IκBζ is a key driver in the development of psoriasis

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Psoriasis is a common immune-mediated, chronic, inflammatory skin disease characterized by hyperproliferation and abnormal differentiation of keratinocytes and infiltration of inflammatory cells. Although TNFα- and IL-17A–targeting drugs have recently proven to be highly effective, the molecular mechanism underlying the pathogenesis of psoriasis remains poorly understood. We found that expression of the atypical IκB member IκB (inhibitor of NF-κB) ζ, a selective coactivator of particular NF-κB target genes, was strongly increased in skin of patients with psoriasis. Moreover, in human keratinocytes IκBζ was identified as a direct transcriptional activator of TNFα/IL-17A–inducible psoriasis-associated proteins. Using genetically modified mice, we found that imiquimod-induced psoriasis-like skin inflammation was completely absent in IκBζ-deficient mice, whereas skin inflammation was still inducible in IL-17A– and TNFα-deficient mice. IκBζ deficiency also conferred resistance against IL-23–induced psoriasis. In addition, local abrogation of IκBζ function by intradermal injection of IκBζ siRNA abolished psoriasis-like skin inflammation. Taken together, we identify IκBζ as a hitherto unknown key regulator of IL-17A–driven effects in psoriasis. Thus, targeting IκBζ could be a future strategy for treatment of psoriasis, and other inflammatory diseases for which IL-17 antagonists are currently tested in clinical trials.

psoriasis | IκBζ | inflammation | cytokines

Psoriasis is a common chronic, inflammatory skin disease of unknown etiology affecting millions of people worldwide (1). Its pathogenesis is still not fully understood; however, infiltrating subsets of T cells, including Th1, Th17, and γδ T cells are known to play an important role. These T cells secrete an array of specific cytokines, including TNFα, IFNγ, IL-17A, IL-17F, and IL-22 (2, 3). TNFα and IL-17A play a key role in disease pathogenesis, not only in psoriasis but also in other autoimmune diseases such as rheumatoid arthritis (4–7). Whereas drugs targeting TNFα have been used in the treatment of these diseases for a number of years, secukinumab, an antibody targeting IL-17A, was just recently (January 2015) approved for the treatment of psoriasis in the European Union and the United States (8). Furthermore, in two phase III clinical trials, secukinumab has shown significant and sustained efficacy compared with placebo in patients with psoriatic arthritis (9). Although the significance of TNFα and IL-17A in the pathogenesis of psoriasis is indisputable, the underlying molecular mechanisms are not fully understood. Combined IL-17A and TNFα stimulation of human keratinocytes synergistically induces several psoriasis-associated genes, such as *IL-8*, *IL-17C*, *IL-19*, *CCL20*, and *DEFB4* encoding human β-defensin 2. Overall, there is a clear correlation between TNFα- and IL-17A–induced genes and the psoriasis gene signature and disease pathogenesis (10). Thus, the molecular mechanism leading to this synergistic induction of specific genes is only sparsely elucidated.

The transcription factor NF-κB has been implicated in several inflammatory diseases, including psoriasis, by activating a huge number of target genes (11, 12). Indeed, recent evidence suggests that the activation of particular NF-κB target genes is highly complex and dependent on selective gene regulation in distinct pathological settings (13). Whereas the rapid activation of primary response genes is directly induced by the classical NF-κB pathway, expression of so-called secondary response genes is delayed and requires prior protein synthesis of additional coregulators (13). IκBζ is an atypical nuclear IκB protein encoded by the *NFKBIZ* (nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, zeta) gene. IκBζ is not regulated by phosphorylation-induced degradation, but can act as an activator of selective target genes (14, 15). For instance, it was recently demonstrated that IκBζ controls TNFα/IL-17A–mediated induction of lipocalin 2 (LCN2) in human alveolar epithelial cells (16). IκBζ itself is a primary response target gene and, by association with the NF-κB subunit p50, it is thought to exert its transcription-enhancing activity on secondary response genes mainly at the level of chromatin remodeling (13, 17). It is rapidly induced by certain inflammatory stimuli, including IL-17A, but only to a minor extent by TNFα stimulation (10, 14, 16). IκBζ is known to play a pivotal role in the development of Th17 cells (18), and recently *NFKBIZ* was identified as a new psoriasis susceptibility locus (19). In the present study, we show for the first time to our knowledge that IκBζ is critically involved in the pathogenesis of psoriasis by mediating downstream effects of IL-17A.

