CIKS/Act1-Mediated Signaling by IL-17 Cytokines in Context: Implications for How a CIKS Gene Variant May Predispose to Psoriasis

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Psoriasis is a relapsing skin disease characterized by abnormal keratinocyte proliferation and differentiation and by an influx of inflammatory immune cells. Recently, IL-17 cytokines have been strongly implicated as critical for the pathogenesis of this disease. IL-17A (also known as IL-17) and IL-17F are the signature cytokines of Th17 cells, but are also produced by innate cells, including γδ T cells present in skin, whereas epithelial cells, including keratinocytes, may produce IL-17C. IL-17 cytokines signal via the adaptor protein connection to IκB kinase and stress-activated protein kinases (CIKS)/Act1. Psoriasis is a disease with a strong genetic predisposition, and the gene encoding CIKS has recently been identified as a susceptibility locus. Unexpectedly, one predisposing gene variant features a mutation that impairs rather than enhances CIKS-mediated IL-17 cytokine signaling, counter to the predicted role for IL-17 cytokines in psoriatic inflammation. In this study, we demonstrate, however, that this mutant adaptor does not impair the IL-17–specific contributions to the genetic response when combined with TNF-α, a cytokine also prominent in psoriatic inflammation. Interestingly, TNF-α signals compensate IL-17 signaling defects imposed by this mutant adaptor even for genes that are not induced by TNF-α alone, including the transcription factors CCAAT/enhancer binding protein δ and IκBγ, which help regulate secondary gene expression in response to IL-17. Based on these findings we discuss a scenario in which the mutant adaptor may interfere with homeostatic maintenance of epithelial barriers, thereby potentially enabling the initiation of inflammatory responses to insults, whereas this same mutant adaptor would still be able to mediate IL-17–specific contributions to inflammation once TNF-α is present.

To explain how an impaired CIKS adaptor might increase the risk to develop psoriasis we hypothesize that IL-17 cytokines may have two distinct roles in the skin: one role may be to help maintain barrier functions, such as warding off bacteria under normal, noninflammatory conditions, and a second role may be to help drive inflammation during psoriatic flare-ups. We therefore asked whether the CIKS adaptor mutant linked to psoriasis would impair the contributions of IL-17 cytokines in an inflammatory context that includes TNF-α. TNF-α has been strongly implicated in psoriasis and anti–TNF-α treatments have proven fairly effective (28, 29). We demonstrate that the mutant CIKS adaptor does not impair the responses to the combined action of these two cytokines, whereas it does impair responses to IL-17 alone, as assessed not only with primary mouse embryo fibroblasts, but also with primary keratinocytes and primary dermal fibroblasts. We show that TNF-α signaling appears to fully compensate for the inability of this CIKS mutant to recruit TRAF6. Most surprisingly, TNF-α signals in concert with IL-17 compensate for the defects in the IL-17–only response even for genes that are induced exclusively by IL-17, but not TNF-α. These genes include the transcription factors IkBβ and CCAAT/enhancer binding protein (C/EBP)β factors that are rapidly induced and that help shape the overall response to IL-17 signaling. We discuss how the CIKS mutant and the thus impaired response to IL-17 alone may promote the initiation of psoriatic inflammation, whereas this mutant adaptor would still be able to mediate IL-17–specific inflammatory signals in the presence of TNF-α, a cytokine with which IL-17 can synergize in induced expression of many genes.

