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Inducible T Cell Tyrosine Kinase Regulates Actin-Dependent Cytoskeletal Events Induced by the T Cell Antigen Receptor

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The tec family kinase, inducible T cell tyrosine kinase (Itk), is critical for both development and activation of T lymphocytes. We have found that Itk regulates TCR/CD3-induced actin-dependent cytoskeletal events. Expression of Src homology (SH) 2 domain mutant Itk transgenes into Jurkat T cells inhibits these events. Furthermore, Itk−/− murine T cells display significant defects in TCR/CD3-induced actin polymerization. In addition, Jurkat cells deficient in linker for activation of T cells expression, an adaptor critical for Itk activation, display impaired cytoskeletal events and expression of SH3 mutant Itk transgenes reconstitutes this impairment. Interestingly, expression of an Itk kinase-dead mutant transgene into Jurkat cells has no effect on cytoskeletal events. Collectively, these data suggest that Itk regulates TCR/CD3-induced actin-dependent cytoskeletal events, possibly in a kinase-independent fashion. *The Journal of Immunology, 2003, 170: 3971–3976.

The activation of T lymphocytes through the Ag receptor/CD3 molecular complex (TCR/CD3) is regulated by several protein tyrosine kinases, one of which is the inducible T cell tyrosine kinase (Itk). Itk is a protein of 72 kDa whose expression is restricted to T lymphocytes, NK, and mast cells (1, 2). Itk-deficient mice display decreased numbers of mature thymocytes, reduced levels of phospholipase Cγ1 activation, inability to open membrane Ca2+ channels, suppressed cytokine production, reduced proliferative responses, and impaired resistance to infection (3–6).

Itk is organized in discrete modular domains, which include Src homology (SH) 2 (SH2), SH3, and kinase domains, as well as a pleckstrin homology (PH) domain (1, 2). Previous studies from our laboratory have demonstrated that upon anti-CD3ε or anti-CD28 Ab-mediated cross-linking, Itk colocalizes with TCR/CD3 and CD28, respectively, in a manner dependent on the PH domain of Itk (7). Furthermore, for its inducible activation Itk must associate with the linker for activation of T cells (LAT) in an interaction dependent on the SH2 domain of Itk (8).

The formation of distinct clusters of signaling molecules localized at the T cell-APC contact site, known as the immunological synapse, is a process dependent on the reorganization and polymerization of the actin cytoskeleton and it is believed to be critical for the activation of T cells through the TCR-CD3 complex (9, 10). However, the mechanism(s) that regulates TCR/CD3-induced actin-dependent events is not clearly understood. In the present investigation, we provide evidence suggesting that Itk is involved in the regulation of TCR/CD3-induced cytoskeletal events and actin polymerization.

Materials and Methods

Mice, Itk constructs, cells, Abs, and other reagents

The Itk−/− mice have been previously described (3). They were provided by Dr. D. Littman (New York University School of Medicine, New York, NY) through Dr. T. Kawakami (La Jolla Institute for Allergy and Immunology, La Jolla, CA) and bred in our facility. Itk and LAT constructs, the Jurkat cell lines and culture conditions, as well as the Abs used in this study have been previously described (7, 8, 11). Itk constructs were tagged with a peptide sequence (aa 319–333) of the HIV gp120 protein. Recombinant Itk proteins were immunoprecipitated with the mAb H902 that is specific for the gp120 tag (National Institutes of Health AIDS Research and Reference Reagent Program, Rockville, MD). Expression of Itk transgenes was 2- to 3-fold of endogenous protein, as assessed by Western blotting analysis.

Bead endocytosis

Jurkat cells, 1 × 106 in 0.5 ml of RPMI 1640, expressing Itk- or LAT-green fluorescent protein (GFP) transgenes or GFP alone were incubated with 2 × 106 latex beads (6-μm diameter) that had been precoated either with anti-human CD3ε Ab OKT3 or isotype control (UPC10) Ab (10 μg/106 beads). Cells and beads were centrifuged (100 × g) and incubated for 7 min at 37°C (optimal as assessed by time kinetics). The cells were then fixed on slides (3.7% paraformaldehyde at room temperature for 30 min) and analyzed under a Zeiss epifluorescence microscope (Zeiss, Oberkochen, Germany). Positive cells were those GFP (transgene)-expressing cells extending protrusions around the beads as to endocytose them (for example, see Fig. 1A). For each transfected cell line, multiple fields were visualized from which several (30–100 depending on the experimental group) cell-bead conjugates were assessed.