Results

IκBζ Is a Key Regulator of a Number of Psoriasis-Related Genes. To characterize the role of IκBζ in the regulation of specific psoriasis-associated genes, siRNA (small interfering RNA) was used to knock down IκBζ. Transfection of cultured human keratinocytes with IκBζ siRNA significantly reduced the mRNA and protein

Significance

Psoriasis is an inflammatory skin disease affecting 2–3% of the population. IL-17A plays a key role in psoriasis pathogenesis, and antibodies targeting IL-17A have recently been approved for psoriasis treatment. Here we demonstrate that IκBζ is a key protein for IL-17A–driven effects in psoriasis. IκBζ was demonstrated essential for both TNFα/IL-17A– and IL-17A–induced expression of a panel of psoriasis-associated proteins. Moreover, IκBζ expression was increased in psoriatic skin, and in two different psoriasis mouse models we showed that psoriasis development was completely absent in IκBζ-deficient mice, but inducible in IL-17A– and TNFα-deficient mice. Finally, local abrogation of IκBζ function in mouse skin abolished psoriasis development. These findings uncover a novel crucial regulatory mechanism involved in psoriasis development.

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expression of IκBζ in TNFα/IL-17A–stimulated cells compared with cells transfected with control siRNA (Fig. S1). In the same cells, we analyzed a panel of key inflammatory genes known to be synergistically induced by TNFα and IL-17A and to be implicated in the pathogenesis of psoriasis (10). Interestingly, siRNA-mediated knockdown of IκBζ significantly reduced TNFα/IL-17A–induced expression of six studied major psoriasis-associated genes, including *CCL20*, *IL-8*, *IL-19*, *IL-17C*, *CXCL5*, and *DEFB4*, compared with control siRNA-transfected cells (Fig. 1*A*). IκBζ has previously been reported to be up-regulated by IL-17A in various cell types (16, 20). To characterize the molecular mechanisms involved in TNFα/IL-17A–induced expression in human keratinocytes, we next analyzed the expression profile of IκBζ. At 1.5 h poststimulation of the cells, it was revealed that *NFKBIZ* mRNA levels were only slightly increased after TNFα administration, whereas IL-17A stimulation yielded an 18-fold increase (Fig. 1*B*). We also examined the protein level of IκBζ and found that 1.5 h of IL-17A stimulation clearly increased IκBζ protein expression, whereas TNFα stimulation only weakly induced IκBζ (Fig. 1*C*). Stimulation of the keratinocytes with a combination of TNFα and IL-17A also led to an increased IκBζ protein level, which could be seen for all three time points examined. Because these results demonstrate that IL-17A is the main inducer of IκBζ expression in human keratinocytes, we next