Materials and Methods

Cell culture and reagents
Primary mouse embryo fibroblast (MEF) cultures were established from wild-type (WT) and CIKS-deficient (knockout, KO) mice as described previously (10). Primary keratinocytes and dermal fibroblasts were isolated from 1-d-old CIKS KO mice exactly as published in a detailed protocol (30). Briefly, pups were euthanized and the skin was carefully removed. The dermis and epidermis were separated manually following trypsin digestion. Keratinocytes were extracted from the epidermal layer and dermal fibroblasts were extracted from the dermal layer. Keratinocytes were grown under low calcium conditions and cultures of epidermal keratinocytes and dermal fibroblasts were analyzed by phase-contrast microscopy to confirm typical cobblestone morphology of the former cells and typical more elongated spindle-like morphology of the latter cells. Cells were grown for a total of up to 5 d prior to stimulation (see below). Mice were bred and housed at the National Institute of Allergy and Infectious Diseases Animal Care and Use Committee and in accordance with all relevant institutional guidelines. Immortalized NF-kB essential modulator (NEMO)-deficient MEFs were donated by Dr. Manolis Papanaklis. IkBβ-deficient mice were provided by Dr. Shizuo Akira. MEFs were generated from C/EBPβ and C/EBPβ-deficient mice, which have been described previously (31, 32). C/EBPβ doubly deficient MEFs were generated from doubly deficient mice generated by crossbreeding of C/EBPβ and C/EBPβ singly deficient mice. Recombinant IL-17 (100 ng/ml; R&D Systems) and/or TNF-α (2 or 10 ng/ml; PeproTech) were used for stimulation of cells.

Plasmids and lentivirus
Full-length human CIKS and the CIKSΔT6 mutant (lacking aa 10–25; see Ref. 26) were cloned into a Gateway Entry vector (Invitrogen) and subcloned into a lentiviral FLAG Tag vector by Gateway LR recombination using the manufacturer’s protocols to generate expression clones. To ensure low-level constitutive expression, the standard CMV promoter in pCDH-EF1α (Invitrogen) following the manufacturer’s instructions. Cells were transduced with virus during an overnight incubation and 24 or 48 h later cells were stimulated with cytokines. In parallel experiments, GFP-expressing lentivirus transductions revealed efficiencies of at least 60% (keratinocytes, dermal fibroblasts) and up to 80% (MEFs). Approximately equal transduction efficiencies for matched samples were confirmed with Western blots for expression of transduced proteins in all experiments.

RNA isolation and real-time PCR
RNA was isolated using the RNeasy RNA isolation kit (Qiagen) according to the manufacturer’s instructions. cDNA was synthesized using oligo(dT) and SuperScript III (Invitrogen). Expression of Il1b, Zc3h12a, Il-6, Cxcl1, Cxcl2, Cxcl5, S100a8, Saa3, Len2, and β-actin was quantified by TaqMan quantitative PCR using primers from Applied Biosystems. All results are expressed as 2−ΔΔCt, where ΔΔCt = (Ct目标 − Ctβ-actin) for stimulated samples − (Ct目标 − Ctβ-actin) for unstimulated controls. Data are shown as the means ± SEM.

Western blots, quantification of chemokines/cytokines, and Abs
Whole-cell extracts were isolated, loaded on 10% SDS-polyacrylamide gel, electrophoresed and transferred to polyvinylidene difluoride membrane (Millipore). The following Abs were used for Western analysis: anti-FLAG (BioLegend); anti–OCT1-1 anti-C/EBPβ, anti-C/EBPβ, and anti–β-actin (Santa Cruz Biotechnology).

Nuclear extract and DNA binding assay
Nuclear extracts were isolated from nearly confluent 15-cm dishes using the CellLytic NuCLEAR extraction kit (Sigma-Aldrich) according to the manufacturer’s instructions. The ability of C/EBPβ to bind DNA was measured using the TransAM C/EBP α/β kit (Active Motif) according to the manufacturer’s instructions.

Statistical analysis
All data were analyzed as paired samples using the Student t test to determine statistical significance.