Actin polymerization index

Jurkat cells that had been transfected with Itk-GFP were treated as above with the difference that incubation was performed only for 5 min at 37°C because this minimized extension of cytoplasmic protrusions around the beads, but still allowed for optimal actin polarization at the cell-bead interface. The cells were then permeabilized in PBS/1% BSA containing 0.05% saponin (30 min at 4°C). Then, phalloidin-tetramethylrhodamine isothiocyanate (TRITC, 100 ng/ml; Sigma-Aldrich, St. Louis, MO) was added and cells were incubated for 30 min at 4°C in the dark. After washing, samples were fixed on slides as described above and analyzed on a laser scanning confocal microscope (Leica TCS SP2; Leica, Heidelberg, Germany). Images were acquired and analyzed with the Leica confocal...
software. To determine the actin polymerization index, a transect was drawn across each cell-bead conjugate that included the cell-bead contact site and the portion of the cell membrane diametrically opposite to the contact site. After compensation of signal intensity to minimize spectral overlap, pixel intensity representing phalloidin binding was quantified within an equal area both at the contact and opposite sites using the Leica confocal software. The actin polymerization index represents the ratio of pixel intensity at the contact site to the pixel intensity of an equal area on the opposite site.

Actin polymerization indices were also assessed and compared between splenocytes from Itk+/− and strain-, sex-, and age-matched control mice in a manner similar to that described above for Jurkat cells with the difference that beads coated with the anti-murine CD3ε Ab 2C11 (BD PharMingen, San Diego, CA) were used, incubation of cells and beads was for 10 min at 37 °C (determined to be optimal by time kinetic analysis), and phalloidin-TRITC concentration was at 50 ng/ml.

**Quantification of actin polymerization by flow cytometry**

Splenic cells from Itk−/− or strain-, sex-, and age-matched control mice were stimulated at 37 °C with Ab 2C11 followed by cross-linking with FITC-conjugated goat anti-armenian hamster secondary Ab (Jackson Immunoresearch, West Grove, PA) for various periods of time. The cells were permeabilized and stained with phalloidin-TRITC (50 ng/ml) as described above and then analyzed on a FACSCalibur flow cytometer (BD Immunocytometry Systems, San Jose, CA). Using appropriate controls, signal intensity was compensated to minimize spectral overlap and then FITC-positive cells (Ab reactive), defined by an electronic gate, were analyzed for red fluorescence (phalloidin-TRITC). The data are expressed as fold increase in actin polymerization that was calculated by the ratio of median fluorescence intensity at each time point to the nonstimulated zero point.

**Immunoprecipitation and Western blotting**

These were performed as previously described (7).

**Results**

**SH2 mutant Itk interferes with TCR/CD3-induced cytoskeletal events**

Previous observations from our laboratory had indicated that a mutation, R265K, in the SH2 domain of Itk interferes with the inducible SH2-mediated interaction of Itk with tyrosine-phosphorylated LAT (8). In addition, this mutation also inhibits the TCR/CD3-induced transphosphorylation and enzymatic activation of Itk (8). We noticed that overexpression of this Itk mutant into Jurkat T cells interferes with the capping of the TCR-CD3 complex upon cross-linking with anti-CD3ε Abs (data not shown). Since capping of the T cell Ag receptor depends on the polymerization of the actin cytoskeleton (12), we further investigated the role of Itk in this phenomenon. To this end, we used a conjugate formation assay previously reported by Lowin-Kropf et al. (13), where incubation of Jurkat cells with anti-TCR/CD3 Ab-coated latex beads induces cytoskeletal changes at the bead-cell attachment site such as reorientation of the microtubule-organizing center (MTOC) and localized actin polymerization. When Jurkat cells are incubated with OKT3-coated latex beads, they extend cytoplasmic processes around the beads as to endocytose them (see Ref. 14 and Fig. 1A, left panel). This action is dependent on the reorganization and polymerization of the actin cytoskeleton (14). In sharp contrast, Jurkat cells incubated with isotype control Ab-coated beads do not extend cytoplasmic protrusions around the beads (Fig. 1A). It should be noted that in these experiments Jurkat cells have been transfected with wild type (WT)-Itk-GFP. Using this bead endocytosis assay, we quantified the effects that expression of the R265K SH2 mutant of Itk has on TCR/CD3-induced cytoskeletal events. Jurkat cells expressing equal amounts (Fig. 1B) of transiently transfected GFP-chimeric constructs of either WT- or R265K-Itk were incubated with latex beads that were coated either with OKT3 or isotype control Abs. The presence of GFP does not interfere with the activity of Itk (7, 8). After an optimal incubation period (7 min), determined by time kinetic analysis, the cells were fixed on slides and analyzed by fluorescence microscopy for GFP-positive cells that extended protrusions around the beads. Eighty-six percent of Jurkat cells transfected with WT-Itk extended protrusions around the beads (Fig. 1C). In contrast, 29% of Jurkat-expressing R265K-Itk displayed bead endocytosis (Fig. 1C), a statistically significant reduction (p < 0.05, Student’s t test). The percentage of bead endocytosis displayed by WT-Itk transfectants was similar to that seen with nontransfected Jurkat (81 ± 8% or Jurkat transfected with GFP alone (84 ± 9%). Thus, the reduction in bead endocytosis is specific to R265K-Itk expression and not due to nonspecific overexpression of the protein. This defect cannot be explained by delayed time kinetics because the percentage of bead endocytosis displayed by the SH2 mutant transfectants is not altered at longer incubation times (up to 30 min). Furthermore, Jurkat cells transfected with an SH2 deletion (ASH2) mutant of Itk display a similarly reduced ability in bead endocytosis (data not shown).

