A Novel ERK-dependent Signaling Process That Regulates Interleukin-2 Expression in a Late Phase of T Cell Activation*

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Engagement of the T cell antigen receptor (TCR) rapidly induces multiple signal transduction pathways, including ERK activation. Here, we report a critical role for ERK at a late stage of T cell activation. Inhibition of the ERK pathway 2–6 h after the start of TCR stimulation significantly impaired interleukin-2 (IL-2) production, whereas the same treatment during the first 2 h had no effect. ERK inhibition significantly impaired nuclear translocation of c-Rel with a minimum reduction of NF-AT activity. Requirement for sustained ERK activation was also confirmed using primary T cells. To induce sustained activation of ERK, T cells required continuous engagement of TCR. Stimulation of T cells with soluble anti-TCR antibody resulted in activation of ERK lasting for 60 min, but failed to induce IL-2 production. In contrast, plate-bound anti-TCR antibody activated ERK over 4 h and induced IL-2. Furthermore, T cells treated with soluble anti-TCR antibody produced IL-2 when phorbol 12-myristate 13-acetate, which activates ERK, was present in the culture medium 2–6 h after the start of stimulation. Together, the data demonstrate the presence of a novel activation process following TCR stimulation that requires ERK-dependent regulation of c-Rel, a member of the NF-κB family.

The T cell receptor (TCR) initiates signal transduction through the intracellular regions of the CD3 and ζ molecules via the sequence referred to as the immunoreceptor tyrosine-based activation motif (1). Antigenic stimulation induces Src family kinase-mediated phosphorylation of both tyrosines in immunoreceptor tyrosine-based activation motifs and creates a binding site for the cytoplasmic protein-tyrosine kinase ZAP-70 (2, 3). Following this recruitment, ZAP-70 is activated and initiates a downstream signaling cascade.

Downstream of ZAP-70, the MAPK family of kinases has been shown to play critical roles in T cell activation and differentiation (reviewed in Refs. 1 and 4). Biological outcomes that have been reported to be controlled by ERK, a member of the MAPK family, include cytokine production, apoptosis, proliferation, positive and negative selection, and cytolysis. A major target of ERK has been postulated to be Elk1, which in turn up-regulates expression of c-Fos (5). c-Fos, when dimerized with the Jun family of transcription factors, forms AP-1. A number of AP-1-binding sites have been identified in promoter regions for genes induced by TCR stimulation, such as IL-2 (6). A function of AP-1 in IL-2 gene activation is to form a complex with another transcription factor, NF-AT (7, 8). Formation of this complex has been shown to play critical roles in IL-2 gene activation.

Recently, we identified a Jurkat T cell-derived mutant cell line (J.SL1) that has lost expression of the adaptor molecule Shc (9). In this cell line, TCR activation leads to impaired IL-2 production and ERK activation, whereas AP-1 and NF-AT activation is unaffected. Detailed analysis revealed that nuclear translocation of an NF-κB transcription factor, c-Rel, is severely reduced in this cell line, whereas RelA, another member of the NF-κB family, is activated normally. Loss of IL-2 promoter activity in J.SL1 cells could be rescued by activation of c-Rel using estrogen receptor fusion protein, indicating that loss of IL-2 production in J.SL1 cells is partly due to lack of c-Rel activation.

Although studies using c-rel knockout mice showed that c-Rel is essential for IL-2 production (10, 11), it is not well understood how TCR triggers c-Rel activation. Other studies have shown that protein kinase Cδ is required for TCR-induced NF-κB activation (12–14). This function of protein kinase Cδ appears to be required for activation of IκB kinase (15, 16). The activation of protein kinase Cδ and IκB kinase takes place within minutes after engagement of TCR, and nuclear translocation of RelA is observed within 15 min after stimulation. In contrast, nuclear translocation of c-Rel takes place 3–4 h after stimulation and also requires de novo synthesis of protein (6, 17, 18). This suggests that TCR-induced c-Rel nuclear translocation requires a signaling pathway distinct from the one that activates RelA.

