Nuclear Translocation of MEK1 Triggers a Complex T Cell Response through the Corepressor Silencing Mediator of Retinoid and Thyroid Hormone Receptor

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Nuclear Translocation of MEK1 Triggers a Complex T Cell Response through the Corepressor Silencing Mediator of Retinoid and Thyroid Hormone Receptor

Lei Guo,* Chaoyu Chen,* Qiaoling Liang,* Mohammad Zunayet Karim,* Magdalena M. Gorska,**† and Rafeul Alam*††

MEK1 phosphorylates ERK1/2 and regulates T cell generation, differentiation, and function. MEK1 has recently been shown to translocate to the nucleus. Its nuclear function is largely unknown. By studying human CD4 T cells, we demonstrate that a low level of MEK1 is present in the nucleus of CD4 T cells under basal conditions. T cell activation further increases the nuclear translocation of MEK1. MEK1 interacts with the nuclear receptor corepressor silencing mediator of retinoid and thyroid hormone receptor (SMRT). MEK1 reduces the nuclear level of SMRT in an activation-dependent manner. MEK1 is recruited to the promoter of c-Fos upon TCR stimulation. Conversely, SMRT is bound to the c-Fos promoter under basal conditions and is removed upon TCR stimulation. We examined the role of SMRT in regulation of T cell function. Small interfering RNA-mediated knockdown of SMRT results in a biphasic effect on cytokine production. The production of the cytokines IL-2, IL-4, IL-10, and IFN-γ increases in the early phase (8 h) and then decreases in the late phase (48 h). The late-phase decrease is associated with inhibition of T cell proliferation. The late-phase inhibition of T cell activation is, in part, mediated by IL-10 that is produced in the early phase and, in part, by β-catenin signaling. Thus, we have identified a novel nuclear function of MEK1. MEK1 triggers a complex pattern of early T cell activation, followed by a late inhibition through its interaction with SMRT. This biphasic dual effect most likely reflects a homeostatic regulation of T cell function by MEK1.

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In this manuscript, we examined nuclear translocation of MEK1 and its consequences following activation of human CD4 T cells. We specifically investigated the interaction of MEK1 with SMRT and the effect of SMRT inhibition on T cell function. We show that MEK1 interacts with SMRT in the nucleus. Both MEK1 and SMRT bind to the c-Fos promoter and regulate its transcription. SMRT knockdown results in an early-phase stimulation, followed by a late-phase inhibition of T cell activation. IL-10 and β-catenin signaling, induced in the early stimulation phase, play an important role in the late-phase negative feedback inhibition of T cell activation.

Materials and Methods

Human subjects

The protocol for human blood draw and T cell staining studies was approved by the Institutional Review Board of National Jewish Health (Denver, CO). Blood was drawn from healthy subjects upon written consent. Blood was anticoagulated with EDTA. In some experiments, buffalo coat (red cell-depleted leukocyte pack) was obtained from the blood bank donor through the Bonfils Blood Center.

Reagents

The mouse monoclonal anti-human CD3 (clone OKT3) and mouse monoclonal anti-CD28 (clone CD28.2) were obtained from eBioscience (San Diego, CA). A concentration of 1–5 μg/ml of each Ab was used for stimulation. Anti-human IL-2, IL-4, IL-10, and IFN-γ ELISA kits were obtained from BD Biosciences. The dilution of each Ab was performed according to manufacturer’s instructions. Rabbit monoclonal anti-p-ERK1/2, anti-pp38, and anti-MEK1 (clone 47E6, catalog 9126) Abs were obtained from Cell Signaling Technology (Danvers, MA), and a dilution of 1:1000 was used for each Ab, as instructed by each Ab data sheet. For Western blotting purpose, a concentration of 4 μg/ml was used. All other Abs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and a final concentration of 0.5–2 μg/ml was used for Western blotting purpose. The PPAR-γ inhibitor T0070907 and the MEK1 inhibitor T0066519 were numbered as follows: 1, 463; 2, 444; 3, 211; 4, 2; and 5, 665; 2, 463; 3, 444; 2, 211; and 5, 665, respectively. Production of retroviruses, T cell infection, and sorting was done as described (31, 32).