When K390R-Itk is expressed in Jurkat cells at levels equivalent to those of R265K and WT-Itk (Fig. 1B), there is no effect on
TCR/CD3-mediated cytoskeletal events (Fig. 1C). This is interesting, since the K390R protein carries a point mutation in the SH1 domain that inactivates the enzymatic activity of Itk (8).

Using the same bead conjugate assay, Lowin-Kropf et al. (13) found that the src tyrosine kinase, Lck, is important for the TCR/CD3-induced cytoskeletal events, as Jurkat mutants (JCaM1.6) lacking expression of Lck were deficient in both MTOC reorientation and localized actin polymerization. Comparison of the behavior of JCaM1.6 to R265K transfectants in the bead endocytosis assay reveals that both cell types are similarly deficient in TCR/CD3-induced actin events (Fig. 1C). The small difference seen is not statistically significant ($p > 0.05$, Student’s $t$ test).

**SH2 mutant Itk interferes with TCR/CD3-induced actin polymerization**

The extension of cytoplasmic protrusions around the OKT3-coated beads depends on localized actin polymerization at the bead-cell attachment site (13). To further explore the effects of SH2 mutant Itk on TCR/CD3-induced cytoskeletal events, we used the bead-conjugate assay to quantify the localized actin polymerization at the contact sites between Jurkat cells and latex beads. To this end, Jurkat cells expressing equal amounts of GFP-R265K Itk or GFP-WT-Itk (Fig. 2B) were incubated with latex beads that had been coated with either OKT3 or isotype control Abs. Incubation was conducted for 5 min, as this minimized extension of cytoplasmic protrusions around the beads, but still allowed for optimal actin polymerization at the cell-bead interface. Following incubation, the cells were permeabilized, stained with TRITC-phalloidin to visualize polymerized actin, and then analyzed by confocal microscopy. Actin polymerization at the cell-bead contact sites was visualized by pseudocoloring (Fig. 2A). The degree of polymerization relates to the color scale shown on top of Fig. 2A, with blue representing the highest and red the lowest intensities, respectively. Pixel intensity at the GFP (transgene)-positive cell-bead contact sites were quantified and expressed as actin polymerization index as described in Materials and Methods. Jurkat cells expressing WT-Itk transgene display a 2-fold increase in the actin polymerization index when stimulated with OKT3-coated beads as compared with isotype control Ab-coated beads (Fig. 2C). This difference is statistically significant ($p < 0.05$, Student’s $t$ test). Similar results were obtained with nontransfected Jurkat cells (data not shown). Importantly, when cells expressing the R265K mutant are stimulated with OKT3-coated beads, they display a very small increase in the actin polymerization index (Fig. 2C) as compared with stimulation with isotype control Ab-coated beads. This small increase is barely significant ($p = 0.04$, Student’s $t$ test). Consistent with the data shown in Fig. 1C, expression of the kinase-dead K390R-Itk mutant has no effect on the actin polymerization index (Fig. 2C).

