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IL-17 Signaling for mRNA Stabilization Does Not Require TNF Receptor-Associated Factor 6

Justin Hartupee, CaiNi Liu, Michael Novotny, Dongxu Sun, Xiaoxia Li, and Thomas A. Hamilton

IL-17 alone is a relatively weak inducer of gene expression, but cooperates with other cytokines, including TNF-α, to generate a strong response in part via prolongation of mRNA t1/2. Because TNFR-associated factor 6 (TRAF6) has been reported to be essential for signaling by IL-17, we examined its involvement in IL-17-mediated mRNA stabilization. Although overexpression of TRAF6 in HeLa cells activates NF-κB, it does not stabilize transfected KC mRNA. Furthermore, a dominant-negative TRAF6 abrogates NF-κB activation, but does not block IL-17-induced chemokine mRNA stabilization. IL-17 can stabilize KC and MIP-2 mRNAs comparably in TNF-α-treated mouse embryo fibroblasts from TRAF6+/+ and TRAF6−/− mice. TRAF6 is known to couple upstream signals with activation of p38 MAPK and mitogen activated protein kinase activated protein kinase 2, both of which have been shown to be important for Toll/IL-1R-mediated mRNA stabilization in various cell types. Inhibition of p38 MAPK, however, does not block IL-17-induced KC mRNA stabilization, and IL-17 can stabilize KC mRNA equally in mouse embryo fibroblasts from both wild-type and mitogen activated protein kinase activated protein kinase 2/3 doubly-deficient mice. Finally, IL-17 can amplify the levels of multiple TNF-α-stimulated mRNAs in wild-type and TRAF6-deficient cells, but not in cells from Act1−−/− mice. Collectively, these findings demonstrate the existence of a TRAF6/p38 MAPK-independent pathway that couples the IL-17R with enhanced mRNA stability. Because the most potent effects of IL-17 on gene expression are obtained in cooperation with other cytokines such as TNF-α, these findings suggest that this pathway is a major contributing mechanism for response to IL-17. The Journal of Immunology, 2009, 182: 1660–1666.
In the present report, we have examined the requirement for TRAF6 in IL-17-induced mRNA stabilization. Surprisingly, using multiple experimental approaches, we find that TRAF6 is not required in signaling for stabilization of TNF-α-induced chemokine mRNAs. In addition, IL-17-induced mRNA stabilization did not depend upon the action of p38 MAPK or downstream kinases MK2 and MK3. These results further highlight the importance of mRNA stabilization in IL-17-mediated amplification of TNF-α-stimulated gene expression and identify a TRAF6/p38/MK2/MK3-independent pathway operating downstream of Act1. Because the potency of response to IL-17 is markedly greater when used in combination with TNF-α, this pathway may represent the predominant means through which IL-17 promotes inflammatory gene expression.

Materials and Methods

Reagents

G418, formamide, MOPS, salmon sperm DNA, and diethylpyrocarbonate were purchased from Sigma-Aldrich. DMEM, Dulbecco’s PBS, penicillin, and streptomycin were obtained from Central Cell Services of the Lerner Research Institute. FBS was purchased from BioWhittaker. PolyFect Transfection Reagent was obtained from Qiagen, and Tri-Reagent was purchased from Sigma-Aldrich. DMEM, Dulbecco’s PBS, penicillin, streptomycin, and 5% FBS were obtained from Central Cell Services of the Lerner Research Institute. DMEM, Dulbecco’s PBS, penicillin, streptomycin, and 5% FBS were obtained from Central Cell Services of the Lerner Research Institute. DMEM, Dulbecco’s PBS, penicillin, streptomycin, and 5% FBS were obtained from Central Cell Services of the Lerner Research Institute. DMEM, Dulbecco’s PBS, penicillin, streptomycin, and 5% FBS were obtained from Central Cell Services of the Lerner Research Institute.

Preparation of peritoneal macrophages

C57BL/6 mice were purchased from Jackson ImmunoResearch Laboratories and were housed in microisolator cages with autoclaved food and bedding to minimize exposure to viral and microbial pathogens. All procedures were approved by the Institutional Animal Care and Use Committee. Thioglycollate-elicited peritoneal macrophages were prepared, as described previously (27), and cultured overnight in RPMI 1640 medium containing 10% FBS, penicillin, streptomycin, and 5% FBS before treatment, as described in the text.