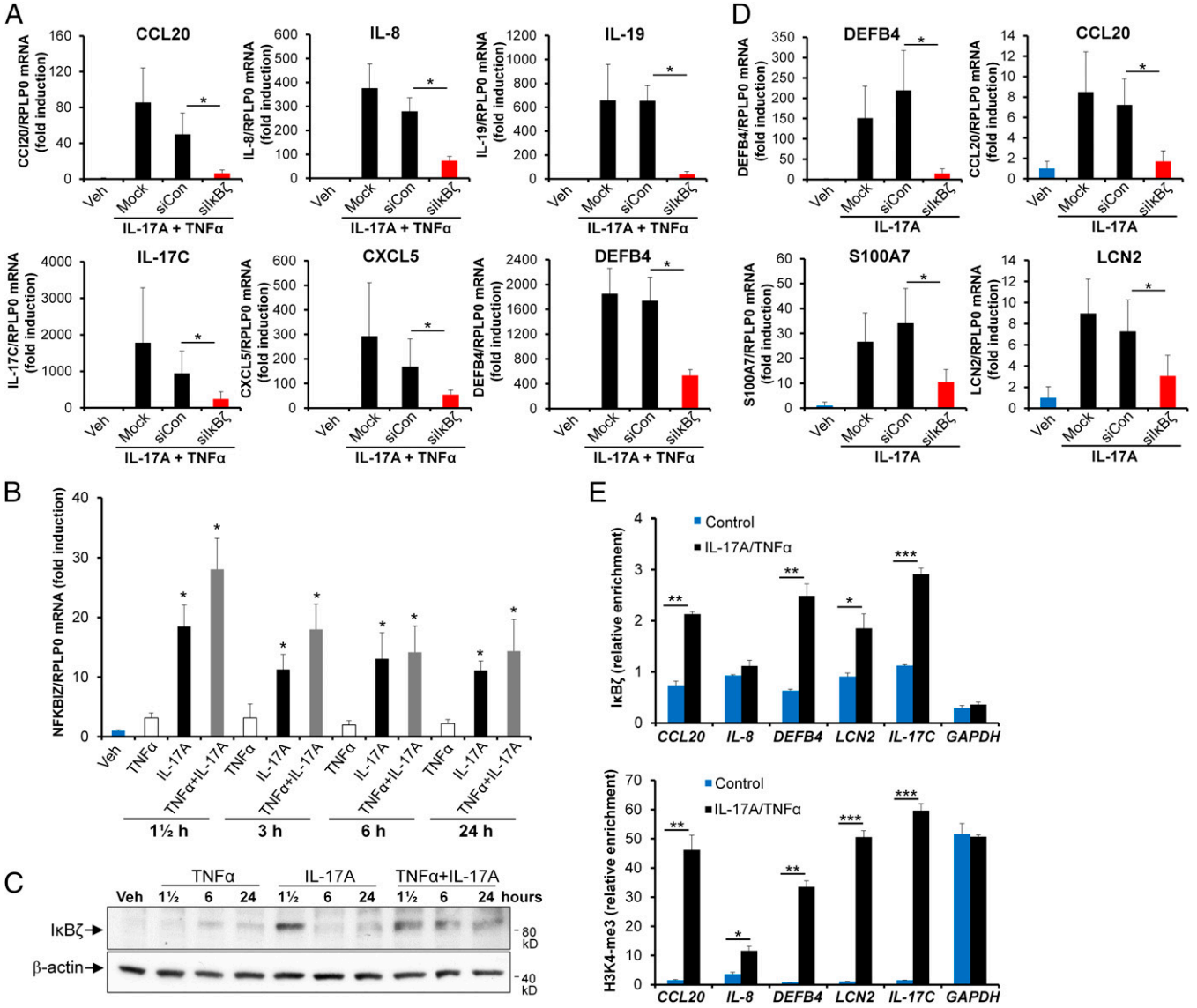

Fig. 1. IκBζ regulates the expression of key psoriasis-associated proteins. (*A*) Cultured human keratinocytes were transfected with IκBζ siRNA (siIκBζ), control siRNA (siCon), or transfection reagent alone (mock) before combined stimulation with TNFα and IL-17A for 24 h. The mRNA expression of *CCL20*, *IL-8*, *IL-19*, *IL-17C*, *CXCL5*, and *DEFB4* was analyzed by qPCR ($n = 3$). *$P < 0.05$, Student's *t* test. (*B* and *C*) Cultured human keratinocytes were stimulated with TNFα and/or IL-17A for the indicated time points. (*B*) *NFKBIZ* mRNA expression was determined by qPCR ($n = 4$). *$P < 0.05$ compared with vehicle-treated cells, one-way repeated measures analysis of variance followed by a Holm–Sidak test. (*C*) IκBζ protein expression was examined by Western blotting ($n = 3$). (*D*) Human keratinocytes were transfected as in *A* and then stimulated with IL-17A for 24 h. *DEFB4*, *CCL20*, *S100A7*, and *LCN2* mRNA expression was measured by qPCR ($n = 4$). In the qPCR experiments, *RPLP0* mRNA expression was used for normalization. Results are expressed as mean ± SD *$P < 0.05$, Student's *t* test. (*E*) ChIP analyses of cultured human keratinocytes that were either untreated or stimulated with TNFα and IL-17A for 3 h. ChIP analyses were performed with antibodies against IκBζ (*Upper*) or H3K4me (*Lower*). Bound DNA was analyzed in triplicate by qPCR for the indicated promoter gene regions. The results are expressed as relative enrichment and are shown as mean ± SD of three keratinocyte cultures from different donors. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Student's *t* test.

analyzed the role of IκBζ in IL-17A–induced gene expression. Silencing of IκBζ by siRNA significantly reduced the IL-17A–induced expression of *DEFB4*, *CCL20*, *S100A7*, and *LCN2*, all of which are known to be IL-17A downstream genes (Fig. 1*D*) (10). Because these data clearly demonstrate an impact of IκBζ on IL-17A–induced expression of key psoriatic proteins, we next investigated if this impact was mediated through a direct interaction of IκBζ on the promoter of these genes. Chromatin immunoprecipitation (ChIP) analyses using an IκBζ-specific antibody indeed revealed that the relative occupancies of the promoters of