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In Vivo Anergized CD4+ T Cells Have Defective Expression and Function of the Activating Protein-1 Transcription Factor

Anette Sundstedt* and Mikael Dohlsten†

The transcription factor activating protein-1 (AP-1) contributes significantly to the regulation of IL-2 gene expression during T cell activation and has been suggested to play a unique role in T cell anergy in vitro. In this study we have used the superantigen staphylococcal enterotoxin A to investigate the regulation of AP-1 in T cell anergy in vivo. Repeated injections of staphylococcal enterotoxin A induce a state of anergy in CD4+ T cells, characterized by reduced expression of IL-2 at mRNA and protein levels. The perturbed IL-2 response in anergic T cells correlated with reduced DNA binding activity of the transcription factors AP-1 and Fos/Jun-containing NF-AT. Using AP-1-luciferase reporter transgenic mice, we now demonstrate the lack of AP-1-dependent transcription. AP-1 activity is controlled by synthesis of its subunits Fos and Jun and by posttranslational phosphorylations. Analysis of Fos and Jun protein levels revealed no major differences in the expression of Jun proteins, but a marked decrease in c-Fos in anergic T cells. Experiments in transgenic mice overexpressing c-Fos (H2-c-fos) showed reconstituted AP-1 DNA binding. In contrast, the AP-1-driven transcription and IL-2 production remained suppressed. The Jun N-terminal kinase is known to play a critical role in regulating AP-1 trans-activation. Analyses of Jun N-terminal kinase demonstrated normal protein amounts, but reduced enzymatic activity, in anergic compared with activated CD4+ T cells. This suggests that in vivo anergized T cells have defects in the AP-1 pathway due to both reduced protein expression and perturbed posttranslational modifications. The Journal of Immunology, 1998, 161: 5930–5936.

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Abbreviations used in this paper: AP-1, activating protein-1; TFE, 12-O-tetradecanoylphorbol-13-acetate-responsive element; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MEK, MAPK/ERK kinase; SEK, stress-activated protein kinase; SEK, p38 mitogen-activated protein kinase; JNK, Jun N-terminal kinase; FRK, Fos regulating kinase; MEK, MAPK/ERK kinase; SEA, staphylococcal enterotoxin A; GST, glutathione S-transferase; EMSA, electrophoretic mobility shift assay.

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protein levels and lack of JNK-mediated posttranslational modifications of AP-1 protein. We suggest that the perturbation of the JNK pathway may be a critical factor in anergic CD4+ T cells in vivo.

Materials and Methods

Animals and treatment

Transgenic C57BL/6 mice expressing a rearranged TCR-Vβ3 gene under the influence of an inserted Ig heavy chain enhancer (25) were provided by Dr. M. Davis (Stanford, CA). AP-1-luciferase reporter transgenic mice (AP-1-luc) expressing the firefly luciferase gene driven by four human collagenase TREs (7) were provided by Dr. R. Flavell (Howard Hughes Medical Institute, Yale University, New Haven, CT). c-fos transgenic mice (H2-c-fos) carrying the mouse c-fos gene under the control of the H-2Kb gene promoter (26) were provided by Dr. T. Tokuhisa (Chiba University School of Medicine, Chiba, Japan). The AP-1-luc and the H2-c-fos mice were crossed with TCR-Vβ3 transgenic mice and used for experiments. Recombinant SEA was expressed in Escherichia coli and purified to homogeneity as described previously (27). Various doses of SEA in PBS with 1% normal syngeneic serum or PBS alone were injected i.v. at 4-day intervals. The TCR-Vβ3 transgenic mice expressed 90–95% of TCR-Vβ3 in the CD3+ T cell population before and after SEA treatment.

Reagents

mAbs directed to murine CD3, CD4, B220, CD19, and TCR-Vβ3 were purchased from PharMingen (San Diego, CA). Recombinant murine IL-2 and mAbs to murine IL-2 (JES6-1A12 and JES6-5H4) were obtained from PharMingen. PMA and ionomycin were purchased from ICN Pharmaceuticals (Costa Mesa, CA). A GST-c-Jun,-9g-expressing plasmid was provided by Dr. M. Karin (University of California-San Diego). Production and purification of GST-c-Jun protein were performed as previously described (28).

