IκBζ is a key player in the antipsoriatic effects of secukinumab

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Background: IκBζ plays a key role in psoriasis by mediating IL-17A–driven effects, but the molecular mechanism by which IL-17A regulates IκBζ expression is not clarified.
Objective: We sought to explore the molecular transformation in patients with psoriasis during anti-IL-17A (secukinumab) treatment with a focus on IκBζ.
Methods: The study was an open-label, single-arm, single-center secukinumab treatment study that included 14 patients with plaque psoriasis. Skin biopsy specimens and blood samples were collected on days 0, 4, 14, 42, and 84 and processed for microarray gene expression analysis. Furthermore, *in vitro* experiments with human keratinocytes and synovial fibroblasts were conducted.
Results: Secukinumab improved clinical scores and histologic psoriasis features. Moreover, secukinumab altered the skin transcriptome. The major transcriptional shift appeared between day 14 and day 42 after treatment initiation, although 80 genes were differentially expressed already at day 4. Expression of nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor (IκB) ζ (*NFKBIZ*, the gene encoding IκBζ) was reduced already after 4 days of treatment in the skin. *NFKBIZ* expression correlated to Psoriasis Area and Severity Index score, and *NFKBIZ* mRNA levels in the skin decreased during anti–IL-17A treatment. Moreover, specific *NFKBIZ* signature genes were significantly altered during anti–IL-17A treatment. Finally, we identified NF-κB activator 1 (Act1), p38 mitogen-activated protein kinase (MAPK), Jun NH2-terminal kinase (JNK), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) as key signaling pathways in *NFKBIZ*/IκBζ regulation.
Conclusion: Our results define a crucial role for IκBζ in the antipsoriatic effect of secukinumab. Because IκBζ signature genes were regulated already after 4 days of treatment, this strongly indicates that IκBζ plays a crucial role in the antipsoriatic effects mediated by anti–IL-17A treatment. (J Allergy Clin Immunol 2019;■■■:■■■-■■■.)

Key words: *Secukinumab, psoriasis, IL-17A,* NFKBIZ, *IκBζ, keratinocytes, c-Jun, NF-κB activator 1, p38 mitogen-activated protein kinase, nuclear factor κB*

Psoriasis is a chronic immune-mediated inflammatory skin disease affecting 2% to 3% of the population, varying according to age and geographic origin.[1] Psoriasis is characterized by hyperproliferation of keratinocytes and increased infiltration of inflammatory cells, including T cells. Increased numbers of active T cells, especially T_H1 and T_H17 cells, contribute to an altered and increased expression of proinflammatory cytokines, such as TNF-α, IFN-γ, IL-17A, and IL-22.[2,3] T_H17 cells and their production of the proinflammatory cytokine IL-17A play an essential role in the pathogenesis of psoriasis, and studies have shown an increased number of T_H17 cells, as well as an increased expression of IL-17A, in psoriatic skin compared with nonlesional psoriatic and normal skin.[4,5] In addition to T_H17 cells, other cells also contribute to increased production of IL-17A, including γδ T cells, neutrophils, and mast cells.[6] Although the essential role of IL-17A in the pathogenesis of psoriasis is indisputable, the underlying molecular mechanisms by which IL-17A regulates gene expression in patients with psoriasis are still not fully understood.

IL-17A binds and signals through a heterodimer receptor consisting of IL-17 receptor A (IL-17RA) and IL-17 receptor C.[7] The IL-17RA/IL-17 receptor C complex associates with the adaptor protein NF-κB activator 1 (Act1) through a SEFIR domain, making Act1 essential for IL-17A signaling.[8,9] IκBζ is a nuclear protein encoded by the nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor ζ *(NFKBIZ)* gene. IκBζ is slightly expressed in most resting cells, but on various inflammatory stimuli, including IL-17A, IL-1β, IL-36, and LPS, its expression is highly upregulated.[10-12] The exact mechanism by which IκBζ is regulated on IL-17A stimulation is not known, although Act1 has been demonstrated to play an essential role in human keratinocytes.[13] The facts that *NFKBIZ* is identified as a psoriasis susceptibility locus[13] and that IκBζ is demonstrated to be important in the development of psoriasis by mediating IL-17A downstream effects[14,15] highly suggest that IκBζ plays an important role in the pathogenesis of psoriasis.

Secukinumab is a human mAb directed against IL-17A, which is approved to treat moderate-to-severe plaque psoriasis,[16] as well as psoriatic arthritis and ankylosing spondylitis.[17] Although treatment of psoriasis with secukinumab has proved highly effective, the underlying molecular mechanisms by which secukinumab mediates its antipsoriatic effects are still to be determined.

Here we investigated the molecular transformation in the skin and blood from patients with psoriasis during 84 days of

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Abbreviations used
Act1: NF-κB activator 1
DEG: Differentially expressed gene
DMSO: Dimethyl sulfoxide
ERK: Extracellular signal-regulated kinase
IL-17RA: IL-17 receptor A
JNK: Jun NH2-terminal kinase
MAPK: Mitogen-activated protein kinase
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells
NFKBIZ: Nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor ζ
PASI: Psoriasis Area and Severity Index
SF: Synovial fibroblast
siRNA: Small interfering RNA

secukinumab treatment and characterized the molecular mechanisms by which IL-17A regulates IκBζ expression in human keratinocytes and synovial fibroblasts (SFs).

METHODS

The study design was an open-label, single-arm, single-center descriptive study. Fourteen patients with severe plaque psoriasis were treated with 300 mg of secukinumab administered subcutaneously at weeks 0, 1, 2, 3, and 4 and then every 4 weeks. Four-millimeter skin punch biopsy specimens and blood samples were obtained on days 0, 4, 14, 42, and 84. A target lesion was chosen, and biopsy specimens were collected from lesional and nonlesional psoriatic skin at baseline. In total, 18 biopsy specimens were obtained from each patient. At each visit, clinical scores (Psoriasis Area and Severity Index [PASI], Physician's Global Assessment, and body surface area) were evaluated, and photos were taken. The work described was carried out at Aarhus University Hospital in Denmark in accordance with the Declaration of Helsinki. Informed consent was obtained from each patient. Patients' demographics are described in Table I, and study visits are described in Fig E1 in this article's Online Repository at www.jacionline.org).

For transcriptomic profiling, the Affymetrix Clariom D microarray (Affymetrix, Santa Clara, Calif), which covers more than 542,000 transcripts, was used (performed according to the manufacturer's protocol at Eurofins, Aarhus, Denmark). In this study we focused only on actual genes, thus excluding the AceView predicted transcripts. One hundred thirty-four thousand seven hundred genes were probed by using the Clariom D array. Microarray data were summarized by applying the signal space transformation–robust multiarray average gene level method, as implemented in Transcriptome Analysis Console 4.0 software (Thermo Fisher Scientific, Waltham, Mass). Differentially expressed genes (DEGs) were identified by means of ANOVA (cutoff: 2-fold change and $P < .05$), and significance was adjusted for multiple testing by estimating false discovery rates.[18]

Data were visualized in Qlucore Omics Explorer version 3.4 (Qlucore AB, Lund, Sweden), including principal component analysis, heat maps, and unsupervised hierarchical clustering. Functional analysis, including pathway, upstream regulator, and network analysis, was performed in Ingenuity Pathway Analysis (Qiagen, Redwood City, Calif). Expression data are deposited in the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE137221. Additional details on the methods used in this study are described further in the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

Secukinumab improved clinical (PASI, Physician's Global Assessment, and body surface area) scores and histologic results in all patients included

Patients' demographics are described in Table I, and study visits are described in Fig E1. Eight of the 14 patients included in the study had a 100% reduction in the Psoriasis Area and Severity Index (PASI100) response. Four had a 90% reduction in the Psoriasis Area and Severity Index (PASI90) response, 1 had a 75% reduction in the Psoriasis Area and Severity Index (PASI75) response, and 1 had a response just below PASI75, with a clearance of 73.4% at week 12. The mean baseline PASI score was 26 (range, 14-52), which reduced to 1 (range, 0-8) after 12 weeks of treatment. Clinically, only traces of improvement were observed on day 4, with a small decrease in erythema and scaling in most patients (mean PASI score at day 4 was 23). A more noticeable PASI score reduction was seen at day 14 (mean PASI score, 14; range, 7-27), but the major shift in the skin was observed at day 42 (mean PASI score, 5; range, 0-18; Fig 1, *A* and *B*). In all patients the target lesion had cleared at day 84, and only hyperpigmentation/hypopigmentation remained (Fig 1, *A*). However, some patients had minor residual psoriasis elsewhere. Hematoxylin and eosin staining of skin biopsy specimens showed no histologic changes after 4 days of treatment, whereas a reduction in epidermal thickness and inflammatory infiltrates was observed at day 14 and forward, resembling nonlesional skin at days 42 and 84 (Fig 1, *C*, upper panel). Ki-67 staining was used as a proliferation marker and decreased during secukinumab treatment (Fig 1, *C*, middle panel). In addition, CD3 staining showed a reduced number of T cells during secukinumab treatment (Fig 1, *C*, lower panel).