Plasmids

Plasmids encoding KC cDNA under control of a tetracycline (tet)-regulated promoter (pTRE2 KCΔ3) and the NF-κB reporter 5× κB luciferase have been previously described, as have the plasmids and methods used to prepare radiolabeled CXCL1 (KC), GAPDH, (CXCL2) MIP-2, and IκBζ cDNAs (20, 28–30). Expression plasmids encoding epitope (FLAG)-tagged Act1, TRAF6, and dominant-negative (dn) TRAF6 have been previously described, as have the plasmids and methods used to prepare radiolabeled CXCL1 (KC), GAPDH, (CXCL2) MIP-2, and IκBζ cDNAs (20, 28–30). Expression plasmids encoding epitope (FLAG)-tagged Act1, TRAF6, and dominant-negative (dn) TRAF6 have been previously described (31, 32).

Cell culture and transfection

HeLa tet-off cells were purchased from BD Clontech; cultured in DMEM containing 10% FBS, penicillin, and streptomycin; and kept under selection with G418. For transfection, HeLa tet-off cells were plated in 100-mm dishes and allowed to grow for 24 h to 70% confluency. Transfection was performed using PolyFect Transfection Reagent, according to the manufacturer’s protocol (Qiagen). Wild-type, TRAF6−/−, and Act1−/− MEFs have been previously described and were cultured in DMEM containing 10% FBS, penicillin, and streptomycin (21, 33). MEFs from MK2/MK3 doubly-deficient mice were provided by M. Gaestel (Institute of Biochemistry, Medical School Hannover, Hannover, Germany) (17).

Northern blot, real-time PCR, Western blot, and luciferase assay

Total RNA was isolated using TRIzol reagent, according to the manufacturer’s protocol. Northern hybridization was performed, as previously described (28). To calculate mRNA t1/2, the autoradiographs were quantified using NIH Image software. mRNA levels for each gene of interest were normalized to GAPDH mRNA levels and plotted as log of the percentage of remaining mRNA vs time. The best fit to linear decay was determined, and the t1/2 was calculated from the intersection of the point corresponding to 50% residual RNA. Quantitative real-time PCR was performed, as previously described (21). The cDNAs were synthesized with random hexamers using Moloney murine leukemia virus reverse transcriptase (Promega) and real-time PCR conducted using SYBER Green PCR.
Luciferase assays were performed according to the manufacturer’s protocol (6679939a1). Western blots were conducted, as described previously (35).

Although KC mRNA rapidly decays following addition of dox, transcription via the activation of NF-κB and stabilization of the constitutively unstable mRNA (14, 36). We have used the tet-off system to control transcription of transgenic KC mRNA, enabling the analysis of mRNA decay in the absence of the requirement for a transcriptional stimulus (28, 37). HeLa cells stably expressing the tet-controlled trans activator are transiently transfected with reporter plasmid (pTRE2 KCΔ3) containing the KC 5’ untranslated region (UTR), coding region, and a portion of the 3’ UTR that confers both instability and stimulus-induced stabilization. In the absence of tet, or its analog doxycycline (dox), the tet activator will drive strong transcription of the KC transgene, but following addition of dox to the culture medium transcription is abolished and the decay of the mRNA can be followed (Fig. 1A).

Furthermore, reflecting the constitutive instability of the mRNA, addition of IL-17 along with dox is able to prolong the t1/2 of the message.

We have previously shown that overexpression of Act1, the adaptor linking directly to the IL-17R, results in enhanced stability of KC mRNA (20). This finding indicates that signals originating from this adaptor are sufficient to initiate the stabilization response. As a first test of the role of TRAF6 in mRNA stabilization, we determined whether overexpression of this molecule would also be sufficient to engage the mRNA stabilization process. Surprisingly, whereas overexpression of Act1 stimulates KC mRNA stabilization, overexpression of TRAF6 does not alter the t1/2 of the message (Fig. 1, B and C). Although TRAF6 overexpression did not affect KC mRNA stability, it promoted strong activation of NF-κB, as measured by a cotransfected luciferase reporter (Fig. 1D) demonstrating the functional activity of the overexpressed molecule. Western blot analysis indicated that each of the adaptor proteins demonstrating the functional activity of the overexpressed molecule.