the *CCL20*, *DEFB4*, *LCN2*, and *IL-17C* genes by IκBζ were significantly increased by the combined treatment of keratinocytes with IL-17A and TNFα (Fig. 1*E*). In contrast, no recruitment of IκBζ was observed upon IL-17A/TNFα treatment to the promoter of *IL-8* as well as *GAPDH*, which was used as a negative control. Similar results were obtained in ChIP analyses with an antibody against trimethylated histone H3 lysine 4 (H3K4me3), which marks promoters with active transcription (Fig. 1*E*). Upon combined stimulation with IL-17A and TNFα H3K4me3 occupancy was strongly enriched at the promoters of all target genes. The enrichment of H3K3me3 was more efficient than the IκBζ ChIP, which can be explained by different ChIP efficiencies of the antibodies and a more stable nature of histone marks compared with IκBζ binding, which requires association with the NF-κB subunits p50 or p52 for chromatin recruitment. Our results, however, clearly demonstrate that IκBζ interacts with the promoters of the psoriasis-associated genes.

IκBζ Expression Is Increased in Psoriatic Skin. Because our in vitro data suggested that IκBζ is a transcriptional regulator of IL-17A–driven effects, and thus could play a role in the pathogenesis of psoriasis, and because previous transcriptome analyses of psoriasis biopsies have shown *NFKBIZ* to be up-regulated in psoriatic skin (21, 22), we next examined the expression level of IκBζ in skin biopsies taken from 17 patients with psoriasis and from six healthy controls. We demonstrated that the mRNA expression of *NFKBIZ* was significantly increased in lesional psoriatic skin where we observed an ∼2.5-fold increase compared with nonlesional psoriatic skin from the same patient. We also found a significant increased *NFKBIZ* mRNA expression in nonlesional psoriatic skin compared with normal healthy controls (Fig. 2*A*). To examine whether the increased mRNA expression of *NFKBIZ* was paralleled by an increased level of the corresponding protein, Western blotting on keratome biopsies from patients with psoriasis was conducted. The protein level of IκBζ was increased in lesional psoriatic skin compared with nonlesional psoriatic skin from the same patient (Fig. 2*B*), supporting a potential role of IκBζ in the pathogenesis of psoriasis.

