Roles of C-terminal Src Kinase in the Initiation and the Termination of the High Affinity IgE Receptor-mediated Signaling*

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Zen-ichiro Honda†, Takeshi Suzuki, Naoto Hirose, Makoto Aihara§, Takao Shimizu§,
Shigeyuki Nada¶, Masato Okada¶, Chisei Raí, Yutaka Morita, and Koji Ito

From the Departments of Internal Medicine and Physical Therapy and of Biochemistry, Faculty of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan, and the Division of Protein Metabolism, Institute of Protein Research, Osaka University, Suita, Osaka 565, Japan, and the Department of Immunology, Juntendo University, School of Medicine, 2-1-1, Hongo Bunkyo-ku Tokyo 113, Japan

As an attempt to analyze the roles of C-terminal Src kinase (Csk) in the high affinity IgE receptor (FcεRI)-mediated signaling, we overexpressed Csk, a membrane-targeted form of Csk (mCsk), and a kinase-defective, membrane-targeted form of Csk (mCsk(−)) in rat basophil leukemia (RBL) 2H3 cells. Specific activity of Lyn at the basal state was decreased in Csk-expressing cells, and further decreased in mCsk-expressing cells. In mCsk(−)-expressing cells, basal specific activity of Lyn was increased, thereby indicating that mCsk(−) functions as a dominant negative molecule. The onset of FcεRI-mediated Lyn activation was delayed in Csk-expressing cells, and further delayed in mCsk-expressing cells. In mCsk(−)-expressing cells, Lyn activation was rapid and quite long lasting. These findings indicate (i) Csk negatively regulates rapid FcεRI/Lyn coupling, and (ii) Csk activity is potentially required for its termination. The onsets of the series of events including tyrosyl phosphorylation of Syk, mitogen-activated protein (MAP) kinase activation, elevation of intracellular calcium concentration ([Ca^2+]), and histamine release were all stepwisely delayed in Csk-expressing cells and in mCsk-expressing cells. The durations of Syk phosphorylation and MAP kinase activation also closely correlated with those of Lyn activation, but [Ca^2+] elevation and histamine release followed different temporal patterns: the delayed responses in Csk-expressing cells and in mCsk-expressing cells lost to sustained [Ca^2+] oscillation and histamine release, while the prompt responses in parent cells and mCsk(−)-expressing cells rapidly subsided. These findings provide further evidence that the initiations of the FcεRI-mediated signals are upstreamly regulated by Src family protein tyrosine kinases and revealed that their terminations are regulated by Lyn-dependent (Syk and MAP kinase) and independent ([Ca^2+] elevation and histamine release) mechanisms.

The high affinity IgE receptor (FcεRI) belongs to a family of multi-subunit receptors for antigen, which include T cell receptor (TCR), B cell receptor (BCR), Fcy, and Fce receptors (1–4). FcεRI is composed of one α subunit possessing IgE binding site and one β and two γ subunits functioning as signal transducers (3, 5–8). The cytoplasmic tails of the β and the γ subunits possess characteristic tyrosine-based amino acid sequence motifs (immunoreceptor tyrosine-based activation motif (ITAM)) (4, 9), which are also found in the signal transducing subunits of TCR and BCR (4). Tyrosine residues in the ITAM sequences are rapidly phosphorylated after the triggering of the antigen receptors (10–13), and the phosphorylated ITAM sequences serve as sites for recruiting signaling molecules mainly through their interaction with Src homology (SH) 2 domains (5, 6, 14–16). Several lines of evidence have implicated Src family PTKs in the primary phosphorylation of the ITAM tyrosine (4, 13, 17–19). In the case of FcεRI, Lyn, Src, and Yes (members of Src family PTKs) are rapidly activated after the receptor triggering (20), Lyn possibly associates with the the β subunit of FcεRI (14, 20, 21), and deletion of the cytoplasmic region of the β subunit abrogates FcεRI signalings in murine mast cells (21). Most notably, recent heterologous expression studies have demonstrated that Lyn expression is required for the efficient phosphorylation of the β and the γ subunits as well as phosphorylation and activation of Syk (15, 22), another cytoplasmic PTK.

