Interleukins (IL)-2 and IL-15 regulate natural killer (NK) cell proliferation, survival, and cytolytic activity. Ets1 is a transcription factor expressed early in NK cell differentiation. Because IL-2Rβ, IL-2Rγ, IL-15, and Ets1 knock-out mice similarly lack NK cells, we explored a molecular connection between IL-2R signaling and Ets1. Here we report the post-transcriptional regulation of Ets1 by IL-2R signaling in human NK cells. IL-2 and IL-15 stimulation leads to increased Ets1 protein levels with no significant change in mRNA levels. Pulse and pulse-chase experiments show that IL-2 stimulation results in both a marked increase in the nascent translation of Ets1 and an increased protein half-life. Pharmacological inhibition of MEK specifically blocks IL-2- and IL-15-induced translation, whereas p38, phosphatidylinositol 3-kinase, and mTOR inhibitors had no effect on Ets1 levels. Fii1, an Ets family member, exhibited a different mechanism of regulation, illustrating the specificity of IL-2R β and γ subunit signaling on the regulation of Ets1 expression. Expression of a dominant negative form of MNK1, a regulator of the translation initiation factor eIF4E, blocks the expression of Ets1 as do the dominant negative forms of the common IL-2R β and γ chains. Expression of Ets1 is regulated similarly in normal peripheral human NK cells. Taken together, our findings provide a direct link between IL-2R subunit signaling and Ets1 expression and helps to explain the interdependence of the IL-2R subunits and Ets1 for NK cell development and function.

Interleukins 2 and 15 Regulate Ets1 Expression via ERK1/2 and MNK1 in Human Natural Killer Cells*

Received for publication, July 23, 2004, and in revised form, September 23, 2004
Published, JBC Papers in Press, November 23, 2004, DOI 10.1074/jbc.M408356200

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Interleukins (IL)-2 and IL-15 regulate natural killer (NK) cell proliferation, survival, and cytolytic activity. Ets1 is a transcription factor expressed early in NK cell differentiation. Because IL-2Rβ, IL-2Rγ, IL-15, and Ets1 knock-out mice similarly lack NK cells, we explored a molecular connection between IL-2R signaling and Ets1. Here we report the post-transcriptional regulation of Ets1 by IL-2R signaling in human NK cells. IL-2 and IL-15 stimulation leads to increased Ets1 protein levels with no significant change in mRNA levels. Pulse and pulse-chase experiments show that IL-2 stimulation results in both a marked increase in the nascent translation of Ets1 and an increased protein half-life. Pharmacological inhibition of MEK specifically blocks IL-2- and IL-15-induced translation, whereas p38, phosphatidylinositol 3-kinase, and mTOR inhibitors had no effect on Ets1 levels. Fii1, an Ets family member, exhibited a different mechanism of regulation, illustrating the specificity of IL-2R β and γ subunit signaling on the regulation of Ets1 expression. Expression of a dominant negative form of MNK1, a regulator of the translation initiation factor eIF4E, blocks the expression of Ets1 as do the dominant negative forms of the common IL-2R β and γ chains. Expression of Ets1 is regulated similarly in normal peripheral human NK cells. Taken together, our findings provide a direct link between IL-2R subunit signaling and Ets1 expression and helps to explain the interdependence of the IL-2R subunits and Ets1 for NK cell development and function.

Natural killer (NK) cells have the critical innate immune function of independently recognizing and lysing tumor and virally and bacterially infected cells (1). In addition to NK cell direct cytotoxicity, they also act to stimulate other cells of the immune system by secretion of immunoregulatory cytokines such as tumor necrosis factor, chemokines, and interleukins that stimulate T cell and B cell responses (2). Interestingly, NK cells participate in antigen-specific immune responses by responding to IL-2 secreted by T cells and dendritic cells (3). Therefore, NK cells are central to both the innate and acquired immune responses. Indeed, there is an impressive correlation between low NK cell function and susceptibility to viral and other microbial infections (4, 5). Patients infected with human immunodeficiency virus have an overall reduction in the number and function of NK cells (6, 7). Also, NK cell activity is inversely proportional to cancer stage (8), and increased survival times correlate with high levels of NK cell activity (9–11). Cytokine therapies that target NK cell activation provide a potential means by which to boost immune surveillance and clear both infected cells and cancer. Currently, an active area of translational research is the administration of cytokines, such as IL-2 and/or IL-15, or transfer of NK cells activated and cultured in vitro for adoptive immunotherapy (12, 13).