Immunofluorescence staining

Immunofluorescence staining was performed, as described previously (34, 35). CD4 T cells were fixed with 2% paraformaldehyde, and then placed and dried on slides. Slides were blocked for 1 h in 5% serum, 0.03% Triton X-100, and then incubated at 4˚C overnight with primary Ab diluted at the supplier’s recommendation or isotype control Ab. Alexa Fluor fluorochrome-conjugated secondary Ab was used at 1 μg/ml. Cells were analyzed by a Nikon TE 2000 inverted microscope using a ×100 objective. The microscope is equipped with a z-motor (Prior Scientific, Rockland, MA), excitation and emission filter wheels, and a CoolSnap HQ camera (Roper Scientific-Photomicro, Tuscon, AZ). The data acquisition and analysis were done by Metamorph, version 7.1 (Molecular Devices, Downingtown, PA). Images of experimental groups to be compared were acquired using the same software setting (e.g., same exposure time). The pixel level of the images was raised to an arbitrary level (a process called thresholding) for quantitative analyses to eliminate the input from the background fluorescence. The same threshold was applied to all images in comparison studies. Thresholded areas within the selected regions were used for integrated fluorescence intensity measurement. The integrated intensity is defined as a sum of intensities of all selected pixels.

Real-time PCR

Real-time PCR was done as described previously (35). RNA purified from TRIzol (Invitrogen) lysates was reverse transcribed using ImProm-II Reverse Transcription System (Promega), according to the manufacturer’s protocol. Absolute SYBR Green ROX Mix (Thermo Fisher Scientific) was used for DNA amplification. Real-time PCR was performed with the ABI Prism 7000 Sequence Detection System (Applied Biosystems).

Chromatin immunoprecipitation assay

The chromatin immunoprecipitation (ChIP) assay was performed using the ChIP-IT Express Enzymatic kit from Active Motif. We used 5–10×10^6 CD4 T cells per ChIP assay, as described (36). We stimulated cells with PMA (50 ng/ml) and ionomycin (1 μM) for 15 min and then cross-linked with formaldehyde for 10 min. Cells were treated immediately with glycine to stop cross-linking, washed three times, and then frozen at −80˚C. The thawed cells were subjected to the enzymatic shearing step, as per the kit instruction. Immunoprecipitation was performed with ChIP-grade mouse anti-SMRT (Thermo Fisher Scientific), anti-MEK1 (Santa Cruz Biotechnology), anti-phosphohistone H3 Abs (Millipore, Upstate, NY), isotype control IgGs (Jackson ImmunResearch Laboratories) (2 μg each), and protein A/G magnetic beads overnight at 4˚C. The next day, the beads were washed three times with the provided buffer. DNA was eluted after reversal of cross-linking and used as a template for subsequent PCR. We designed five sequential primer sets spanning the proximal c-Fos promoter from +3 to −899 bp position and used them in the PCR. The primer sets were numbered as follows: 1, −899 to −665; 2, −680 to −444; 3, −463 to −210; 4, −348 to −141; and 5, −155 to +3.

Data analysis

Each experiment was done 3–10 times using blood lymphocytes from different donors. The statistical significance of difference between two samples was calculated by paired t test. Results are shown as mean ± SEM. The p value <0.05 was considered statistically significant.

Results

Nuclear translocation of MEK1

MEK1 and MEK2 are two threonine–tyrosine protein kinases that play a crucial role in T cell activation by activating ERK1/2. MEK1, but not MEK2, has a noncanonical nuclear localization motif, Threonine-Proline-Threonine (TPT) (residues 386–388) (11). Phosphorylation of the threonine residues in this motif causes its translocation to the nucleus. MEK1 shuttles between the
nucleus and cytosol at a low level under basal conditions. The nuclear translocation increases upon stimulation (12). We examined the expression of MEK1 and MEK2 in human CD4 T cells using an anti-MEK1 and an anti-MEK1/2 Ab. The anti-MEK1 Ab detected a major band with an approximate molecular mass of 45 kDa (Fig. 1A). There was a faint band below the 45-kDa species. The anti-MEK1/2 Ab did not reveal any additional band. Based upon these findings, we conclude that the 45 kDa is the major MEK1 species in human CD4 T cells. The expression of MEK2 in this cell type is negligible. Next, we examined the nuclear translocation of MEK1 in resting and activated CD4 T cells by Western blotting and immunofluorescence staining. Resting CD4 T cells from healthy human subjects had a low level of MEK1 in the nucleus (Fig. 1B–D). Stimulation of CD4 T cells with anti-CD3 and anti-CD28 Abs increased the overall expression of MEK1 and its nuclear translocation. Treatment of cells with leptomycin B, a known inhibitor of nuclear export, further increased the nuclear level of MEK1.