The kinase activity of Src family PTKs has been shown to be regulated by the phosphorylation level of the C-terminal tyrosine conserved among the family members (23, 24). It is postulated that the phosphorylated tyrosine associates with the intramolecular SH2 domain (23) and that the "closed" conformation (25) prevents the catalytic domain from interacting with external kinase substrates. Disruption of the intramolecular interaction, presumably through the dephosphorylation of the C-terminal tyrosine or through the replacement of the phosphorylated C-terminal tyrosine with high affinity protein ligands (23), results in augmented kinase activity ("open" conformation). In hematopoietic cells, C-terminal Src kinase (Csk) and CD45 tyrosine phosphatase are implicated in the phosphorylation and the dephosphorylation of the regulatory tyrosine, respectively (4, 23). Csk, first purified as a PTK that specifically phosphorylates the regulatory tyrosine (26), has structural similarities with Src family PTKs but is devoid of the C-terminal regulatory tyrosine and the N-terminal myristylation signal required for membrane localization (26–28). Cre-
ation of Csk-deficient mice, which could not survive embryonic stages, has revealed that Src family PTKs are commonly activated in Csknull cells, thereby indicating that Csk is one of the negative molecules. By using these cell lines, we show herein that (i) Csk is involved in the initiation and the termination of FcεRI-mediated Lyn activation, (ii) the initiations of the series of events leading to granule release followed Lyn activation, and (iii) the termination of the signals are regulated by both Lyn-dependent and -independent mechanisms.

**EXPERIMENTAL PROCEDURES**

**Materials**—All the culture media and Geneticins® were purchased from Life Technologies Oriental (Osaka, Japan). Fetal calf serum was obtained from Equitech-Bio (Ingram, TX). Protein G-Sepharose beads were from Pharmacia-LKB (Uppsala, Sweden). Enolase and mouse monoclonal anti-dinitrophenyl IgE (anti-DNP IgE) were from Sigma. 2,4-dinitrophenylated bovine serum albumin (DNP-BSA, 30 ± 5 mol of DNP/mol of BSA) was from LSL (Tokyo, Japan). [γ−32P]ATP was from Amersham Corp. (Bucksinghamshire, United Kingdom). All other chemical reagents were of analytical grade.

**Generation of RBL2H3 Cells Overexpressing Csk and Mutated Forms of Csk**—cDNAs of rat Csk and a kinase-defective Csk (Csk(−)), in which Lys-222 is replaced with Arg, were prepared as described (27). Csk and Csk(−) chimera possessing N-terminal 16 amino acids of rat c-Src could not survive embryonic stages, has revealed that Src family PTKs are commonly activated in Csknull cells, thereby indicating that Csk is one of the negative molecules. By using these cell lines, we show herein that (i) Csk is involved in the initiation and the termination of FcεRI-mediated Lyn activation, (ii) the initiations of the series of events leading to granule release followed Lyn activation, and (iii) the termination of the signals are regulated by both Lyn-dependent and -independent mechanisms.

**Antibodies**—Polyclonal antibody against amino acids 44–63 of human Csk were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-Csk polyclonal antibody (27, 29) were prepared as described. Generation of RBL2H3 Cells Overexpressing Csk and Mutated Forms of Csk—cDNAs of rat Csk and a kinase-defective Csk (Csk(−)), in which Lys-222 is replaced with Arg, were prepared as described (27). Csk and Csk(−) chimera possessing N-terminal 16 amino acids of rat c-Src (c-Src tag: [M]GSNKSKPKDASQRRR), harboring a myristylation signal of rat c-Src (28) was fused to the entire coding sequence of rat Csk (mCsk). A kinase defective mCsk (mCsk(−)) chimeras possessing N-terminal 16 amino acids of rat c-Src and the N-terminal 7 amino acids of rat Csk were selected in the medium containing 1.2 mg/ml Geneticin, and clones mCsk(−) 1 to 4 were obtained. Of these, we mainly used Csk 5 and 9, mCsk 1 and 3, and mCsk(−) 1 and 3, with similar expression levels of these molecules. The data described below were reproducible in the two sets of clones for each cDNA.