Cytokines act en masse to control NK cellular responses through the activation of signaling pathways, including the MAP kinase pathways (ERK1/2, p38, and c-Jun N-terminal kinase), as well as the PI3K pathway (14). These signaling pathways transmit external stimuli to the nucleus and activate numerous transcription factors, resulting in both the temporal and spatial changes in gene expression required for cellular proliferation, cytokine secretion, or cytotoxicity (15). Thus, a variety of cytokines act in concert to coordinately regulate NK cell processes.

Interestingly, both IL-2 and IL-15 selectively induce survival and proliferation of NK cells and other CD56+ cells such as NKT cells but not conventional T cells or B cells (16). Both the IL-2 and IL-15 receptors (IL-2R and IL-15R) are composed of three different subunits (α, β, and γ) that are involved in differential signaling and ligand binding specificities. The IL-2Rα and IL-15Rα subunit specifically binds IL-2 or IL-15, respectively (17). Importantly, only the β subunit participates in binding IL-2 and IL-15, whereas the γ subunit participates in binding IL-2, -4, -7, -9, and -15 (15). In addition to IL-2 and IL-15, NK cells respond to other cytokines such as IL-18 and IL-12 that do not share receptor subunits with IL-2 or IL-15 (14). The common signaling components and functions of IL-2 and IL-15 in NK cells suggest common regulation of a shared set of downstream target genes.

The founding member of the Ets family of transcription factors, Ets1, plays a pivotal role in the regulation of NK cell function (18). The earliest NK cell precursor in bone marrow is characterized by Ets1 transcript expression (19). Ets1-deficient mice have severe defects in the NK cell lineage showing marked reductions in NK cell number and NK cell cytolytic activity, have reduced interferon γ secretion, and develop tumor-bearing mice with NK cell susceptible tumor cells (18). In addition to their defects in NK cells, the thymocytes of Ets1−/− mice do not produce normal levels of IL-4 after anti-CD3 stimulation (20). Moreover, their peripheral T cells display a severe proliferative defect in response to multiple acti-
IL-2 and IL-15 Regulate Ets1 Protein Synthesis

Ets1 expression is regulated at the post-transcriptional level via the activation of the mTOR pathway, which increases Ets1 translation initiation. We also demonstrated that MEK/ERK activation correlates with the activation of the translation initiation factor, eIF4E, and phospho-eIF4E (Ser209) antibodies were purchased from Cell Signaling. Antibodies directed against GST and PI3K were purchased from New England BioLabs (NEB)

MEK/ERK signaling regulates Ets1 transcriptional activity by allowing appropriate protein partners (22, 23, 24–26), we report here that IL-2 signaling positively regulates Ets1 expression on the post-transcriptional level via the activation of MEK > ERK1/2. Induction of Ets1 by IL-2R activation correlates with the activation of the translation initiation factor, eIF4E, and phospho-eIF4E (Ser209) antibodies were purchased from Cell Signaling. Antibodies directed against GST and PI3K were purchased from New England BioLabs (NEB)

Ets1 expression is regulated by an Erk1 > MNK1 > eIF4E-dependent pathway that leads to an increased Ets1 translation initiation. We also show that both IL-2 and IL-15 similarly regulate Ets1 and that blockade of either the common β or γ chain of the IL-2R results in a decreased expression of Ets1. These results demonstrate a molecular link between IL-2R signaling and Ets1 and a possible explanation for the commonality of knock-out phenotypes.