**MEK1 physically interacts with and regulates the transcriptional corepressor SMRT**

Next, we examined the nuclear function of MEK1. MEK1 has previously been shown to phosphorylate SMRT and prevents its interaction with the nuclear receptor–thyroid hormone receptor and the transcription factor PLZF (19). The foregoing studies were performed in overexpression models, so their physiological implications remain unknown. We examined physical interaction of MEK1 with SMRT using nuclear extract from anti-CD3/CD28 Ab-stimulated CD4 T cells. MEK1 coprecipitated with SMRT from the nuclear extract (Fig. 2A). SMRT exists in multiple molecular mass forms due to alternative splicing (19, 21, 22). We examined the presence of various molecular mass forms of SMRT in CD4 T cells by Western blotting using a polyclonal Ab that has been used in other laboratories (28, 37). We observed a prominent high molecular mass form ~170 kDa and a low molecular mass form ~120 kDa (Fig. 2B) in the nucleus and only the high molecular mass form in the cytosol. There were two faint bands just below and above the 170-kDa molecular mass form. In most experiments, we detected a doublet of the 170-kDa form and its slightly higher molecular mass form. The 170-kDa doublet was consistently detected by Abs obtained from three different vendors, Affinity Bioreagents/Thermo Fisher Scientific (Waltham, MA), Santa Cruz Biotechnology (Santa Cruz, CA), and Abcam (Cambridge, MA). Its mRNA expression was reduced by SMRT siRNA (Fig. 3D). For these reasons, we will present data on the 170-kDa doublet. Next, we studied the effect of TCR stimulation on SMRT mRNA expression by real-time PCR. Anti-CD3/CD28 stimulation decreased the expression of mRNA for SMRT in the first 4 h and then increased its expression at 24 h (Fig. 2C). Immunofluorescence staining showed a predominant nuclear localization of SMRT under basal conditions (Fig. 4). Anti-CD3/CD28 stimulation for 1 h reduced the nuclear level and increased the cytosolic level of SMRT, suggesting a nuclear export (Fig. 4). Thus, TCR stimulation resulted in an immediate reduction in nuclear SMRT through two distinct mechanisms, inhibition at the transcriptional level and nuclear export at the protein level. The latter most likely occurs due to the action of MEK1 as its inhibition with U0126 resulted in increased nuclear SMRT, as shown by Western blotting (Fig. 2D) and immunofluorescence staining (Fig. 4). We examined this issue further by overexpressing MEK1 using a bicistronic GFP-expressing retroviral vector. Overexpression of MEK1 increased its level in the nucleus (Fig. 2E). This was associated with a reduction in the nuclear level of SMRT.

**MEK1 and SMRT bind to the promoter of c-Fos and reciprocally regulate its expression**

MEK1 along with ERK1/2 has been shown to bind the promoter of c-Fos and insulin in a ChIP assay (38). The exact role of MEK1 at the promoter site is unknown, but could include phosphorylation of transcriptional activators and gene repressors, and the removal of the latter from the nucleus. We examined the binding of MEK1 and SMRT to the c-Fos promoter because of its importance in IL-2 production. We performed ChIP studies using five different primer sets spanning the promoter site from +3 to −889 bp position. We detected the presence of SMRT at the distal site (primer set 1) of the c-Fos promoter in nonstimulated CD4 T cells and its removal after stimulation of cells with PMA and ionomycin (Fig. 3A, 3B). MEK1 was present at a low level under basal conditions and was recruited further upon stimulation (Fig. 3B). As a positive control,

![Figure 1](http://www.jimmunol.org/)