Secukinumab treatment normalized the psoriatic transcriptome in the skin

To explore the molecular transformation during anti–IL-17A treatment, we took advantage of a deep and broad transcriptome analysis covering more than 542,000 transcripts. A clear treatment effect was observed with time (see Fig E2 in this article's Online Repository at www.jacionline.org). During treatment, more than 4000 DEGs were detected. The major transcriptional shift appeared between day 14 and day 42 (Fig 2, *A*), and some patients seemed to shift from a lesional gene expression profile to a more nonlesional-like profile already at day 14 (Fig 2, *A*). Indeed, even at day 4, we observed 80 DEGs after only 1 secukinumab treatment (Fig 2, *B* and *C*).

When comparing the gene expression profile of our patient cohort with a psoriasis-specific gene signature based on other independent populations with psoriasis,[19] we demonstrated a clear overlap, thus validating that our patients were representative of the general population with psoriasis (see Fig E3 in this article's Online Repository at www.jacionline.org).

Secukinumab did not change the transcriptome in PBMCs during treatment

We observed very few DEGs in blood samples when testing the isolated PBMCs, suggesting that neither secukinumab nor the degree of psoriasis alters the blood transcriptome (see Fig E4 in this article's Online Repository at www.jacionline.org). Although there seems to be a shift in the expression profile at day 84, this variation mostly reflects noncoding RNAs and large individual differences, including sex-specific genes (see Fig E4).

NFKBIZ mRNA and IκBζ signature gene expression decreased significantly already after 4 days of secukinumab treatment

The fact that IκBζ was demonstrated recently to be a key driver in psoriasis by mediating IL-17A–driven effects[14] prompted us to

investigate IκBζ/*NFKBIZ* expression during anti–IL-17A treatment *in vivo*. *NFKBIZ* mRNA expression in psoriatic skin was significantly downregulated at days 4, 14, 42, and 84 after secukinumab treatment compared with that in lesional skin at day 0 (Fig 3, *A*). No alteration in *NFKBIZ* mRNA expression was observed in PBMCs of the patients with psoriasis during secukinumab treatment (Fig 3, *B*). Furthermore, *NFKBIZ* expression was demonstrated to correlate with PASI scores, indicating that *NFKBIZ* expression is related to the degree of psoriasis (Fig 3, *C*). The microarray network analysis supported that *NFKBIZ* expression was increased in lesional compared with nonlesional skin on day 0 and downregulated in lesional psoriatic skin on days 4, 14, 42, and 84 compared with lesional skin on day 0, confirming that *NFKBIZ* expression is influenced by secukinumab treatment (Fig 3, *D*, and see Figs E5 and E6 in this article's Online Repository at www.jacionline.org).

Next, we investigated specific IκBζ signature genes during anti–IL-17A treatment. *LCN2*, *IL19*, *DEFB4*, *S100A7/8/9*, *IL8*, *CCL20*, *IL17C*, *CXCL3/5/8*, *CHI3L1*, *CSF2/3*, *IL6*, *IL10*, *IL23A*, *IL17A*, *IFNG*, and *IL36G* were all regulated by IκBζ according to previous publications or based on Ingenuity Pathway Analysis (IPA).[14,15,20] These IκBζ downstream genes were upregulated in lesional skin at day 0 compared with nonlesional skin (Fig 3, *D*). During secukinumab treatment, most of these IκBζ signature genes became significantly downregulated already at day 4, which emphasizes that IκBζ plays a crucial role in the early antipsoriatic effects of secukinumab *in vivo* (Fig 3, *D*, and see Fig E5). When comparing the expression profile of specific IκBζ signature genes from the patients, the major shift appeared between day 14 and day 42 (see Fig E6).

IL-17A–induced IκBζ expression is regulated by a p38 mitogen-activated protein kinase, Jun NH2-terminal kinase, and c-Jun–dependent mechanism

Recently, we demonstrated increased expression of IκBζ on IL-17A stimulation in human keratinocytes and that IκBζ regulates downstream psoriasis-associated genes.[14] However, the specific downstream pathways involved in IL-17A–mediated induction of IκBζ are unknown. To examine the signaling pathways involved in IL-17A–mediated expression of IκBζ, we conducted a screening analysis in cultured human keratinocytes using Proteome Profiler Arrays (see Fig E7 in this article's Online Repository at www.jacionline.org). Based on their increased activation on IL-17A stimulation and known importance in the pathogenesis of psoriasis,[21,22] p38 mitogen-activated protein kinase (MAPK) and c-Jun were selected for further investigation. A time-course study was conducted to validate the observed phosphorylation/activation of p38 MAPK and c-Jun, as demonstrated by using the Proteome Profiler Array. An increase in phosphorylation/activity of p38 MAPK was seen after 5 minutes of IL-17A stimulation compared with that seen in vehicle-treated cells (Fig 4, *A*). In addition, we found an increase in phosphorylation/activity of c-Jun after 15 minutes compared with that in vehicle-treated cells (Fig 4, *A*).

Because IL-17A was found to increase the phosphorylation/activity of p38 MAPK and c-Jun, we next characterized the role of p38 MAPK and c-Jun in IL-17A–induced IκBζ expression. Preincubation with a p38 MAPK inhibitor (SB202190) significantly reduced IL-17A–induced *NFKBIZ* mRNA expression (approximately 60%) compared with the dimethyl sulfoxide (DMSO) control (Fig 4, *B*). In addition, p38 MAPK inhibition (SB202190) reduced the IL-17A–induced phosphorylation of p38 MAPK (see Fig E8 in this article's Online Repository at www.jacionline.org). We also investigated the effect of p38 MAPK inhibition on the IκBζ protein level and demonstrated a reduction in IκBζ protein levels in cells preincubated with the p38 MAPK inhibitor (Fig 4, *C*).

To examine the role of c-Jun in IL-17A–induced IκBζ expression, keratinocytes were transfected with c-Jun small interfering RNA (siRNA) before stimulation with IL-17A. Transfection of human keratinocytes with c-Jun siRNA reduced protein levels of c-Jun compared with those in control siRNA-transfected cells (Fig 4, *D*, and see Fig E9 in this article's Online Repository at www.jacionline.org). In addition, knockdown of c-Jun significantly decreased IL-17A–induced *NFKBIZ* mRNA expression (approximately 35%) compared with that in control siRNA-transfected cells (Fig 4, *E*). The effect of c-Jun knockdown on *NFKBIZ* mRNA expression was paralleled by a reduced IκBζ protein level compared with that seen in the control siRNA-transfected cell (Fig 4, *D*, and Fig E9).

To further characterize IL-17A–induced c-Jun phosphorylation, cultured human keratinocytes were preincubated with a Jun NH2-terminal kinase (JNK) inhibitor (SP600125) before IL-17A stimulation. Inhibition of JNK significantly decreased IL-17A–induced c-Jun phosphorylation compared with the DMSO control (Fig 4, *F*). In contrast, p38 MAPK inhibition (SB202190) did not affect IL-17A–induced phosphorylation of c-Jun (Fig 4, *F*). Because JNK was involved in IL-17A–induced phosphorylation/activation of c-Jun, we further characterized the role of JNK in IL-17A–induced IκBζ expression. Inhibition of JNK significantly reduced both IL-17A–induced *NFKBIZ* mRNA expression (approximately 40%) and IκBζ protein levels compared with those in the DMSO control (Fig 4, *G* and *H*). These data demonstrate that IL-17A regulates expression of IκBζ by a p38 MAPK- and JNK/c-Jun–dependent mechanism in human keratinocytes. This also correlates with the increased JNK activity after IL-17A stimulation, as found in the Proteome Profiler Arrays (see Fig E7).

IL-17A–induced IκBζ expression involves nuclear factor kappa-light-chain-enhancer of activated B cells signaling

Previous studies have suggested that nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) plays an essential role in IL-17A downstream signaling.[8,23,24] Human keratinocytes were preincubated with an NF-κB inhibitor (SC-514) before stimulation with IL-17A to characterize the role of NF-κB in IL-17A–induced IκBζ expression. Inhibition of NF-κB significantly reduced IL-17A–induced *NFKBIZ* mRNA expression compared with that seen in the DMSO control (Fig 5, *A*). Moreover, reduced *NFKBIZ* mRNA expression was paralleled by a decreased IκBζ protein level, as demonstrated by using Western blotting (Fig 5, *B*).