Here, we report evidence that the ERK signaling pathway is essential for c-Rel nuclear translocation in the TCR signaling pathway. Most interestingly, ERK activity is required for c-Rel activation 2–6 h after the start of TCR stimulation. This late phase ERK activity is also required for IL-2 production.

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The abbreviations used are: TCR, T cell antigen receptor; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; IL-2, interleukin-2; CHO, Chinese hamster ovary; ER, estrogen receptor; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; NF-κB, nuclear factor-κB; PMA, phorbol 12-myristate 13-acetate; ELISA, enzyme-linked immunosorbent assay; JNK, c-Jun N-terminal kinase.

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MATERIALS AND METHODS

Cells—Jurkat cells (a gift from Dr. A. Weiss, University California, San Francisco, CA) and 2B4 and CHO cells (gifts from Dr. M. M. Davis, Stanford University, Stanford, CA) were maintained in RPMI 1640 medium supplemented with 5% (for Jurkat cells) or 10% (for 2B4 and CHO cells) fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 50 μM -mercaptoethanol (2B4 and primary T cells). The 2B4 cell line, which stably expresses c-Rel/estrogen receptor (ER) fusion protein (9), was established by retroviral transduction using the MIGR1 retroviral vector and BOSC23 packaging cell line (gifts from Dr. W. S. Pear, University of Pennsylvania, Philadelphia, PA) as described (19). Primary T cells were purified from splenocytes of C57BL/6 mice (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) or TCR A1(M) transgenic mice (transgenic for the TCR of an I-Ek-restricted H-Y antigen-specific T cell clone) (20) using nylon wool (Robbins Scientific Corp., Sunnyvale, CA) or goat anti-mouse IgG antibody-based panning (21), respectively.

Antibodies and Reagents—Anti-Jurkat TCR antibody (C305, a kind gift from Dr. A. Weiss) was purified from mouse ascites using an Immunopure IgM purification kit (Pierce). Anti-c-Rel and anti-ER polyclonal antibodies were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). Anti-phospho-ERK polyclonal antibody was from Promega. Anti-p65 NF-κB (RelA; sc-8008) and anti-estrogen receptor (sc-543) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Fluorescein isothiocyanate-labeled anti-CD69 and anti-CD154, biotinylated anti-CD3 (2C11), and phycoerythrin-labeled anti-CD25 monoclonal antibodies were from Pharmingen. Horseradish peroxidase-labeled anti-mouse and anti-rabbit polyclonal antibodies and the MEK inhibitor PD98059 were purchased from New England Biolabs Inc. (Beverly, MA). Actinomycin D and estrogen (β-estradiol) were from Sigma. PMA and ionomycin were from Calbiochem. Goat anti-mouse Ig polyclonal antibody was from Jackson ImmunoResearch Laboratories, Inc.

Stimulation and Inhibitor Treatment of Cells—For stimulation with plate-bound anti-TCR antibody, tissue culture plates and polystyrene beads (Polyscience, Warrington, PA) were incubated with antibody