**FIGURE 1.** (A) Expression of MEK1 and MEK2 in human CD4 T cells. Purified human CD4 T cells from a healthy subject were Western blotted using an anti-MEK1 and an anti-MEK1/2 Ab (n = 3). Nuclear expression of MEK1. (B) Western blotting of nuclear extract. Purified human CD4 T cells from a healthy subject were incubated in medium alone or with anti-CD3/CD28 Abs for 1 h and then were processed for nuclear and cytosolic extracts and Western blotted for MEK1. Nuclear and cytosolic extracts were reprobed for JunD and tubulin, respectively (n = 4). (C and D) Immunofluorescence staining. Purified CD4 T cells were incubated in medium alone or with anti-CD3/CD28 Abs ± leptomycin B (LMB; 10 ng/ml) for 1 h and then stained for MEK1 (green). Nuclei were stained blue with DAPI. Original magnification ×100. All images were thresholded at the same upper and lower limits of the pixel count for comparison across the experimental groups. Integrated fluorescence intensity of nuclear (blue colored region) MEK1 from 25 cells per experiment and from four separate experiments was analyzed statistically (C). *p < 0.05, paired t test.
we used an anti–phospho-H3 Ab. As anticipated, phospho-H3 was absent under basal conditions, but became detectable at the distal promoter after stimulation. Its dissociation from the c-Fos promoter upon T cell stimulation suggests that SMRT regulates the expression of c-Fos. To test this possibility, we developed two approaches to knockdown SMRT in

FIGURE 2. (A) Coprecipitation of MEK1 with SMRT. We prepared nuclear extract from anti-CD3/CD28–stimulated CD4 T cells, immunoprecipitated with an anti-SMRT or control IgG Ab and then Western blotted for MEK1. Because of disparate molecular masses of MEK1 (45 kDa) and SMRT (170 kDa), the membrane was not reprobed for SMRT. Instead we show equal loading of IgH and IgL chains. The immunoprecipitate was separately Western blotted for SMRT (bottom panel) (n = 3). (B) Identification of isoforms of SMRT in CD4 T cells. Nuclear and cytosolic extracts from CD4 T cells were Western blotted for SMRT (n = 6). (C) Early inhibition and late induction of SMRT by CD3/CD28 stimulation. CD4 T cells were stimulated with anti-CD3/CD28 Abs for the indicated periods of time and then used to isolate RNA and assay for mRNA for SMRT and β-actin by real-time PCR. Data are presented as normalized ratio of SMRT to β-actin (n = 3, *p < 0.05, paired t test). (D) MEK inhibition increases the level of nuclear SMRT. CD4 T cells were stimulated with anti-CD3/CD28 Abs for 1 h in the presence or absence of the MEK inhibitor U0126 (10 μM) and then Western blotted for SMRT using the nuclear and cytosolic extracts. The membrane with the nuclear extract was reprobed for the nuclear repressor ZEB1 (n = 3). The pixel density is shown above the bands. (E) Effect of overexpression of MEK1 on SMRT expression. CD4 T cells were infected with a GFP-expressing bicistronic retrovirus containing cDNA for MEK1 (MEK1) or the empty retrovirus (RV). Cells were sorted for GFP and then Western blotted for SMRT and MEK1 (n = 3). A 50-kDa nonspecific band in the MEK1 blot shows equal protein loading.