To further characterize the role of NF-κB in IL-17A–induced *NFKBIZ*/IκBζ expression, human keratinocytes were transfected with NF-κB p50 siRNA (sip50) and NF-κB p65 siRNA (sip65) before stimulation with IL-17A. Knockdown of the NF-κB p50 subunit or the p65 subunit significantly reduced IL-17A–induced *NFKBIZ* mRNA expression compared with that in siRNA controls (Fig 5, *C*). However, single knockdown of the p50 or

TABLE I. Demographics and disease characteristics of patients with psoriasis included in the study

Patient no.	1	2	3	4	5	6
Age (y)	59	53	42	54	26	49
Sex	Male	Male	Female	Male	Female	Female
BMI (kg/m^2)	29	—	39.5	27.5	44.1	43.9
Smoker	Ex-smoker	Ex-smoker	Yes	No	No	Yes
Cholesterol (mmol/L)	5.0	—	5.0	6.2	5.1	5.2
HbA1C (mmol/mol)	—	—	5.7	6.3	37	40
Comorbidities	Depression	DM, hypercholesterolemia	None	None	PCO	Hypercholesterolemia
Psoriatic arthritis	Yes	No	No	Yes	No	No
Age of psoriasis onset (y)	30	29	10	—	13	10
PASI score, baseline	45	19.2	32.4	17.2	18.3	20.3
Prior treatments	Topical steroids, Humira, Remicade, Stelara, mtx	Topical steroids, tar, bucky, UVB, mtx	Topical steroids, tar, cyclosporine, mtx	Topical steroids, mtx	Topical steroids, UVB, mtx	Topical steroids, bucky, UVB, mtx

DM, Diabetes; *mtx*, methotrexate; *NASH*, non-alcoholic streatohepatitis; *PCO*, polycystic ovaries; *PTSD*, post-traumatic stress disorder.

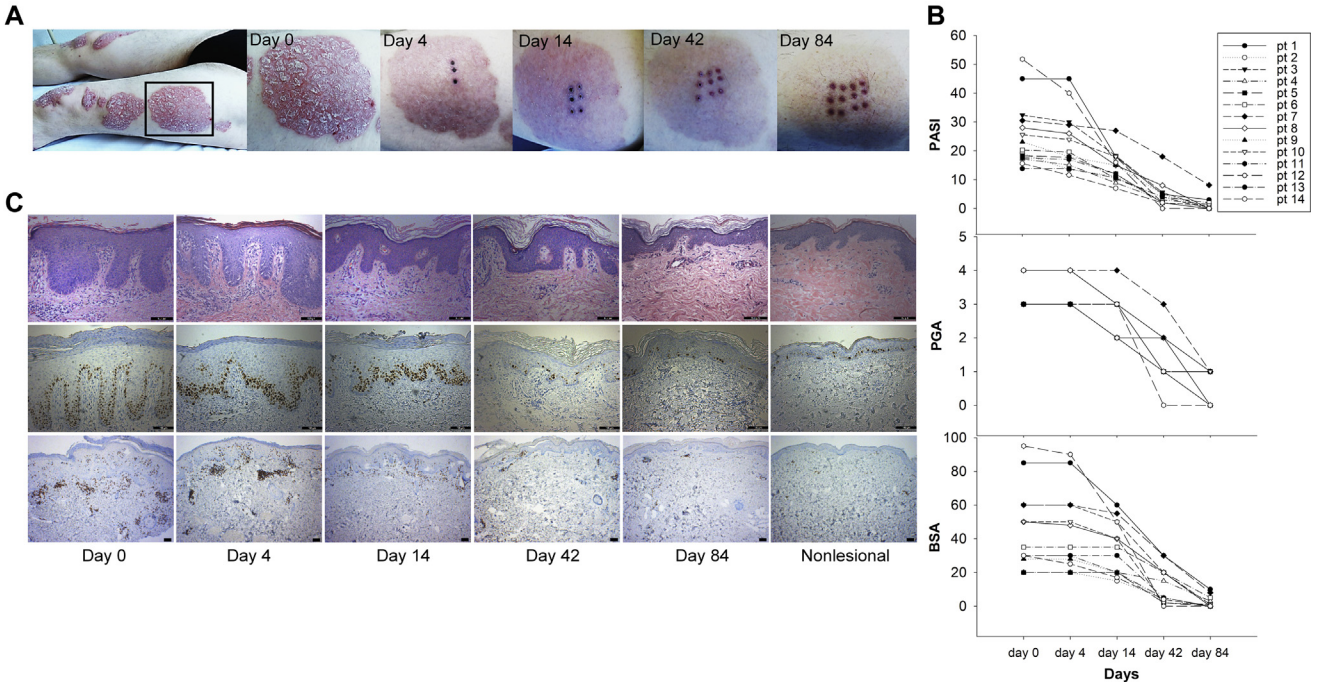


FIG 1. Clinical pictures, clinical scores, and histology during anti–IL-17A treatment in 14 patients with psoriasis. **A,** Clinical pictures were taken at each visit. One representative patient is shown. The same target lesion was followed over time. **B,** Clinical scores of each patient during 84 days of anti–IL-17A treatment: PASI, Physician's Global Assessment *(PGA)* and body surface area *(BSA)* scores based on clinical evaluation. **C,** *Top,* Hematoxylin and eosin staining showing histologic changes. *Middle,* Ki67 staining. *Bottom,* CD3 staining. One representative patient is shown. *Scale bars* = 100 μm.

p65 subunit did not significantly reduce the IκBζ protein level (Fig 5, *D*, and see Fig E10 in this article's Online Repository at www.jacionline.org). Only when both subunits were knocked down was the IκBζ protein level reduced (Fig 5, *D*, and see Fig E10). These data demonstrate that in human keratinocytes both the p50 and p65 subunits of NF-κB are important for IL-17A–induced IκBζ expression.

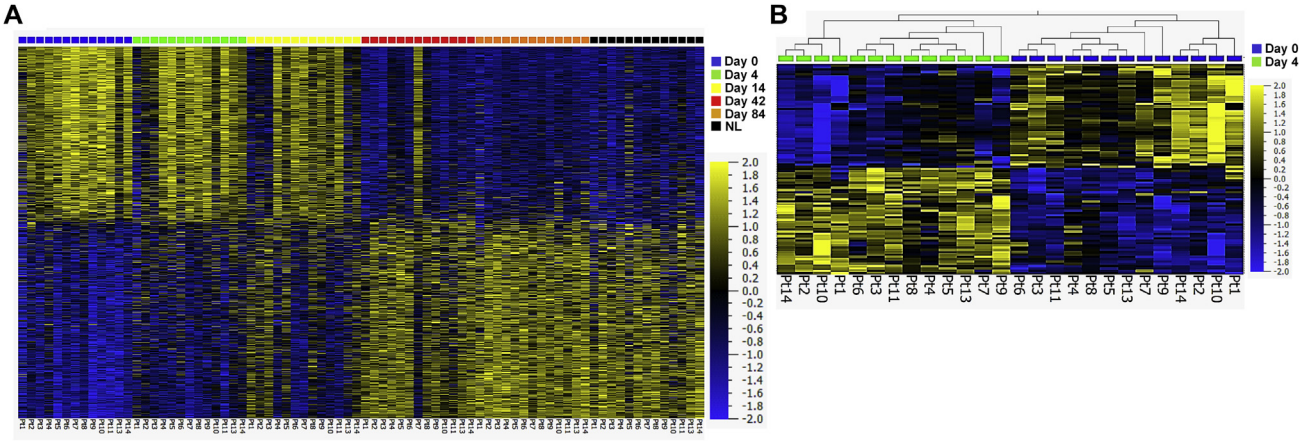
Moreover, to investigate whether there was an interrelationship between c-Jun, p38 MAPK, and NF-κB, we inhibited the pathways of each of these signaling molecules at different time points. Interestingly, c-Jun activity/phosphorylation was not only affected by inhibiting the JNK/c-Jun pathway (SP600125), as expected, but also by inhibiting the p38 MAPK pathway (SB202190) and the NF-κB pathway (SC-514). In contrast, inhibition of the p38 MAPK pathway (SB202190) did not affect NF-κB/p65 phosphorylation nor did NF-κB inhibition (SC-514) affect p38 MAPK phosphorylation (Fig 5, *E*). To examine whether inhibition of these signaling pathways influenced gene expression, *DEFB4* mRNA expression was analyzed. We found that inhibition of each of these signaling pathways resulted in a significant reduction in *DEFB4* mRNA expression (Fig 5, *F*).