Results

The response to IL-17 plus TNF-α does not require CIKS to recruit TRAF6
We set out to explore why a mutant CIKS allele that is unable to fully mediate signaling by IL-17 cytokines might yet be associated with increased susceptibility to psoriasis, an inflammatory disease thought to critically involve IL-17. Because IL-17 is known to synergize with other inflammatory cytokines, notably TNF-α, and because psoriatic inflammation also critically involves TNF-α, we first examined the effect of the CIKS mutation on the signaling response to the combined action of both cytokines. To address this question we used primary, CIKS-deficient MEFs that were reconstituted, via lentiviral transduction, with either WT CIKS or a mutant CIKS protein in which the region between asp 10 and 25 containing the TRAF6 binding site was deleted (CIKSΔT6); CIKS-deficient (KO) cells were used a negative control. As shown previously by us and others, the CIKS mutant lacking the TRAF6 binding site was impaired in its ability to signal for activation of NF-κB in response to IL-17 and thus was impaired in its ability to induce expression of TRAF6/NF-κB-dependent genes (26, 27). Cells were stimulated with IL-17, TNF-α, or IL-17 plus TNF-α and gene expression was measured by real-time PCR after 1 and 6 h. These time points were chosen so as to be able to survey both immediate-early genes, of which some are induced only transiently, as well as later-induced genes, some of which comprise a secondary response dependent on induced expression of immediate-early transcriptional factors. The results confirm that the interaction between TRAF6 and CIKS controls the IL-17–driven expression of a number of genes, as IL-17 stimulation of MEFs reconstituted with the CIKSΔT6 mutant resulted in a block or significantly reduced expression of all of the test genes shown when compared with stimulation of cells reconstituted with WT CIKS

References

CIKS (Fig. 1A; Fig. 1B shows approximately equal expression of WT CIKS and CIKSΔT6 in transduced cells). As expected, CIKS KO MEFs showed no response to IL-17 and the induction of those genes responsive to TNF-α alone was completely unaffected by the status of CIKS in these cells. Importantly, there were no significant differences in gene induction between MEFs reconstituted with WT CIKS or CIKSΔT6 after stimulation with both TNF-α plus IL-17. This included genes for which the two cytokines together provided a very strong synergistic stimulus (such as Cxcl1, Cxcl5, and late induction of Saa3, Lcn2, and IL-6), but interestingly also genes that were primarily induced by IL-17 (with WT CIKS), but not by TNF-α (Zc3h12a, Lcn2; additional genes below). We confirmed these results by measuring the protein levels of IL-6, CXCL1, and CXCL5 in supernatants after 6 or 24 h stimulation (Fig. 1C). Taken together, these results suggested that the response to the combined actions of TNF-α and IL-17 was not impaired by the inability of the CIKS adaptor to recruit TRAF6 in response to IL-17. Such a result might be expected if the only role of IL-17 in this context was to stabilize TNF-α–induced mRNAs, as this function of IL-17 is independent of TRAF6 (33); Cxcl1, Cxcl2, and Cxcl5 are among the genes whose mRNAs are known to be stabilized by IL-17 (34, 35). However, that IL-17 must make other specific contributions beyond stabilizing mRNAs was revealed by the ability of TNF-α to also compensate for the CIKSΔT6 defect in the induction of genes that responded only to IL-17, not TNF-α. These findings suggested that the CIKSΔT6 mutant, which significantly impaired the genetic response to IL-17 alone, did not impair the combined response of TNF-α plus IL-17.

**FIGURE 1.** TRAF6 binding motif in CIKS is required for IL-17, but not IL-17 plus TNF-α signaling. CIKS deficient primary MEFs were reconstituted via lentiviral transduction with WT CIKS or the CIKSΔT6 mutant lacking aas 10–25. Nontransduced cells were used as a negative control (KO). (A) Real-time PCR analyses for indicated genes with RNAs isolated from cells stimulated for 1 or 6 h with IL-17, TNF-α, IL-17 plus TNF-α, or left unstimulated. Fold induction is in reference to unstimulated cells. (B) Western blot analysis of cell lysate collected from a representative experiment used in (A) to show approximately equal expression levels of transduced FLAG-tagged CIKS proteins, with β-actin serving as a loading control. (C) Concentrations of proteins in supernatants of MEF cultures were measured after 6 (CXCL1) or 24 h (IL-6 and CXCL5) of stimulation as indicated. Data are shown as the means ± SEM for four independent experiments. *p < 0.05 (Student t test).
IkBζ is an important component of the IL-17–induced response

To gain a more comprehensive answer to the question whether the CIKSDΔT6-imposed defects in IL-17–induced, TRAF6-dependent signaling might be fully compensated by TNF-α-initiated events, we began to focus on transcription factors that are induced by IL-17; such factors are involved in secondary gene induction and thus have an impact on the expression of many genes. If these factors were also fully induced by TNF-α plus IL-17 in the presence of the CIKSDΔT6 mutant, then it would be reasonable to suggest that the conclusions based on the more limited analyses shown in Fig. 1 are likely valid for the entire genetic response in cells carrying this mutant CIKS adaptor.

