A Guanine Nucleotide Exchange Factor-independent Function of Vav1 in Transcriptional Activation*

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T cell antigen receptor (TCR) stimulation induces the tyrosine phosphorylation of several intracellular proteins including the protooncogene Vav1. Vav1 expression is necessary for normal T cell development and activation. We previously showed that overexpression of Vav1 in Jurkat T cells potentiates the activity of the transcription factor nuclear factor of activated T cells (NF-AT). The mechanism by which Vav1 participates in TCR signaling events is not clear. Vav1 contains a guanine nucleotide exchange factor (GEF) domain that has specificity for Rac and other Rho GTPases that have been recently implicated in T cell activation events. Significantly, in vitro tyrosine phosphorylation of Vav1 by Lck activates its exchange activity. This Lck-mediated phosphorylation of Vav1 has been reported to depend upon Tyr-174 in Vav1, a site implicated in Vav1 function by other studies as well. In this report, we demonstrated that Tyr-174 is not required for the TCR-induced phosphorylation of Vav1 in vivo. Moreover, mutation of Tyr-174 abrogated the ability of Vav1 to stimulate NF-AT activation as well as the Vav1 GEF function leading to Rac activation. However, we also showed that the GEF activity of Vav1 was neither sufficient nor necessary for potentiation of NF-AT, and thereby we identify a GEF-independent role of Vav1 in potentiating NF-AT-driven transcription. Oncogenic Vav1 in which the amino-terminal 67 amino acids were deleted had elevated GEF activity but did not potentiate NF-AT when overexpressed in Jurkat cells. We also showed that a GEF mutant form of Vav1 that had impaired GEF function could still potentiate NF-AT. These studies reveal a previously unrecognized negative regulatory function of Tyr-174 in Vav1 and suggest that domains other than the Vav1 GEF domain contribute to TCR signals leading to NF-AT activation.

Engagement of the T cell antigen receptor (TCR)1 with antigen or with cross-linking antibodies initiates a signaling cascade that leads to activation of the T cell. One of the proximal events triggered by TCR engagement is the activation of protein-tyrosine kinases, which results in the tyrosine phosphorylation of several substrates. Previous work has demonstrated that activation of the Src tyrosine kinase Lck is necessary to initiate the signaling cascade. Lck is required to phosphorylate the cytoplasmic tails of the CD3 complex (γδε) and the ζ chain of the TCR on tyrosines within motifs designated as immunoreceptor tyrosine-based activation motifs (ITAMs). Phosphorylation of the ITAMs provides docking sites for the Src homology-2 (SH2) domains of the Syk family protein-tyrosine kinases, ZAP-70, and Syk. Recruitment of ZAP-70 or Syk to the phosphorylated ITAMs leads to the activation of these protein-tyrosine kinases and subsequent tyrosine phosphorylation of multiple intracellular substrates.

One substrate that is rapidly tyrosine-phosphorylated in response to TCR engagement is the 95-kDa protooncogene Vav1. Vav1 is expressed exclusively in hematopoietic cells. However, a homologous protein, Vav2, is ubiquitously expressed. Vav1 is a multi-domain-containing protein consisting of a calponin homology domain, an acidic domain, a guanine nucleotide exchange factor (GEF) domain, a pleckstrin homology domain, a cysteine-rich (CR) domain, and an SH2 domain surrounded by two Src homology-3 (SH3) domains. Vav1 was initially identified as an oncogene based on its ability to transform fibroblasts in a gene transfer experiment (1). However, the expression, structure and the inducible tyrosine phosphorylation of Vav1 suggests it has an important role in antigen receptor-mediated signal transduction. The importance of Vav1 in lymphocyte development and activation was demonstrated in gene-targeted deletion experiments (2–5). These studies revealed that mice lacking Vav1 display a profound defect in the positive selection of T cells and undergo negative selection inefficiently. Furthermore, the single positive T cells that develop have impaired calcium mobilization, decreased activation of Erk kinases, decreased activation of NFκB, a failure to form TCR caps following activation, a reduced expression of activation marker CD69, and a reduced expression of CD5 (6–8).