Results

Overexpression of Act1, but not TRAF6, mediates stabilization of KC mRNA

Robust expression of KC mRNA from the endogenous gene requires both transcription via the activation of NF-κB and stabilization of the constitutively unstable mRNA (14, 36). We have used the tet-off system to control transcription of transgenic KC mRNA, enabling the analysis of mRNA decay in the absence of the requirement for a transcriptional stimulus (28, 37). HeLa cells stably expressing the tet-controlled trans activator are transiently transfected with reporter plasmid (pTRE2 KCΔ3) containing the KC 5’ untranslated region (UTR), coding region, and a portion of the 3’ UTR that confers both instability and stimulus-induced stabilization. In the absence of tet, or its analog doxycycline (dox), the tet trans activator will drive strong transcription of the KC transgene, but following addition of dox to the culture medium transcription is abolished and the decay of the mRNA can be followed (Fig. 1A).

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TRAF6 is not required for IL-17-induced mRNA stabilization

Two experimental strategies were used to determine whether TRAF6 was required for IL-17-induced mRNA stabilization. First, we tested the ability of a dn version of TRAF6 (31) to interfere

![FIGURE 2](https://example.com/figure2.png)

**FIGURE 2.** The dnTRAF6 does not block IL-17-induced mRNA stabilization. A, HeLa tet-off cells were transfected with 2 μg of pTRE2 KCΔ3, 2 μg of 5X κB luciferase reporter, 1 μg of pcDNA3, and 1 μg of dnTRAF6 or empty vector (pcDNA3). One set of cultures was treated with dox alone or in combination with IL-17 (25 ng/ml) for the indicated times. Total RNA was collected, and KC and GAPDH mRNAs were determined by Northern hybridization. NT, Represents untreated cells and serves as the zero time point for all treatment conditions. B, The autoradiographs from four separate experiments similar to A were quantified, as described in the legend to Fig. 1, and t1/2 for each condition were determined. The mean ± 1 SD for each condition was determined and presented. C, A second set of cultures transfected as in A was treated for 6 h with IL-1α (10 ng/ml) or IL-17 (100 ng/ml), or left untreated. Cell lysates were prepared, and luciferase activity was determined. Values represent the mean of duplicate samples.

![FIGURE 3](https://example.com/figure3.png)

**FIGURE 3.** TRAF6 is not required for IL-17-induced mRNA stabilization. A, Wild-type and TRAF6-deficient MEFs were treated for 1 h with TNF-α (10 ng/ml). Fresh medium was then added containing ActD (5 μg/ml) alone or with IL-17 (10 ng/ml). Total RNA was collected at the indicated times, and KC and GAPDH levels were determined by Northern hybridization. NT, Represents cells treated with TNF-α for 1 h and serves as the zero time point for all treatment conditions. B, The autoradiographs in A were quantified, as described in the legend to Fig. 1, and the percentage of remaining KC mRNA relative to GAPDH is shown for each experimental condition. C, Data from three independent experiments were analyzed for KC mRNA t1/2, and the mean ± 1 SD is shown.
with IL-17-induced KC mRNA stabilization. TRAF6 has previously been linked to NF-κB activation in response to both IL-17 and IL-1α (26, 33). IL-17 treatment stabilized KC mRNA in HeLa tet-off cells transfected with pTRE2 KC(Δ3) in the presence of either empty vector or an expression vector encoding dnTRAF6 (Fig. 2A). A similar result was obtained in four separate experiments, which collectively show that IL-17 prolongs the $t_{1/2}$ of KC mRNA by a mean value of more than 2-fold in the presence or absence of dnTRAF6 (Fig. 2B). Under the same conditions, even high doses of IL-17 (100 ng/ml) serve as a very weak stimulus for NF-κB activation compared with lower doses of IL-1α (10 ng/ml) (Fig. 2C). Consistent with prior reports, however, NF-κB activation following IL-17 or IL-1α treatment was diminished in cells expressing the dnTRAF6.