FIGURE 3. (A and B) ChIP studies with c-Fos promoter. CD4 T cells were stimulated (stim) with and without (basal) PMA (50 ng/ml)/ionomycin (1 μM). DNA was cross-linked to proteins using formaldehyde and sheared by enzymatic processing using the ChIP-IT Express Enzymatic kit. ChIP grade rabbit anti-MEK1, anti-SMRT, and anti–phospho-H3 Abs, and control rabbit IgG were used to immunoprecipitate the bound chromatin. Following reversal of cross-linking and digestion of the proteins, the DNA was used for PCR. Five sets of primers (1–5, in which 5 is proximal to the start codon site) were designed from the proximal promoter sequence of c-Fos and used in PCR. An image of the electrophoresed amplicons is shown in (A). The input DNA was electrophoresed at 1:50 dilution. The density of the bands generated with the 1 primer set was analyzed and presented as enrichment over the input. Results from three separate experiments are shown in Fig. 5B. (C) Expression of SMRT mRNA following siRNA- and shRNA-mediated knockdown. Purified CD4 T cells were either transfected with the Smartpool siRNA or infected with the shRNA retrovirus for SMRT. After resting overnight, cells were stimulated with anti-CD3/CD28 Abs for 30 min. Purified RNA was used for real-time PCR (n = 3). (D and E) CD4 T cells were transfected with siSMRT or control siNT and stimulated as above for 0.5, 2, and 4 h. An aliquot of cells that were stimulated for 0.5 h was Western blotted for SMRT, c-Fos, MEK1, and β-actin (control) (D). The other aliquots were used to isolate RNA and real-time PCR for c-Fos (E) and c-Jun (F). n = 3, *p < 0.05, paired t test.
the cells. We used a smartpool siRNA for SMRT and a control nontargeting siRNA pool (siNT) and transfected cells with these siRNA using the Amaxa protocol. We also constructed bicistronic GFP-expressing retroviral vectors encoding shRNA against luciferase (negative control) and SMRT. Short interfering SMRT (siSMRT) and shRNA for SMRT, but not the nontargeting siNT and shRNA, reduced the expression of SMRT by >65% (Fig. 3C, 3D). The siSMRT-mediated knockdown was associated with an increase in c-Fos protein (Fig. 3D) and mRNA (Fig. 3E) within 30 min. SMRT knockdown did not affect the expression of mRNA for c-Jun (Fig. 3F).

**SMRT knockdown results in an early increase, followed by a late decline in cytokine production**

Next, we studied the effect of SMRT knockdown on T cell function. We measured cytokine production by ELISA following stimulation of CD4 T cells with anti-CD3/CD28 stimulation. Because we observed a biphasic (an early-phase inhibition and a late-phase upregulation) effect of TCR stimulation on SMRT expression (Fig. 2C), in preliminary studies we examined T cell cytokine production at multiple time points following anti-CD3/CD28 stimulation. We observed an initial rise and a late fall in IL-2 production in SMRT knockdown CD4 T cells (Fig. 5A). In subsequent studies, we studied cytokine production at an early (8 h) and a late (48 h) time point. We confirmed the biphasic profile of IL-2 production in a larger study group. We also observed a similar early rise and a late fall in the production of IL-4, IL-10, and IFN-γ in SMRT knockdown T cells as compared with the control cells (Fig. 5B, 5C). SMRT knockdown was associated with an inhibition of T cell proliferation, as demonstrated by reduced CFSE dilution (Fig. 5D, 5E). The effect on proliferation most...
likely results from the inhibition of IL-2 production that commences by ~24 h. The late decline in IL-4 and IFN-γ is secondary to the dramatic reduction in the level of IL-2. Supplementation with IL-2 reversed the inhibitory effect of SMRT knockdown on IL-4 and IFN-γ production (Fig. 5F). The supplementation also reversed the inhibition of T cell proliferation with siSMRT (Fig. 5G). To understand the phenotype of SMRT knockdown T cells, we studied activation of three signaling pathways that are involved in T cell activation, ERK1/2, p38, and p65 NF-κB. We also examined the expression of JunD, a negative regulator of T cell activation (37). There was no difference in ERK1/2 activation after TCR stimulation of SMRT knockdown T cells (Fig. 6A). The phosphorylation of p38 was mildly elevated. There was no difference in the nuclear expression level of p65 NF-κB and JunD (Fig. 6B). We recognize that the p65 NF-κB is just one of many members of the NF-κB family, and our result does not rule out the involvement of other NF-κB members in SMRT knockdown cells.