IκBζ Is Essential for Development of Imiquimod-Induced Psoriasis-Like Skin Lesions in Mice. To further characterize IκBζ in the pathogenesis of psoriasis, we took advantage of a mouse model in which psoriasis-like skin inflammation was induced by topical application of the Toll-like receptor (TLR) agonist imiquimod. Although being a mouse inflammatory skin model, this model in many ways resembles human psoriasis (23). Daily topical application of imiquimod to the back skin of the mice led to significant redness, thickening, and scaling of the skin with a maximum cumulative score after 3–4 d of treatment (Fig. S2). Likewise, RNA analysis showed a rapid and significant induction of *Nfkbiz* after imiquimod treatment with an ∼3.5-fold larger increase than in vehicle-treated mice (Fig. S3). This finding supports the idea that IκBζ plays a role in the induction of the psoriasis-like skin changes in this model. To assess the functional role of IκBζ in this psoriasis model, we used genetically modified mice. Imiquimod-induced skin inflammation was completely absent in IκBζ-deficient mice compared with wild-type mice as measured by an increase in ear thickness (Fig. 3*A*). The model was also conducted on TNFα- and IL-17A–deficient mice. Although both TNFα- and

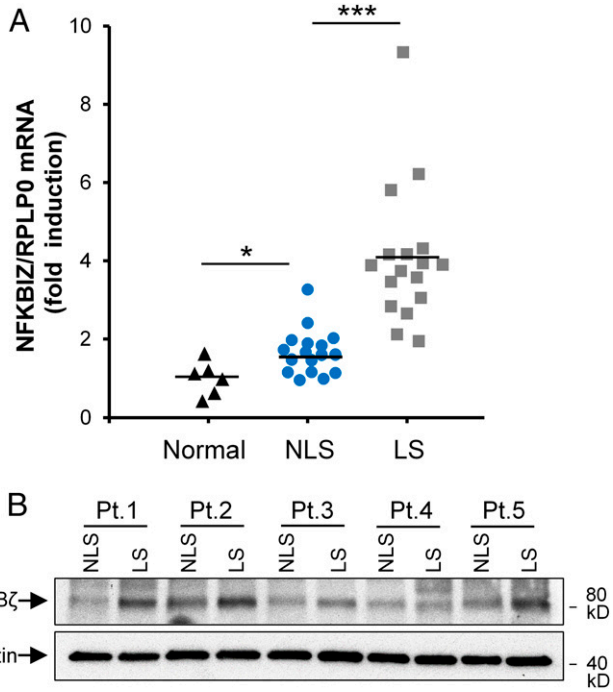

Fig. 2. The mRNA and protein level of IκBζ are increased in psoriatic skin. (*A*) *NFKIBZ* mRNA expression was analyzed in biopsies obtained from normal healthy volunteers as well as lesional and nonlesional psoriatic skin by qPCR. *RPLP0* mRNA expression was used for normalization. Scatterplot shows the result from 6 healthy volunteers and 17 patients with psoriasis. $*P < 0.05$, $***P < 0.001$, Student's *t* test. (*B*) IκBζ protein expression was examined in paired lesional (LS) and nonlesional (NLS) biopsies from five patients with psoriasis (Pt. 1–Pt. 5) by Western blotting.

IL-17A–deficient mice developed significantly less inflammation than wild-type mice, imiquimod-induced skin inflammation was more pronounced in TNFα- and IL-17A–deficient mice than in IκBζ-deficient mice (Fig. 3*A*). Histological evaluation of skin sections from wild-type mice treated with imiquimod for 5 d showed a characteristic induction of psoriasis-like skin lesions. These included increased epidermal thickness caused by hyperproliferation of keratinocytes as assessed by staining with the proliferation marker Ki67 as well as dermal infiltration of inflammatory cells. Interestingly, histological analysis of skin sections from imiquimod-treated IκBζ-deficient mice showed no signs of epidermal thickening or dermal cell infiltration and was comparable to vehicle-treated mice (Fig. 3 *B* and *C*). In contrast, increased epidermal thickness and inflammatory cell infiltration were observed in both TNFα- and IL-17A–deficient mice treated with imiquimod. Immunofluorescence staining revealed the presence of T cells and neutrophils in skin sections from imiquimod-treated wild-type mice. In contrast, these cells were not observed in skin sections from imiquimod-treated IκBζ-deficient mice (Fig. 3 *D* and *E*).

