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A Costimulation-Initiated Signaling Pathway Regulates NFATc1 Transcription in T Lymphocytes

Roza I. Nurieva,* Sergei Chuvpilo,† Eric D. Wieder,* Keith B. Elkon,‡ Richard Locksley,§ Edgar Serfling,† and Chen Dong2*  

T cell activation and differentiation is accompanied and mediated by transcriptional reprogramming. The NFATc1 transcription factor is strongly induced upon T cell activation and controls numerous genes involved in the T cell effector function. However, its regulation by physiological stimuli in primary T cells has not been well understood. We previously found that ICOS synergizes with TCR and CD28 to greatly enhance NFATc1 expression in primary T cells. In this study, we have examined the signaling mechanisms whereby costimulation regulates NFATc1 expression. We found that CD28 and ICOS regulate sustained PI3K activity in primary T cells, which is required for NFATc1 up-regulation. CD28 and ICOS costimulation, possibly through Itk, a Tec kinase downstream of the PI3K, enhanced phosphorylation of phospholipase Cyt1 and increased and sustained Ca2+ flux in T cells. Costimulation of T cells potentiated transcription of the Nfatc1 gene P1 promoter in a PI3K-dependent manner. This work demonstrates an important role for costimulatory receptors in sustaining T cell activation programs leading to Nfatc1 gene transcription and has implications in our understanding of the immune response and tolerance. The Journal of Immunology, 2007, 179: 1096–1103.

T cell activation and differentiation is accompanied and mediated by transcriptional changes. NFAT represents a family of transcription factors important in T cell activation and differentiation to effector cells. A particular member, NFATC1, is strongly induced upon T cell activation and controls numerous genes that are involved in T cell effector function (1, 2). Multiple isoforms of NFATC1 have been recently identified that are generated by the activity of two different promoters designated as P1 and P2, alternate splicing, and two polyadenylation sites, pa1 and pa2 (3). The activity of the P1 but not the P2 promoter is highly induced after T cell activation. Although the P1 promoter was shown to be autoregulated by NFAT factors resulting in a strong increase in NFATC1 protein levels in activated T cells, the physiological stimuli that regulate NFATC1 expression are not well understood.

The T cell activation program is instructed by the innate immune system, which provides not only Ag peptide-MHC complexes for TCR recognition but also “costimulation” by accessory molecules on APC to engage their corresponding receptors on T lymphocytes. Activation of T cells in the absence of costimulation possibly results in their inactivation and unresponsiveness to subsequent stimulation (4). CD28 is the most prominent costimulatory receptor on T cells. Its ligands B7.1 (CD80) and B7.2 (CD86) are greatly up-regulated on APC by infectious agents. Studies using T cells derived from mice deficient in CD28 or in both B7.1 and B7.2 demonstrated their crucial roles in CD4+ T cell activation and proliferation (5). ICOS is another member of the CD28 superfamily and its expression is rapidly induced after T cell activation (6, 7). The ligand for ICOS, B7h, is constitutively expressed on B cells and other types of APC (6, 8). Studies using mice deficient in ICOS or B7h demonstrated that ICOS-B7h interaction regulates Th2 differentiation and IL-4 production (9, 10). When we examined the transcriptional mechanisms by which ICOS regulates Th2 differentiation, we found that ICOS synergizes with TCR and CD28 signals at an early stage of T cell activation to greatly enhance NFATc1 protein expression, which subsequently leads to induction of the transcription factor c-Maf (11).

In this study, we have examined signal transduction mechanisms that lead to the Nfatc1 gene transcription in T lymphocytes. Our results indicate that a PI3K-Itk-phospholipase C (PLC)γ1-Ca2+ pathway initiated by CD28 and ICOS leads to the induction of Nfatc1 P1 promoter activity.