IL-17A–induced IκBζ expression is mediated through an Act1-dependent mechanism

Previous studies have demonstrated that IL-17A signaling is mediated through the adaptor protein Act1.[13,25] Human keratinocytes were transfected with Act1 siRNA before

TABLE I. *(Continued)*

7	8	9	10	11	12	13	14
43	36	38	62	34	48	45	40
Male	Male	Female	Male	Male	Male	Male	Female
30.9	24.6	24.4	31.8	26.5	31.3	38.4	30.8
No	No	No	Yes	Yes	Ex-smoker	Ex-smoker	No
6.7	4.2	4.2	4.1	6.8	6.0	4.6	4.2
35	39	37	34	39	44	34	32
Hypertension, depression	None	None	Disc prolapse	None	PTSD, hypertension, NASH	Hypertension	None
Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
29	19	18	40	32	—	—	15
30.5	28	23.1	25.6	17.6	51.8	13.8	15.6
Topical steroids, mtx	Topical steroids, mtx, climate therapy	Topical steroids, bucky, mtx, Neotigason	Topical steroids, UVB, mtx	Topical steroids, mtx	Topical steroids, mtx, Imurel, cyclosporine	Topical steroids, UVB, mtx, Neotigason	Topical steroids, UVB, mtx



C

Top downregulated genes D4 vs D0

Gene Symbol	Gene Title	p-value	q-value	Difference	FC
CXCL8	chemokine (C-X-C motif) ligand 8	0.00	0.04	-2.26	4.80
IL19	interleukin 19	0.00	0.02	-2.26	4.79
EPGN	epithelial mitogen	0.00	0.05	-2.13	4.37
IL36A	interleukin 36, alpha	0.00	0.03	-2.01	4.03
DEFB4B	defensin, beta 4B	0.00	0.05	-1.97	3.93
DEFB4A	defensin, beta 4A	0.01	0.07	-1.81	3.51
CLDN17	claudin 17	0.00	0.01	-1.76	3.38
RHCG	Rh family, C glycoprotein	0.01	0.06	-1.73	3.31
SLC6A14	solute carrier family 6	0.00	0.06	-1.70	3.24
STC1	stanniocalcin 1	0.00	0.01	-1.68	3.20
AC055736.4	---	0.01	0.07	-1.66	3.17
S100A12	S100 calcium binding protein A12	0.00	0.05	-1.57	2.97
KRT17P1	keratin 17 pseudogene 1	0.00	0.03	-1.54	2.91
HSPD1P3	heat shock 60kDa protein 1	0.00	0.04	-1.53	2.89
HSPD1P2	heat shock 60kDa protein 1	0.00	0.04	-1.53	2.89
ANXA3	annexin A3	0.00	0.03	-1.43	2.70
KRT17	keratin 17, type I	0.00	0.05	-1.40	2.63
AC055736.2	---	0.01	0.07	-1.38	2.60
HEPHL1	hephaestin-like 1	0.03	0.16	-1.30	2.46
MMP1	matrix metallopeptidase 1	0.02	0.13	-1.25	2.37
ODC1	ornithine decarboxylase 1	0.01	0.07	-1.24	2.36
TCN1	transcobalamin I	0.03	0.14	-1.22	2.33
HMGCS1	3-hydroxy-3-methylglutaryl-CoA synthase 1	0.00	0.02	-1.21	2.31
IL1B	interleukin 1 beta	0.01	0.08	-1.18	2.27
FGFBP1	fibroblast growth factor binding protein 1	0.01	0.10	-1.17	2.25
ANXA1	annexin A1	0.00	0.04	-1.15	2.23
ENDOU	endonuclease, polyU-specific	0.00	0.06	-1.14	2.21
KRT17P2	keratin 17 pseudogene 2	0.00	0.04	-1.14	2.20
KRT19	keratin 19, type I	0.01	0.07	-1.13	2.20
S100A2	S100 calcium binding protein A2	0.02	0.11	-1.11	2.16
DSC2	desmocollin 2	0.01	0.07	-1.11	2.15
KRT16P3	keratin 16 pseudogene 3	0.02	0.11	-1.10	2.15
KRT17P6	keratin 17 pseudogene 6	0.00	0.02	-1.09	2.13
AC055736.3	---	0.00	0.05	-1.08	2.11
DEFB103A /B	defensin, beta 103A	0.00	0.06	-1.05	2.07
NAMPTP1	nicotinamide phosphoribosyltransferase	0.00	0.03	-1.05	2.06
KRT17P7	keratin 17 pseudogene 7	0.01	0.10	-1.04	2.05
SPRR2F	small proline-rich protein 2F	0.01	0.10	-1.03	2.04
DEFB103A	defensin, beta 103A	0.01	0.07	-1.02	2.03
KRT6C	keratin 6C, type II	0.02	0.11	-1.01	2.02
CXCL1	chemokine (C-X-C motif) ligand 1	0.02	0.12	-1.01	2.01
CHAC1	ChaC glutathione-specific -g- glutamyltransferase 1	0.01	0.08	-1.00	2.00

Top upregulated genes D4 vs D0

Gene Symbol	Gene Title	p-value	q-value	Difference	FC
STAT2	signal transducer and activator of transcription 2	0.00	0.01	1.01	2.01
BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)	0.00	0.01	1.01	2.01
SERPING1	serpin peptidase inhibitor, clade G (C1 inhibitor)	0.00	0.01	1.02	2.03
CASP14	caspase 14	0.01	0.06	1.03	2.04
PSAPL1	prosaposin-like 1 (gene/pseudogene)	0.00	0.05	1.04	2.05
AGTR1	angiotensin II receptor, type 1	0.02	0.11	1.04	2.05
CNTNAP3/2P2	contactin associated protein-like 3	0.01	0.08	1.05	2.07
SRGAP2C	SLIT-ROBO Rho GTPase activating protein 2C	0.00	0.01	1.06	2.09
CENPP	centromere protein P	0.00	0.02	1.06	2.09
MFAP4	microfibrillar associated protein 4	0.01	0.08	1.06	2.09
AR	androgen receptor	0.00	0.02	1.06	2.09
GBP2	guanylate binding protein 2	0.00	0.02	1.06	2.09
IL37	interleukin 37	0.02	0.12	1.06	2.09
HIBCH	3-Hydroxyisobutyryl-CoA Hydrolase	0.00	0.02	1.06	2.09
MIR568	microRNA 568 /// zinc finger and BTB domain	0.00	0.03	1.07	2.09
BTN3A2	butyrophilin, subfamily 3, member A2	0.00	0.01	1.08	2.12
CHL1	cell adhesion molecule L1-like	0.00	0.03	1.08	2.12
LCE2B	late cornified envelope 2B	0.00	0.05	1.09	2.12
THEM5	thioesterase superfamily member 5	0.00	0.02	1.09	2.13
CTSH	cathepsin H	0.00	0.02	1.10	2.14
RPTN	repetin	0.02	0.13	1.10	2.15
VIT	vitrin	0.00	0.02	1.11	2.16
CCL5	chemokine (C-C motif) ligand 5	0.00	0.01	1.12	2.17
IRF1	interferon regulatory factor 1	0.00	0.03	1.12	2.17
DLG2	discs, large homolog 2 (Drosophila)	0.00	0.01	1.17	2.26
TXNIP	thioredoxin interacting protein	0.00	0.01	1.17	2.26
SERPINB12	serpin peptidase inhibitor, clade B (ovalbumin)	0.00	0.04	1.21	2.32
FBXO32	F-box protein 32	0.00	0.02	1.24	2.37
GBP4	guanylate binding protein 4	0.00	0.05	1.25	2.38
KRT15	keratin 15, type I /// microRNA 6510	0.00	0.03	1.30	2.46
LCE2D	late cornified envelope 2D	0.00	0.03	1.31	2.49
LOR	loricrin	0.02	0.10	1.32	2.49
CLEC2A	C-type lectin domain family 2, member A	0.00	0.05	1.36	2.57
CXCL9	chemokine (C-X-C motif) ligand 9	0.04	0.16	1.40	2.63
IFI44L	interferon-induced protein 44-like	0.01	0.08	1.43	2.70
SOSTDC1	sclerostin domain containing 1	0.00	0.03	1.49	2.81
FLG2	filaggrin family member 2	0.02	0.14	1.73	3.31
CXCL10	chemokine (C-X-C motif) ligand 10	0.01	0.07	1.78	3.42

FIG 2. DEGs during anti–IL-17A treatment in 14 patients with psoriasis. **A,** Heat map based on 4213 genes identified by using pairwise comparisons ($P < 0.05$, $q = 0.07$-0.28, >2 fold change [FC]). **B,** Expression profile in lesional skin on days 0 and 4 identified by using a paired *t* test ($P < .05$, $q = 0.26$, >2 FC). **C,** Eighty top upregulated and downregulated genes in lesional skin between day 0 (D0) and day 4 (D4) with an FC of 2 or greater.