Cell separation

Spleens were prepared from mice injected i.v. with SEA or PBS at different times before analysis. Purified CD4+ T cells (>95% CD4+ as determined by FACS analysis) and B220+ B cells (>95% CD19+) were obtained by positive selection using magnetic beads coated with anti-CD4 mAb or anti-B220 mAb (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer’s instructions.

Luciferase activity analysis

Purified CD4+ T cells were incubated at 1 × 106 cells/well (24 wells/plate) in the absence or the presence of various stimuli. Purified B220+ B cells were used as APCs at 1 × 105 cells/well. After specific periods of time, cells from each independent well were harvested, washed twice in PBS, and lysed in lysis buffer (luciferase assay, Promega, Madison, WI) for 30 min at room temperature. Lysate was spun down for 5 min, and total supernatant was analyzed using the luciferase reagent (Promega) and measured in a luminometer (MicroLumat LB 96 P, Berthold, Nashua, NH). Background measurement was subtracted from each sample, and experimental values are expressed as recorded light units of luciferase activity.

IL-2 protein levels

Blood samples were drawn at various time points after i.v. injections of SEA or PBS and were tested for IL-2 content by a specific ELISA using mAbs JES6-1A12 and JES6-5H4 according to instructions from the manufacturer. The same method was used for IL-2 measurements in culture supernatants.

Preparation of nuclear extracts

Nuclear extracts were made according to the method described by Schreiber et al. (29). Purified CD4+ T cells (5–10 × 106) were used for extraction, and the nuclear proteins were finally dissolved in 20 mM HEPES (pH 7.9), 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 0.5 μg/ml leupeptin, and 0.5 μg/ml aprotinin. The protein concentration of all samples was measured by the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA), and the extracts were stored in aliquots at −70°C until used.

Western blot analysis

Before analysis by Western blotting, protein extracts were separated on 10% SDS-PAGE and electroblotted onto nitrocellulose membranes (0.2 μM; Bio-Rad). The membranes were probed with specific rabbit antisera.

The immune complexes were detected by a 1/3000 dilution of goat anti-rabbit Ig-horseshadish peroxidase conjugate (Bio-Rad) and were visualized using enhanced chemiluminescence detection (Amersham, Little Chalfont, U.K.). For analyses of Jun and Fos expression, nuclear protein extracts and the following rabbit polyclonal Abs were used: anti-c-Fos (4), anti-JunB (N-17), and anti-JunD (329) purchased from Santa Cruz Biotechnology (Santa Cruz, CA). A mouse anti-c-Jun mAb and a goat anti-mouse Ig-horseshadish peroxidase conjugate for detection were obtained from Transduction Laboratories (Lexington, KY). To examine JNK protein abundance, cellular extracts and JNK-1 (C-17) and JNK-2 (FL) rabbit polyclonal Abs (Santa Cruz Biotechnology) were used.

Electrophoretic mobility shift assay (EMSA)

The AP-1 consensus oligonucleotide used contained the following sequence: 5′-CTAGTGATGAGTCAGCCGGATC-3′. The probe was end labeled with [γ-32P]ATP using T4 polynucleotide kinase (Promega), according to instructions from the manufacturer and was purified on 5% polyacrylamide gels in 1× TBE (89 mM Tris, 89 mM boric acid, and 2 mM EDTA). Binding reactions were performed with the same amount of protein in each reaction (0.5–1 μg) in 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 5% glycerol, and 2 μg of poly(dI-dC) (Pharmacia, Piscataway, NJ). The reactions were incubated at 37°C for 30 min with 15,000 cpm of double-stranded [32P]-labeled oligonucleotides. The samples were electrophoresed on 5% polyacrylamide gels in 1× TBE. The gels were dried under vacuum and exposed to autoradiography at −70°C.

Preparation of cellular extracts

Total cellular extracts for immunocomplex protein kinase assays and Western blots were made from 5–10 × 106 purified CD4+ T cells according to the method of Hibi et al. (28). In individual experiments, the same number of cells from each group was used for extraction. The cells were lysed in 600 μl of cold lysis buffer consisting of 20 mM Tris-HCl (pH 7.7), 250 mM NaCl, 3 mM EDTA, 3 mM EGTA, and 0.5% Nonidet P-40; to which protease and phosphatase inhibitors had been freshly added. After a 30-min incubation on ice, the extracts were spun for 10 min in an Eppendorf centrifuge at 4°C to pellet cellular debris. The supernatants were removed and stored at −70°C.