**FIGURE 2.** Effects of Itk mutants on TCR/CD3-induced actin polymerization. A, Jurkat cells transiently transfected with murine WT-Itk-GFP were incubated (5 min, 37°C) with latex beads coated with either OKT3 (top panels) or UPC10 control (bottom panels) Abs. Cells were then permeabilized, stained with phalloidin-TRITC, and analyzed by laser scanning confocal microscopy. Results are from one of seven replicate experiments. Two representative cell-bead conjugates are shown out of at least 30 analyzed per condition. Results are displayed as phalloidin fluorescence intensity visualized by pseudocoloring (left panels) and differential interference contrast images (right panels). Intensity correlates to the color scale on the top. Beads are marked by asterisks. B, Jurkat cells transiently transfected with Itk-GFP transgenes as indicated were analyzed as described in Fig. 1B. C, Jurkat cells transiently transfected with Itk-GFP transgenes were incubated with OKT3 or UPC10 control Ab-coated beads as indicated and analyzed as described in A. Results are displayed as the actin polymerization index calculated as described in Materials and Methods. Results are averages ($\pm$SEM) of seven replicate experiments per condition.
Itk-deficient mice are defective in TCR/CD3-mediated actin polymerization

The above data suggest that Itk may regulate TCR/CD3-induced, actin-dependent cytoskeletal events. If this is correct, then T cells lacking expression of Itk should display deficiencies in actin polymerization induced through their Ag receptors. To assess this, we used a previously described method (15, 16) to compare TCR/CD3-induced actin polymerization in splenic T cells from Itk-deficient (Itk−/−) mice to age- and sex-matched controls (WT). Cells were stimulated with anti-murine CD3e Ab 2C11 followed by cross-linking with FITC-conjugated secondary Ab for various periods of time, and after staining with phalloidin-TRITC they were analyzed by flow cytometry as described in Materials and Methods. Within 5 min of stimulation, significant differences (p < 0.05, Student’s t test) in TCR/CD3-induced actin polymerization were seen between T cells of Itk−/− and control mice (Fig. 3A). T cells from Itk-deficient animals displayed 30–40% lower TCR/CD3-induced actin polymerization through the 5- to 30-min time points (Fig. 3A). These differences are statistically significant (p < 0.05, Student’s t test).

Even though splenic T cells from Itk−/− mice display significantly lower TCR/CD3-induced actin polymerization, they are not totally devoid of it. This, most likely, is due to the fact that in the experiments of Fig. 3A we measure total actin polymerization. To further extend these experiments, we used the bead conjugate assay and quantified localized actin polymerization at the cell-bead interface as described above for Jurkat cells. In contrast to splenic T cells from WT mice that display a significant increase (p < 0.05, Student’s t test) in the actin polymerization index, T cells from Itk−/− mice displayed no significant (p > 0.05) increase (Fig. 3B). The increase seen with T cells from WT mice is specific to TCR/CD3-induced stimulation, as incubation with beads coated with Abs to H-2Kb did not cause a significant increase in the actin polymerization index (data not shown). These data confirm that splenic T cells from Itk−/− mice have defective TCR/CD3-induced actin polymerization. The actin polymerization index increases seen with splenocytes were smaller than those with Jurkat cells (see Fig. 2C), probably due to the smaller cell sizes and areas of contact between splenic T cells and beads.

**FIGURE 3.** Itk−/− mice display deficient TCR/CD3-induced actin polymerization. A, Spleen cells from either Itk−/− (●) or control WT mice (□) were stimulated with Ab 2C11 followed by cross-linking with FITC-conjugated secondary Ab for various periods of time as indicated. The cells were then stained with phalloidin-TRITC, analyzed by flow cytometry, and fold increase in actin polymerization calculated as described in Materials and Methods. The results are averages (±SEM) of five replicate experiments using different mice. B, Spleen cells from either Itk−/− or WT control mice were incubated with latex beads that were coated either with 2C11 or isotype control (C) Ab. Experiments were performed in a fashion identical to the one described in the legend of Fig. 2, with the exception that incubation of cells and beads was for 10 min at 37°C. Results are cumulative of three experiments using different mice. ○, Different cell-bead conjugates analyzed in the three experiments. WT splenocytes stimulated with 2C11-coated beads; n = 38 for WT splenocytes stimulated with isotype control Ab-coated beads; n = 49 for Itk−/− splenocytes stimulated with 2C11-coated beads; and n = 57 for Itk−/− splenocytes stimulated with isotype control Ab-coated beads. The horizontal lines represent the average actin polymerization index for each group.