The TCR-mediated induction of Vav1 tyrosine phosphorylation may regulate Vav function. Vav1 contains 30 tyrosines. Neither the sites of phosphorylation nor the protein-tyrosine kinases responsible for phosphorylation of specific sites have been definitively established. However, both Syk and Src family tyrosine kinases have been shown to be able to phosphorylate Vav1. Syk and ZAP-70 as well as the Src family kinase Fyn have been implicated in events leading to the phosphorylation of Vav1 in vivo (9–11). Furthermore, we have evidence from Lck-deficient thymocytes that Lck contributes to events leading to the phosphorylation of Vav.2 Moreover, Lck has been shown to directly phosphorylate Vav1 in vitro, specifically at tyrosine 174 (12, 13). In addition, at least two studies have suggested tyrosine 174 to be a good candidate for phosphorylation by Syk and ZAP-70 (10, 14) in vitro.

The tyrosine phosphorylation of Vav1 appears to be impor-

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1 The abbreviations used are: TCR, T cell receptor; GEF, guanine nucleotide exchange factor; CR, cysteine-rich; SH3, Src homology 3; SH2, Src homology 2; NF-AT, nuclear factor of activated T cells; ITAM, immunoreceptor tyrosine activation motif; PAK, p21-activated kinase; mAb, monoclonal antibody; PMA, phorbol 12-myristate 13-acetate.

2 J. Wu and A. Weiss, unpublished observations.
tant in regulating its GEF function. The GEF domain of Vav1 shares considerable homology with that of the oncogene Dbl and is often referred to as a Dbl homology domain. Like Dbl, Vav1 has been shown to catalyze the GDP-GTP exchange for Rho family GTPases in vitro (13, 15). The GEF function of Vav1 appears to exhibit some preference for the Rac GTPase. The Vav1 exchange activity in vitro can be induced by its tyrosine phosphorylation by Lck. Tyr-174 in Vav1 is required for Lck-dependent phosphorylation of Vav1 in vitro (12). Thus, the exchange activity of Vav1 leading to its activation of Rac and other Rho GTPases would appear to depend upon phosphorylation of Tyr-174.

The function of Rac activation in T cells is an area of active investigation. One T-cell receptor-derived signaling event required for T cell activation is induction of the transcription factor nuclear factor of activated T cells (NF-AT). NF-AT plays a critical role in the regulation of many genes including lymphokine genes. NF-AT-directed transcription depends upon Vav1 expression (6, 7) and can be induced by overexpression of Vav1 (16). Moreover, overexpression of Vav1 synergizes with TCR signals and depends upon proximal TCR-signaling machinery. This may reflect a requirement for Vav1 tyrosine phosphorylation. The mechanism by which Vav1 potentiates NF-AT is not understood. However, the effects of its GEF function on Rac or Rho have been implicated based on studies of Vav1-deficient cells (6, 8). Similarly, Rac activation has been implicated in NF-AT-mediated activation in response to FceRI stimulation in mast cells (17). Collectively, these data would suggest the following model. TCR-induced tyrosine phosphorylation of Vav1 leads to activation of its GEF function, which in turn leads to activation of Rac. Rac activation then plays an important, yet ill-defined, role in regulating the activation of NF-AT.

In this report, we test this model by expressing mutants of Vav1. We examine the importance of Tyr-174 in Vav in regulating its ability to influence inducible Vav1 tyrosine phosphorylation, NF-AT activation, and Rac GEF activity. Moreover, we directly test the importance of Vav GEF activation in regulating NF-AT activation in an overexpression system. Surprisingly, our results demonstrate that Tyr-174 is not required for inducible Vav1 phosphorylation, NF-AT activation, or GEF function. Instead, our studies suggest Tyr-174 negatively regulates Vav function. Finally, we show that the ability of Vav1 to activate Rac is neither sufficient nor necessary to stimulate NF-AT-driven transcription.

**EXPERIMENTAL PROCEDURES**

**Cells and Reagents**—The Jurkat T cell line was maintained in RPMI 1640 supplemented with 5% fetal bovine serum, 2 mM glutamine, penicillin, and streptomycin. Cells were stimulated with C305, an anti-TCR-γ chain monoclonal antibody (mAb) (26). The anti-phosphotyrosine and the anti-Myc mAbs were derived from the 4G10 and 9E10 hybridomas, respectively. The NF-AT-luciferase reporter plasmid for luciferase activity (27). For luciferase assays with GEFmtVav, 2 μg of DNA total. 20 μl of lysis buffer. Samples were eluted by heating the column and beads to 95 °C for 5 min, and spinning the eluted samples into the catch tube. Due to the toxicity of the GEFmt form of Vav1 on the cells, the assay was altered slightly by harvesting cells only 6 h after transfection. Eluted samples from immunoprecipitations or from GEF assays were separated by SDS-polyacrylamide gel electrophoresis, and proteins were transferred to Immobilon-P (Millipore) for analysis by Western blotting.