Immunocomplex protein kinase assay

Immunocomplex kinase assays were performed as described by Hibi et al. (28). Clarified cellular extracts were incubated with protein A/G-agarose beads (Santa Cruz) precoated with polyclonal Ab to JNK-1 (C-17; Santa Cruz) for 2 h at 4°C with gentle rotation. Immune complexes were washed and then assayed for enzyme activity as described, using GST-c-Jun,-9g as a substrate. The beads were pelleted and resuspended in 30 μl of kinase buffer (20 mM HEPES (pH 7.6), 2 mM DTT, 10 mM MgCl2, 1 mM EDTA, and 1 mM EGTA, to which phosphate inhibitors had been freshly added) containing 1 μg of GST-c-Jun, 1 μM ATP, and 1 μCi of [γ-32P]ATP. Incubations were conducted for 20 min at 30°C, then reactions were stopped by addition of 10 μl of 4× SDS sample buffer. Phosphorylation of the substrates was analyzed by 10% SDS-PAGE followed by autoradiography of the dried gel. [32P]ATP incorporation was determined by cutting the gel and measuring radioactivity in a beta counter.

Results

Reduced AP-1 transcriptional activity in CD4+ T cells energized by superantigen in vivo

We have previously shown that repeated injections of SEA to TCR-Vβ3 transgenic mice induce a state of IL-2 hyporesponsiveness in the CD4+ compartment that correlates with reduced DNA binding of the AP-1 transcription factor (24). To confirm that AP-1 is also perturbed at the transcriptional level, we used transgenic mice expressing a luciferase reporter gene under the control of AP-1 binding sites (AP-1-luc) (7). Double-transgenic TCR-Vβ3×AP-1-luc mice were treated with one injection of SEA to induce activation and with repeated injections to induce anergy. CD4+ transgenic spleen T cells were purified, stimulated in vitro with SEA-coated syngeneic B220+ B cells, and analyzed for AP-1 transcriptional activity. Activated CD4+ T cells (1× SEA) exhibited significant AP-1 transcriptional activity in response to SEA (Fig. 1A), while no activity could be detected in the anergic CD4+ T cells.
in anergic T cells was due to reduced expression of Jun and Fos proteins, we analyzed nuclear Fos and Jun protein content by Western blotting. We have previously shown that large amounts of AP-1 DNA binding were induced in CD4+ T cells 1 h after a single SEA injection (24). The AP-1 complexes detected by EMSA contained mainly heterodimers between c-Fos and JunB or JunD (24). Using Western blot analysis of the nuclear extracts, we found that JunD was constitutively expressed, and the amount did not change following activation either by a single (1× SEA) or repeated (3× SEA) injections (Fig. 2A). In contrast, c-Fos, JunB, and c-Jun were expressed only after stimulation (Fig. 2, B–D). Interestingly, the induction of c-Fos was defective in anergic CD4+ T cells (Fig. 2D). On the contrary, JunB protein was induced in both groups to a similar extent (Fig. 2B). c-Jun was expressed at low levels, which were further reduced in the anergic cells (Fig. 2C). Taken together, these results suggested that the reduced c-Fos expression may explain the defective AP-1 DNA binding and trans-activation in the anergic CD4+ T cells.