As a second test for the requirement of TRAF6, we compared the ability of IL-17 to induce mRNA stabilization in wild-type and TRAF6-deficient MEFs. We have previously shown that KC mRNA transcribed in response to TNF-α is highly unstable and decays rapidly following the addition of actinomycin D (ActD), whereas the addition of IL-17 along with ActD is able to prolong the $t_{1/2}$ of the TNF-α-induced KC mRNA (20). The ability of IL-17 treatment to prolong the $t_{1/2}$ of KC mRNA remains intact in the TRAF6-deficient cells (Fig. 3, A and B). Similar results were obtained in two additional experiments, as demonstrated by comparison of mean $t_{1/2}$ values in the two different cell populations either with or without IL-17 treatment (Fig. 3C). It is noteworthy that KC mRNA instability was reduced in the TRAF6$^{-/-}$ cells even in the absence of IL-17, but treatment with IL-17 resulted in a quantitatively comparable increase in $t_{1/2}$ in both wild-type and TRAF6-deficient cells. These results support the conclusion that TRAF6 is not required for IL-17-induced chemokine mRNA stabilization.

**IL-17-induced mRNA stabilization does not require p38 or MK2/MK3**

p38 MAPK and its downstream kinase MK2 have been shown to play a critical role in stimulus-induced stabilization of proinflammatory mRNAs in a variety of settings (11, 12, 15–17), including IL-17. TRAF6 is known to link to p38 activation, and thus, the finding that TRAF6 was not required for IL-17-induced mRNA stabilization raised questions regarding the role of the p38 MAPK cascade in the IL-17 response. As a first test to assess the role of p38, we determined the effect of the p38 MAPK inhibitor SB203580 on IL-17-induced KC mRNA stabilization. The presence of the inhibitor did not affect the ability of IL-17 to stabilize KC mRNA in HeLa tet-off cells (Fig. 4A). In contrast, as we have previously reported (38, 39), the ability of LPS to stabilize KC mRNA in mouse macrophages is highly sensitive to the inhibitory effects of SB203580 (Fig. 4B).

To further assess the role of the p38/MK2 pathway in IL-17-induced stabilization, we tested the ability of IL-17 to prolong KC mRNA $t_{1/2}$ in MEFs from MK2/MK3-deficient mice. Wild-type and MK2/MK3-deficient MEFs were stimulated with TNF-α (10 ng/ml) alone or with IL-17 (25 ng/ml) with or without SB203580 (2 μM) for the indicated times. NT, Represents untreated cells and serves as the zero time point for all treatment conditions. Total RNA was collected, and the KC and GAPDH levels were determined by Northern hybridization. Blots were quantified, as described in the legend to Fig. 1, and decay curves are presented. B, Thioglycolate-elicited peritoneal macrophages were treated with LPS for 3 h before the addition of ActD (5 μg/ml) alone or along with SB203580. NT, Represents cells treated with LPS for 3 h and serves as the zero time point for both treatment conditions. Total RNA was prepared at the indicated times, and KC and GAPDH mRNA levels were determined by Northern hybridization and quantified as above. C, Wild-type and MK2/ MK3-deficient MEFs were stimulated with TNF-α (10 ng/ml) alone or with IL-17 (10 ng/ml) for 1 h, followed by the addition of ActD. Total RNA was collected at the indicated times following addition of ActD, and KC and GAPDH mRNA levels were determined by Northern hybridization and quantified as above. NT, Represents cells treated with TNF-α for 1 h and serves as the zero time point for both treatment conditions. The results from three separate experiments were used to determine the mean $t_{1/2}$ of KC mRNA ± 1 SD as shown.

**FIGURE 4.** IL-17-induced mRNA stabilization does not require p38 or MK2 activity. A, HeLa tet-off cells were transfected with 2 μg of pTRE2 KCΔ3 and 4 μg of pcDNA3. Cells were treated with dox alone or with dox plus IL-17 (25 ng/ml) with or without SB203580 (2 μM) for the indicated times. NT, Represents untreated cells and serves as the zero time point for both treatment conditions. The results are presented. B, Thioglycolate-elicited peritoneal macrophages were treated with LPS for 3 h before the addition of ActD (5 μg/ml) alone or along with SB203580. NT, Represents cells treated with LPS for 3 h and serves as the zero time point for both treatment conditions. Total RNA was prepared at the indicated times, and KC and GAPDH mRNA levels were determined by Northern hybridization and quantified as above. C, Wild-type and MK2/ MK3-deficient MEFs were stimulated with TNF-α (10 ng/ml) alone or with IL-17 (10 ng/ml) for 1 h, followed by the addition of ActD. Total RNA was collected at the indicated times following addition of ActD, and KC and GAPDH mRNA levels were determined by Northern hybridization and quantified as above. NT, Represents cells treated with TNF-α for 1 h and serves as the zero time point for both treatment conditions. The results from three separate experiments were used to determine the mean $t_{1/2}$ of KC mRNA ± 1 SD as shown.