**Cell Stimulation and Cell Solubilization**—106 cells in 6-cm dishes were cultured for 36 h and then starved for serum for another 24 h. Cells were sensitized with anti-DNP IgE (a final concentration of 1 μg/ml) for 1 h in serum-free DMEM containing 0.1% fatty acid-free BSA, washed with phosphate-buffered saline, and incubated in trun-}

**RESULTS**

**Changes in the Basal Activity of Lyn in Cells Overexpressing Csk and Its Mutants**—Previous studies have shown that TCR signaling is inhibited by Csk and almost completely blocked by Csk, thus phosphorylating mast cell tyrosine kinase and inducing calcium signals (31, 32). The augmented functions caused by the membrane targeting are presumed to be due to close positioning of these Csk mutants to Src family PTKs (31, 32). We, therefore, prepared a membrane-targeted Csk in which the N-terminal myristylation signal of rat c-Src (28) was fused to the entire coding sequence of rat Csk (mCsk). A kinase defective mCsk...
FIG. 1. Expressions of Csk and its mutants altered the specific activity of Lyn. Total cell lysate (20 μg of protein) was prepared from wild-type RBL2H3 cell line (WT) and from two sets of independent cell lines overexpressing Csk (Csk5 and Csk9), mCsk (mCsk1 and mCsk3), and mCsk(−) (mCsk(−)1 and mCsk(−)3). The cell lysates were subjected to immunoblotting with anti-Csk antibody (Csk:blot). The migrated positions of Csk, mCsk, and mCsk(−) are indicated by arrows. The cell lysates (20 μg of protein) were next subjected to immunoblotting with anti-Lyn antibody (Lyn:blot), and relative amounts of Lyn were densitometrically determined. Lyn was immunoprecipitated with excess anti-Lyn antibody from the cell lysates containing equal amounts of Lyn, and kinase activity in the immunoprecipitates were assayed by 32P incorporation into enolase, followed by autoradiography. Relative specific activity of Lyn (Lyn:spec) was decreased in Csk and mCsk-expressing cells, whereas it was increased in mCsk(−)-expressing cells.

To explore the effects of the overexpression of these Csk-based molecules on the basal activity of Lyn, protein levels of Lyn were first compared. Cells at quiescence were solubilized with the buffer containing 1% Nonidet P-40 and 0.1% deoxycholate, and the lysates were subjected to immunoblotting with anti-Lyn antibody. As shown in Fig. 1 (lane Lyn:blot), the amount of Lyn was reproducibly decreased in mCsk(−) cells as compared with that in parent cells. Lyn was immunoprecipitated from each volume of the cell lysate containing an equal amount of Lyn, and the relative specific activity of Lyn toward enolase was compared. As shown in Fig. 1 (lane Lyn:spec), the specific activity of Lyn was decreased in Csk cells and further decreased in mCsk cells while it was increased in mCsk(−) cells. Decreased abundance of Src family PTKs as seen in mCsk(−) cells has also been noted in Csknull cells (29, 38), where the C-terminal regulatory tyrosine in Src PTKs are underphosphorylated. Although precise mechanisms of the altered Lyn levels in mCsk(−) cells remain to be elucidated, it could be ascribed to the shortened half-lives of Src family PTKs whose C-terminal tyrosine is dephosphorylated (39, 40). The decreased protein level, along with the increased specific activity of Lyn in mCsk(−) cells, strongly indicated that mCsk(−) interfered with intrinsic Csk activity as a dominant negative molecule.