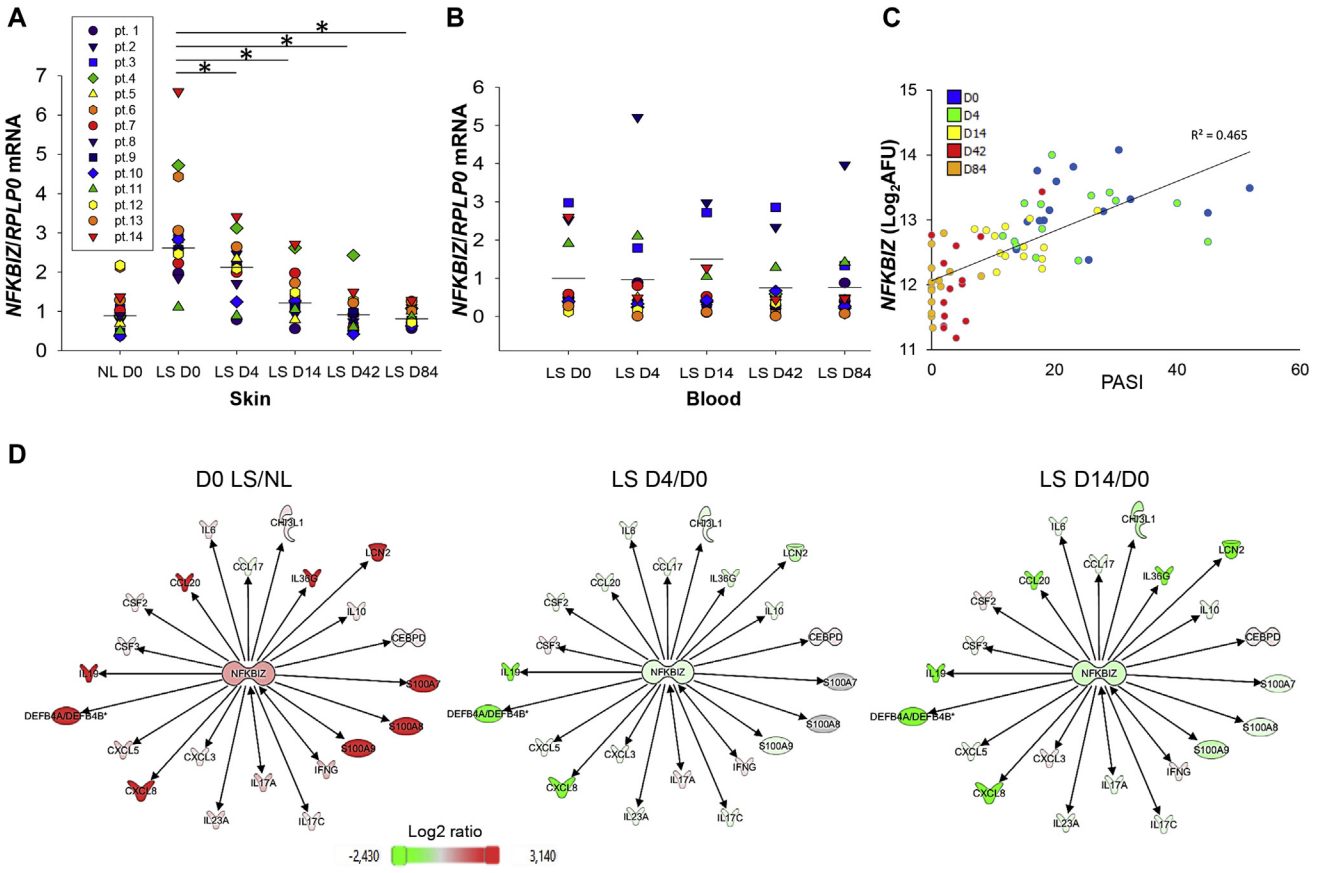


FIG 3. *NFKBIZ* expression during anti–IL-17A treatment. **A,** *NFKBIZ* mRNA expression in punch biopsy specimens from nonlesional *(NL)* and lesional *(LS)* psoriatic skin on day 0 and in lesional skin during secukinumab treatment on days 4, 14, 42, and 84. **B,** *NFKBIZ* mRNA expression in PBMCs during secukinumab treatment. *NFKBIZ* expression was analyzed by using quantitative PCR in Fig 3, *A* and *B* (n = 14). Ribosomal phosphoprotein P0 *(RPLP0)* expression was used for normalization. *$P < .05$. **C,** *NFKBIZ* expression assessed by using microarray analysis and plotted against PASI scores during 84 days of anti–IL-17A treatment. **D,** Expression of IκBζ signature genes in lesional *(LS)* skin on days 0, 4, and 14 and nonlesional *(NL)* skin on day 0 during anti–IL-17A treatment based on microarray data (n = 14) arranged as networks. Colors indicate changes in expression (log_2 ratio).

stimulation with IL-17A to examine the role of Act1 in the IL-17A–induced IκBζ expression. Transfection of human keratinocytes with Act1 siRNA significantly reduced protein levels of Act1 compared with those in keratinocytes transfected with control siRNA (see Fig E11, *A*, in this article's Online Repository at www.jacionline.org). Moreover, knockdown of Act1 significantly decreased *NFKBIZ* mRNA expression (approximately 60%), as well as protein levels of IκBζ, compared with values seen in control siRNA-transfected cells (see Fig E11).

NFKBIZ upstream signaling pathways are regulated during anti–IL-17A treatment in psoriatic skin

Having shown that c-Jun, p38 MAPK, JNK, Act1, and NF-κB are involved in expression of IκBζ *in vitro*, we next conducted a network analysis based on microarray data investigate these pathways. By comparing all gene expression profiles of patients with psoriasis during anti–IL-17A treatment, a clear treatment response in the expression of c-Jun *(JUN)*, p38 MAPK (β = *MAPK11* and α = *MAPK14*), JNK (JNK1 = *MAPK8* and JNK2 = *MAPK9*), Act1 *(TRAF3IP2)*, and NF-κB–inducing kinase *(MAP3K14)* was observed. c-Jun *(JUN)* was downregulated in the network analysis in lesional skin on day 0 compared with nonlesional skin and upregulated on days 4, 14, 42, and 84 compared with day 0 (Fig 6 and see Fig E12 in this article's Online Repository at www.jacionline.org). NF-κB–inducing kinase *(MAP3K14)* showed a similar regulation to c-Jun, but differences in regulation of NF-κB subunits were observed. NF-κB p65 *(RELA)* was downregulated in lesional skin on day 0 compared with nonlesional skin and upregulated on days 4, 14, 42, and 84 compared with day 0. In contrast, NF-κB p50 *(NFKB1)* was upregulated in lesional skin on day 0 compared with nonlesional skin and downregulated on days 4, 14, 42, and 84 compared with day 0. JNK1 and JNK2 (*MAPK8* and *MAPK9*) were upregulated in the network analysis on days 4, 14, 42, and 84 compared with day 0. However, JNK1 *(MAPK8)* was upregulated in lesional skin on day 0 compared with nonlesional skin, whereas JNK2 *(MAPK9)* was downregulated. Act1 *(TRAF3IP2)* expression was increased in lesional skin on day 0 compared with nonlesional skin and on