**Transfection, Stimulation, and Luciferase Assay**—For transient transfections, 2 × 107 Jurkat cells in 400 μl of RPMI 1640 were electroporated at 2500V, 960 microfarads with 30 μg of DNA total. 20 μg of the various Vav1 constructs were used to obtain equal protein expression. TCR stimulation for analysis of Vav1 tyrosine phosphorylation or Rac activation involved incubating phosphate-buffered saline washed cells (2 × 107 cells/ml at 37 °C for 15 min followed by the addition of anti-TCR mAb, C305 (1:500). Cells were incubated for 2 min, immediately pelleted, and lysed. For TCR stimulation in luciferase assays, 18 h after transfection cells were either left untreated or were incubated with anti-TCR mAb C305 (1:500) or PMA (25 ng/ml) and ionomycin (1 μM) for 6 h. Cells were then harvested, lysed, and assayed for luciferase activity (27). For luciferase assays with GEFmtVav, 2 μg of a β-galactosidase reporter under the control of the β-actin promoter were co-transfected into the cells. 6 h after transfection, cells were lysed, and β-galactosidase and luciferase activities were measured.

**Preparation of Lysates, Immunoprecipitates, and Western Blotting**—Cells were lysed at 1 × 107 cells/ml in lysis buffer (50 mM Hepes pH 7.4, 2 mM EDTA, 1% Nonident P-40, 150 mM NaCl, 10% glycerol, and protease and phosphatase inhibitors). After a 15 min incubation on ice with intermittent vortexing, samples were clarified by centrifugation at 14,000 rpm for 10 min at 4 °C. For immunoprecipitations, lysates were incubated with primary antibody for 1 h on ice and then protein G-Sepharose beads with continuous mixing for 2 h at 4 °C. Beads were then washed 3 times in 500 μl of lysis buffer. Samples were eluted by resuspending the beads in reducing SDS sample buffer and heated to 95 °C for 5 min. For the GEF assay, lysates were incubated with 2 μg of glutathione S-transferase-PAR70-106 and glutathione-Sepharose in a spin column for 15 min at 4 °C with continuous mixing as reported by Manser et al. (20). After incubation, lysates were spun away from the beads, and the beads were washed 3 times with 500 μl of lysis buffer and then spun away. Rac was eluted from the beads by transferring the bead-containing spin column to a fresh catch tube, resuspending the beads in reducing sample buffer, heating the column and beads to 95 °C for 5 min, and spinning the eluted samples into the catch tube. Due to the toxicity of the GEFmt form of Vav1 on the cells, the assay was altered slightly by harvesting cells only 6 h after transfection. Eluted samples from immunoprecipitations or from GEF assays were separated by SDS-polyacrylamide gel electrophoresis, and proteins were transferred to Immobilon-P (Millipore) for analysis by Western blotting. Membranes were blocked with 3% bovine serum albumin and incubated with mAb 4G10 or blocked with 5% nonfat milk and incubated with 9E10 followed by goat anti-mouse antibody conjugated with horseradish peroxidase. Reactive proteins were subsequently visualized by enhanced chemiluminescence (Amersham Pharmacia Biotech).

**RESULTS**

**Mutation of Tyrosine 174 to Phenylalanine Does Not Eliminate TCR-induced Tyrosine Phosphorylation of Vav1**—Previous studies have suggested that Tyr-174 is required for Lck-mediated Vav1 phosphorylation in vitro (12) and might play an important role in Syk- or ZAP-70-dependent phosphorylation of Vav in vivo (10, 14). To examine the importance of this site in vivo, a point mutation was introduced into the acidic domain of Vav1 (Fig. 1A), changing tyrosine 174 to phenylalanine...
Y174F Vav1 Potentiates NF-AT Activation—Previous studies from our lab and others reveal that overexpression of Vav1 can potentiate the activation of NF-AT in unstimulated as well as in TCR-stimulated Jurkat cells (16, 18, 19). Since Tyr-174 has been shown to be a requisite site for Lck-dependent Vav phosphorylation in vitro and Lck is required for the inducing effects of Vav overexpression on NF-AT activation, we investigated the effect of this mutation on the ability of Vav1 to potentiate NF-AT. Jurkat cells were transfected with a reporter construct containing three NF-AT binding sites from the IL-2 promoter fused to luciferase with either wild type Vav1 (WT Vav1), or Y174F Vav1. Transfectants were either left untreated, stimulated with anti-TCR mAb C305, or stimulated with PMA and ionomycin. Activity is reported as a percentage of the activity produced from stimulation with PMA and ionomycin. The inset Vav1 blot shows expression levels of Vav1 in vector-, WT Vav1-, and Y174F Vav1-transfected cells. This is a representative experiment independently performed five times.