We first focused on the transcription factor IkBζ because its IL-17–induced expression is significantly dependent on TRAF6/ NF-κB (26) and because knockdown studies in human epithelial cell lines have shown a role for IkBζ in IL-17–induced expression of at least two genes, namely hBD and NGAL (36, 37). Most importantly, although this factor was induced by IL-17, peaking after ~1 h, it was not induced by TNF-α in these primary MEFs (Fig. 2A). Consequently, induction of this factor and all secondary genes regulated by it should comprise part of the IL-17–induced expression is significantly dependent on TRAF6/IKKα/IKKβ (26) and because knockdown studies in human epithelial cell lines have shown a role for IkBζ in IL-17–induced expression of at least two genes, namely hBD and NGAL (36, 37). Most importantly, although this factor was induced by IL-17, peaking after ~1 h, it was not induced by TNF-α in these primary MEFs (Fig. 2A). Consequently, induction of this factor and all secondary genes regulated by it should comprise part of the IL-17–specific contribution in the overall response to TNF-α plus IL-17 in these primary cells. To experimentally determine whether IkBζ is indeed functionally significant in the context of stimulation with both TNF-α plus IL-17 in our experimental system, we generated WT and IkBζ-deficient primary MEFs and assessed gene induction at 1 and/or 6 h after stimulation with IL-17, TNF-α, or IL-17 plus TNF-α; such an analysis has not been reported previously (Fig. 2B, 2C). As expected, loss of IkBζ had no impact on induction of various test genes by 1 h, but led to a significant block in the IL-17 plus TNF-α–induced synergistic expression of, in particular, IL-6 and Lcn2 by 6 h, and there was a trend toward lower expression of Cxcl1 and Cxcl2 under these conditions.

We also investigated the relevance of IkBζ in the regulation of C/EBPβ and C/EBPδ with our primary cells, as these C/EBPs have previously been suggested to play an important role in the response to IL-17 in certain fibroblasts and epithelial cells (38, 39). The induced expression of the C/EBPδ protein, which can be observed as early as 2 h after stimulation, was completely dependent on IkBζ in primary MEFs; in contrast, induced expression of C/EBPβ was independent of this factor (Fig. 2D). This is in line with previous work showing that C/ebpδ induction by LPS is dependent on IkBζ (40). C/EBPδ and C/EBPβ are the only inflammation-relevant C/EBPs expressed in fibroblasts. C/EBPβ is already present at basal levels prior to stimulation and the two protein bands represent the LAP and LAP* isoforms, both of which have transactivating activity and differ in size due to alternative in-frame translational initiation sites (41). Taken together, these data highlight the importance of the IL-17–specific induction of IkBζ in the overall response to stimulation with both IL-17 plus TNF-α.

C/EBPs are induced and/or activated by IL-17 and important for the IL-17–specific response

As shown above and previously by others (39), the levels of both C/EBPβ and C/EBPδ were induced in response to IL-17, and in the case of C/EBPβ, this protein was already present at basal levels prior to stimulation. Both factors have previously been implicated in the genetic response to IL-17 signaling in cell lines as well (38, 39, 42). Although C/EBPβ activity appears to be regulated ex-

![Figure 2](http://www.jimmunol.org/)
clusively via induced expression, C/EBPβ is a self-inhibited transcription factor that also requires posttranslational modification for activation, specifically phosphorylation (41, 43). Beyond that, C/EBPβ expression levels were also increased above basal levels by both IL-17 and TNF-α, and this was dependent on NF-κB/NEMO (similarly, induced expression of C/EBPβ was dependent on NF-κB/NEMO). Furthermore, nuclear levels of C/EBPβ rose in parallel with increased cellular levels (Fig. 3A, 3B); however, DNA binding activity of C/EBPβ appeared to be activated primarily by IL-17, not TNF-α in these primary MEFs (Fig. 3C); this has also previously been suggested by analysis with a stromal cell line (39). These data further highlight unique contributions of IL-17 when compared with TNF-α.