**EXPERIMENTAL PROCEDURES**

**Cell Culture, Cytokines, and Inhibitors**—The nontransformed immortalized human NK cell line, NK92, (35) was obtained from the American Type Tissue Collection and maintained in a minimum essential medium (Invitrogen) supplemented with 12.5% fetal calf serum (Invitrogen), 12.5% horse serum (Invitrogen), 150 units/ml of IL-2, 0.1 mM 2-mercaptoethanol, and penicillin-streptomycin; maintained at 37 °C and 5% CO2; and passaged every 48 h. Recombinant human IL-2 was made available by the Biological Resources Branch, National Cancer Institute Preclinical Repository. Recombinant human IL-15 was obtained from R&D Systems Inc. Recombinant human IL-18 was purchased from Medical & Biological Laboratories. For IL-2 starvation, NK92 cells were plated in the absence of IL-2 for 48–60 h prior to IL-2 stimulation (20 ng/ml). The concentrations of the other cytokines were as follows: PD98059, 100 μM; SB203580, 10 μM NaF; 5 mM trehalose in the presence of 100 μM PD98059. The p38 inhibitor SB203580, the PI3K inhibitor wortmannin, and the FK506-binding protein inhibitor rapamycin were purchased from BIMOL Research Labs, Inc. All of the inhibitors were added 30 min prior to cytokine addition. The concentrations of each are as follows: PD98059, 100 μM; SB203580, 50 μM; wortmannin, 100 μM; and rapamycin, 5 μM.

**Isolation of Normal Peripheral Human NK Cells**—Peripheral blood lymphocytes were isolated from the blood of healthy adult donors by density gradient centrifugation (Histopaque; Sigma). Isolated peripheral blood lymphocytes were removed from the gradient and cultured in complete media containing either 4 ng/ml IL-2 or 2 ng/ml IL-15. After 2 days in culture, peripheral NK cells were isolated using a NK cell negative isolation kit using procedures supplied by the manufacturer (Dynal). NK cells were cultured for 24 h as above in the presence or absence of 100 μM PD98059.

**Antibodies**—Rabbit polyclonal antibodies directed against Ets1 (C-20), Ets2 (C-20), Fli1–1 (C-19), ERK2 (K-23), and cyclin D3 (C-16) were purchased from Santa Cruz Biotechnology. Ets1 monoclonal antibody (C-19) was purchased from BIMOL Research Labs, Inc. All of the antibodies were added 30 min prior to cytokine addition. The concentrations of each are as follows: PD98059, 100 μM; SB203580, 50 μM; wortmannin, 100 μM; and rapamycin, 5 μM.

**Northern Blot Analysis**—Total RNA was isolated using RNA STAT-60 (TEL-TEST, INC.) according to the manufacturer’s protocol. Total RNA (10 μg) was separated on a 1% agarose formaldehyde gel, transferred to Duralon-UV membrane (Stratagene), and subjected to UV cross-linking. The Ets1 probe was derived from a full-length cDNA clone and the β-actin probe from I. Maroulakou (Tufts University). Hybridizations were performed using probes generated using a random priming protocol and QuikHyb in procedures provided by the manufacturer (Stratagene). The blots were quantitated using a Bio-Rad Molecular Imaging System. Northern blotting was repeated at least three independent times for each experiment.

**Flow Cytometry**—Sixty hours post-electroporation, the cells were harvested by centrifugation, washed in ice-cold phosphate-buffered saline supplemented with 2% fetal bovine serum, filtered through a 40-μm nylon cell strainer (BD Falcon), and resuspended in the same medium at 10^6 cells/ml. Sorting was performed on a fluorescence-activated Vantage cell sorter (BD Biosciences). EFYP was excited by Innova 70 laser at 488-nm excitation wavelength. EFYP fluorescence emission was measured using a 530/30-nm band pass filter.