Y174F Vav1 enhances GEF function. Jurkat cells were co-transfected with Myc-tagged Rac and either vector, Myc-tagged wild type Vav1, or Myc-tagged Y174F Vav1 and then either left unstimulated or stimulated for 2 min with C305. Lysates of the transfectants were mixed with beads containing the CD42-Rac interaction and binding domain of p21-activated kinase. A, eluates of the beads were blotted for Myc. Lysates of the transfected cells were blotted with anti-Myc antibody to verify that equivalent levels of Myc-tagged Vav1 proteins (B, top) or Myc-tagged Rac (B, bottom) were expressed. This is a representative experiment independently performed five times.

Dissociation of GEF Activity of Vav1 and NF-AT Potentiation—The observation that the mutation of Tyr-174 resulted in an enhanced GEF activity as well as an increased potentiation of NF-AT activity led us to investigate whether GEF activity is involved in the activation of NF-AT when Vav1 is overexpressed in Jurkat cells. We first reexamined the effects of oncogenic Vav1, which structurally differs from protooncogenic (wild type) Vav1 by lacking the amino-terminal 67 amino acids (21). This amino-terminal truncation of Vav1 was first identified as a result of a gene transfer assay, which revealed its transforming potential when expressed in NIH 3T3 cells (1). Previous work from our lab demonstrated a difference in NF-AT potentiation between wild type Vav1 and oncogenic Vav1 (16). We confirmed here (Fig. 4A) that, although the wild type Vav1 potentiated NF-AT, the oncogenic form of Vav1 did not potentiate NF-AT above the level of the vector transfect
Furthermore, unlike the potentiating effect of the Y174F mutation on the function of protooncogenic Vav1, the Y174F mutation in oncogenic Vav1 did not enhance NF-AT-dependent transcription. These findings indicate that inactivating the putative negative regulatory function of Tyr-174 cannot reverse the lack of function of oncogenic Vav1 in regulating NF-AT activity.

To address whether GEF activity of Vav1 and potentiation of NF-AT are correlated, the GEF activity of oncogenic Vav1 was assayed. The Rac activation assay was performed, as before, using lysates of Jurkat cells transfected with Myc epitope-tagged Rac and either vector, wild type Vav1, Y174F Vav1, or oncogenic Vav1 (Fig. 4C). The cells expressing the oncogenic form of Vav1 demonstrated high levels of GTP-bound Rac in unstimulated Jurkat cells, equivalent to those seen in cells transfected with the Y174F form of Vav1. Oncogenic Vav1 exhibits much greater GEF activity than wild type Vav1 in Jurkat cells, even though this “activated” form of Vav1 failed to up-regulate NF-AT activity. Combined with the results of the NF-AT assay (Fig. 4A), these results indicate that the GEF activity of Vav1 is not sufficient to potentiate NF-AT-driven transcription.

To investigate whether the GEF activity of Vav1 is necessary for NF-AT activation, a series of mutations were introduced to the GEF domain of Vav1 to disrupt its GEF function. Mutations altering amino acids 338–344 from LLLQELV to IIIQDAA (Fig. 4B) and referred to as GEFmtVav1 and then either left unstimulated or TCR-stimulated for 2 min with anti-TCR mAb C305. GEF activity was assayed as described in Fig. 3. Eluates of the beads were blotted for Myc (top). Lysates were blotted for Myc to verify equivalent levels of Myc-tagged Rac expression (bottom). This is a representative experiment independently performed five times. D. Jurkat cells were co-transfected with a reporter construct encoding three NF-AT binding sites from the interleukin 2 promoter fused to luciferase and either vector, wild type Vav1, onco-Vav1, or GEFmtVav1. NF-AT activities of transfectants were normalized to β-galactosidase activity. The inset Vav1 blot shows expression levels of Vav1 in the transfectants. This is a representative experiment independently performed three times.
transfected with vector, wild type Vav1, oncogenic Vav1, or the GEFmt form of Vav1 (Fig. 4C). These results confirmed that GEFmtVav1 does not retain any GEF activity for Rac. The ability of GEFmtVav1 to potentiate NF-AT-dependent transcription was then assayed. Jurkat cells were transfected with the NF-AT reporter construct and co-transfected with either vector, wild type Vav1, oncogenic Vav1, or GEFmtVav1. Due to the observed toxicity of the GEFmtVav1 when expressed in Jurkat cells, the potentiation of NF-AT had to be measured only in unstimulated cells at an earlier time following transfection, at 6 h instead of 18 h following electroporation. For the purpose of normalization, each cell was co-transfected with a reporter construct encoding the constitutively active β-actin promoter fused to β-galactosidase. The reported NF-AT activity was normalized to β-galactosidase. As was seen before, NF-AT activity is enhanced by expression of the wild type form of Vav1, whereas no potentiation of NF-AT was seen with the oncogenic form of Vav1. The GEFmt form of Vav1 potentiated NF-AT activity as well or better than the wild type protein. These results indicate the GEF activity of Vav1 is not necessary for potentiation of NF-AT in Jurkat cells.