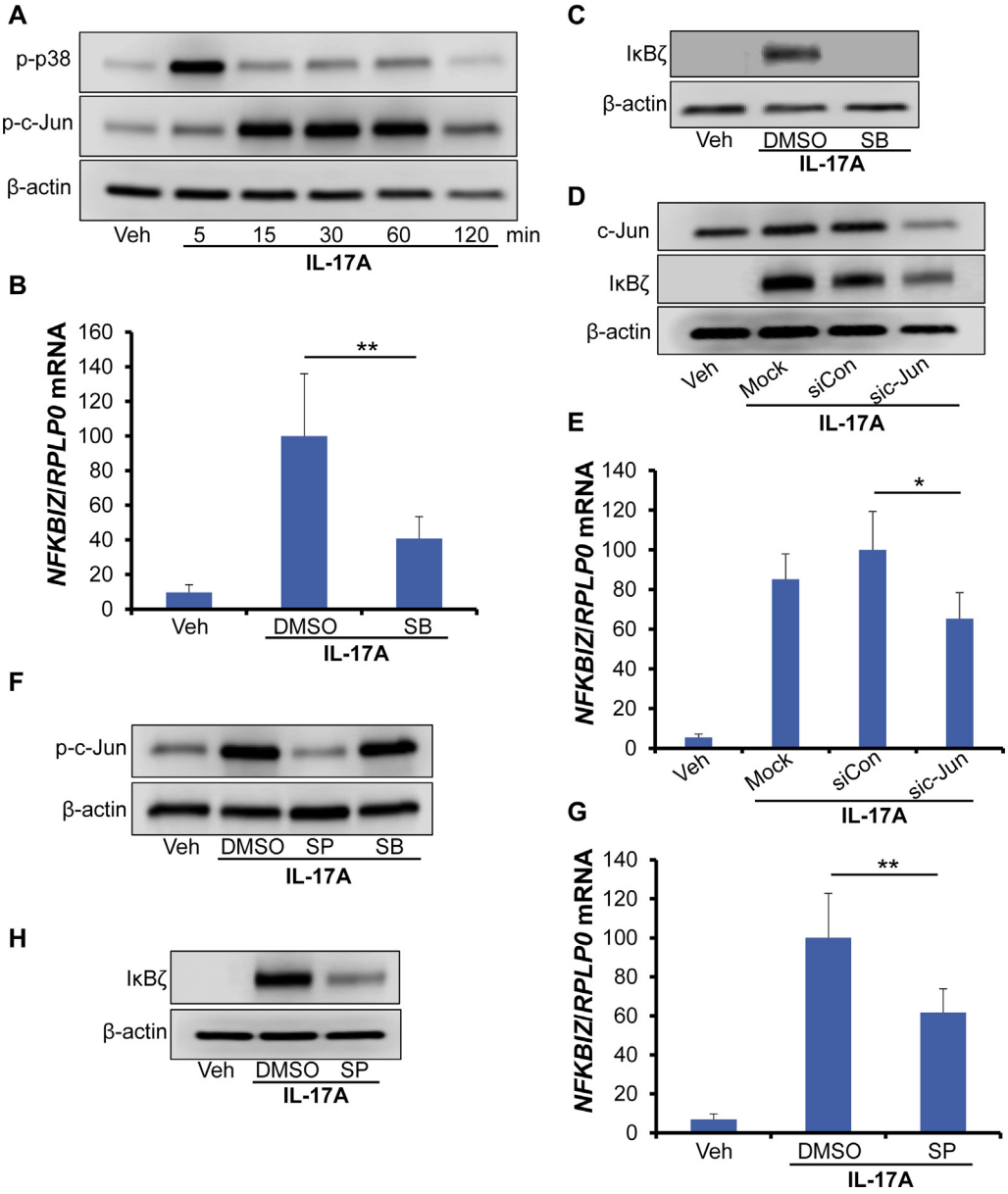


FIG 4. p38 MAPK, JNK, and c-Jun are involved in IL-17A–induced IκBζ expression. **A,** Keratinocytes were stimulated with IL-17A before phosphorylation of p38 MAPK (n = 5) and c-Jun (n = 4) was determined by using Western blotting. **B** and **C,** Keratinocytes were preincubated with SB202190 *(SB)* before stimulation with IL-17A for 1.5 hours. Fig 4, B, *NFKBIZ* expression analyzed by using quantitative PCR (n = 6). Fig 4, *C*, IκBζ protein was examined by using Western blotting (n = 4). **D** and **E,** Keratinocytes transfected with c-Jun siRNA *(sic-Jun)*, control siRNA *(siCon)*, or transfection reagent *(Mock)* before stimulation with IL-17A for 1.5 hours. Fig 4, *D*, c-Jun and IκBζ protein levels (n = 3). Fig 4, *E*, *NFKBIZ* expression analyzed by using quantitative PCR (n = 4). **F,** keratinocytes were preincubated with SP600125 *(SP)* or SB202190 *(SB)* before stimulation with IL-17A for 30 minutes. Phosphorylation of c-Jun was analyzed by using Western blotting (n = 4). **G** and **H,** Keratinocytes were preincubated with SP600125 *(SP)* before stimulation with IL-17A for 1.5 hours. Fig 4, *G*, *NFKBIZ* expression analyzed by using quantitative PCR (n = 4). Fig 4, *H*, IκBζ protein levels (n = 4). In Fig 4, *A*, *C*, *D*, *F*, and *H*, β-actin was used as a control for equal protein loading. **P* < .05 and ***P* < .01. *Error bars* indicate SDs. *Veh*, Vehicle.

days 4 and 14 compared with day 0. A treatment effect on Act1 expression was not observed until after 42 days of anti–IL-17A treatment (Fig 6 and see Fig E12). Together, these data demonstrate that c-Jun, p38 MAPK, JNK, NF-κB, and Act1 were regulated during anti–IL-17A treatment in psoriatic skin.

IκBζ is induced by IL-17A and TNF-α and regulates IL-6 and CXCL1 in fibroblasts

Because secukinumab also is approved for the treatment of psoriatic arthritis, we next investigated IκBζ in SFs. Stimulation with IL-17A for 2 hours significantly increased *NFKBIZ* mRNA expression (Fig 7, *A*). TNF-α stimulation alone did not affect

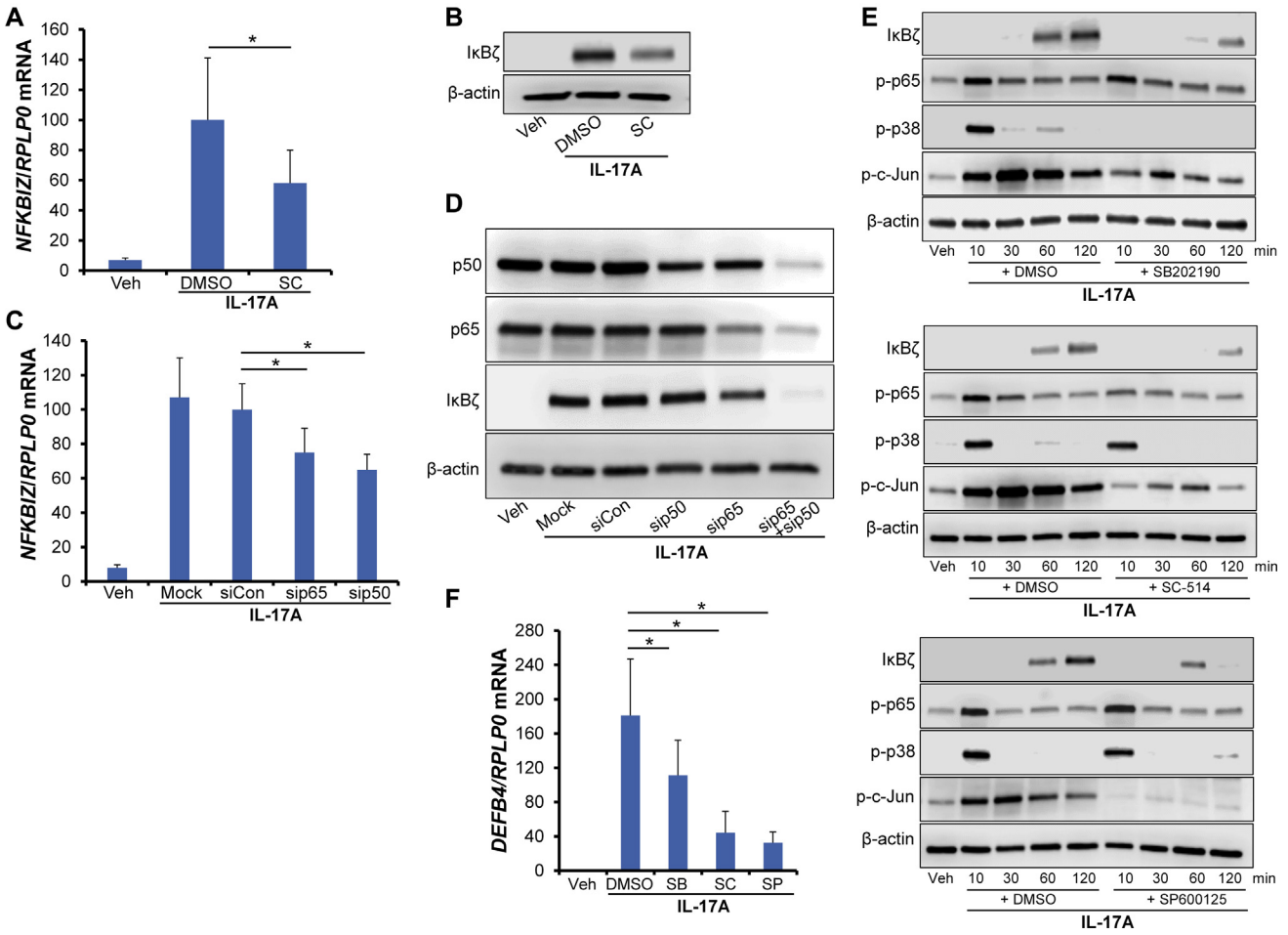


FIG 5. NF-κB is involved in IL-17A–induced expression of IκBζ. **A** and **B,** Keratinocytes were preincubated with SC-514 *(SC)* or DMSO before stimulation with IL-17A for 1.5 hours. Fig 5, *A, NFKBIZ* expression was analyzed by using quantitative PCR (n = 4) and expressed as means ± SDs. **P* < .05. Fig 5, *B,* IκBζ protein levels (n = 4). β-actin was used as a control for equal protein loading. **C** and **D,** Keratinocytes were transfected with NF-κB p50 siRNA *(sip50)* and/or NF-κB p65 siRNA *(sip65)*, control siRNA *(siCon)*, or transfection reagent *(Mock)* before stimulation with IL-17A for 1.5 hours. Fig 5, *C, NFKBIZ* expression was analyzed by using quantitative PCR (n = 4). **P* < .05. Fig 5, *D,* NF-κB p50, NF-κB p65, and IκBζ protein levels. **E,** Keratinocytes were preincubated with different inhibitors (SB202190 *[SB]*, SC-514 *[SC]*, or SP600125 *[SP]*) before stimulation for the indicated time points with IL-17A. Protein levels of IκBζ, phosphorylated p65, phosphorylated p38, and phosphorylated c-Jun were examined by using Western blotting (n = 3). β-Actin was used as a control for equal protein loading. **F,** Keratinocytes were preincubated with SB, SC, SP, or DMSO before stimulation with IL-17A for 24 hours. *DEFB4* expression was analyzed by using quantitative PCR (n = 3). **P* < .05. *Error bars* indicate SDs.