**RESULTS**

IL-2 Regulates Ets1 Post-transcriptionally in NK Cells—The results generated from knock-out animal studies have suggested a molecular link between IL-2R-mediated signaling and the Ets1 transcription factor (18, 32, 34). The human IL-2-dependent natural killer cell line, NK92, was used to dissect the interdependence of the IL-2R and Ets1. This cell line requires IL-2 for proliferation; removal of IL-2 causes a block in the G1 phase of the cell cycle without an increase in apoptosis (data not shown and Ref. 36). To determine whether IL-2 withdrawal

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IL-2 and IL-15 Regulate Ets1 Protein Synthesis and Stability—To determine whether IL-2 regulates Ets1 via increased translation initiation and/or protein stability, pulse and pulse-chase analyses were performed. IL-2-starved NK92 cells were pulse-labeled with $[^{35}S]$methionine/cysteine in the presence or absence of IL-2. As shown by the immunoprecipitations and the quantitation in Fig. 2 (A and B), there is a marked increase in the rate of Ets1 synthesis in the presence of IL-2. To determine whether IL-2 also affected the stability of Ets1 protein, pulse-chase analyses were performed. As shown by the immunoprecipitations in Fig. 2C, IL-2 causes a slight increase in the stability of Ets1; however, the most pronounced effect of IL-2 is the induction of Ets1 protein synthesis in NK cells.

IL-2 Regulates Ets1 Protein Levels through Erk1/2—Several pharmacological inhibitors were used to investigate the pathway(s) by which IL-2 induces the expression of Ets1 protein. NK92 cells growing in IL-2 were treated with PD98059, a MEK inhibitor, the p38 inhibitor SB203580, or the PI3K inhibitor wortmannin. The MEK inhibitor PD98059 had the largest effect on Ets1 steady state levels (Fig. 3A). The steady state expression of another Ets family member expressed in NK cells, Ets2, was unaffected by these pharmacological agents. To determine whether the MEK inhibitor specifically blocks Ets1 induction by IL-2, IL-2-starved cells were pretreated with PD98059 or SB203580 prior to IL-2 induction (Fig. 3B). Similarly, the most marked reduction of induced Ets1 levels was in the presence of the MEK inhibitor PD98059. To assess the specificity of ERK inhibition on Ets1, we evaluated the effect of these inhibitors on a different Ets family member whose expression is also induced by IL-2, Fli1. The induction of Fli1 is unaffected by ERK1/2 inhibition (Fig. 3B). Interestingly, Fli1 induction is blocked almost exclusively by p38 inhibition, suggesting that IL-2 regulates Ets family member expression through different signaling pathways in NK cells. Loss of Fli1, however, does not affect NK cell function.

Because the state of phosphorylation of ERK1/2, p38, and AKT closely mirror their activities, we used phosphospecific antibodies in Western blot analyses to verify the effectiveness of these upstream inhibitors with NK92 cells. As expected, the p38 inhibitor SB203580 caused a reduction in the phosphorylation of p38 (Fig. 3C). Moreover, the phosphorylation of ERK1/2 was reduced by treatment with the MEK inhibitor PD98059 (Fig. 3C). Wortmannin, a PI3K inhibitor, was effective in reducing the phosphorylation status of AKT (Fig. 3C), a downstream effector of PI3K (41). Interestingly, inhibition of the MEK > ERK1/2 pathway or the PI3K > AKT pathway resulted in a slight but reproducible increase in the phosphorylation of p38 (Fig. 3C). These data indicate that PD98059, SB203580, and wortmannin block their expected targets in NK92 cells. Taken together our results suggest that Ets1 pro-
protein levels are significantly regulated by the MEK > ERK1/2 pathway. Because Ets1 protein levels are not completely blocked by the MEK inhibitor, it is possible that secondary mechanisms of Ets1 regulation exist.

To confirm the requirement of ERK1/2 for the IL-2-dependent increased rate of Ets1 translation, IL-2-starved cells were treated with the MEK inhibitor prior to IL-2 stimulation, and the translation rate of Ets1 was measured by pulse label analyses. As shown in Fig. 4A, inhibition of MEK by PD98059 results in a significant decrease in the rate of Ets1 synthesis. The quantitation of these data is shown in Fig. 4B. Taken together, these findings indicate that IL-2 causes an ERK1/2-dependent increase in Ets1 protein expression primarily on the level of translation initiation.