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To further assess the role of the p38/MK2 pathway in IL-17-induced stabilization, we tested the ability of IL-17 to prolong KC mRNA $t_{1/2}$ in MEFs from MK2/MK3-deficient mice. Wild-type and MK2/MK3-deficient MEFs were stimulated with TNF-α alone or with IL-17 for 1 h, followed by the addition of ActD. The TNF-α-induced KC mRNA was unstable in both cell populations, although the magnitude of response to TNF-α was markedly reduced in MK2$^{-/-}$ cells (Fig. 4C). However, in both cell populations, the $t_{1/2}$ of KC message induced by the combination of TNF-α and IL-17 was significantly and comparably prolonged in comparison with that seen in cells stimulated by TNF-α alone (Fig. 4C). Comparable results were obtained in three similar experiments, as demonstrated by comparison of the mean $t_{1/2}$ values (Fig. 4C). These findings provide further support for the conclusion that IL-17-induced mRNA stabilization does not require the p38 MAPK pathway, including MK2 and MK3.

**TRAF6 is not required for IL-17-mediated amplification of TNF-α-induced gene expression**

Prior reports demonstrate that the modest ability of IL-17 alone to induce activation of NF-κB and the transcription of select target genes is dependent upon TRAF6 (22, 26). The present study, however, shows that IL-17-induced stabilization of chemokine mRNA
transcribed in response to TNF-α is TRAF6-independent. The requirement for TRAF6 in NF-κB activation, but not mRNA stabilization, enables a comparison of the relative contribution of the two mechanisms by examining a broader spectrum of IL-17-induced genes in TRAF6-deficient cells. Using quantitative real-time PCR, we determined the expression of a set of five genes that have been previously identified as sensitive to IL-17-mediated amplification based upon oligonucleotide microarray analysis of gene expression in fibroblasts stimulated with TNF-α alone or with IL-17 (5, 20). Wild-type and TRAF6-deficient MEFs were stimulated for 3 or 9 h with TNF-α alone or in combination with IL-17. For comparison, the same treatments were also performed on Act1+/− and Act1−/− MEFs because IL-17-induced gene expression has previously been reported to be fully dependent on Act1 (21). In four of the five genes studied, the response to IL-17 in the TRAF6-deficient MEFs was comparable to that seen in wild-type cells (Fig. 5). A single dramatic exception was observed for behavior of the gene encoding cxcl5. cxcl5 mRNA levels, however, although modestly induced in response to TNF-α alone in wild-type cells, were below the level of detection in all samples from TNF-α-treated TRAF6-deficient cells. Hence, there appears to be a deficiency in TNF-α-stimulated cxcl5 gene transcription in the TRAF6-deficient cell population rather than a requirement for TRAF6 in IL-17-mediated enhancement of cxcl5 expression. Although IL-6 mRNA levels are reproducibly higher in the TRAF6−/− cells than in wild-type cells, it is clear that in both cell populations IL-17 is able to amplify the TNF-α-induced response. These results are in dramatic contrast to the behavior of the same genes in Act1-deficient MEFs, in which the response to IL-17 is completely lost. The magnitude of responses in the different MEF cell populations (wild type, TRAF6−/−, Act1+/−, or Act1−/−) shows variability, but the effects of IL-17 are qualitatively similar for the TRAF6 pair and qualitatively different for the Act1 pair.

To further assess the IL-17-mediated amplification of TNF-α-stimulated gene expression in the presence or absence of TRAF6, we compared the $t_{1/2}$ of two additional mRNAs (MIP-2 and IL-8). As was seen for KC, the $t_{1/2}$ in TNF-α-stimulated cells are short

![Figure 5](http://www.jimmunol.org/)

**FIGURE 5.** TRAF6 is not required for IL-17-mediated amplification of TNF-α-induced gene expression. MEFs from wild-type, TRAF6−/−, Act1+/−, or Act1−/− mice were left untreated or were stimulated with TNF-α (10 ng/ml) alone or in combination with IL-17 (10 ng/ml). Total RNA was isolated following treatment for 3 or 9 h, and relative mRNA levels for the indicated genes were determined by quantitative real-time PCR. Results are presented as the mean ± half of the range of duplicate determinations. Similar results were obtained in two separate experiments.