To assess the contributions of C/EBPs in the response to IL-17 plus TNF-α we generated primary MEFs lacking C/EBPβ, C/EBPδ, or both factors; we then stimulated these cells with IL-17, TNF-α, or IL-17 plus TNF-α to measure induced expression of various genes over time, an analysis with primary cells that extends prior, more limited studies with cell lines (38) (Fig. 4A). C/EBPβ appeared to have a partial role in the immediate-early response to IL-17 or IL-17 plus TNF-α, as loss of C/EBPβ alone (or loss of both C/EBPs) trended toward or caused a significant, albeit partial, reduction in the induced expression of IkBz and Zc3h12a. As expected, loss of C/EBPδ alone had little or no effect on these immediate-early events, which are presumably mediated by IL-17–induced activation of basal C/EBP protein levels. C/EBPs appeared to have no role in the induced expression of Cxcl1, Cxcl2, and Cxcl5 (measured at 1 and 6 h stimulation), but C/EBPs were critical for the IL-17 plus TNF-α–induced expression of Lcn2, Saa3, and IL-6 after 6 h stimulation, as loss of both C/EBPβ and C/EBPδ largely abrogated expression of these genes at that time; loss of C/EBPδ alone had little effect, whereas loss of C/EBPβ trended toward or resulted in a partial reduction in the case of Saa3 and IL-6, respectively. Therefore, the functions of the two C/EBP factors in the induced expression of these three later-induced genes were largely, although not completely, redundant. Taken together, these data highlight the importance of IL-17–induced expression/activation of C/EBPs in the overall response to IL-17 plus TNF-α in our primary MEF cultures.

We also examined C/EBPβ and C/EBPδ levels in WT MEFs and the various C/EBP-deficient MEFs before and after 2 h stimulation with IL-17 (Fig. 4B). The data confirm the loss of one or the other or of both C/EBPs in the corresponding KO MEFs; additionally, they show an apparently higher level of induced C/EBPδ in the C/EBPβ KO MEFs, which may represent a compensatory effect.

**FIGURE 3.** IL-17–induced activation of C/EBPs. (A) Western blot analysis of C/EBPβ and C/EBPδ expression in whole-cell lysates isolated from WT and NEMO-deficient MEFs (KO) after 2 h stimulation with cytokines as indicated. Control is unstimulated cells; β-actin served as a loading control. (B) Western blot analysis for expression levels of C/EBPβ in nuclear extracts isolated from WT MEFs subjected to stimulation with cytokines for 2 h as shown. Oct1 served as loading control. All Western blots were repeated three times and representative blots are shown. (C) C/EBPβ DNA binding assay with nuclear extracts used in (B). Data are shown as the means ± SEM for five independent experiments and fold induction is reference to unstimulated cells. *p < 0.05 (Student t test).

TNF-α compensates for defective IL-17–specific induction/activation of IkBz and C/EBP in cells with CIKS lacking the TRAF6 recruitment domain

The results above indicate that the transcription factors IkBz, C/EBPβ, and C/EBPδ are important not only in the overall response to IL-17 alone, but also in the response to IL-17 plus TNF-α. Furthermore, the results show that the induced activity of these particular transcription factors is largely due to IL-17, not to TNF-α. We thus wanted to determine whether the IL-17–induced expression of these factors was impaired in cells carrying the CIKSΔT6 mutant, and, if so, whether TNF-α would be able to compensate for this defect even though TNF-α alone could not induce either IkBz or C/EBPβ or further activate C/EBPβ (it was able to increase levels of C/EBPδ). If so, this would allow us to extrapolate findings in Fig. 1 to suggest that the CIKSΔT6 mutant does not appear to impair the overall response to IL-17 plus TNF-α. Loss of the TRAF6 recruitment domain in CIKS significantly impaired IL-17–induced expression of IkBz (Fig. 5A) and abolished IL-17–induced expression of C/EBPβ (which is dependent on IkBz; see Ref. 40) (Fig. 5B). In contrast, cells carrying the CIKSΔT6 mutant showed no significant reductions in the IL-17 plus TNF-α–induced expression of IkBz and C/EBPβ or the enhanced induction of C/EBPβ. Therefore, one would not expect this mutant CIKS protein to impair downstream effects of these transcription factors in the context of IL-17 plus TNF-α stimulation, as already evidenced by those test genes in Fig. 1 for which induction was dependent on these early-induced transcription factors. Based on these novel findings, we thus conclude that the mutant CIKSΔT6 adaptor is capable of transmitting IL-17–specific signals that are not activated downstream of TNF-α alone, signals that are important for the overall genetic response to the combined action of both cytokines; furthermore, TNF-α signals can compensate for the inability of the mutant CIKSΔT6 adaptor to transmit TRAF6-dependent signals downstream of IL-17 alone, required to induce many of the IL-17 target genes.