Phosphorylation of MNK1 and eIF4E Is Regulated by IL-2 in NK Cells and Is Blocked by MEK Inhibition—Our results suggest that IL-2 signaling through MEK may regulate the rate of Ets1 translation. Multiple signaling pathways including PI3K/AKT and MEK/ERK have roles in the regulation of translation (42, 43). ERK1/2 has been shown to directly phosphorylate MNK1/2 (44). MNK1/2 are serine/threonine kinases that directly phosphorylates eIF4E (45), a cap-binding protein that regulates translation initiation by recruiting mRNAs to the ribosome (46). Phosphorylation by MNK1 serves to activate eIF4E independent of the 4E-binding proteins that bind and inhibit eIF4E. To determine whether IL-2 leads to the increased activity of MNK1 and its downstream substrate eIF4E in NK92 cells, their phosphorylation status was tested by Western blot analyses. As shown in Fig. 5A, treatment with the MEK inhibitor, PD98059 and not p38 inhibitor, SB203580 blocks the generation of phospho-MNK1. Likewise, eIF4E phosphorylation is induced by IL-2 but blocked by PD98059. Therefore, induction of MNK1 and eIF4E activity correlates with IL-2-induced MEK activation in NK92 cells. Consequently, IL-2-induced MNK1 and eIF4E activity may be linked to Ets1 induction in NK cells.

Our pharmacological data suggest that Ets1 translation is independent of PI3K/AKT. AKT activation signals through mTOR to increase translation initiation (42). To further investigate the role of this pathway on Ets1 levels, IL-2-stimulated NK92 cells were treated with rapamycin. Rapamycin suppresses mRNA translation through direct inhibition of mTOR (47). Cyclin D3 translation is dependent on mTOR (42, 48). As expected, rapamycin significantly blocks the induction of cyclin D3 in IL-2-stimulated NK92 cells (Fig. 5B). However, rapamycin treatment of IL-2-stimulated NK92 cells does not block induction of Ets1 protein levels. These data indicate that the translation of Ets1 via ERK activation is not due to signals mediated by mTOR and is consistent with our finding that PI3K/AKT signaling does not affect Ets1 expression in IL-2-induced NK cells.

To confirm the regulation of Ets1 protein expression by a MNK1 > eIF4E-dependent pathway, NK92 cells were transiently transfected with an expression construct containing a dominant negative, kinase-dead form of MNK1 that lacks Erk1-specific phosphorylation sites (49). As shown in Fig. 5C, expression of this dominant negative MNK1 causes a reduction in the expression of Ets1, whereas expression of an unrelated protein (EYFP) has no effect. Because the MNK1 dominant negative is tagged with GST, the blot was stripped and reprobed with an antibody directed against GST as a control for its expression. Taken together, these findings suggest a pathway whereby activation of the IL-2R results in a post-transcriptional increase in the expression of Ets1 via an ERK1/2 > MNK1 > eIF4E-dependent mechanism of translation initiation.

IL-15 Regulates Ets1 Translation Initiation Similarly to IL-2—In contrast to IL-18, IL-2 and IL-15 induced Ets1 expression
(Fig. 1A). Because IL-2 and IL-15 share the IL-2R β and γ subunits (40), we determined whether IL-15 similarly stimulates the translation of Ets1. To this end, NK92 cells were deprived of IL-2, treated with or without PD98059, and induced with IL-15. Levels of phosphorylated ERK1/2 are significantly decreased by PD98059, but total ERK1/2 levels are unaffected. PD98059 significantly blocked induction of Ets1 by IL-15 (Fig. 6A). Thus, IL-15 may control the expression of Ets1 through a similar mechanism as IL-2.

To assess whether IL-15 induces Ets1 protein levels through a post-transcriptional mechanism, we examined Ets1 mRNA levels in the presence or absence of IL-15. As shown by the Northern blot in Fig. 6B, Ets1 mRNA levels are not significantly affected either by IL-2 or IL-15 induction or in the presence of the MEK inhibitor, PD98059, although PD98059 treatment may result in a slightly increased steady state level of Ets1 message. Thus, IL-15 induction of Ets1 protein levels, similar to that shown for IL-2, is regulated primarily at the post-transcriptional level.