Itk reconstitutes TCR/CD3-induced actin-dependent events in LAT-negative cells

We and others have previously demonstrated that Itk fails to become activated upon TCR/CD3 engagement of Jurkat cells deficient in the expression of LAT (8, 17). Therefore, we reasoned that these cells should be deficient in actin-mediated events. In fact, we find that LAT-deficient JCaM2.5 cells fail to extend cytoplasmic protrusions around OKT3-coated latex beads (GFP-transfected group in Fig. 4). This defect in LAT-negative cells has been also independently demonstrated by Bunnell et al. (18), who used an

**FIGURE 4.** SH3 mutants of Itk reconstitute actin polymerization-deficient Jurkat cells. LAT-deficient JCaM2.5 cells were transiently transfected with the indicated GFP chimeric constructs. Cells were then stimulated with either UPC10- or OKT3-coated latex beads as indicated and the percentages of cells extending cytoplasmic protrusions around the beads were assessed as described in the legend of Fig. 1. Results are averages (±SEM) of four replicate experiments. Inset, Typical example of Itk transgene expression assessed by immunoprecipitation and Western blotting analyses.
anti-TCR-coated coverslip assay to assess Ag receptor-induced actin-dependent cytoskeletal events. Evidence that the lack of LAT expression is responsible for this deficiency displayed by JCaM2.5 cells is the fact that reconstitution with the LAT transgene renders JCaM2.5 cells capable of mediating a significant (p < 0.05, Student’s t test) degree of endocytosis of OKT3-coated beads as compared with control Ab-coated beads (Fig. 4).

To test whether Itk could reconstitute this deficiency in LAT-negative cells, we transfected either WT-Itk or an SH3 domain mutant (W208K-Itk) into JCaM2.5 cells. The W208K mutation interferes with the SH3-dependent interaction of Itk with proline-rich motifs of other signaling molecules (19). However, this mutation does not interfere with the TCR/CD3-induced transphosphorylation and enzymatic activation of Itk (8), neither does it affect the inducible colocalization of this mutant with TCR/CD3 (our unpublished observations). Expression of WT-Itk partially reconstitutes the ability of JCaM2.5 cells to endocytose OKT3-coated beads (Fig. 4). Albeit small, this increase is statistically significant (p < 0.05, Student’s t test). Interestingly, expression of W208K-Itk results in significant reconstitution (p < 0.05, Student’s t test) of bead endocytosis at levels equivalent to those seen with the LAT-reconstituted cells (Fig. 4). The fact that the increase in bead endocytosis occurs only when the cells encounter OKT3-coated beads and not control beads indicates that this increase is not a nonspecific phenomenon, but it requires events that are induced by the engagement of the TCR-CD3 complex. The specificity of this phenomenon is indicated by the lack of bead endocytosis in JCaM2.5 cells transfected with the nonrelated protein GFP (Fig. 4).

Discussion

The above data suggest that the tec family kinase Itk plays an important role in TCR/CD3-induced, actin-dependent cytoskeletal events. This has been demonstrated using both the Jurkat cell system and T cells from Itk−/− mice. We have used a previously published (13) conjugate formation assay where Jurkat cells are stimulated with latex beads coated with anti-CD3e Abs and actin-mediated events are measured either by the extension of cytoplasmic protrusions around the beads or by quantification of actin polymerization at the cell-bead interface. We found that overexpression of an SH2-Itk mutant (R265K) inhibits these actin-mediated events through a mechanism that is not well understood. Previous studies in our laboratory have demonstrated that R265K-Itk does not become activated upon Ag receptor engagement (8). The analogous mutation at the SH2 domain of another tec family kinase, Bruton’s tyrosine kinase (Btk), disrupts the ability of this kinase to interact with its cellular targets (20). Furthermore, mutations in this position of Btk have been found in patients with X-linked agammaglobulinemia, suggesting the functional importance of this residue (21). The lack of TCR/CD3-induced activation of R265K-Itk is probably attributable to its inability to interact with LAT (8), as this linker protein is known to be critical for the activation of Itk (8, 17).

For its activation, Itk undergoes two tyrosine phosphorylation events: transphosphorylation by theSrc family kinase Lck followed by autophosphorylation (22). Thus, interaction with LAT could be critical in bringing Itk to the proximity of Lck for subsequent activation. This novel mechanism of LAT-dependent activation of Itk positions this kinase in a pathway critical in the TCR/CD3-induced reorganization and polymerization of actin. Upon TCR/CD3 engagement, the Syk kinase Zap-70 becomes activated and subsequently phosphorylates LAT at multiple tyrosine residues (23). This modification of LAT provides docking sites for many important signaling molecules among which is Itk (8). The signaling complex formed on the LAT platform contains proteins that play critical roles in the reorganization and polymerization of the actin cytoskeleton. Among these are SLP-76, Grb2, Nck, Wiskott-Aldrich syndrome (WASP), and others (14, 24).