At the molecular level, the expression of selected psoriasis-related transcripts in the skin was analyzed in the different mice strains. IL-17 signature genes including *S100a7a*, *Lcn2*, *S100a9*, and *Defb4*, as well as key psoriasis-associated genes including *Il23a*, *Il17c*, *Il22*, and *Il19*, were all found to be expressed at significantly lower levels in imiquimod-treated IκBζ-deficient mice than in wild-type mice (Fig. 3*F*). Although TNFα and IL-17A both are proven to be effective treatment targets in psoriasis (4, 7), the transcript of all of the genes analyzed was clearly higher in imiquimod-treated TNFα and IL-17A–deficient mice than in imiquimod-treated IκBζ-deficient mice.

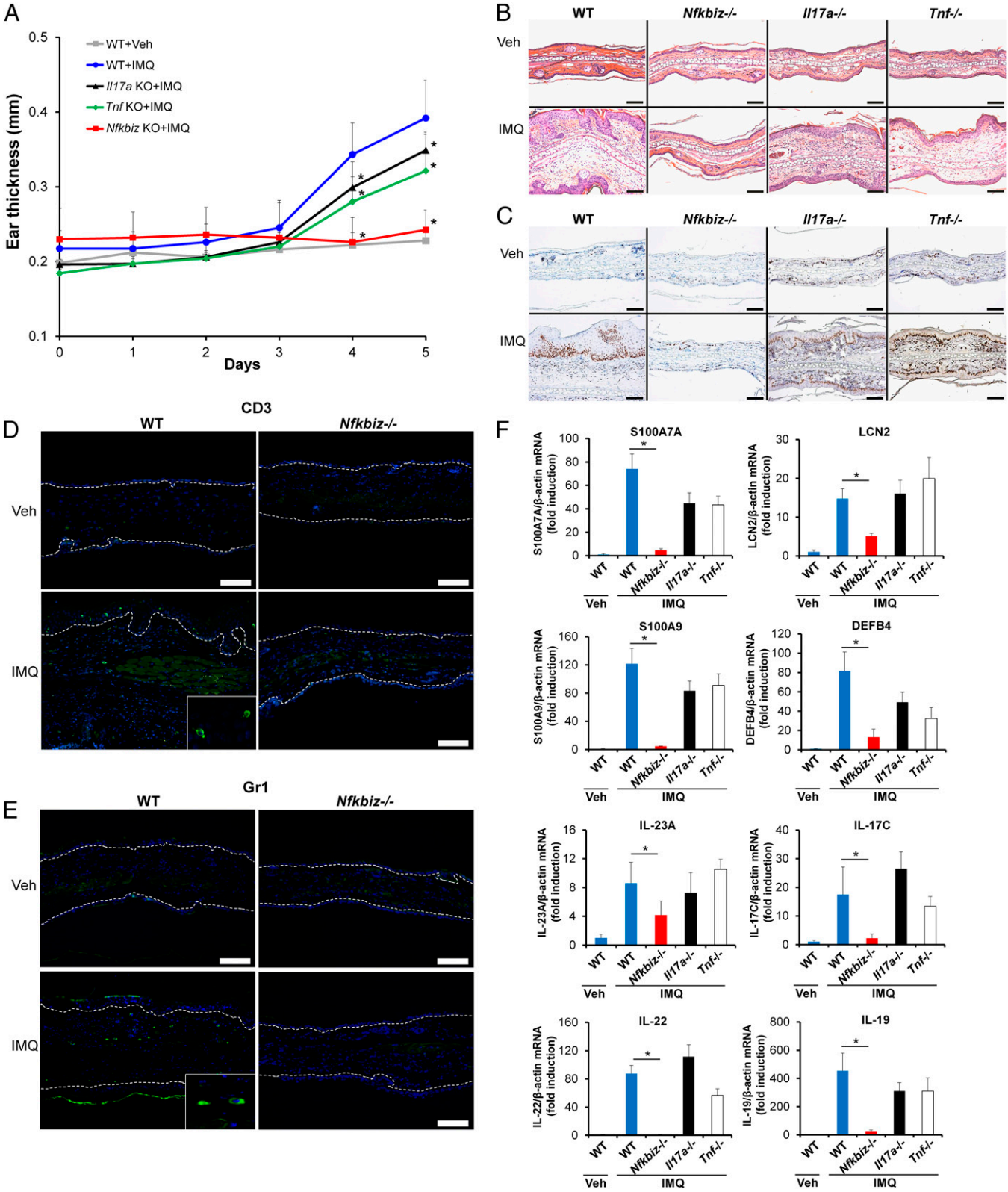

Fig. 3. IκBζ is essential for imiquimod-induced psoriasis-like skin inflammation in mice. (*A*) Ear thickness of wild-type (WT), *Il17a* knockout (KO), *Tnf* KO, and *Nfkbiz* KO mice treated daily with imiquimod (IMQ) or vehicle (Veh) cream. Data points represent the mean of 10 (WT and *Il17a* KO), 7 (*Tnf* KO), and 5 (*Nfkbiz* KO) mice ± SD **P* < 0.01 compared with imiquimod-treated WT mice, one-way repeated measures analysis of variance followed by a Holm–Sidak test. (*B* and *C*) Ear biopsies from WT, *Il17a* KO, *Tnf* KO, and *Nfkbiz* KO mice treated daily with imiquimod or vehicle cream for 5 d and stained with (*B*) H&E or (*C*) for Ki67. (*D* and *E*) Sections of imiquimod-treated ears from WT and *Nfkbiz* KO mice following 5 d of treatment were analyzed for (*D*) T cells (CD3), and (*E*) neutrophils (Gr1) by immunofluorescence staining. (Scale bars, 100 μm.) (*F*) qPCR analyses for indicated cytokines and antimicrobial peptides in ear biopsies from WT and *Nfkbiz* KO mice harvested on day 5 of imiquimod treatment. *β-Actin* was used for normalization. Results are expressed as mean ± SD from 5 mice. **P* < 0.05, Student's *t* test.