IL-10 establishes a negative feedback loop to inhibit T cell activation

Because SMRT knockdown CD4 T cells produced high quantities of IL-10 at the early time point, we asked whether this early rise in IL-10 mediated the late inhibition of cytokine production. To this goal, we infected CD4 T cells with the control shRNA- or SMRT shRNA-encoding bicistronic retrovirus. Infected cells were sorted for expression of GFP, rested overnight in the medium alone, and then cultured on an anti-CD3/CD28–coated plate with a neutralizing anti–IL-10 or an isotype control Ab. The anti–IL-10 Ab partially reversed the inhibitory effect of SMRT knockdown on the late-phase IL-2 production (Fig. 7A). We also checked FOXP3 expression in SMRT knockdown cells. There was no difference in FoxP3 expression following SMRT knockdown (Fig. 7B). Next, we examined whether IL-10 exerted its inhibitory effect on CD4 T cells through the corepressor SMRT. We cultured CD4 T cells with anti-CD4/CD28 Abs for 48 h in the presence and absence of IL-10 and then examined the expression of SMRT by immunofluorescence staining and Western blotting. Anti-CD3/CD28 stimulation significantly upregulated SMRT expression (Fig. 7C–E). This expression was further amplified by the addition of IL-10. Cells cultured with IL-10 alone showed a negligible effect.

β-catenin signaling contributes to the late-phase inhibition of T cell IL-2 production

SMRT antagonizes gene transcription induced by β-catenin (39, 40) and PPAR-γ (41). The latter molecules are known to regulate T cell function (42, 43). We applied two pharmacologic inhibitors to explore their involvement in inhibition of IL-2 production in SMRT knockdown T cells. T0070907 is a specific inhibitor of PPAR-γ and its nuclear reduction plays a permissive role (Fig. 8). In the late phase, SMRT functions as an activator of T cells. SMRT antagonizes two inhibitory signals that arise in the late phase, IL-10 and β-catenin. By antagonizing these two signals, SMRT promotes sustained IL-2 production in SMRT knockdown T cells.

The translocation of MEK1 to the nucleus has previously been demonstrated in other cells (10). MEK1 is translocated to the nucleus in an importin 7-dependent manner. Two distinct mechanisms have been identified for nuclear translocation (11). Translocation following a mitogenic stimulation requires MEK1 kinase activity. MEK1 also translocates to the nucleus at a low level under basal conditions. This is largely independent of MEK1 kinase activity. It requires phosphorylation of a unique TPT (residues 386–388) motif for translocation. ERK2 has been shown to phosphorylate the TPT motif. We have shown that MEK1 directly binds to the c-Fos promoter in T cells. This is in agreement with a previous report in which human pancreatic β cells were studied (38). We have also shown that the recruitment of MEK1 to the promoter involves an interaction with a specific stimulatory signal (44). FH535 inhibits both β-catenin and PPAR-γ (45).

Effect of SMRT knockdown on cytosolic and nuclear signaling. (A) CD4 T cells were transfected with siSMRT and siNT Smartpool shRNA; stimulated with and without (medium) anti-CD3/CD28 Abs for 10 min; and then Western blotted for p-p38 and pERK1/2. The p-p38 membrane was reprobed for p38, and the pERK1/2 membrane was reprobed for β-actin. (n = 4). (B) CD4 T cells were transfected with siSMRT and siNT shRNA and then stimulated with anti-CD3/CD28 Abs for 8 and 48 h. Nuclear extract was isolated and Western blotted for p65 NF-κB and JunD. The membrane was reprobed for HDAC3 (n = 4).

Discussion

In this work, we have shown that MEK1 translocates to the nucleus following TCR stimulation and interacts with the nuclear corepressor SMRT in CD4 T cells. MEK1 is bound to the c-Fos promoter at a low level under basal conditions and is further recruited following T cell activation. In contrast, SMRT is bound to the c-Fos promoter under basal condition and is removed following stimulation. These reciprocal changes in MEK1 and SMRT binding to the promoter site result in increased c-Fos expression and cytokine production in the early stage of T cell activation. The role of SMRT in T cell activation is far more complex than would be expected from a simple corepressor function. In the early stage of T cell activation, SMRT functions as a corepressor and its nuclear reduction plays a permissive role (Fig. 8). In the late phase, SMRT functions as an activator of T cells. SMRT antagonizes two inhibitory signals that arise in the late phase, IL-10 and β-catenin. By antagonizing these two signals, SMRT promotes sustained IL-2 production and T cell proliferation.