NFKBIZ mRNA expression significantly (*P* = .21), whereas IL-17A and TNF-α costimulation significantly increased *NFKBIZ* expression similar to what was observed with IL-17A alone (Fig 7, A). *IL6* mRNA expression was investigated as a secondary target gene and was increased only moderately on IL-17A and TNF-α stimulation at this early time point (Fig 7, *A*). *NFKBIZ* mRNA expression was paralleled by IκBζ protein levels, which peaked after 2 hours of stimulation with IL-17A alone or in combination with TNF-α (Fig 7, *B*). At 6 hours of stimulation, IκBζ protein levels were decreasing, indicating a short half-life (Fig 7, *B*).

To investigate secondary target genes regulated by IκBζ, SFs were transfected with 2 individual siRNAs to knockdown IκBζ. A strong knockdown of *NFKBIZ*/IκBζ was observed by both siRNAs (Fig 7, *C* and *D*). IL-6 and CXCL1 expression were regulated by an IκBζ-dependent mechanism in SFs, as demonstrated by decreased mRNA and protein expression after knockdown of IκBζ (Fig 7, *E*). Similar results were obtained in SFs derived from patients with psoriatic arthritis, in whom IL-6 and CXCL1 levels were increased by combined IL-17A and TNF-α stimulation (see Fig E13 in this article's Online Repository at www.jacionline.org). Moreover, in dermal fibroblasts derived from healthy subjects, IL-17A/TNF-α–induced IL-6 and CXCL1 protein levels were regulated through an IκBζ-dependent mechanism (see Fig E14 in this article's Online Repository at www.jacionline.org). Both *IL6* and *CXCL1* expression were also observed to be regulated early during anti–IL-17A treatment in skin from the patients with psoriasis included in the *in vivo* study (Figs 2, *C*, and 3, *D*).

Cells were preincubated with secukinumab before IL-17A and TNF-α stimulation to examine the effect of anti–IL-17A treatment in cultured SFs. Secukinumab preincubation clearly

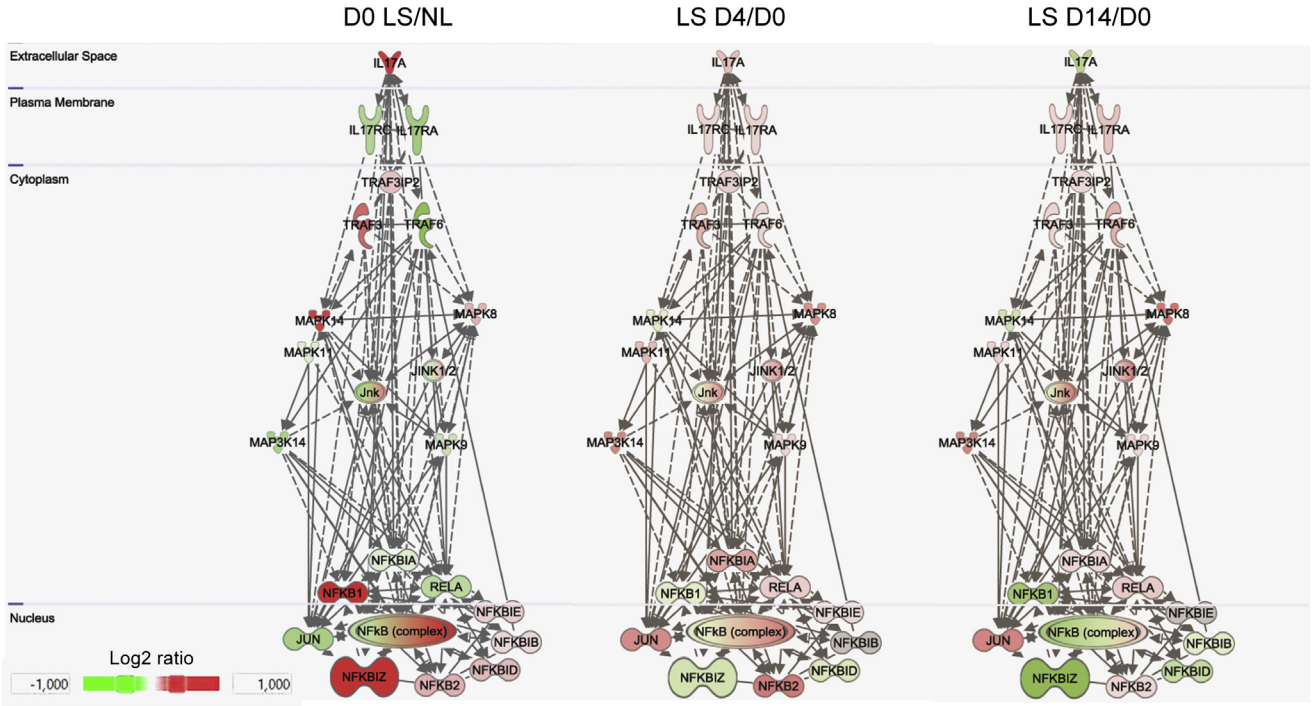


FIG 6. Upstream regulation of *NFKBIZ* in skin biopsy specimens from patients with psoriasis. Supervised network analysis based on microarray data during anti–IL-17A treatment are shown (n = 14). Gene expression of regulators is arranged according to location: JNK1 = *MAPK8*, JNK2 = *MAPK9*, p38β = *MAPK11*, p38α = *MAPK14*, NF-κB p65 = *RELA*, and NF-κB p50 = *NFKB1*. Time comparisons are as indicated. Upregulated and downregulated genes (referred equally to simply being up or down in the expression analysis) are colored according to the $\log_2$ fold change ratio for the lesional *(LS)*/nonlesional *(NL)*, day 4 *(D4)*/day 0 *(D0)*, and day 14 *(D14)*/day 0 *(D0)* contrast.

decreased not only IκBζ expression but also protein levels of the secondary proteins IL-6 and CXCL1 (Fig 7, *F* and *G*).

To further examine the IL-17A/TNF-α downstream signaling pathways, we conducted a time study investigating phosphorylation of NF-κB, p38 MAPK, and extracellular signal-regulated kinase 1/2. A definite increase over time in activity/phosphorylation of p38 MAPK, NF-κB (p65), and extracellular signal-regulated kinase 1/2 was observed after stimulation with IL-17A, TNF-α, or both (Fig 7, *H*).

DISCUSSION

Psoriasis is a chronic inflammatory skin disease with a complex pathogenesis, which is still not fully understood. The proinflammatory cytokine IL-17A plays a pivotal role in driving psoriasis,[26-28] as also evidenced by the high efficacy of IL-17A– and IL-17RA–targeting drugs.[29,30] However, the underlying molecular mechanism by which anti–IL-17A–targeting drugs mediate their antipsoriatic effect is not fully elucidated. Here we demonstrate that secukinumab, an anti–IL-17A antibody, mediates some of its antipsoriatic effects by rapidly inhibiting IκBζ and subsequently IκBζ signature genes, which highly suggests that IL-17A/IκBζ signaling is a key driver of the complex psoriatic phenotype. However, *NFKBIZ* is not the only target gene that is differentially expressed after treatment. The association between disease remission and downregulation of *NFKBIZ* expression is important but does not rule out that this observation could be due to secondary effects. On anti–IL-17A treatment, many of the genes with early regulation observed in this study were keratinocyte-associated genes. This indicates that keratinocytes play an important role in the early response mediated by anti–IL-17A, which is in agreement with previously reported data.[31-36] Although antagonizing IL-17A had an extensive effect on gene expression in psoriatic skin lesions, only a sparse effect was observed in gene expression profiles measured in PBMCs during anti–IL-17A treatment. This emphasizes the importance of localized keratinocytes in psoriasis pathogenesis and recovery. In line with these observations, recent data have shown that skin-localized IκBζ plays a pivotal role in the pathogenesis of psoriasis. Local inhibition of IκBζ in the skin by means of intradermal injection of siRNA directed against IκBζ clearly diminished imiquimod-induced psoriasis-like skin inflammation in mice and significantly decreased expression of key psoriasis-associated markers.[14] In addition, isolated keratinocytes from skin of patients with psoriasis have displayed significant alterations in their transcriptome.[36] Together, these data strengthen the hypothesis that local IL-17A/IκBζ signaling in keratinocytes plays a crucial role in the pathogenesis of psoriasis.