Most notably, WASP, through its interaction with the Arp2/3 complex, plays a very important role in actin polymerization (25) and patients carrying mutations in the WASP coding gene display severe immunodeficiency characterized by recurrent infections and hematopoietic malignancies (26). Previously published data indicate that Itk, WASP, and Arp2/3 colocalize with TCR/CD3 upon stimulation (7, 14). Furthermore, Bunnel et al. (27) have provided in vitro evidence that Itk interacts with several of the members of the actin polymerization machinery. Thus, Itk interacts with Grb2 via its proline-rich region and with SLP-76 through a cooperative interaction of its SH2 and SH3 domains (27). The same investigators uncovered an interaction between the Itk-SH3 domain and WASP by screening phage display libraries for optimal Itk-SH3-binding motifs (19). Notably, this interaction was detected under conditions of increased detergent stringency (27). Preliminary experiments from our laboratory indicate that there exists an inducible association between Itk and WASP (our unpublished data). Intriguingly, Labno et al. (28) found no WASP activation in T cells from Itk−/− mice upon stimulation through their Ag receptors, assessed with an Ab that can distinguish between active and inactive WASP. These observations are consistent with the data presented here where T cells from Itk−/− mice display significantly reduced TCR/CD3-mediated actin polymerization.

In view of the above observations, a dominant-negative mechanism could account for the inhibitory properties of R265K-Itk, which could compete with endogenous WT-Itk for binding to a signaling complex, such as the one assembled by phosphorylated LAT, thus inhibiting the formation of this complex or subsequent signaling events critical to TCR/CD3-induced actin polymerization. One of the ways Itk might interact with this complex is through SH3-dependent binding to a polyproline-rich region on WASP (19). Additional interactions of Itk with other members of the complex are also possible.

The Lck-mediated transphosphorylation of Itk as well as its subsequent autophosphorylation play key roles in the activation of this kinase (1). However, additional observations have suggested that through its various domains Itk may interact with other signal transducers and in this fashion become conformationally altered and activated. In particular, the analysis of a fragment of Itk by multidimensional nuclear magnetic resonance reveals an intramolecular association between the SH3 domain and a proline-rich sequence located between the PH and SH3 domains of Itk (28). The authors of this study suggested that this association might regulate the interaction of Itk with its targets/substrates and its subsequent activation (28). Thus, it is possible that the ability of the SH3 mutants of Itk to reconstitute TCR/CD3-induced actin-dependent events in LAT-deficient cells might be due to the disruption of the SH3-polyproline interaction and the conformational alteration of Itk into an active state. However, even if this is the case the participation of the SH3 mutant Itk in actin-mediated events still requires the engagement of the TCR/CD3 for its recruitment to relevant sites (see Fig. 4). It is interesting that comparison of GFP-tagged W208K- and R265K-Itk mutants by confocal microscopy reveals the ability of the former, but not the latter

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29. Donnadieu et al. (29) who used a similar SH1 mutant of Itk and found it to have no effect on Ag receptor-induced cytoskeletal events. Thus, it is possible that either the kinase activity of Itk is not important for the regulation of actin-dependent events or that inactivating it by itself may not be sufficient to affect actin polymerization. Recently, several investigators have reported on effects mediated by different protein kinases in a manner independent of their enzymatic activity. Thus, the IPN-inducible protein kinase PKR can induce NF-κB activation through a mechanism independent of its kinase activity (30), and the p210 BCR-ABL tyrosine kinase can modulate cell-extracellular matrix interactions independent of its catalytic activity (31). Interestingly, however, Woods et al. (32) have reported that a similar kinase-dead mutant of Itk can inhibit TCR/CD3-induced β1-integrin-mediated adhesion to the extracellular matrix when transfected in Jurkat cells. This is an interesting finding because it raises the possibility that Itk may behave differently in “inside-out” signaling pathways. However, additional experiments will be needed to elucidate this possibility.

In conclusion, we have presented evidence that strongly supports an important role for Itk in TCR/CD3-induced cytoskeletal events perhaps in a kinase-independent fashion. Since Itk is known to regulate TCR/CD3-induced intracellular Ca2+ mobilization through its ability to phosphorylate phospholipase Cγ1 (4, 5), the present data support the hypothesis that Itk is an important link between TCR/CD3-mediated Ca2+ mobilization and cytoskeletal reorganization (33).

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