The response to IL-17 plus TNF-α is not impaired in primary keratinocytes or primary dermal fibroblasts carrying the CIKSΔT6 mutant adaptor

Psoriasis is an inflammatory skin disease also characterized by aberrant keratinocyte functions and differentiation (14–17). Because keratinocytes are known targets of IL-17 cytokines and because these cells might be wired differently for signaling by these cytokines than MEFs, we wanted to determine whether primary keratinocytes behaved similarly to primary MEFs when carrying the CIKSΔT6 mutant adaptor. In addition to epidermal keratinocytes, we also wanted to investigate signaling in dermal fibroblasts, because these cells are likely targets of IL-17 cyto-
kines as well, given that the dermis harbors γδ T cells, a major source of IL-17 cytokines in mouse psoriasis models (44). To carry out these investigations we isolated primary keratinocytes from the epidermis and fibroblast from the dermis of the skins of 1-d-old CIKS-deficient mice and then reconstituted these cell types with either WT CIKS or the CIKS<sup>D</sup>T6 mutant via lentiviral transduction.

**FIGURE 4.** C/EBPs are essential for a subset of IL-17–induced genes. (A) Real-time PCR analyses for indicated genes with RNAs isolated from WT, C/EBPβ-deficient (βKO), C/EBPδ-deficient (δKO), and C/EBPβδ doubly deficient (βδKO) MEFs stimulated for 1 or 6 h with IL-17, TNF-α, or IL-17 plus TNF-α. Data are shown as the means ± SEM of four independent experiments and fold induction is in reference to unstimulated cells. *p < 0.05 (Student t test). (B) Western blot analysis of cell lysate collected from a representative experiment shown in (A) after 2 h IL-17 stimulation to confirm absence or show expression levels of C/EBPβ and C/EBPδ; β-actin served as a loading control.

**FIGURE 5.** Costimulation with TNF-α compensates for lack of TRAF6-mediated IL-17 signaling in expression of critical IL-17–specific genes. CIKS-deficient primary MEFs were reconstituted via lentiviral transduction with WT CIKS or the CIKSΔT6 mutant. Nontransduced cells were used as a negative control (KO). Cells were stimulated with IL-17, TNF-α, or IL-17 plus TNF-α. (A) Real-time PCR analysis for <i>IkBζ</i> with RNAs isolated from cells stimulated for 1 h. Data are shown as the means ± SEM for four independent experiments and fold induction is in reference to unstimulated cells. *p < 0.05 (Student t test). (B) Western blot analysis for expression of C/EBPβ, C/EBPδ, and FLAG-tagged CIKS in cell lysate collected after 2 h stimulation, with β-actin serving as a loading control. The blot is representative of three independent experiments.
transduction, prior to stimulating these cells with cytokines. Primary keratinocytes carrying the CIKSΔT6 mutant showed significantly impaired expression of some key target genes in response to IL-17 alone when compared with cells carrying the WT CIKS protein; in contrast, there was no difference after stimulation with both TNF-α and IL-17 (Fig. 6A, 6B). The target genes included Lcn2, which exhibited strong synergistic induction by these two cytokines, as well as IκBζ, for which TNF-α merely corrected for the partial defect imposed by CIKSΔT6 in the IL-17–only mediated response. We also tested for induction of target genes in dermal fibroblasts reconstituted with either the WT or the CIKSΔT6 adaptor and again found that none of the responses was impaired when both cytokines were present (Fig. 6C, 6D), whereas IL-17–only signaling was impaired (Fig. 6C). Therefore, the conclusions we were able to draw from the more extensive analysis of primary MEFs also appear to hold true for primary keratinocytes and primary dermal fibroblasts. In all of these cells the CIKSΔT6 mutant only impaired responses to signaling by IL-17 cytokines alone, but did not impair responses to the combined action of IL-17 plus TNF-α.

Discussion

In this study, we have shown that a mutant CIKS protein lacking the N-terminal TRAF6 binding domain significantly impaired IL-17–induced expression of target genes, but had no impact on the response to IL-17 plus TNF-α. This was the case even though the genetic response to IL-17 plus TNF-α included genes that were induced by IL-17, but not TNF-α; furthermore, these genes were induced by IL-17 in a manner dependent on the TRAF6 recruitment domain of CIKS. Importantly, among the proteins encoded by these latter genes were transcription factors such as IκBζ and C/EBPα, which in turn are involved in the expression of later-induced genes in response to both IL-17 and IL-17 plus TNF-α. Therefore, our results may be extrapolated to suggest that the overall genetic response to IL-17 plus TNF-α appears to be largely unaffected by loss of the TRAF6 recruitment domain in CIKS. Consequently, such a mutant CIKS adaptor would not be expected to interfere with IL-17–mediated contributions in an inflammatory context, such as in psoriasis, where TNF-α is also present and critical for disease pathogenesis (28, 29). In addition to IL-17(A), IL-17F and IL-17C may contribute to psoriasis as well (5, 6, 22, 45, 46), and the results described here for IL-17 signaling via the mutant CIKS adaptor are likely to apply to these cytokines as well.

Although the bulk of these studies were conducted with primary mouse embryo fibroblasts, we also confirmed key findings with primary keratinocytes and primary dermal fibroblasts. Both cell types are targets of IL-17 cytokines in skin, and aberrant functions along with disturbed differentiation/proliferation of keratinocytes are prominent features of psoriatic lesions in both human patients and in mouse models of this disease (16, 19, 22, 47). Taken together, our findings provide strong evidence for why a mutant CIKS adaptor unable to recruit TRAF6 should not impair inflammatory responses downstream of IL-17 cytokines in the context of psoriasis, contrary to expectations.

How might TNF-α signals compensate for the lack of TRAF6 recruitment in mediating IL-17–specific responses? Recruitment of TRAF6 to CIKS is required for IL-17–induced NF-κB activation, which plays an important role in the overall genetic response to IL-17. TRAF6 may also be involved, via TAK1, in the activation of MAPKs by IL-17, although little is presently known about how this cytokine activates various MAPKs. Whereas ERK activation appears to be largely independent of TRAF6, activation of JNK appears to be dependent (26, 27, 48). TNF-α is a very potent activator of NF-κB (49) and this is likely an important mechanism by which TNF-α compensates for CIKS mutant-dependent defects in IL-17 signaling. Because our data suggest

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

The CIKSΔT6 mutant only impairs IL-17– but not IL-17 plus TNF-α–induced target gene expression in primary keratinocytes and primary dermal fibroblasts. CIKS KO primary epidermal keratinocytes (A, B) and primary dermal fibroblasts (C, D), reconstituted with WT CIKS, the CIKSΔT6 mutant, or left untransduced (KO) were stimulated with IL-17, TNF-α, IL-17 plus TNF-α, or left unstimulated for 2 (A, C) or 24 h (B, D) before harvesting cells and real-time PCR analyses for induced target gene expression, as indicated. Data are shown as the means ± SEM for at least four independent experiments and fold induction is in reference to unstimulated cells. *p < 0.05 (Student t test).
that TNF-α can fully compensate for impaired IL-17 signaling imposed by this CIKS mutant, TNF-α must be able to compensate for all TRAF6-mediated activities, even though TNF-α itself does not signal via TRAF6, but instead signals via TRAF2 and TRAF5 (49). The precise and full details of the compensatory mechanisms will require further study.

How might TNF-α and IL-17 cytokines synergize to induce expression of certain target genes and why might this be preserved in the presence of the CIKSΔT6 mutant? One mechanism likely involves IL-17–induced mRNA stabilization, which is independent of TRAF6 (33, 50, 51). Transcription of a given gene might be strongly induced by TNF-α, but only weakly by IL-17, but its mRNA might be very unstable unless stabilized by IL-17; in this scenario the two cytokines would synergize even in the presence of the CIKSΔT6 mutant. Another mechanism likely involves synergistic interactions of IL-17–induced transcription factors such IκBζ with transcription factors activated by TNF-α. Because TNF-α also corrects for the partial defect in IL-17–induced, CIKSΔT6-mediated expression of IκBζ, synergy would be preserved in the presence of this mutant adaptor.

Our results clearly show that the combined action of IL-17 and TNF-α is not impaired by the inability of the CIKS mutant to recruit TRAF6, so IL-17 should continue to drive psoriatic inflammation in patients carrying this mutation. An important remaining question however is whether this CIKS mutant might in fact compromise epithelial skin barriers under homeostatic, noninflammatory conditions, conditions in which TNF-α is not expressed. If barrier functions were compromised this may lead to heightened responses to insults, for example by allowing pathogens or their products to more easily penetrate skin, thereby causing inflammation. In such an inflammatory context IL-17 cytokines would then once again be able to fully contribute to disease pathogenesis, because this particular mutant CIKS adaptor does not interfere with the responses to IL-17 plus TNF-α, as shown in this study. Some prior reports have indeed suggested a role for IL-17 in maintaining barrier functions under homeostatic conditions (1, 52, 53). Although TNF-α is presumably not expressed in homeostatic, noninflammatory conditions, there is evidence that IL-17 cytokines might be produced, albeit at low levels. Dermal γδ T cells are poised to produce large amounts of IL-17 under inflammatory conditions (47), but a recent report suggests that they already constitutively express low levels of both IL-17A and IL-17F (44). A similar situation may apply to IL-17C, which is reportedly produced by keratinocytes in a mouse psoriasis model (5, 6), but which might also be expressed at low levels under noninflammatory conditions. Low levels of these IL-17 cytokines could be envisioned to help induce sufficient levels of antimicrobial peptides to keep pathogens and even commensals at bay; low levels of IL-17 cytokines may also induce sufficient levels of chemokines to attract cell types with antimicrobial activity or with the ability to insulate skin from various other insults. It is these activities of IL-17 cytokines that we suspect of being compromised in host skin carrying a mutant CIKS adaptor lacking the ability to recruit TRAF6. To fully prove our hypothesis in vivo will require the generation of mice expressing the CIKSΔT6 mutant in place of the WT gene; such mutant mice could then be tested for impaired barrier functions as well as for their ability to still develop full psoriatic phenotypes in appropriate animal models of psoriasis.

Importantly, the genome-wide association studies showed that the CIKS susceptibility allele carrying a mutation in the TRAF6 recruitment domain is often found in a homozygous state in patients, albeit not always (23–25). Whether such a mutant might have similar functional consequences in the heterozygous state will need to be addressed in future studies. It is of course possible that patients carrying just one such mutant allele could carry additional genomic changes that predispose to psoriasis.

Collectively, our data provide a possible explanation for how a mutant CIKS allele impaired in TRAF6 recruitment may promote and sustain psoriasis, contrary to initial expectations. Such a mutant would be fully capable of transmitting potent IL-17–mediated signals in an inflammatory context with TNF-α, as shown in this study. At the same time we conjecture that such a mutant CIKS adaptor might actually increase the risk of inflammation. We hypothesize that this mutant may impair low-level IL-17 signaling under homeostatic conditions, thereby weakening barrier functions; this in turn might increase entry of microbes or microbial products and thus lead to inflammation. Our work also suggests that treatment of psoriasis with a combination of drugs that suppress both IL-17 and TNF-α signaling might be particularly beneficial, with doses that may be suboptimal if given individually.

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Disclosures

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References