![Figure 6](http://www.jimmunol.org/)

**FIGURE 6.** Stabilization of MIP-2 and IL-8 mRNAs by IL-17 does not require TRAF6. A, MEFs from wild-type and TRAF6−/− mice were treated with TNF-α for 2 h before addition of ActD (5 µg/ml) or ActD + IL-17 (25 ng/ml). MIP-2 and GAPDH mRNA levels were determined after the indicated times by Northern hybridization. Zero time corresponds to cells treated for 2 hrs with TNFα. The autoradiographs were quantified, as described in the legend to Fig. 1, and the decay curves for each cell population are shown. B, MEFs from wild-type and TRAF6−/− mice were treated with TNF-α (10 ng/ml) alone or in combination with IL-17 (25 ng/ml) for 2 h. ActD (5 µg/ml) was added to all cultures, and the levels of IL-8 and GAPDH mRNAs were determined by Northern hybridization after the indicated incubation times. Zero time corresponds to cells treated for 2 h with TNFα alone or with IL-17. The autoradiographs were quantified, as described in the legend to Fig. 1, and the decay curves for each cell population are shown. Similar results were obtained in two separate experiments.
and markedly prolonged for both mRNAs in the presence of IL-17 (Fig. 6). As seen with KC mRNA, IL-17 stabilizes the MIP-2 message effectively even when added in the presence of ActD in both cell populations. For IκBζ mRNA, we compared decay following stimulation with TNF-α either alone or in combination with IL-17 because starting mRNA levels were significantly higher. Although the levels of IκBζ mRNA in cells treated with TNF-α alone are low and our measure of t1/2 may be an underestimate, the results demonstrate substantive stabilization of the IκBζ mRNA in the presence of IL-17, and this response is comparable in both cell populations.

Discussion

IL-17-induced inflammatory gene expression has been linked with both transcriptional and posttranscriptional mechanisms, although the signaling pathways that couple IL-17R engagement with such responses remain poorly understood. In the present study, we undertook to determine whether signaling events linked with the activation of NF-κB via TRAF6 were also requisite for the prolongation of specific mRNA t1/2. The data presented establish that the ability of IL-17 to stabilize a selection of TNF-α-induced mRNAs does not require the participation of TRAF6. This conclusion is based upon three complementary experimental observations. First, although overexpression of TRAF6 can stimulate the activation of NF-κB, it cannot stimulate mRNA stabilization. Second, a dominant-negative version of TRAF6 can effectively block activation of NF-κB, but does not block mRNA stabilization in response to stimulation with IL-17. Finally, IL-17 remains fully capable to stabilize TNF-α-induced KC, MIP-2, and IκBζ mRNAs in TRAF6-deficient cells. Hence, the current findings identify a pathway distinct from NF-κB activation that leads from the IL-17R to enhanced stability of specific mRNAs.

TRAF6 has been shown to be involved in TLR-mediated activation of MAPKs, including p38 through the action of TAK1 (33, 40, 41), p38 MAPK and its downstream targets MK2 and MK3 have well-established roles in stimulus-induced mRNA stabilization and have been reported to participate in such responses to IL-17 (11, 12, 15–17, 23, 42). The observation that TRAF6, however, was not necessary for the stabilization of KC and MIP-2 mRNAs in response to IL-17 in the present studies raised the possibility that this response did not involve the activation of p38. More direct tests of this hypothesis using a pharmacologic inhibitor of p38 or cells genetically deficient in MK2 and MK3 further support the conclusion that IL-17-induced stabilization does not require the p38/MK2 pathway. Thus, although KC mRNA stabilization in response to TLR ligands requires p38 in mouse macrophages (38, 39), the response to IL-17 in the nonmyeloid cell populations studied in this work is both p38 and MK2/3 independent. The differences between the current findings and prior work both in TLR- and IL-17-induced mRNA stabilization are likely to reflect distinct mechanisms that may function differentially depending upon the cell type. In this regard, we have recently reported that IL-1α-induced stabilization of KC and MIP-2 mRNA is also independent of both TRAF6 and the p38/MK2 pathway in these same cell populations (35). Collectively, these findings suggest a common pathway downstream of Act1 and IL-1-α-associated kinase 1 that mediates mRNA stabilization in nonmyeloid cells in response to IL-17 and IL-1α, respectively.