Materials and Methods

Mice

B7-deficient mice previously analyzed on a C57BL/6 × 129Fl background (10) were subsequently backcrossed for more than six generations with C57BL/6 (B6) mice. CD80/CD86 doubly deficient mice on a B6 background were purchased from The Jackson Laboratory and bred with B7-deficient animals (12). GFP-protein kinase B (PKB)-pleckstrin homology (PH)-transgenic mice (13), provided by Dr. D. Cantrell at University of Dundee (Dundee, U.K.), were bred with OT-II TCR transgenic

3 Abbreviations used in this paper: PLC, phospholipase C; AM, acetoxymethyl ester; B6, C57BL/6; PIP3, phosphatidylinositol 3,4,5-triphosphate; PH, pleckstrin homology; PKB, protein kinase B.

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*Department of Immunology, M.D. Anderson Cancer Center, Houston, TX 77030;  
†Institute of Pathology, Department of Molecular Pathology, University of Würzburg, Würzburg, Germany;  
‡Division of Rheumatology, Department of Medicine, University of Washington, Seattle, WA 98195; and  
§Department of Medicine and Department of Microbiology/Immunology, Howard Hughes Medical Institute, University of California, San Francisco, CA 94110

Roza I. Nurieva,† Sergei Chuvpilo,‡ Eric D. Wieder,* Keith B. Elkon,‡ Richard Locksley,§ Edgar Serfling,† and Chen Dong2*  

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Address correspondence and reprint requests to Dr. Chen Dong, Department of Immunology, M.D. Anderson Cancer Center, 7455 Fannin, Unit 906, Houston, TX 77030. E-mail address: cdong@mndanderson.org

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mice. All animal work has been approved by the Institutional Animal Care and Use Committee (M.D. Anderson Cancer Center, Houston, TX).

**T cell isolation and stimulation**

CD4⁺ T cells from the lymph nodes and spleens of 6- to 8-wk-old mice were isolated and stimulated as reported previously (9, 11). In brief, the CD4⁺ T cells were first enriched by immunomagnetic negative selection using Abs against CD8, MHC class II, and NK1.1 markers and magnetic beads conjugated with goat anti-mouse and anti-rat Ig (Polysciences). The CD62L⁻ naïve cells were further purified by an autoMACS sorter (Miltenyi Biotech). Purified naïve CD4⁺ T cells were stimulated with plate-bound anti-CD3 Ab in the presence of anti-CD28 and/or anti-ICOS Abs for 24 h before analysis of NFATc1 expression.

**Measurement of phosphatidylinositol 3,4,5-triphosphate (PIP3) levels in T cells**

CD4⁺ T cells were purified from the spleen and lymph nodes of GFP-PKB-PH⁺ OT-II mice by positive selection using anti-CD4-conjugated microbeads (Miltenyi Biotech). Spleenic APC from B6, B7⁻/−, B7h⁻/−, and B7⁻/−B7h⁻/− mice were prepared by complement-mediated lysis of Thy1⁻ T cells. Purified naïve CD4⁺ T cells were stimulated with OVA peptide and APC for 1, 3, 5, and 8 h. T cell-APC conjugates and membrane localization of the GFP fusion protein were analyzed by fluorescence microscopy (NIKON Eclipse E400).

**GST pull-down, immunoprecipitation, and Western blot analyses**

EL-4 cells were lysed in Triton lysis buffer (20 mM Tris (pH 7.4), 137 mM NaCl, 0.25 mM β-glycerophosphate, 0.2 mM sodium pyrophosphate, 2 mM EDTA, 1% Triton X-100, 10% glycerol, 1 mM sodium pyrophosphate, 2 mM EDTA, 1% Triton X-100, 10% glycerol, 1 mM Na3VO4, 0.1 mM PMSF, 10 μg/ml leupeptin, 5 μg/ml aprotinin, and 0.5 mM DTT). EL-4 lysates were incubated with immobilized GST fusion proteins (unphosphorylated or phosphorylated wild-type or mutant GST-ICOS) on glutathione beads and the precipitates were subjected to SDS-PAGE and immunoblotted with an anti-p85 antisemur. Whole cell lysates were prepared by lysing cells in Triton lysis buffer. The amounts of proteins were determined by a Bio-Rad protein assay to ensure equal protein loading before Western blot analysis with Abs raised against pPLCγ1, NFATc1, or β-actin (Santa Cruz Biotechnology).