The N-terminal nuclear export signal allows MEK1 to exit the nucleus. MEK1 has previously been shown to cause nuclear export of SMRT (19) and PPAR-γ (14). We have demonstrated a direct
interaction of endogenous MEK1 with SMRT in CD4 T cells in this study. Inhibition of MEK1 increases the nuclear content of SMRT, suggesting that MEK1 regulates gene repression by controlling the nuclear level of the corepressor SMRT. MEK1 is one of a number of molecules that regulate the nuclear level of SMRT. The others include MEK kinase 1 (19), 14-3-3, and PIN1 (46). T cell stimulation results in activation and engagement of these molecules (47–49). The individual contribution of these molecules in regulating nuclear export of SMRT following T cell activation will require further studies.

SMRT has been shown to antagonize the transcriptional activity of β-catenin (39, 40). β-catenin is important for T cell development and function. β-catenin positively regulates thymopoiesis (50) and Th2 cell differentiation in the mouse model (51, 52). However, it inhibits differentiation of CD4 Th17 cells (53) and CD8 effector cells (54). β-catenin induces T cell anergy in mature T cells (55). In human T cells, β-catenin signaling inhibits IL-2 and IFN-γ production and blocks upregulation of the IL-2R (56). As a result, T cell proliferation is blocked, β-catenin impairs maturation of CD45RA naïve T cells into CD45RO memory cells. Our results are in agreement with the foregoing studies. β-catenin modestly, but significantly inhibited IL-2 production in human CD4 T cells (Fig. 7F). This inhibition was remarkably pronounced in SMRT knockdown T cells, as uncovered in our inhibitor studies with FH535. The results suggest that SMRT antagonizes β-catenin signaling in T cells. Although FH535 inhibits both β-catenin and PPAR-γ, we think PPAR-γ is unlikely to play any role in our model. The PPAR-γ-specific inhibitor T0070907 actually inhibited IL-2 production. IL-2 production in SMRT knockdown T cells was unchanged in the presence of T0070907.

Previously, SMRT was studied in Jurkat T cells (57). Through the use of reporter genes, it was shown that CD3/CD28 ligation increased the association of SMRT with nuclear RAR, RXR, and thyroid hormone receptor. This association resulted in silencing the target gene expression. The increase in SMRT–nuclear re-
ceptor association was protein kinase C-0 dependent. RAR, RXR, and LXR are negative regulators of T cells, although their effect on a specific Th cell subtype may vary. A conductive T cell activation signaling requires silencing these receptors through SMRT. The late effect of SMRT knockdown in our study is in agreement with the foregoing study. We speculate that in the absence of SMRT, gene transcription through RAR, RXR, and LXR is uninhibited, which also contributes to the inhibition of cytokine production and T cell proliferation.

In addition to NFs, SMRT targets certain transcription factors and regulates the expression of their target genes. In macrophages, SMRT regulates the expression of 87 LPS-inducible and LXR-regulated genes (25). Along with NCoR, it coregulates another 80 genes. We have observed SMRT recruitment to the c-Fos promoter. NF-kB has been reported to be a target of SMRT (26). We have not observed any difference in the nuclear level of p65 NF-kB in SMRT knockdown T cells. The results suggest that the nature of the gene targeted by SMRT varies depending upon the type of the cell. Our studies have revealed a biphasic effect of SMRT knockdown in human T cells. We believe that the early-phase rise in cytokine production results from the absence of co-repressor activity of SMRT for c-Fos and possibly other SMRT-regulated genes. SMRT interacts with many histone deacetylases belonging to the class I and class II HDACs (58). Absence of SMRT could lead to the dissociation of HDACs from the gene promoters and initiation of gene transcription. The disruption of HDAC11 has been shown to induce IL-10 production (59). SMRT is a broad-spectrum co-repressor. It represses not only inhibitory, but also stimulatory genes (25). Based upon this tenet, we have anticipated a complex function of SMRT in T cells. Accordingly, we have observed early-phase repressive and late-phase stimulatory effects of SMRT in CD4 T cells. Our results suggest that a complete deficiency would be detrimental to CD4 T cells. However, a low-level upregulation and downregulation of SMRT would most likely affect a wide spectrum of T cell functions.

Disclosures
The authors have no financial conflicts of interest.

References