The TRAF6/p38 pathway is believed to promote the stabilization of AU-rich mRNAs by targeting tristetraprolin, an RNA-binding protein that promotes rapid decay of mRNAs containing multiple copies of the pentameric sequence AUUUA (43–45). Indeed, KC mRNA decay (and LPS-mediated stabilization) in peritoneal macrophages depends upon this mechanism (38). It is noteworthy, however, that IL-17- and IL-1α-mediated stabilization of KC in nonmyeloid cells does not require tristetraprolin and depends upon a tristetraprolin-insensitive sequence in the 3′ UTR that contains no AUUUA motifs (S. Datta, M. Novotny, P. Pavicic, C. Zhao, T. Herjan, J. Hartupee, and T. Hamilton, manuscript in preparation). This different sequence requirement provides an additional criterion distinguishing this IL-17 signaling pathway.

The variable dependence of responses to IL-17 on TRAF6 appears to relate directly to the mechanism involved; activation of NF-κB requires TRAF6, whereas stabilization of mRNA does not. Thus, the assessment of several well-recognized IL-17-inducible mRNAs for dependence on TRAF6 provides some measure of the relative contribution of the two mechanistic pathways. It should be noted, however, that our study is limited to examining the response to IL-17 in the context of costimulation with TNF-α. Because TNF-α is a potent transcriptional activator, but a poor stimulus for mRNA stabilization, this feature of our experimental design focuses attention on the mRNA stabilization pathway by overriding the requirement for transcriptional stimulation. The results demonstrate that many of the IL-17-mediated responses are TRAF6 independent and are, therefore, likely to have some dependence upon the mRNA stabilization pathway. It is, however, worth considering whether transcriptional versus posttranscriptional mechanisms are involved in controlling the expression of each of these mRNAs. Of the five TRAF6-independent responses demonstrated in Figs. 5 and 6, three clearly appear to involve mRNA stabilization, as follows: KC (CXCL1), MIP-2, and IκBζ (20, 24). IL-6 is known to be unstable and sensitive to mRNA stabilization (16), but the IL-17 response may also use an indirect transcriptional mechanism via the induction of C/EBPβ and C/EBPδ (46, 47). Lipocalin 2 (Lcn2) has been shown to be inducible by the combination of TNF-α and IL-17 (5) and involves transcription, but not mRNA stabilization (48). Interestingly, several recent reports demonstrate convincingly that Lcn2 expression is dependent upon both NF-κB and the expression of IκBζ (49, 50). This latter finding, along with prior work (24) and that reported in this study, suggests that transcriptional induction of the lcn2 gene by IL-17 represents a downstream consequence of the stabilization of IκBζ. Thus, it appears that multiple mechanisms are contributing to the pattern of enhanced gene expression in IL-17-stimulated cells through both direct and indirect means.

Previous reports showing that the response to IL-17 is dependent upon TRAF6 were focused on the action of IL-17 alone (18, 26), whereas those reported in this study reflect the IL-17-mediated amplification of response to TNF-α. It is noteworthy that the former responses are, in fact, relatively modest in magnitude, whereas the combination of TNF-α and IL-17 often induces robust increases in gene expression that are much greater than the response to either cytokine alone (see Fig. 5) (5, 20, 21). It seems unlikely that IL-17 will be encountered alone at inflammatory sites in vivo, and hence, the cooperative effects seen with TNF-α are more likely representative of physiologic responses. The finding that most of the cooperative responses that we assessed were TRAF6-independent suggests that the mRNA stabilization pathway is at least one major mechanism by which IL-17 signals the amplification of inflammatory gene expression. Because this mechanism appears to function in a cell type-dependent fashion, there may be circumstances in vivo in which TRAF6-dependent, noncooperative IL-17-mediated responses occur, and this remains to be fully explored.

Disclosures

The authors have no financial conflict of interest.
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