**Restored AP-1 DNA-binding in anergic CD4+ T cells from H2-c-fos transgenic mice**

In an attempt to restore the possible c-Fos defect in the anergic T cells, we used transgenic mice that express c-fos under an MHC class I promoter (H2-Kb; H2-c-fos) (26). These mice were crossed with the TCR-Vβ3 transgenic mice and used for anergy experiments. Constitutive expression of c-fos was confirmed by RT-PCR (data not shown). Double-transgenic mice were injected one or three times with SEA. Nuclear extracts from purified CD4+ T cells were analyzed by EMSA for AP-1 DNA binding. Interestingly, CD4+ T cells from H2-c-fos×TCR-Vβ3 transgenic mice anergized by SEA (3× SEA) contained significantly more AP-1 than anergic CD4+ T cells from control mice (Fig. 3A). The levels of AP-1 in H2-c-fos transgenic mice anergized by SEA corresponded to the levels observed in CD4+ T cells from control mice activated by a single injection of SEA (1× SEA), suggesting that c-Fos indeed was a limiting factor for obtaining AP-1 band shifts. Supershift analysis of the obtained AP-1-binding protein complex in H2-c-fos transgenic mice demonstrated the same constitution as that previously described for SEA-treated TCR-Vβ3 transgenic mice (24), consisting mainly of heterodimers between c-Fos and JunB or JunD (data not shown). In contrast, analysis of IL-2 production in serum from double-transgenic mice revealed a perturbed IL-2 response similar to that in control mice (Fig. 3B). This suggests that there is an additional defect in the AP-1 pathway, possibly due to a perturbation in the posttranslational modifications of Fos and Jun.

**Lack of AP-1 transcriptional activity in c-Fos-overexpressing mice**

To exclude the possibility that AP-1 transcriptional activity was restored in the H2-c-fos mice and that the absence of IL-2 production instead represented additional defects in other transcription factors, we crossed the H2-c-fos mice with the AP-1-luc mice on TCR-Vβ3 background. Triple-transgenic mice were activated (1× SEA) or anergized (3× SEA) by SEA in vivo. Purified CD4+ T cells were stimulated with SEA-coated syngeneic B220+ B cells and PMA in vitro and analyzed for AP-1 transcriptional activity. Activated H2-c-fos CD4+ T cells (1× SEA) exhibited significant AP-1 transcription in response to SEA and PMA (Fig. 4A), while the anergic H2-c-fos CD4+ T cells failed to respond (Fig. 4A). Addition of ionomycin restored the response in the anergic cells (Fig. 4A), as shown above (Fig. 1A). Similarly, the AP-1 transcriptional activity correlated with the ability to synthesize IL-2 (Fig. 4A).
FIGURE 2. Reduced expression of c-Fos in anergic CD4\(^+\) T cells. TCR-V\(\beta\)3 transgenic mice were injected i.v. one or three times with 10 \(\mu\)g of SEA or PBS (untreated control) at 4-day intervals. Spleens were removed 1 h after the last SEA injection, and purified CD4\(^+\) T cells were prepared. Two micrograms of nuclear extracts were loaded on a 10% SDS-PAGE gel; transferred to a nitrocellulose filter; probed with anti-JunD antiserum (A), anti-JunB antiserum (B), and anti-c-Fos antiserum (D); and detected by chemiluminescence. The specific proteins and m.w. (MW) standards are indicated in the figure. One of three similar experiments is shown.

Reduced JNK activity in anergic CD4\(^+\) T cells

Synthesis of Fos and Jun proteins is not sufficient to induce AP-1 activity. In addition, these proteins need to be phosphorylated to become transcriptionally active (30). Protein kinase activities that phosphorylate and activate Jun (JNK) and Fos (FRK) have been described (28, 31). However, the c-Fos kinase FRK is poorly understood. To investigate whether JNK activity was reduced in the anergic CD4\(^+\)T cells, we used a specific immunocomplex protein kinase assay. TCR-V\(\beta\)3 transgenic mice were injected one or three times with SEA to induce activation or anergy, respectively. Analysis of JNK activity in purified CD4\(^+\) T cells revealed that a significant response was rapidly induced in activated CD4\(^+\) T cells (1× SEA), with peak activity after 0.5 h in vivo (Fig. 5A). In contrast, the anergic cells exhibited significantly lower basal JNK activity before the last SEA injection and a minor activation-induced increase (Fig. 5A). Analysis of JNK protein levels by Western blotting showed that these proteins were constitutively expressed (Fig. 5B) in conformity with earlier studies (21). Thus, the defective AP-1 activity in anergized CD4\(^+\) T cells may be the result of deficient JNK activation.