Csk-mediated Changes in the Kinetics of Protein Tyrosine Phosphorylation—As an initial experiment, these cell lines were sensitized with a saturating concentration of anti-DNP IgE (1 μg/ml) (41), stimulated with various concentrations of DNP-BSA (1–1,000 ng/ml) for 30 min, and released histamine was measured. Contrary to the expectation that Csk and mCsk might inhibit histamine release, all the clones responded well to these concentrations of antigen after the long incubation periods, with an optimal concentration of 100 ng/ml (not shown, and see Fig. 6B). These findings suggest that Csk or mCsk does not substantially suppress the overall cell activation. To further assess the influence of these molecules, we followed the temporal patterns of FceRI-mediated signaling under the optimal stimulation conditions (1 μg/ml anti-DNP IgE, and 100 ng/ml DNP-BSA).

FceRI-mediated changes in the tyrosine phosphorylation of cellular proteins are shown in Fig. 2A. Under the quiescent conditions, two sets of doublets with molecular masses of
around 55 kDa (identified as two isoforms of Lyn (not shown)) and 180 kDa (not identified in this study) were already tyrosyl phosphorylated in all the clones, and tyrosyl phosphorylation of other proteins was minimal. After the triggering of FcRI, multiple proteins were tyrosyl phosphorylated in all the clones, but their temporal patterns were different. Among the tyrosyl-phosphorylated proteins, those around 70 kDa (indicated by arrows) gave strongest signals in all the clones. In the wild-type cells, the signal became rapidly detectable within 30 s, peaked at around 5 min, and then declined. The time span of the signal was delayed in Csk-expressing cells (peaked at around 10–20 min) and was further delayed in mCsk-expressing cells (peaked at around 30–60 min) (Fig. 2A). In mCsk(−) expressing cells, it was as rapidly detectable as in the wild-type cells, and, intriguingly, its duration was markedly prolonged (Fig. 2A). The time-dependent changes in the intensity of the 70-kDa phosphoproteins were densitometrically determined in the two sets of clones (WT, Csk 5 and 9, mCsk 1 and 3, and mCsk(−) 1 and 3), and the average was depicted in the graph (Fig. 2B). The stepwisely delayed responses in Csk- and mCsk-expressing cells and the rapid response in mCsk(−)-expressing cells clearly indicated that Csk negatively regulates the initiation of FcεRI-mediated protein tyrosine phosphorylation in a manner dependent on its catalytic activity. In addition, the prolonged response in mCsk(−)-expressing cells suggested that the dominant negative Csk delayed the termination of the signal.

Csks-mediated Changes in the Kinetics of FcεRI-mediated Lyn Activation—To examine if the Csk-based molecules altered cell signaling by directly affecting Lyn activation, time-dependent changes in the Lyn activity were followed. Lyn was immunoprecipitated from the cell lysate at each time after FcεRI stimulation, and kinase activity in the immunoprecipitate was asssayed using an external substrate, enolase. The reaction mixtures were subjected to SDS-polyacrylamide gel electrophoresis, and radioactive bands were visualized using Fuji image analyzer (FUJI BAS 3000). Note that the exposure of each gel was arbitrarily set so that the signal intensities at basal (unstimulated) states became almost comparable in the four clones. Lyn activation followed complex and oscillatory time courses. Overexpression of Csk delayed and that of mCsk further delayed the onset of Lyn activation. In mCsk(−)-expressing cells, Lyn activation was rapid and the most sustained. These data are representative of three independent experiments using Csk 5, mCsk 3, and mCsk(−) 3 clones. The results were reproducible in the other set of clones (Csk 9, mCsk 1, and mCsk(−) 1).