IκBζ-Deficient Mice Are Protected from Development of IL-23–Induced Psoriasis. IκBζ was found to be crucial for the development of psoriasis in a model where psoriasis was induced by topical application of the TLR agonist imiquimod on mice ears. To support these data, we next examined the role of IκBζ in another skin inflammatory mouse model where a psoriasis-like skin inflammation was induced by intradermal injection of IL-23 into the mice ears, a model that has been demonstrated to be highly dependent on IL-17A (24). As shown in Fig. 4*A*, IκBζ-deficient mice developed no skin inflammation upon IL-23 treatment as measured by ear thickness, and their response was comparable to that of vehicle-treated mice (Fig. 4*A*). In contrast, ear thickness was significantly augmented in IL-23–injected wild-type mice compared with vehicle-treated mice. These data were supported by histological staining of the ears, which showed no epidermal thickening or inflammatory cell infiltration in IL-23–injected IκBζ-deficient mice compared with wild-type mice (Fig. 4*B*). Furthermore, Ki67 staining demonstrated less keratinocyte proliferation in IL-23–treated IκBζ-deficient mice than in wild-type mice treated with IL-23 (Fig. 4*C*). Likewise, the expression of the inflammatory markers *Defb4*, *Lcn2*, *S100a9*, and *Il17c* was significantly lower in IκBζ-deficient mice than in wild-type mice, which reflects their overall lower disease state (Fig. 4*D*). These data support the important role of IκBζ in the development of psoriasis-like skin inflammation.

Local Abrogation of IκBζ in the Skin Inhibits Imiquimod-Induced Psoriasis. