respect to CD43-mediated cell proliferation remain to be understood, our study could pro-

vide one insight into the understanding of a mechanism of hematopoiesis and leukemogenesis.

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