FIG. 1. Requirement for ERK activation by IL-2 production and c-Rel nuclear translocation. A, inhibition of IL-2 production and NF-AT activity by PD98059. Jurkat cells were stimulated with anti-TCR antibody in the presence of various concentrations of PD98059. Percent inhibition of IL-2 secretion (■) and NF-AT luciferase activity (□) is presented (samples with no inhibitor were taken as 100%). B, ERK activation in PD98059-treated cells. Jurkat cells were stimulated with PD98059 for 30 min prior to the start of stimulation. Cells were then stimulated in the presence of PD98059 with soluble anti-TCR antibody. Cell lysates were made 5 min after the start of stimulation and analyzed by Western blotting with anti-phospho-ERK antibody (pERK; upper panel) or by an in vitro kinase assay using myelin basic protein as the substrate (lower panel). S, stimulated; NS, not stimulated; pMBP, phosphorylated myelin basic protein. C, effect of PD98059 treatment on the induction of surface antigens. Jurkat cells were stimulated with anti-TCR antibody in the absence or presence of 25 μM PD98059 as indicated. Expression of CD25, CD69, and CD154 was analyzed using a fluorescence-activated cell sorter. The solid lines show the profiles of stimulated cells, and the dashed lines those of unstimulated cells. D, inhibition of c-Rel nuclear localization by PD98059. Jurkat cells were stimulated with anti-TCR antibody for 4 or 6 h in the absence or presence of PD98059 (2 or 25 μM). Nuclear extracts (N; upper panel) and cytoplasmic fractions (C; lower panel) were isolated and analyzed by Western blotting using anti-c-Rel antibody. The relative amount of each band was determined by densitometry and is shown below each lane. The amount detected at 4 h of stimulation with no PD98059 treatment was taken as 100%. U, unstimulated. E, comparison of PD98059 effect on c-Rel and RelA. Jurkat cells were stimulated with anti-TCR antibody for 2, 4, or 6 h in the absence or presence of PD98059 (2 or 25 μM). Nuclear extracts were isolated and analyzed by Western blotting with anti-c-Rel (upper panel) and anti-RelA (lower panel) antibodies. The relative amount detected for each sample is shown below each lane. The amount detected at 4 h of stimulation with no PD98059 treatment was taken as 100%.
ERK Requirement in a Late Phase of T Cell Activation

ERK Activation Is Required for c-Rel Nuclear Translocation—Because lack of Shc in Jurkat cells results in partial loss of ERK activation and significant impairment of c-Rel activation and IL-2 production, we hypothesized that a high level of ERK activity is required for c-Rel nuclear translocation and IL-2 production. To test this, we examined the effect of ERK inhibition on Jurkat cells stimulated with anti-TCR antibody in the presence of varying amounts of a MEK-specific inhibitor, PD98059. As shown in Fig. 1A, treatment of Jurkat cells with 25 \( \mu \text{M} \) PD98059 blocked IL-2 production by 80%, and 5 \( \mu \text{M} \) PD98059 still inhibited IL-2 production by 50%. In contrast, NF-AT-dependent transcriptional activity was less sensitive to PD98059 (Fig. 1A). 25 \( \mu \text{M} \) PD98059 inhibited activity by >50% whereas 5 \( \mu \text{M} \) PD98059 had little effect, if any.

To examine the effectiveness of treatment with PD98059, cell lysates were isolated from samples to which differing doses of PD98059 were added 30 min before the start of 5 min of TCR stimulation. As shown in Fig. 1B, PD98059 blocked TCR-induced ERK activation in a dose-dependent manner. Thus, the lack of NF-AT inhibition with low concentrations of PD98059 treatment was not due to ineffective ERK inhibition.

To examine whether other T cell responses are sensitive to inhibition of ERK, we tested the effect of PD98059 on activation-induced expression of the cell-surface antigens CD69, CD154 (CD40 ligand), and CD25 (IL-2 receptor \( \alpha \)-chain). Jurkat cells were stimulated with anti-TCR antibody in the presence of 25 \( \mu \text{M} \) PD98059, and the surface expression of these molecules was determined. The expression patterns of CD69, CD154, and CD25 were only slightly impaired even though 25 \( \mu \text{M} \) PD98059 had been shown to substantially inhibit IL-2 production (Fig. 1C).

Next, we determined whether low doses of the MEK inhibitor affect c-Rel activation. Cells were stimulated with anti-TCR antibody in the presence of 2 or 25 \( \mu \text{M} \) PD98059, and their nuclear and cytoplasmatic proteins were analyzed by Western blotting with anti-c-Rel antibody. As shown in Fig. 1D, treat-
ment of Jurkat cells with either 2 or 25 μM PD98059 reduced the amount of nuclear c-Rel in a dose-dependent manner (upper panel). No significant change was observed for cytoplasmic c-Rel (lower panel). In contrast, treatment with 5 μM PD98059 had very little effect on RelA nuclear localization as shown in Fig. 1E. Even with 25 μM, reduction of nuclear localization was limited to 50% of untreated samples. It should be noted that RelA translocated to the nucleus much earlier than c-Rel (see 2-h samples) and that the RelA levels in the nucleus decreased after 4 h. These data suggest that c-Rel and RelA are under the control of two different signaling pathways and that ERK plays a more significant role in c-Rel activation.