**RNase protection assays**

For RNase protection assays, total RNA extracted using TRizol reagent (Invitrogen Life Technologies) was analyzed using the RiboQuant multi-probe RNase protection kit and specific probes (BD Pharmingen). For analysis of *Nfatc1* or *Pl1* promoter activity, a 386-bp fragment cloned in pKS was used for the synthesis of an antisense riboprobe (3).

**RT-PCR analysis of NFATc1 isoforms**

Total RNA extracted from T cells using TRizol (Invitrogen Life Technologies) was reverse-transcribed to cDNA using oligo(dT) (Invitrogen Life Technologies). The following PCR primers were used to detect specific NFAT isoforms: NFATc1α forward, 5’-ATGCCAACATCCACCTTTCCACGTCCTC-3’; NFATc1α reverse, 5’-CCTGAGTTAAGGTGTAAAGACAGATGTTAC-3’; NFATc1β forward, 5’-ATGCCAACATCCACCTTTCCACGTCCTC-3’; NFATc1β reverse, 5’-CCTGAGTTAAGGTGTAAAGACAGATGTTAC-3’; NFATc1γ forward, 5’-ATGCCAACATCCACCTTTCCACGTCCTC-3’; NFATc1γ reverse, 5’-CCTGAGTTAAGGTGTAAAGACAGATGTTAC-3’.

**Luciferase assays**

EL-4 cells (2 × 10⁶) were cotransfected with 2 μg of NFAT/AP1-Luc or NFAT-P1-Luc plasmid and 0.25 μg of a control reporter plasmid (pPRL-null; Promega). Jurkat cells (2 × 10⁶ cells) were cotransfected with 2 μg of NFAT/AP1-Luc or NFAT-P1-Luc reporter plasmid, 1 μg of pcDNA3, pcDNA3-ICOS, or pcDNA3-ICOSmut (Y-F), and 0.25 μg of a control reporter plasmid (pPRL-null; Promega). Cells were pulsed using a Bio-Rad Gene Pulser at 260 volts and 960 farads in 10% FCS. After 16 h, cells were stimulated with plate-bound anti-CD3, anti-CD28, and/or anti-ICOS Abs for 6 h and lysed. Luciferase activity was detected using the Dual luciferase system (Promega). Luciferase units of the experimental vector were normalized at the level of the control vector in each sample.

**Calcium flux**

EL-4 cells were resuspended at 10⁷/ml in medium (RPMI 1640, 10% FBS, 1-glutamine, and penicillin/streptomycin) containing 5 μg of indol-1-acetoxyethyl ester (AM) (Sigma-Aldrich) and incubated for 1 h at 37°C. Light exposure was minimized to prevent photobleaching. Cells were then washed, incubated with the indicated stimulated Abs for 3 h at 4°C, and washed again. Then, 200-μl aliquots containing 5 × 10⁶ cells/ml were diluted with 800 μl of complete RPMI 1640 medium prewarmed at 37°C and the sample was applied to a FACSVantage flow cytometer (BD Biosciences) for 45 s to measure the basal intracellular Ca²⁺ concentration. Hamster IgG1 isotype control was added at a final concentration of 10 μg/ml and cytoplasmic Ca²⁺ levels were measured for 10 min. Changes in intracellular calcium were assessed as the ratio of calcium-bound (FL5) to calcium-unbound (FL4) fluorescence. Primary CD4⁺ T cells were activated with Con A for 48 h. Activated cells were loaded with Fluor-4-AM and Fura Red-AM (Invitrogen Life Technologies), activated with polystyrene beads coated with a combination of the indicated Abs, and analyzed.
on a FACS Aria flow cytometer (BD Biosciences). Calcium flux data was analyzed using FlowJo (Tree Star) software.