FIG. 3. Time-dependent changes in the kinase activity of Lyn in RBL2H3 cell lines overexpressing Csk and its mutants. Cell lysate (400 µg of protein) was prepared from the cell lines at each time after FcεRI triggering and immunoprecipitated with anti-Lyn polyclonal antibody (Santa Cruz) with the aid of protein G-Sepharose (Pharmacia Biotech Inc.). Kinase activity in the immunoprecipitates was measured using an external substrate, enolase. The reaction mixtures were subjected to SDS-polyacrylamide gel electrophoresis, and radioactive bands were visualized using Fuji image analyzer (FUJI BAS 3000). Note that the exposure of each gel was arbitrarily set so that the signal intensities at basal (unstimulated) states became almost comparable in the four clones. Lyn activation followed complex and oscillatory time courses. Overexpression of Csk delayed and that of mCsk further delayed the onset of Lyn activation. In mCsk(−)-expressing cells, Lyn activation was rapid and the most sustained. These data are representative of three independent experiments using Csk 5, mCsk 3, and mCsk(−) 3 clones. The results were reproducible in the other set of clones (Csk 9, mCsk 1, and mCsk(−) 1).

activation in the four kinds of clones. Syk was immunoprecipitated at each time after FcεRI stimulation, and the extent of tyrosyl phosphorylation of Syk was determined by immunoblotting with anti-phosphotyrosine antibody. Erk 2 activation was assessed by detecting a slowly migrating band of Erk 2 in SDS-polyacrylamide gel electrophoresis, which corresponds to the activated form of Erk 2 (33, 46). As seen in Fig. 4, these signals also followed the variously altered temporal patterns found in Lyn activation and in protein tyrosine phosphorylation. In the wild-type cells, tyrosyl phosphorylation of Syk was detected within 1 min, peaked at around 5–10 min, and then declined (Fig. 4A). Syk phosphorylation was stepwisely delayed in Csk-expressing cells (detected at 3 min, peaked at 30 min) and in mCsk-expressing cells (detected at 10 min, peaked at 60 min) (Fig. 4A). In mCsk(−)-expressing cells, it was detectable at 1 min and peaked at around 5–10 min, and the peak intensity was maintained for 120 min (Fig. 4A). Recovery of Syk protein in the immunoprecipitate was not changed during the incubation periods (not shown). As shown in Fig. 4B, the appearance of the activated Erk 2 (indicated as pp42), which exhibited retarded electrophoretic mobility as compared with the nonactivated form (indicated as p42), was stepwisely delayed in Csk-expressing cells (detected at 5 min) and in mCsk-expressing cells (detected at 10 min) as compared with that in the wild-type cells (detected at 3 min). In mCsk(−)-expressing cells, pp42 is slightly visible at quiescence, probably due to the increased basal Lyn activity in the cells, and the triggering-dependent appearance of pp42 was rapid (detected at 3 min)
and most sustained. These temporal alterations were reproducible in two independent sets of clones.

Csk-mediated Changes in the Kinetics of Intracellular Calcium Elevation and Histamine Release—FceRI-mediated spatial and temporal changes in \([\text{Ca}^{2+}]_i\) was next compared. Time-dependent changes in the spatial distribution of \([\text{Ca}^{2+}]_i\) were shown as pseudocolor images (Fig. 5, top squares in each panel). Real time recordings of changes in \([\text{Ca}^{2+}]_i\) in three representative subcellular areas (depicted as white squares in the ratio images) are shown in the bottom graphs. The time of antigen addition is indicated by arrows. Initial \([\text{Ca}^{2+}]_i\) spikes were delayed in Csk-expressing cells and were further delayed in mCsk-expressing cells. The initial responses in these cells were followed by long lasting \([\text{Ca}^{2+}]_i\) oscillations. In wild-type cells and in mCsk(-)-expressing cells, \([\text{Ca}^{2+}]_i\) was as rapidly elevated after FceRI stimulation and promptly subsided. These results are representative of four independent experiments using the two sets of clones (Csk 5 and 9, mCsk 1 and 3, and mCsk(-) 1 and 3).
were 4.0 ± 1.1 min (mean ± S.D. of 16 traces, 8 for Csk 5, and 8 for Csk 9) and 12.0 ± 3.4 min (mean ± S.D. of 16 traces, 8 for mCsk 1 and 8 for mCsk 3), respectively. Unexpectedly, the delayed initial [Ca\textsuperscript{2+}]\textsuperscript{i} rises were followed by quite long lasting repetitive calcium spikes (Fig. 5, Csk and mCsk), which usually lasted for more than 30 min. In mCsk(−)-expressing cells, [Ca\textsuperscript{2+}]\textsuperscript{i} elevation was rapidly detected within 1 min, and despite the most sustained Lyn activation/Syk phosphorylation in these cells, it was as rapidly subsided as that in the wild-type cells. These results were reproducible in the two independent sets of clones. These findings indicate that the initial rises in [Ca\textsuperscript{2+}]\textsuperscript{i} followed the onset of Lyn activation but that the duration of the calcium signal was not solely regulated by Lyn activity. As shown in Fig. 6, temporal patterns of histamine release correlated well with those of [Ca\textsuperscript{2+}]\textsuperscript{i} elevation. Histamine release from the wild-type cells and that from mCsk(−)-expressing cells followed almost identical time courses. It became rapidly detectable within 2 min, the rate of release was gradually slowed down, and it almost reached plateau levels at around 10–20 min (Fig. 6). In Csk- and mCsk-expressing cells, the onset of histamine release was stepwisely delayed, the rate of release was gradually accelerated, and it lasted even after 20 min (Fig. 6).