c-Rel Activation Partially Rescues Inhibition of IL-2 Production by PD98059—To test whether loss of c-Rel nuclear translocation was responsible for reduced IL-2 production following treatment with PD98059, we established T cell lines constitutively expressing a c-Rel/ER fusion protein. This fusion protein accumulated both in the cytoplasm and nucleus in the presence of estrogen as shown in Fig. 2A. This increase in cytoplasmic and nuclear c-Rel/ER was also observed in 2B4 mouse T cell hybridoma cells in an estrogen dose-dependent manner (Fig. 2B). When Jurkat T cells expressing c-Rel/ER were stimulated with anti-TCR antibody in the presence of 25 μM PD98059, IL-2 production was reduced by 50% compared with 70% for control Jurkat cells (Fig. 2C). Treatment with estrogen alone showed almost no effect on IL-2 production in both Jurkat cells. This shows that nuclear accumulation of c-Rel is not sufficient for IL-2 production. When estrogen was added along with PD98059, c-Rel/ER-transfected Jurkat cells (but not parental Jurkat cells) showed a significant increase in IL-2 production over cells treated with only PD98059.

We also established transfectants of the 2B4 mouse T cell hybridoma cells (specific for I-Ek plus moth cytochrome c) (22) expressing c-Rel/ER. Transfected cells were stimulated with antigen peptide presented by I-Ek-positive CHO cells. When 2B4 T cells were treated with PD98059, antigen-induced IL-2 production was almost abrogated (Fig. 2D). However, when estrogen was added to the culture along with PD98059, c-Rel/ER transductants (but not parental 2B4 cells) showed significant levels of IL-2 production, as observed with control Jurkat cells. No IL-2 production was observed with estrogen treatment alone (data not shown). The data suggest that the reduced IL-2 production caused by inhibition of ERK is partially due to the loss of c-Rel nuclear translocation.

c-Rel Activation and IL-2 Production Require Sustained ERK Activation—Because nuclear localization of c-Rel is a slow event (peaks at 4 h after the start of TCR stimulation), we examined which time point of ERK activity is most important...
for c-Rel activation and IL-2 production. We first determined the critical time point of ERK activation in IL-2 production. Jurkat cells were stimulated with plate-bound anti-TCR antibody, and the level of IL-2 production was determined in untreated and PD98059-treated samples. When PD98059 was added at the beginning of stimulation (with a pretreatment for 30 min) and removed 2 or 4 h later, IL-2 production was not reduced (Fig. 3A). However, when PD98059 was present for 6 h, a significant reduction was observed. To our surprise, when the inhibitor was added 2 h after the start of stimulation and present for up to 6 h, IL-2 production was almost eliminated. Addition of the inhibitor even 4 h after the start of stimulation caused a significant reduction in IL-2 production. NF-AT activity was affected much less (maximum 30% reduction) by PD98059. IL-2 production by antigen peptide-stimulated 2B4 cells showed similar kinetic characteristics compared with Jurkat cells (Fig. 3B), although the amounts of IL-2 produced were much higher.

The requirement for sustained ERK activation was also tested with primary T cells. Purified CD4⁺-enriched mouse splenic T cells (from TCR A1(M) transgenic mouse, >90% CD4⁺) (data not shown) were stimulated with anti-CD3 antibody for 24 h (Fig. 3C). PD98059 was added to the culture at various time points. As shown, addition of PD98059 12 h after the start of stimulation still blocked IL-2 production as effectively as addition at the start of culture.