Several biological agents can suppress the IL-17A signaling pathway, some indirectly, such as anti–IL-12/IL-23 (p40), anti–IL-23 (p19), or anti–TNF-α agents, which interfere with T-cell activity and development or by reducing synergistic costimulation.[37-39] Other biologics suppress the IL-17A pathway directly, such as brodalumab, an anti–IL-17 receptor antibody, or ixekizumab, another anti–IL-17A antibody. Gene expression profiling and proteomics during brodalumab, secukinumab, and ixekizumab treatment have been reported.[31,33-35,40,41] However,

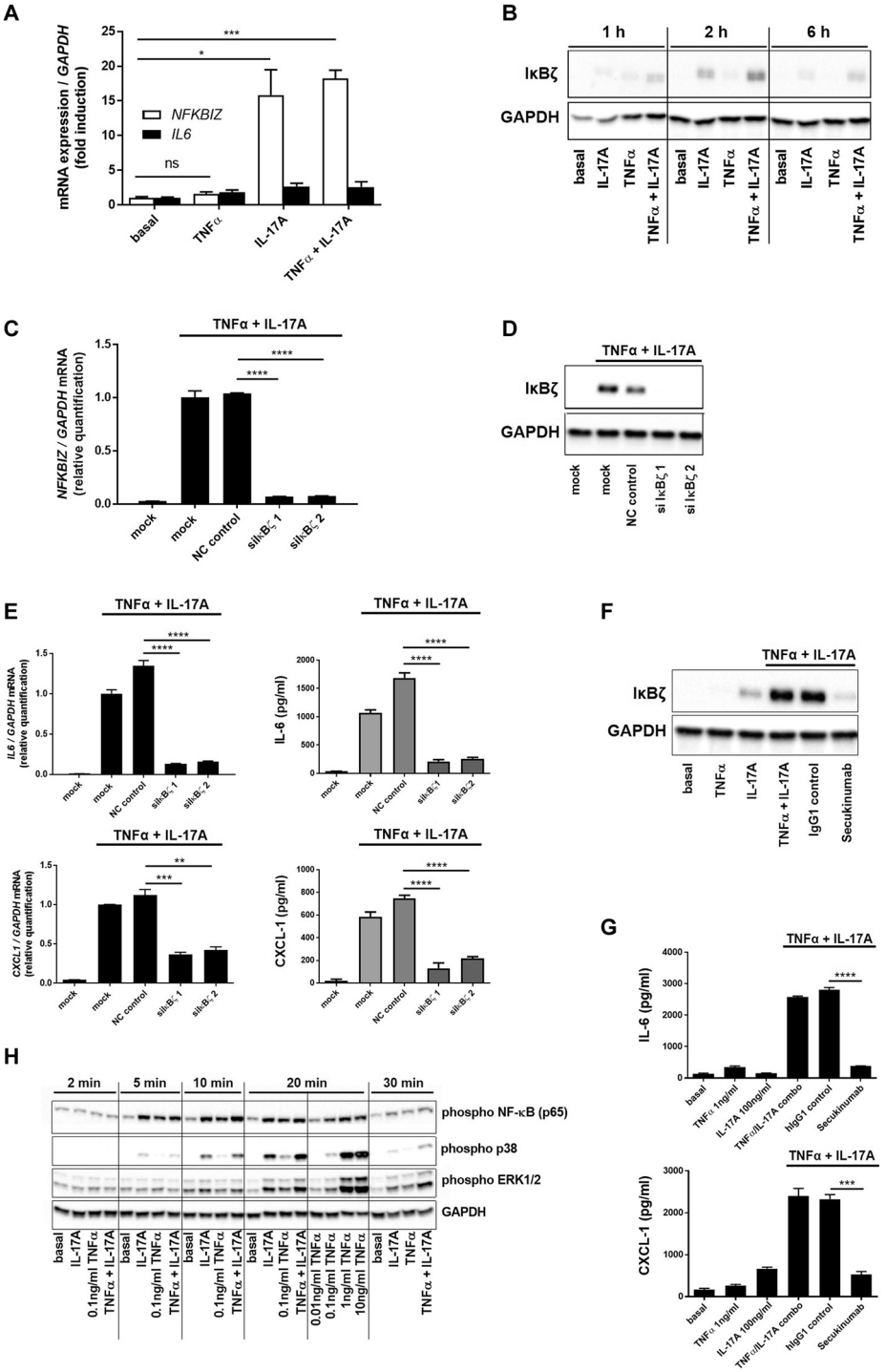


FIG 7. IL-17A/TNF-α–induced IκBζ regulates IL-6 and CXCL1 in SFs. **A,** SFs were stimulated with IL-17A (100 ng/mL), TNF-α (1 ng/mL), or both for 2 hours before *NFKBIZ* and *IL6* expression was analyzed by using quantitative PCR (n = 2). **B,** IκBζ protein levels in SFs stimulated with IL-17A, TNF-α, or both. **C-E,** SFs were transfected with IκBζ siRNA or a nontargeting control *(NC)* 24 hours before stimulation with IL-17A/TNF-α for 2 hours (Fig 7, *C* and *D*) or 22 hours (Fig 7, *E*; n = 2). Fig 7, *C*, *NFKBIZ* expression was analyzed by using quantitative PCR (n = 2). Fig 7, *D*, IκBζ protein levels (n = 2). Fig 7, *E*, *IL6* and *CXCL1* mRNA expression *(left)* and protein *(right)* analyzed by using quantitative PCR and Homogeneous Time Resolved Fluorescence (HTRF), respectively (n = 2). **F** and **G,** SFs were treated with secukinumab (5 μg/mL) or IgG_1 immediately before stimulation with IL-17A, TNF-α, or both. Fig 7, *F*, IκBζ protein levels after 2 hours of stimulation (n = 1). Fig 7, *G*, IL-6 and CXCL1 release measured after 22 hours by using HTRF (n = 2). **H,** SFs were stimulated with IL-17A, TNF-α, or both before phosphorylation of NF-κB, p38 MAPK, and extracellular signal-regulated kinase 1/2 *(ERK1/2)* were examined (n = 2). Graphs show data from a representative experiment measured in technical triplicates. **P* < .05, ***P* < .01, ****P* < .001, and *****P* < .0001, unpaired *t* test. *Error bars* indicate SDs. *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase.

here we extend our current knowledge by exploring the molecular transformation during secukinumab treatment with additional time sampling and, moreover, a deeper and broader transcriptome analysis. Unique to this study, we characterized the gene expression profile in skin biopsy specimens taken already after 4 days of anti–IL-17A treatment. Although no histologic changes were observed after 4 days of treatment, we found that at the molecular level, 80 genes were differentially expressed already 4 days after treatment initiation, demonstrating the rapid mode of action of anti–IL-17A treatment. Interestingly, IκBζ, along with many described IκBζ signature genes,[14,15,20,42] were included in the 80 genes that were differentially expressed after 4 days of treatment. These data strongly indicate that an important and very early mechanism of action of anti–IL-17A therapy in patients with psoriasis is a reduction in IκBζ expression and a concomitant reduction in expression of IκBζ signature genes.

Secukinumab and ixekizumab are highly effective not only in the treatment of psoriasis but also in the treatment of psoriatic arthritis.[32] Thus it is possible that the IL-17A/IκBζ signaling axis also plays a role in the pathogenesis of psoriatic arthritis. Although IL-17A has been demonstrated to be a potent inducer of IκBζ expression in various cell types,[13,14,43] the effect of IL-17A on IκBζ expression in fibroblasts has not been investigated. Here we demonstrated that IL-17A alone or in combination with TNF-α strongly induced IκBζ expression in cultured SFs. In addition, IκBζ was found to be essential for IL-17A/TNF-α–induced expression of IL-6 and CXCL1 in these cells. Thus, based on these data, it is possible that the IL-17A/IκBζ signaling axis also plays an important role in psoriatic arthritis, although further studies are needed to clarify this.

p38 MAPK activity has previously been demonstrated to play an important role in the pathogenesis of psoriasis,[21] and IL-17A has been found to induce the activation of p38 MAPK in different cell types.[44-46] Our study supports this notion, both *in vitro* and *in vivo*, and also demonstrates that p38 MAPK is regulated in psoriatic skin during anti–IL-17A treatment. Interestingly, the 2 subtypes, p38α/*MAPK*14 and p38β/*MAPK*11, were inversely regulated, which suggests an individual role of each p38 MAPK subtype. In this study we further present an essential role of NF-κB in IL-17A–mediated IκBζ expression *in vivo*. IL-17A has previously been demonstrated to activate NF-κB through phosphorylation of the NF-κB subunit p65.[8,12,24] Interestingly, our results demonstrate involvement of NF-κB in IL-17–induced IκBζ expression, which involves both the p65 and p50 subunits in keratinocytes. In addition, our results indicate cross-activation of the JNK/c-Jun pathway by the p38 MAPK and NF-κB pathways in regulation of IκBζ expression after IL-17A stimulation. This indicates that the JNK/c-Jun pathway might be a key pathway in IL-17A–mediated IκBζ expression in human keratinocytes.

In summary, blockade of IL-17A by secukinumab leads to clinical, histologic, and molecular resolution of psoriasis along with normalization of *NFKBIZ*. Because *NFKBIZ* is regulated already at day 4 after treatment initiation and reduces the expression of several psoriasis-associated genes, this indicates that clearance of psoriasis in the skin is driven in part by IκBζ. This suggests that future small-molecule treatments targeting IκBζ could be effective in the treatment of psoriasis.

D. Baeten kindly provided SFs derived from patients with psoriatic arthritis. A. Bregnhøj helped with the collection of patient materials.

Key messages

- **Secukinumab treatment rapidly decreases IκBζ expression and subsequently IκBζ signature gene expression in patients with psoriasis.**
- ***In vitro* IL-17A regulates IκBζ expression through a mechanism involving c-Jun, Act1, p38 MAPK, JNK, and NF-κB.**

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