Results

Costimulatory receptors regulate sustained PI3K activity

How NFATc1 expression is regulated by physiological stimuli in primary T cells has not been understood. Previously, we found that during early T cell activation ICOS synergizes with TCR and CD28 in the up-regulation of NFATc1 protein expression (11). We thus seek to elucidate the signaling mechanisms through which CD28 and ICOS regulate NFATc1 expression. Although CD28 signaling has been extensively studied, the mechanism whereby ICOS transduces intracellular signaling is relatively unclear. Like CD28, ICOS contains an evolutionarily conserved YMXYT sequence motif in its cytoplasmic region. In CD28 this motif associates with the p85 subunit of PI3K. Several studies indicate that ICOS constitutively associates with the p85 subunit of PI3K and that ICOS ligation led to PI3K activation (14–16). Consistent with these observations, we also found that the association of ICOS with p85 is dependent on the tyrosine residue of the YMXYT motif.
because the mutation of tyrosine 181 to phenylalanine (ICOS/Y181F) abolished any association with p85 (Fig. 1A). These results suggest that, similar to CD28, ICOS might signal by activating PI3K, which regulates the production ofPIP3. How these two receptors collectively regulate PI3K activity in primary T cells has not been understood. A recent work indicated that PIP3 production is maintained in primary naive CD8+ T cells for up to 9 h after they encounter APC carrying the appropriate Ag, and a sustained PI3K activity is required for optimal T cell proliferation (13). To analyze the role of costimulation in PI3K regulation in T cells, we purified CD4+ T cells from OT-II mice carrying the OVA peptide presented by APC from wild-type mice or control BALB/c mice were activated with the indicated combination of anti-CD3, anti-CD28, and anti-ICOS Abs for 24 h. The amount of NFATc1 and pPLCy1 in the whole cell extract was examined by Western blot analysis. The data shown are consistent with an additional experiment giving similar results. 

**Sustained PI3K activity is required for NFATc1 protein expression**

We showed previously that CD28 and ICOS synergize in the regulation of NFATc1 protein expression and nuclear translocations (11). To assess the role of PI3K downstream of CD28 and ICOS in NFATc1 induction, we measured the amount of NFATc1 protein in CD4+ T cells activated with plate-bound anti-CD3 and anti-CD28 and/or anti-ICOS for 24 h (Fig. 2A). As we showed previously, the expression of NFATc1 is most strongly up-regulated by TCR, CD28, and ICOS agonists (11). NFATc1 up-regulation was completely blocked by LY-294002, a PI3K inhibitor (Fig. 2A). In this culture, cell death remained unaffected by the inhibitor (data not shown). These results suggest that PI3K activity is required for NFATc1 expression upon the activation of T cells.

PTEN is an antagonistic phosphatase that hydrolyzes PIP3 (17). To further substantiate the role of PI3K in NFATc1 expression, we activated naive CD4+ T cells from PTEN+/− mice or wild-type mice and assayed the expression of IL-2 and NFATc1 24 h after activation (Fig. 2B). As we showed previously, wild-type cells activated with anti-CD3 plus anti-ICOS Abs produced the same levels of NFATc1 and IL-2 as those stimulated only with anti-CD3; anti-ICOS only had an effect on NFATc1 expression in the presence of anti-CD28, further supporting the notion that CD28 is the predominant costimulatory receptor in CD4+ cells (11). Interestingly, there was a strong effect on IL-2 production and NFATc1 up-regulation by anti-ICOS on PTEN+/− T cells activated with anti-CD3. Because PTEN+/− and PTEN−/− cells up-regulated ICOS to a similar extent (data not shown), PTEN appears to be a negative regulator of ICOS signaling; its haploinsufficiency allows T cells to respond strongly to ICOS stimulation.