To examine whether the effect of PD treatment was at the level of synthesis or secretion, we next tested the level of IL-2 production by intracellular staining (Fig. 3D). A1(M) splenic T cells were stimulated with anti-CD3 and anti-CD28 antibodies and showed clear expression of intracellular IL-2 (left panel). Addition of PD98059 for the last 6 h of culture resulted in IL-2 production similar to the levels generated by unstimulated samples. Culture supernatants from cells treated in this manner did not contain IL-2 when examined by ELISA (data not shown). Thus, PD98059 blocks the synthesis and not the secretion of IL-2. As observed with Jurkat cells, CD25 and CD69 expression was at comparable levels in untreated and PD98059-treated samples (data not shown).

Next, we examined the effect of the same treatment on nuclear localization of c-Rel (Fig. 4A). Treatment of TCR-activated Jurkat cells with PD98059 for the first 2 h (with 30 min of pretreatment) had only a minor effect on c-Rel nuclear localization. However, when the inhibitor was present for 4 h, nuclear localization of c-Rel was significantly reduced. Inhibition of ERK 2 or 4 h after the start of stimulation was equally effective.

It was previously reported that de novo synthesis of protein is required for nuclear translocation of c-Rel (17). The ERK requirement in the late phase of activation raised the question of whether ERK is required for de novo protein synthesis. To test this, we examined whether the kinetics of de novo protein synthesis required for c-Rel activation are the same as those of ERK. RelA was also analyzed in this experiment to examine whether the ERK requirement is specific for c-Rel. Jurkat cells were treated with actinomycin D to inhibit mRNA/protein synthesis at different points of activation, and nuclear localization of c-Rel was measured by Western blotting. As shown in Fig. 4B, actinomycin D treatment of Jurkat cells inhibited c-Rel nuclear translocation very effectively when it was added to the culture for the first 2 h. Treatment between the 4- and 6-h time points of stimulation also showed a significant inhibitory effect. This pattern differs from that of PD98059 inhibition and indicates that protein synthesis required for c-Rel activation at an early stage (0–2 h) is ERK-independent. In contrast, inhibition of ERK showed very little effect on nuclear localization of RelA (Fig. 4B). Moreover, RelA nuclear translocation was only mildly affected by 6 h of actinomycin D treatment and even increased with limited treatment between 4 and 6 h. This increase may reflect the loss of IκB expression, which plays a critical inhibitory role in nuclear localization of RelA.

To confirm the effect of ERK inhibition, the levels of phospho-ERK were compared among samples treated with PD98059 at different time points. As shown in Fig. 4 (C and D),
when the level of phospho-ERK in cells stimulated with anti-TCR antibody was taken as 100%, treatment with PD98059 for the first 2 h caused a slight increase in ERK phosphorylation. This is likely due to inhibition of ERK phosphatase induction (24). In contrast, treatment of cells 0–6 or 2–6 h after stimulation resulted in effective inhibition of ERK activity at the 6-h time point.

**Activation of ERK at Late Stages Can Restore IL-2 Production by Soluble Anti-TCR Antibody**—It has been well documented that stimulation with antibody against TCR in soluble forms does not induce lymphokine production even though early biochemical events appear to represent what occurs with full T cell activation (25, 26). Because we found that sustained activation of ERK is essential for IL-2 production, we tested whether soluble anti-TCR antibody induces sustained ERK activation. Jurkat T cells were stimulated with either soluble or plate-bound anti-TCR antibody. When we compared the state of the activated form of ERK, soluble anti-TCR antibody induced robust activation of ERK (Fig. 5A, left panels). This activation lasted for 30 min, but quickly declined thereafter. In contrast, plate-bound antibody stimulation induced ERK activation slowly (peaked at 30 min), but the level of activation was sustained and remained high 6 h after the start of stimulation. A similar pattern was observed for activation of MEK (right panels). NF-AT activity induced by soluble antibody was ~30% of that induced by plate-bound antibody.2

To test whether activation of ERK could be the missing element required for IL-2 production induced by soluble antibody treatment, we used a pharmacological agent to stimulate ERK in a TCR-independent manner. PMA induces strong activation of Ras and downstream molecules, including ERK, in T cells (27, 28). When ERK was activated by addition of PMA to the culture medium, soluble anti-TCR antibody induced a significant amount of IL-2 (Fig. 5B). Stimulation by ionomycin together with soluble anti-TCR antibody had no significant effect on IL-2 production. Treatment of Jurkat cells with PMA also induced rapid c-Rel and RelA nuclear translocation (Fig. 5C). Interestingly, soluble antibody alone induced an early (30 min), but not late (6 h), increase in nuclear RelA.