DISCUSSION

This study was undertaken to explore the roles of Csk in Fc\textsubscript{e}RI signaling. To this end, we prepared RBL2H3 cell lines overexpressing Csk, a membrane-targeted form of Csk (mCsk) and a kinase-defective mCsk (mCsk(−)). We first showed that specific activity of Lyn at basal states was decreased by Csk overexpression and further decreased by mCsk overexpression. These findings suggest that a part of the C-terminal regulatory tyrosine of Lyn is dephosphorylated in RBL2H3 cells at quiescent, as observed in DT40 B cell line (39, 40), and have confirmed that mCsk is a gain of function mutant of Csk (31, 32). In mCsk(−)-expressing cells, the basal specific activity of Lyn was increased, thereby indicating that mCsk(−) functioned as a dominant negative molecule. In mCsk(−) cells, protein levels of Lyn were found to be significantly decreased (see Fig. 1). Although the precise mechanisms of the altered protein levels remain to be elucidated, these observations are at least consistent with the notion that the half-lives of Src family PTKs are shortened when their C-terminal tyrosines are dephosphorylated (39, 40).

We next showed that these cell lines with varying Csk activities exhibited critically altered temporal patterns of Fc\textsubscript{e}RI-mediated signaling. First, overexpression of Csk delayed and that of mCsk further delayed Fc\textsubscript{e}RI-mediated Lyn activation. The relative effects of Csk and mCsk were correlated with their abilities to suppress basal specific activity of Lyn, and the initial Lyn activation was as rapidly observed in mCsk(−) cells as in parent cells. Thus, the delayed responses in Csk and mCsk-expressing cells do not seem to be due to nonspecific effects of overexpression, such that these molecules compete for β-ITAM with Lyn, but rather due to the augmented catalytic activity of Csk. Although substrates for Csk are not strictly confined to Src family PTKs (47), the most probable explanation for the delayed responses are that Csk and mCsk exerted these effects by increasing the phosphorylation levels of the C-terminal tyrosine of the Src family PTKs. Csk might delay the initial Lyn activation by decreasing the probability of open conformation of Lyn, which could readily participate in ITAM phosphorylation, or by actively rephosphorylating the free C-terminal tyrosine in once activated Lyn (48). In either case, Csk activity appears to be critically involved in the initiation of Fc\textsubscript{e}RI-mediated Lyn activation. Second, overexpression of mCsk(−) did not delay the onset of Lyn activation but did significantly prolong its duration. In light of the dominant negative effect of mCsk(−), these findings suggest that Csk activity participates in the termination of Fc\textsubscript{e}RI-mediated Lyn activation. It is not likely that the prolonged Lyn activation is simply a consequence of higher peak activity of Lyn in these cells since the peak activity was kept at a fairly constant level. Possible roles of Csk in the feedback inhibition of Src PTKs have also been suggested in other experimental systems (25, 49–52). For instance, integrin-mediated activation and inactivation of e-Src are chronologically correlated with dephosphorylation and phosphorylation of the C-terminal tyrosine, respectively (25). Csk is recruited to membrane in the vicinity of Src PTKs through the association with several tyrosyl-phosphorylated membrane proteins, such as paxillin and IRS-I (49–52). Csk co-localizes with Src in the membrane podosome like structures in v-Src-expressing fibroblasts (52). These observations have raised the possibility that Csk regulates, at least in part, the feedback inhibition of the signals mediated by Src family PTKs, presumably through the changes in its localization. These possibilities are now further investigated by using immunocytochemical techniques.