**FIGURE 3.** Activation of Itk and PLCγ1 and an increase in Ca2+ levels in costimulation signaling. A, Naive CD4+ T cells from Itk+/− mice or control BALB/c mice were activated with the indicated combination of anti-CD3, anti-CD28, and anti-ICOS Abs for 24 h. The amount of NFATc1 and pPLCγ1 in the whole cell extract was examined by Western blot analysis. The data shown are consistent with those from an additional two experiments with the similar results. B, Indo-1-loaded EL-4 cells or Con A-activated CD4+ T cells preloaded with Fluo4-AM and Fura Red-AM were stimulated with the indicated Abs and Ca2+ mobilization was analyzed by flow cytometry. The data shown are consistent with an additional two experiments with the similar results.
Because CD28 and ICOS signals resulted in sustained PIP3 production, we next assessed the question of whether sustained PI3K activity is required for NFATc1 expression. LY294002 was added at various time points after CD4/H11001 T cells were activated by anti-CD3 and splenic APC. Adding LY294002 0–6 h after T cell activation completely inhibited IL-2 production and NFATc1 expression (Fig. 2C). However, the addition of LY294002 at a later time point (10 h) was less effective, indicating a time window during which PI3K activity was required for the induction of NFATc1 and IL-2.

Itk is required for PLCγ/H11001 phosphorylation and NFATc1 up-regulation

PI3K has many important downstream targets. In T cells, PI3P recruits the PH domain-containing Akt/PKB and the Tec protein kinases to the cell membrane. Interestingly, activated Akt restored T cell proliferation but not the Th2 defect in CD8+/− T cells (18). In contrast, Itk was shown to be important for full activation of PLCγ1, Ca2+ mobilization, the nuclear localization of NFATc1, and IL-4 expression (19). Because ICOS and CD28 synergize to enhance NFATc1 expression and early IL-4 production (11), we focused our research on the role of Itk in costimulation-mediated NFATc1 induction. Naïve CD4+ T cells purified from Itk-deficient mice and their appropriate controls were activated as described above and NFATc1 expression was determined by Western blotting. Itk deficiency led to impaired NFATc1 up-regulation, indicating that Itk is essential for NFATc1 expression mediated by costimulation (Fig. 3A).

Lyk, like other Tec kinases, plays an important role in PLCγ phosphorylation and Ca2+ mobilization (19). Accordingly, we found that the phosphorylation of PLCγ1, which was enhanced in T cells receiving costimulatory signals, was greatly reduced in the absence of Itk (Fig. 3A). We next examined whether the costimulation of T cells increases Ca2+ mobilization. EL-4 is a thymoma that constitutively expresses TCR/CD3, CD28, and ICOS. To measure Ca2+ mobilization in T cells, EL-4 cells loaded with Indo-1 as well as Con A-activated CD4+ T cells preloaded with Fluo4-AM and Fura Red-AM were stimulated with anti-CD3 in the presence of anti-CD28 with or without anti-ICOS. CD28 costimulation increased Ca2+ mobilization in EL4 and primary CD4+ T cells. ICOS stimulation, along with TCR and CD28 stimulation, led not only to an increased magnitude but also to a more sustained Ca2+ mobilization (Fig. 3B). These results indicate a crucial role for CD28 and ICOS costimulation in Ca2+ signaling.

Costimulation regulates Nfatc1 gene transcription

Our previous and current data have indicated a crucial role of costimulation in the regulation of NFATc1 protein expression (11). The transcriptional initiation of the Nfatc1 gene is controlled by
two different promoters, P1 and P2 (3), which mediate the expression of individual isoforms. Although P1-mediated transcription from exon 1 generates an N-terminal \( \alpha \) peptide of 42 aa, the P2 promoter initiates transcription from exon 2, which results in the \( \beta \) peptide of 29 aa. In addition, alternate RNA splicing events and two poly(A) addition sites, pA1 and pA2, control NFATc1 expression in six individual NFATc1 isoforms, \( \alpha A, \alpha B, \alpha C, \beta A, \beta B, \) and \( \beta C \) (Refs. 3 and 20 and our unpublished data). To examine which promoter of the \( Nfatc1 \) gene is the target of costimulation-dependent regulation, we hybridized RNA from EL-4 or primary CD4 \( ^+ \) cells activated under various conditions with RNA probes specific for the detection of mRNA transcripts initiated at the P1 or P2 promoter. We found that the \( Nfatc1 \) P1 promoter activity was minimal upon anti-CD3 stimulation alone but was enhanced by CD28 costimulation (Fig. 4A). Anti-ICOS treatment did not affect NFATc1 P1 activity mediated in anti-CD3-treated T cells. The combination of anti-CD3, anti-CD28, and anti-ICOS Abs strongly enhanced the transcription of the \( Nfatc1 \) P1 promoter (Fig. 4A), correlating with NFATc1 protein expression. In contrast, P2 activity was not found to be different in these cells (data not shown). These data suggest that costimulation may regulate the transcription of the P1 promoter.