Discussion

The AP-1 transcription factor is intimately involved in the regulation of IL-2 gene transcription (6–10) and may be a unique target in T cell clonal anergy (5, 22). We have previously shown that CD4\(^+\) T cells anergized by superantigen in vivo contained reduced AP-1 and NF-AT/AP-1 DNA binding (24). In this study we have further characterized the in vivo expression and function of AP-1 in the anergic CD4\(^+\) T cells. Using AP-1-luciferase reporter transgenic mice, we conclude that the AP-1 transcriptional activity in SEA-anergized CD4\(^+\) T cells was repressed and correlated strongly with the inability to synthesize IL-2. Earlier studies in these mice by Rincón and Flavell (7) demonstrated a clear dissociation between the signals required for AP-1 DNA binding and transcriptional activity in primary T cells in vitro. While protein kinase C activation was sufficient to induce DNA binding activity, an additional calcium increase was required to induce AP-1-driven transcription and IL-2 production (7).

AP-1 protein activity is regulated both at the level of gene transcription and by posttranslational modifications, which are largely controlled by protein kinases of the MAPK family (30). c-Fos transcription is controlled by ERK-dependent phosphorylation of the ternary complex factor Elk-1 that regulates the c-fos promoter (32). Activation of JNK results in the phosphorylation of c-Jun and JunD, which augments their transcriptional activities (28, 33). In addition, JNK phosphorylates ATF-2, which together with c-Jun bind to a nonconventional TRE site in the c-Jun promoter and induce c-jun transcription (34). It was recently demonstrated that c-Fos, FosB, and JunB proteins are reduced in Th1 clones anergized by anti-CD3 treatment in vitro (22), which correlated with impaired activities of the MAPK family members ERK and JNK (21). In this study, we observed that in vivo anergized CD4\(^+\) T cells expressed less c-Fos protein than activated CD4\(^+\) T cells. In contrast, both JunB and JunD proteins were expressed at similar levels in activated and anergic CD4\(^+\) T cells. Thus, a potential explanation for defective AP-1 trans-activation in anergic cells in vivo would be poor activation of MAPK and subsequent suboptimal Fos protein expression. However, overexpression of c-Fos protein in H2-c-fos transgenic mice restored AP-1 DNA binding, but failed to induce IL-2 production and proper AP-1-dependent transcription. Thus, in addition to the observed defect in synthesis of the Fos/Jun family of proteins observed here and by others (22), anergic T cells lack posttranslational modifications of AP-1 proteins required for AP-1-dependent trans-activation.

Protein kinase activities that phosphorylate and activate Jun (JNK) and Fos (FRK) have been described. Whereas the c-Fos kinase FRK is poorly understood, the c-Jun kinase JNK has been studied in detail. The JNK protein kinases bind Jun proteins at a docking site between amino acids 30 and 60 (30) and subsequently phosphorylate the NH\(_2\)-terminal activation domain of c-Jun, causing increased transcriptional activity (28, 33). JunD is also a substrate for JNK, but contains a weaker docking site for JNK than c-Jun (33). Interestingly, full activation of JNK1 and JNK2 has
been reported to require two signals in T cells, i.e., ligation of both TCR and CD28 (17). Studies of T cell cultures in vitro have further shown that the activity of JNK correlates to the ability of T cells to synthesize IL-2 (17). In contrast, MAPK ERK1 and ERK2 were activated by TCR stimulation alone and were not affected by CD28 (17). Hence, integration of signals that lead to full T cell activation may occur at the levels of JNK activation. In this study rapid induction of JNK activity in CD4\(^+\) T cells was observed after SEA priming in vivo. In contrast, we observed inhibition of JNK activation in SEA-anergized T cells. This could explain the absence of AP-1 transcriptional activity in the c-Fos-overexpressing cells, although prominent AP-1 DNA binding was recorded. Supershift analysis of the induced AP-1 complexes showed that both the activated and anergic T cells contained mainly heterodimers between c-Fos and JunB or JunD (24) (data not shown). We did, however, detect c-Jun protein by Western blotting and found reduced protein levels in the anergic cells (Fig. 2). Thus, it could be speculated that c-Jun may be present at other important regulatory elements of the IL-2 promoter, such as the CD28RE/AP-1 (35) and NF-AT (9) sites, and that a defect in JNK activation may result in reduced transcriptional activity of those sites. Indeed, a dominant negative mutant form of c-Jun was shown to inhibit NF-AT-driven transcription and to prevent IL-2 gene expression (36).