As a consequence of variously altered Csk activity, we obtained RBL2H3 cell lines exhibiting four different kinetics of Lyn activation. It was (i) rapid and transient in parent cells, (ii)
delayed in Csk-expressing cells, (iii) further delayed in mCsk-expressing cells, and (iv) rapid and most sustained in mCsk(−) expressing cells (schematically depicted in Fig. 7). These cell lines provided a novel experimental system to test the chronological correlations between Lyn activation and the other signals in the context of mast cells. We showed that the onsets of tyrosyl phosphorylation of cellular proteins including Syk, Erk 2 activation, [Ca^{2+}]i elevation, and granule release all followed Lyn activation (Fig. 7). Preliminary experiments showed that co-expression of an active human Lyn A mutant (Tyr-508 is replaced with Phe) in mCsk cells rescued the delayed calcium signaling.2 These observations support the notion that Src family PTKs regulate the onsets of the series of events and that Csk and mCsk exerted those negative influences through the inhibition of Lyn activity. Furthermore, the durations of tyrosyl phosphorylation of Syk, and Erk 2 activation were most prolonged in mCsk(−)-expressing cells (Fig. 7). Those findings provide strong evidence that Syk phosphorylation and Erk 2 activation are under the control of Lyn activity.

In contrast to these biochemical signals, the terminations of [Ca^{2+}]i elevation and histamine release did not correlate with those of Lyn activation. First, [Ca^{2+}]i elevation in mCsk(−)-expressing cells was as rapidly subsided as in parent RBL2H3 cells, despite the most sustained biochemical signals in the cells. Secondly, the delayed initial [Ca^{2+}]i rises in Csk and mCsk-expressing cells were followed by disproportionately long lasting [Ca^{2+}]i oscillations (see Figs. 5 and 7). Those observations have revealed that the duration of [Ca^{2+}]i elevation is not solely controlled by elevated Lyn activity but is mainly regulated by other desensitization mechanisms. Although the mechanisms of the differential desensitization should be investigated by further studies, similar observations in TCR (53), and in unrelated G protein-coupled receptors (54), may provide a clue to explain these situations. In CD45null T lymphocytes, in mast cells, and in unrelated G protein-coupled receptors (54), may provide strong evidence that Syk phosphorylation and Erk 2 activations are under the control of Lyn activity.

In summary, we show here that Csk is a negative regulator of basal Lyn activity, as well as initial coupling of FcεRI with Lyn, and that Csk activity is also potentially required for the termination of the FcεRI-mediated Lyn activation. Temporal comparison of FcεRI signals in parent cells and in the clonal cells overexpressing Csk, mCsk, and mCsk(−) provided further evidence that Src family PTKs are upstream regulators of the initiation of the series of events leading to granule release. However, the terminations of the signals appeared to be regulated by Src family PTK-dependent and independent pathways. In addition, these cell lines with varying Csk activities would provide a unique experimental system for analyzing the roles of Src family PTKs in the other pro-inflammatory receptors, such as G protein-coupled receptors (58, 59) or integrins (25).