To substantiate these results, we performed RT-PCR analyses for the detection of individual isoforms generated from either the P1 or the P2 promoter. Transcripts generated from the P1 promoter encoding \( \alpha \) peptides with short (A) or long (B plus C) C termini were induced after T cell activation (Fig. 4B). Expression of the \( \alpha A \) isoform and, to a less extent, the \( \alpha B \) plus C isoforms, is highly responsive to CD28 and ICOS costimulation, consistent with a role for NFATc1 \( \alpha \) proteins in T cell proliferation (3). In contrast, the synthesis of short and long \( \beta \) isoforms appeared not to be stimulated upon T cell activation.

We further examined costimulation regulation of P1 promoter using a P1-driven luciferase reporter gene. In EL-4 cells, the P1 promoter was only weakly activated by stimulation with anti-CD3 in the absence or presence of anti-CD28 or anti-ICOS. However, anti-CD3, anti-CD28 and anti-ICOS synergized to induce significant P1 activation (Fig. 5A). Therefore, from our above three experiments it appears that P1 is a target of costimulation-dependent regulation.

The \( Nfatc1 \) P1 promoter has been previously reported to be autoregulated by NFATc1 itself (3). When we transfected an NFAT-AP1 reporter construct carrying multiples of the distal NFAT/AP1 site from the IL-2 promoter into EL4 cells, we found that it was induced in a similar fashion as that of NFATc1 protein or the P1 transcript by TCR and costimulation (Fig. 5A). Furthermore, the induction of the NFATc1 protein (11) or P1 transcript (Fig. 4A) was strongly inhibited by cyclosporin A. Therefore, costimulation by CD28 and ICOS resulted in NFATc1 nuclear translocation and possibly autoregulation of the NFATc1 P1 promoter.

FIGURE 5. Regulation of NFAT activity by ICOS. A, NFAT/AP1-Luc or \( Nfatc1 \) P1-Luc constructs and a pPRL-null construct were cotransfected into EL-4 cells by electroporation. After 16 h cells were treated with the indicated combination of anti-CD3, anti-CD28, and anti-ICOS for 6 h and lysed. Luciferase activity was detected by using a Dual luciferase system (Promega). Results from one of four independent experiments were shown as relative luciferase activity (RLA). N/A, Nonactivated. B, Jurkat cells were cotransfected with an NFAT/AP1-Luc reporter plasmid and various expression vectors (pCDNA3, pCDNA3-ICOS, or pCDNA3-ICOSmut (Y-F)) and a control reporter plasmid, pPRL-null. After 16 h, cells were treated with the indicated combination of anti-CD3, anti-CD28, and anti-ICOS for 6 h and luciferase activity was detected by a Dual luciferase assay. A representative of three independent experiments with similar results is shown.
PI3K activity is required for NFAT activation

Because CD28 and ICOS function to regulate PI3K activity, we next examined the importance of PI3K activity in transcription of the Nfatc1 gene. We first treated EL-4 cells with LY294002 and found that the transcription of the Nfatc1 P1 promoter that was induced by TCR and costimulation was inhibited with this treatment (Fig. 4A). Second, we used human Jurkat cells that do not express ICOS for transfections with constructs expressing wild-type mouse ICOS or ICOS harboring a Y181F mutation. These two forms of ICOS were expressed at similar levels as determined by flow cytometry analysis (data not shown). Anti-CD3 activated a NFAT-AP1 reporter, which was enhanced by anti-CD28 (Fig. 5B). Because Jurkat cells are deficient in PI3K, cells transfected with wild-type ICOS, anti-ICOS enhanced the NFAT-AP1 activity activated by anti-CD3 with or without CD28 costimulation (Fig. 5B), which correlates with our previous data on PTEN+/− cells (Fig. 2B). Cells transfected with mutant ICOS/Y181F did not respond to anti-ICOS in enhancing NFAT reporter activity. Therefore, PI3K activated by ICOS appears crucial in the regulation of NFAT activity.