Using this function of PMA, we determined the time period when PMA treatment is required for IL-2 production. Jurkat cells were stimulated with soluble anti-TCR antibody. PMA was added to the culture at the start of stimulation and was removed at four different time points. As shown in Fig. 5D, when PMA was removed as late as 4 h after stimulation, the effect of PMA on IL-2 production was not observed. However, if PMA was present in the culture for 6 h or longer, soluble anti-TCR antibody stimulation induced IL-2 production at a level comparable to that of plate-bound antibody.

Next, Jurkat cells were first stimulated with soluble anti-TCR antibody, and then PMA was added later to the culture to test how long PMA stimulation can be delayed for the induction

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Fig. 5. Production of IL-2 by cells stimulated with soluble anti-TCR antibody and PMA. A, kinetics of ERK and MEK activation by soluble and plate-bound anti-TCR antibodies. Jurkat cells were stimulated with soluble anti-TCR antibody (S; upper panels) or plate-bound (for ERK) or bead-bound (for MEK) anti-TCR antibody (F; lower panels). Cells were lysed at various time points (indicated above each lane) and analyzed by Western blotting with antibodies against phospho-ERK (pERK; left panels) or phospho-MEK (pMEK; right panels). B, IL-2 production by Jurkat cells stimulated with soluble anti-TCR antibody and PMA. Jurkat cells were stimulated with soluble (Ab/s) or plate-bound (Ab/b) anti-TCR antibody. PMA (10 ng/ml) or ionomycin (Iono; 1 μM) was added to the culture. The levels of secreted IL-2 for each sample 24 h after the start of stimulation were determined by ELISA. C, nuclear translocation of c-Rel and RelA in cells stimulated with soluble anti-TCR antibody and PMA. Nuclear translocation of c-Rel (upper panels) and RelA (lower panels) was determined for Jurkat cells stimulated as described for B. Nuclear (N) and cytoplasmic (C) fractions were isolated 30 min (left panels) or 6 h (right panels) after the start of stimulation. D, IL-2 production by Jurkat cells stimulated with soluble anti-TCR antibody with limited PMA treatment. Jurkat cells were stimulated with soluble anti-TCR antibody, and PMA (10 ng/ml) was added to the culture for limited periods as shown below each bar. After removal of PMA-containing medium, cells were washed, and anti-TCR antibody-containing medium was added back to the culture. 90% of the medium of each sample was replaced with fresh medium 6 h after the initial stimulation. IL-2 production was determined by ELISA.

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2 T. Koike and M. Iwashima, unpublished data.
of IL-2. As shown in Fig. 5E, we observed no reduction of IL-2 production by soluble anti-TCR antibody when PMA was added as late as 4 h after the start of stimulation. When PMA was added 6 h after TCR stimulation, a slight decrease in IL-2 production was observed. All these results indicate that activation of ERK at late time points (4–6 h) is essential for the production of IL-2.

To confirm that the function of PMA involves activation of ERK, we added PD98059 to the cells that were stimulated with soluble anti-TCR antibody and PMA. As shown in Fig. 5F, addition of PD98059 (25 μM) abrogated IL-2 production that could be induced by soluble anti-TCR antibody plus PMA treatment. This strongly suggests that ERK activation is an essential requirement for IL-2 production induced by PMA stimulation in this system.

**DISCUSSION**

The data presented here demonstrate that TCR-induced activation of ERK must be sustained for the production of IL-2. Most strikingly, treatment of both human and mouse T cells with an ERK inhibitor was not effective in blocking subsequent IL-2 production during the initial phase of TCR-induced activation, but rather exerted its effect in a late phase (2–6 h). The loss of c-Rel translocation is, in part, responsible for the loss of IL-2 production because activation of c-Rel/ER partially counteracts the reduction in IL-2 production caused by ERK inhibition.