**DISCUSSION**

Vav1 contains 30 tyrosine residues and is inducibly tyrosine-phosphorylated in response to TCR stimulation. Recent reports have implicated the phosphorylation of Vav1 in its ability to function as a GEF for Rac. In particular, Tyr-174 has been indicated to be a site for phosphorylation by Lck or by Syk kinases, to bind the SH2 domain of Lck, and to play a role in regulating the GEF activity of Vav1 (10, 12, 13). Moreover, mutation of this single site eliminated the ability of Lck to phosphorylate Vav1 in vitro (12). We had also previously demonstrated that the activating effects of Vav1 depended upon the proximal TCR-regulated signaling machinery, specifically including Lck (16). In light of these reports, we asked whether phosphorylation of this residue in Vav1 regulated 1) the ability of Vav1 to be tyrosine-phosphorylated following TCR stimulation; 2) the transcriptional activity of NF-AT, or 3) its GEF activity in vivo. Our data suggest that Tyr-174 is not the sole site of tyrosine phosphorylation in Vav, and phosphorylation of this site is not required for TCR-induced Vav1 tyrosine phosphorylation. Our transcriptional and Rac GEF studies suggest Tyr-174 is a negative regulatory site of phosphorylation. Quite surprisingly, further studies to examine the role of the GEF function of Vav1 demonstrate that the GEF function of Vav is neither sufficient nor necessary for its NF-AT activating function when overexpressed in the Jurkat T cell system.

Phosphotyrosine blots of Vav1 immunoprecipitates revealed the Y174F mutation did not eliminate TCR-induced tyrosine phosphorylation of Vav1 (Fig. 1). Therefore, we can conclude that Tyr-174 is not the only site of TCR-induced Vav1 tyrosine phosphorylation. This result differs from the in vitro phosphorylation studies by Han et al. (12) and a model proposed by Deckert et al. (10). Those reports examined the in vitro phosphorylation of Vav1 with Lck or Syk and Zap-70 protein-tyrosine kinases, respectively. Han et al. (12) report that the Y174F mutation eliminated all tyrosine phosphorylation of Vav1 when phosphorylated by Lck in vitro. Our report has analyzed the in vitro phosphorylation of Vav1. Deckert et al. (10) suggest that phosphorylation of Tyr-174 by a Syk protein-tyrosine kinase could allow for the recruitment of Lck via an interaction of its SH2 domain with Tyr-174. This would suggest that Tyr-174 might be a requisite initiating phosphorylation event. Our studies demonstrate that the phosphorylation of Tyr-174 is neither the sole site of Vav1 phosphorylation nor is it required for phosphorylation of other sites in response to TCR stimulation. It is likely that protein-tyrosine kinases other than those used for in vitro phosphorylation and interaction studies as well as the intracellular milieu may contribute to the tyrosine phosphorylation pattern observed.

Surprisingly, overexpression of Vav1 containing the Y174F mutation resulted in an even greater NF-AT response than that seen with wild type Vav1. This was an unexpected result since others suggested this was a critical phosphorylation site for the activation of Vav GEF function (12). Our findings suggest that tyrosine 174 in Vav1 is a site of negative regulation, presumably via phosphorylation, that influences the ability of Vav1 when overexpressed to induce as well as up-regulate TCR-mediated NF-AT-driven transcription. The loss of negative regulatory function appears to be specific for Tyr-174, since a similar mutation altering tyrosine 209 to phenylalanine did not result in a gain of function (data not shown). In an attempt to identify candidate negative regulators that could associate with this site, tyrosine 174 was recognized as a potential docking site for the SH2 domains of the soluble phosphotyrosine phosphatase SHP-1 (18), a phosphatase known to down-regulate signaling. Indeed, an interaction between SHP-1 and Vav1 has been previously reported (24), and we were able to confirm this interaction (data not shown). However, co-immunoprecipitation of SHP1 with Vav1 was maintained in immunoprecipitations of Y174F Vav1 (data not shown) and, thus, SHP1 is not considered to be the effector associating with phosphorylated Tyr-174. At present, the mechanism by which Tyr-174 negatively regulates Vav function is not clear.