Inhibition of JNK activation may also explain the observed defective expression of c-Fos protein in the anergic T cells. In addition to its ability to activate Jun synthesis and transcriptional activity, it was recently demonstrated that JNK activates the c-fos promoter through phosphorylation of TCF/Elk-1 (37). Furthermore, expression of the protein kinase MEKK-1 in the JNK cascade induced c-fos transcription and Elk-1 transcriptional activity in the absence of considerable ERK activation (37). Interestingly, induction of MEKK-1 activity also requires two signals in T cells (38), suggesting similar activation requirements for JNK and
MEKK-1 proteins. However, the roles of ERK and JNK in the induction of Fos and Jun proteins and in the control of AP-1 transcription remain to be completely established in T cells. The absence of AP-1 transcriptional activity in anergized CD4+ T cells could in addition include a failure to phosphorylate c-Fos. However, little information is available about FRK (31). FRK is a protein kinase of the MAPK family that is stimulated via the p21ras pathway and phosphorylates c-Fos at Thr232 (31). The role of FRK in regulation of AP-1 activity in T cells is not known. Interestingly, when analyzing the induced c-Fos protein using higher resolution electrophoresis, we observed the presence of a slower migrating band in the activated CD4+ T cells that was absent in the anergic cells (data not shown). Whether this represents a phosphorylated form of c-Fos remains to be determined.

In a recent study by Li et al., it was demonstrated that the block in ERK and JNK activation upon restimulation of anergic T cells in vitro could be completely overcome by stimulation with phorbol ester (21). They further showed that triggering of anergic cells resulted in dephosphorylation and translocation of the calcium/calmodulin-dependent transcription factor NF-AT (21). Taken together, these results suggested that the block in anergic T cells was not due to a global defect in TCR/CD3 signal transduction, but occurred specifically at the level of p21ras. In T cells, PMA activates protein kinase C, which, in turn, activates p21ras by inhibiting Ras GTPase-activating protein (39). A defect in Ras activation was directly demonstrated by Fields et al., who showed that conversion of GDP-p21ras to the activated GTP-bound form was prevented in the anergized cells (39). In this study we were unable to restore AP-1 transcription by PMA alone. A rise in intracellular calcium by ionomycin treatment was necessary to induce AP-1 activity in the anergized T cells. Thus, in addition to the defect in Ras activation observed in vitro (21, 40), the in vivo anergized CD4+ T cells seem to mobilize insufficient levels of calcium to trigger AP-1 transcription.

Rincón and Flavell (7) demonstrated the occurrence of DNA binding, but transcriptionally inactive AP-1 in primary T cells in response to PMA. Furthermore, they reported that blocking of the calcium-dependent pathway by cyclosporin A, which inhibits the protein phosphatase calcineurin (41), had minor effect on AP-1 DNA binding, but completely inhibited the transcriptional activity (7). In a recent study on B cells by Goodnow and co-workers, it was demonstrated that the amplitude and duration of calcium signals contribute to selective activation of NF-AT, NF-κB, and JNK (42). NF-κB and JNK required a large transient Ca2+ rise for activation, whereas NF-AT was activated by a low sustained Ca2+ plateau (42). Furthermore, stimulation of B cells tolerized by self Ag was shown to trigger low calcium increases and activated NF-AT and ERK but not NF-κB and JNK (43). The need for activation of the calcium- and calcineurin-dependent pathway by ionomycin to restore AP-1 transcription may imply that suboptimal calcium mobilization contributes to the insufficient response in vivo anergized CD4+ T cells. Indeed, studies on T cell clones have implicated that low amplitude Ca2+ responses are involved in the induction of T cell anergy by altered peptide ligands (44).

In conclusion, we have demonstrated that in vivo anergized CD4+ T cells contain multiple defects in the AP-1 pathway, which strongly correlated to the inability of these cells to induce NF-κB activity and to synthesize IL-2. The requirements for both PMA and ionomycin to restore AP-1 and IL-2 responses led us to propose that a block in p21ras activation and suboptimal increase in intracellular Ca2+ may be involved in the induction of anergy in vivo. Identification of the upstream targets responsible for regulation of calcium amplitude and uncoupling of the JNK pathway in anergic T cells will be a major focus for future studies.

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References

DEFECTIVE EXPRESSION OF AP-1 IN ANERGIC T CELLS