This result indicates that there is a novel signaling pathway that connects ERK and c-Rel at a late time point in T cell stimulation. As an NF-κB family member, c-Rel is regulated by the IκB family of proteins, and their regulation is controlled by the IκB kinase family of kinases (29). Although MEKK1 (JNK kinase) has been shown to activate IκB kinase, no involvement of MEK or ERK has been detected (30). Our data show that RelA is much less sensitive to inhibition by MEK inhibitors, indicating that the MEK/ERK pathway is specifically involved in the regulation of c-Rel. The possibility of a c-Rel-specific regulatory mechanism is also supported by the fact that it takes 2–4 h for TCR stimulation to induce nuclear translocation of c-Rel, whereas RelA translocates into the nucleus within 15 min after stimulation.

A model regarding how ERK controls c-Rel nuclear translocation is that ERK is involved in de novo synthesis of c-Rel. This model is based on previous findings showing that de novo synthesized c-Rel translocates to the nucleus after TCR stimulation (31). However, the period when ERK is most required for IL-2 production and c-Rel activation does not coincide with the time when de novo RNA/protein synthesis is required. This raises another possibility that ERK is required for regulation of newly synthesized molecules that are involved in c-Rel activation. The target may be newly synthesized c-Rel itself or its regulator. Indeed, c-Rel phosphorylation in Jurkat cells has been reported recently (32). These two possibilities are not mutually exclusive, and ERK may be required for both the synthesis and phosphorylation of activating molecules.

Recently, a number of studies reported that sustained TCR engagement is required for T cell activation (reviewed in Ref. 33). Formation of the supramolecular activation clusters (SMAC)/immunological synapse could provide the source of continued receptor engagement. Such sustained receptor engagement could enable the prolonged ERK activation that is required for T cell activation as presented here. In this sense, ERK could play a role in determining the threshold for full versus partial activation. Lack of sustained TCR engagement may attenuate activation of ERK at the late stage and thus inhibit IL-2 production.

It is not yet clear how ERK activation can be sustained for several hours after the start of stimulation. There are at least two pathways known that can activate Ras and the MEK/ERK pathway: the SOS-dependent pathway and the RasGRP pathway (34, 35). Recent analysis using LAT mutants showed that it is a phospholipase Cγ-1-binding site of LAT that mainly regulates ERK activation in T cells (36, 37). This suggests that RasGRP may play a major role in Ras activation after TCR engagement downstream of LAT. Recently, we found that Ras is activated weakly but in a sustained manner in the absence of LAT.³ She is clearly tyrosine-phosphorylated in the absence of LAT and appears to play a critical role in this pathway. Because She has been implicated in the SOS activation pathway in many receptor systems (38), it is likely that SOS-dependent Ras activation can be carried out via She. Our recent study also showed that loss of She causes loss of c-Rel activation and IL-2 production in Jurkat cells (9). Together, the data imply that TCR utilizes two pathways to activate Ras via LAT/phospholipase Cγ-1/RasGRP and She/Grb2/SOS and these two Ras activation pathways may play essential roles in enabling early and sustained activation of the Ras/MAPK pathway.

An alternative possibility for how ERK is activated at a later time point is by the contribution of secreted lymphokines such as macrophage migration inhibitory factor (39, 40). It has been shown that macrophage migration inhibitory factor can activate ERK in a sustained manner and plays a role in IL-2 production. Because secretion of macrophage migration inhibitory factor occurs in a relatively early phase of stimulation, it is possible that this lymphokine provides sustained activation of ERK.

In summary, the data presented here show a novel function for ERK in TCR signaling. Activation of ERK is essential for nuclear translocation of c-Rel, and this role for ERK occurs during a late period of T cell stimulation. The data also suggest the possibility that ERK activation may contribute to determining the threshold between sustained agonistic stimulation and temporary partial agonist stimulation.

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A Novel ERK-dependent Signaling Process That Regulates Interleukin-2 Expression in a Late Phase of T Cell Activation
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