Discussion

Costimulation critically determines T cell activation and tolerance. In this study, we demonstrate a signaling pathway activated by CD28 and ICOS leading to activation of the Nfatc1 P1 promoter. ICOS is a novel costimulatory receptor important in Th2 cytokine production (9, 21, 22). However, the signal transduction mechanisms of ICOS have not been well characterized. Several studies have previously shown that ICOS, like CD28, could associate with p85 and activate PI3K (14). In the current study, we have characterized the function of the YMXM motif in the ICOS cytoplasmic region. We found that the Y181 residue in this motif is required for association with p85 (Fig. 1A) and for ICOS-dependent NFAT activation (Fig. 5B). Thus, the YMXM motif, commonly found in costimulatory receptors in leukocytes, confers at least one important aspect of ICOS signaling. Interestingly, PTEN phosphate limits the response by CD4+ T cells to ICOS, so that ICOS can only enhance the signals induced by TCR and CD28. The loss of one allele of PTEN resulted in a hyperresponsiveness of T cells to ICOS costimulation. It has been previously reported that PTEN+/− mice developed fatal autoimmune disease (23). It is not clear whether this results from increased response to ICOS. It is noteworthy that the disruption of an ICOS repressor, roquin (Rc3h1) E3 ubiquitin ligase, also resulted in autoimmune diseases in mice. Thus, maintaining the signaling strength of ICOS is an important regulation on immune tolerance.

Costimulation is critical in regulating T cell activation and tolerance. Recently, we found that absence of both CD28 and ICOS costimulation resulted in T cell clonal tolerance (12, 24). In vitro, T cells activated in the absence of both CD28 and ICOS costimulation were able to up-regulate activation markers and undergo 2–3 rounds of division but were unable to differentiate into effector cells (11). In the current study, we found that in the absence of costimulation T cells were able to produce PIP3 within 1 h of activation, which was rapidly reduced to background within 3–5 h of T cell activation (Fig. 1B). Thus, we propose that costimulation is not required for the initial T cell activation events but rather to enhance and sustain this activation, at least in part, via signaling through the PI3K pathway. Sustained T cell activation mediated by costimulation is needed for productive T cell activation, because the expression of IL-2, an important T cell growth factor, and the induction of the NFATc1 transcription factor required at least 6 h of PI3K activity (Fig. 2C).

NFATc1 expression is induced after naive T cell activation; the physiological signals that regulate NFATc1 induction has not been understood. Previously, we showed that T cell activation led to a strong induction of the P1 promoter while the synthesis of other NFATc1 isoforms driven by the P2 promoter and of NFATc2 remained unaffected (3, 20). The autoregulation of the P1 promoter by NFAT factors results in strongly increased NFATc1 protein expression. In our current study, we found that signals from CD28 and ICOS costimulatory receptors synergize with TCR, resulting in the activation of the Nfatc1 P1 promoter activity. This work illustrates a physiological context of Nfatc1 transcriptional regulation. NFATc1 is important in T cell proliferation and Th2 differentiation (1, 2). In addition to CD28 and ICOS, OX40 (25) and IL-25 (26) have been shown to enhance NFATc1 nuclear translocation in activated T cells. This effect is associated with potentiation of cytokine-independent early IL-4 production and Th2 differentiation. It is not clear whether the NFATc1 P1 promoter is regulated by these factors. NFATc1 likely functions where the multiple signaling pathway is integrated. The mechanisms illustrated in the current study may be applied to other systems, which will further elucidate the molecular mechanisms governing T cell activation and functional differentiation.

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Disclosures

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