To confirm that IL-15 required the ERK1/2 pathway to increase in the rate of Ets1 translation, NK92 cells were pulse-labeled in the presence and absence of the MEK inhibitor PD98059. As shown in Fig. 7A (and the quantitation in Fig. 7B), inhibition of the MEK/ERK1/2 pathway greatly reduces the IL-15 increase in Ets1 protein synthesis. Taken together these results indicate that IL-2 and IL-15 regulate Ets1 protein levels through a common mechanism, possibly through their shared IL-2R subunits by an ERK1/2-dependent pathway in human NK cells.

Because both IL-15 and IL-2 share the β and γ subunits of the IL-2R, dominant negative constructs of these subunits were tested for their ability to inhibit the expression of Ets1. These receptor constructs contain deletions within their cytoplasmic domains and are therefore incapable of transmitting a signal (50, 51). As shown in Fig. 8, transient transfection of the dominant negative forms of IL-2R β and γ subunits results in a reduction in the expression of Ets1. These results indicate that Ets1 expression is dependent on the common IL-2 receptor subunits in NK cells.

Ets1 Expression Is ERK1/2-dependent in Normal Peripheral NK Cells—Our findings suggest that IL-2 and IL-15 through their common IL-2 receptor subunits positively control the expression of Ets1 via a pathway that is dependent on ERK1/2 in NK92 cells. To confirm that Ets1 was regulated in a similar
fashion in normal peripheral NK cells, NK cells isolated from normal donors were cultured in the presence of either IL-2 or IL-15 followed by treatment with the MEK inhibitor PD98059. As shown by the Western blot analysis in Fig. 9, inhibition of the MEK > ERK1/2 pathway results in a marked decrease in the expression of Ets1 in peripheral NK cells.

The findings presented above support the model, shown in Fig. 10, that IL-2 and IL-15 through the shared receptor signaling directly impacts Ets1 protein synthesis in NK cells through the activation of the MEK > ERK1/2 > MNK1 pathway and provides a mechanism that explains commonality in the NK phenotypes generated by the receptor/effecter knock-out animals.

**DISCUSSION**

The most profound phenotype of the Ets1 knock-out mice is a reduction in the number and activity of NK cells (18). Given that knock-out animal studies have revealed roles for IL-2, IL-15, IL-15Rα, IL-2/15Rβ, and IL-2/15Rγ chain in the regulation of NK cells (18, 31–34, 52, 53), we sought to determine whether there was a link between IL-2R signaling and the Ets1 transcription factor. Here we demonstrate for the first time that IL-2 and IL-15 stimulation results in the post-transcriptional increase in Ets1 protein expression in the IL-2-dependent human NK cell line, NK92. Withdrawal of IL-2 results in a decrease in the expression of Ets1 protein that is induced upon the addition of either IL-2 or IL-15. Both IL-2 and IL-15 treatment results in a marked increase in the rate of Ets1 translation with a concurrent affect on protein stability and little affect on Ets1 mRNA expression. Elucidation of the signaling pathways elicited by these cytokines has revealed that Ets1 translation is dependent on MEK/ERK1/2 activation by a pathway that is PI3K- and mTOR-independent. The increase in Ets1 expression correlated with the phosphorylation of MNK1 and eIF4E in NK cells. Indeed, transfection with dominant negative forms of MNK1 and the common IL-2R β and γ chains allowed for the demonstration of a direct link between the common IL-2R β and γ subunits, MNK1, and Ets1 expression. These findings suggest a signaling pathway leading from the activation of the IL-2R by IL-2 or IL-15 to an MEK > ERK1/2 > MNK1 > eIF4E pathway that results in an increase in Ets1 protein in K cells. These results provide a molecular link between IL-2R-mediated signaling and Ets1 and potentially explain the common phenotypes observed in the knock-out mice. A hallmark of growth factor/cytokine stimulation is the induction of protein synthesis, a requirement for cellular proliferation and survival. In this report we show that the IL-2 induction of Ets1 protein expression in NK cells is controlled by an ERK-dependent pathway and not through PI3K/AKT/mTOR. AKT activation has been shown to cause the phosphorylation and inactivation of the eIF4E-binding proteins, which sequester the 5’ cap-binding protein eIF4E (54). eIF4E is a crucial mediator of translation initiation, recruiting translation initiation factors to the 5’ cap of particular mRNAs (55). eIF4E is required for the translation of proteins involved in cell cycle, cellular survival, and oncogenesis (56). Although much attention has been given to the role of PI3K/AKT activation in the regulation of protein synthesis, recent evidence suggests that signaling through the MAP kinases also converge on this important mediator of translational control (43). ERK1 and ERK2 activate cap-dependent translation by direct phosphorylation of the kinase MNK1 (44). Subsequently, the MNK1 kinase directly phosphorylates and activates eIF4E. In this report we show that Ets1 expression correlates with MNK1 and eIF4E phosphorylation. In addition to its involvement in translation initiation, a recent report has suggested a role for eIF4E in the regulation of mRNA nuclear export (57). We were unable to detect any change in nuclear versus cytoplasmic localization of the Ets1 message in the presence or absence of IL-2 (data not shown). Taken together our results suggest that the IL-2-mediated effect on Ets1 translation is controlled by ERK1/2-dependent activation of eIF4E.