The mechanism used by Vav1 to potentiate NF-AT-dependent transcription is not known. Efforts to identify the mechanism by which Vav1 potentiates NF-AT have focused on Vav1 as a GEF for Rac. Previous reports, however, have only partially addressed this by examining the isolated GEF domain of Vav1 (6) or by monitoring events dependent on transcription factors other than NF-AT (25). Here, we assessed the GEF activity of full-length Vav1 by analyzing Rac-GTP levels in vivo. Our assay shows that Rac is activated in Jurkat cells by the stimulation of the TCR and that this activation is enhanced when Vav1 is overexpressed. Unexpectedly, the levels of activated Rac are further enhanced when Y174F Vav1 is expressed. This suggests that Tyr-174 is not required for Vav1 GEF function, and instead, phosphorylation of this site may negatively regulate its GEF activity.

This GEF gain of function combined with the enhanced NF-AT activity resulting from the mutation of Tyr-174 suggested that Vav1 GEF function plays a role in potentiating NF-AT in cells overexpressing Vav1. Rac is one target of Vav GEF function, and it has been implicated in the activation of NF-AT in T cells yet is not enough to induce NF-AT-driven transcription (6). Moreover, it has been reported that GTP-bound-Rac or a mutant form of Rac that cannot hydrolyze GTP, RacV12, is sufficient to induce translocation of NF-AT from the cytoplasm to the nucleus yet is not enough to induce NF-AT-driven transcription (17). This prompted us to determine whether GEF activity of Vav1 is essential for its role in potentiating NF-AT in Jurkat cells. To address whether the GEF activity of Vav1 is sufficient to potentiate NF-AT-driven transcription, we examined the GEF activity of oncogenic Vav1. We have demonstrated here and previously that oncogenic Vav1, in which the first 67 amino acids are deleted, is not capable of potentiating NF-AT (16). However, expression of oncogenic Vav1 significantly enhances the levels of activated Rac recovered from cell lysates, indicating a robust GEF activity of the transfected oncogenic Vav1. Therefore, we conclude the GEF activity of Vav1 is not sufficient for activation of NF-AT in Jurkat cells overexpressing Vav1. These results suggest the amino-terminal 67 amino acids, possibly along with other do-
mains, may be responsible for Vav1-mediated NF-AT potentiation. We did not address whether increased levels of nuclear NF-AT are induced by oncogenic Vav1. However, to address whether the GEF activity of Vav1 is necessary for potentiation of NF-AT, a series of point mutations were introduced in the GEF domain of Vav1 (GEFmtVav1) to abolish its GEF activity. Quite surprisingly, although GEFmtVav1 does not induce Rac activation, it still retains the ability to potentiate NF-AT-driven transcription. The NF-AT potentiation of GEFmtVav1 along with the GEF activity of oncogenic Vav1 indicates the GEF activity of Vav1 is neither necessary nor sufficient for Vav1, when Vav1 is overexpressed, to potentiate NF-AT-driven expression.

The mechanism by which Vav1 potentiates NF-AT remains unanswered. Although it could involve increased nuclear localization of NF-AT, as suggested by studies of Rac function in mast cell (17), it is clear from our studies that a GEF-independent function, and presumably Rac-independent function, of Vav1 can contribute to NF-AT regulation. This is not a surprising finding given the multiple protein interaction domains, besides the GEF domain, present in Vav1. Furthermore, although we have shown here that the GEF activity of Vav1 can be dissociated from its ability to potentiate NF-AT when overexpressed in Jurkat cells, our studies cannot rule out an important role of the GEF domain for the normal function of Vav1. All of our studies were performed in the context of cells expressing endogenous wild type Vav1. Thus, Vav1 GEF function could be provided in trans by endogenous Vav1 in our experiments. Nevertheless, our studies have revealed an important negative regulatory function of Tyr-174 and the independent contribution of domains other than the GEF domain in the regulation of signaling pathways leading to NF-AT activation by Vav1. Other assay systems or studies in a Vav1 null background will be required to establish the precise function of the amino terminus, Tyr-174, and the Vav1 GEF domain.

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