Ets1 is a member of a large family of transcription factors that exhibit some functional redundancy. As suggested by the knock-out phenotype, no other family member can compensate for Ets1 activity in NK cells. We show that both Ets2 and Fli1 are expressed in NK cells and that IL-2 also induces Fli1 expression. The IL-2 induction of Fli1 is apparently regulated by a different mechanism than Ets1. Our pharmacological data suggest that Fli1 is regulated via the stress-induced MAP kinase, p38. Interestingly, Fli1 expression is, in part, regulated via alternative translation initiation sites within its 5’-UTR (58). Such alternative translation initiation sites are considered to be cap-independent (59). The 5’-UTR of Ets1 does not contain alternative translational start sites or internal ribosome entry sites (data not shown). Furthermore, the Ets1 5’-UTR does not confer IL-2-dependent translation of a chimeric message (data not shown), indicating that IL-2 responsive cis-acting elements may be located within the 3’-UTR of Ets1.
Given that certain polymorphisms within the 3′-UTR of the Ets1 gene are linked with clinical symptoms associated with systemic lupus erythematosus (60), localization of these regulatory regions could identify sequence elements related to aberrant Ets1 expression in particular disease states.

Ets1 is not only essential for the NK cell lineage in targeted mutant mice but also plays an important role in the regulation of other cell lineages, including B cells, T cells, and NKT cells. In B cells, Ets1 is apparently required for class switching and/or negatively controlled by Ets1 remains an open question.

NK cell function and the particular target genes positively regulated with the notion that Ets1 plays differential roles within different hematopoietic lineages. It has become increasingly apparent with the evidence that Ets1 plays differential roles within the TCR stimulation (21). Ets1 is also required for development of NKT cells (20). These findings are consistent in response to TCR stimulation (21).

Future directions will undoubtedly include identification of Ets1-specific target gene selection in NK cells. The exact role for Ets1 as a central regulator of cytokine gene selection. The exact role for Ets1 as a central regulator of cytokine gene selection. The exact role for Ets1 as a central regulator of cytokine gene selection.

Acknowledgments—We thank Drs. Alexander Agwuiletswit, Makio Ogawa, and Vincent Damiani for scientific insight, Dr. Haifun Zeng for technical assistance (Hollings Cancer Center cell sorting facility), and Dr. H. L. Grimes for critical reading of the manuscript.
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J. Biol. Chem. 2005, 280:4772-4778.
doi: 10.1074/jbc.M408356200 originally published online November 23, 2004

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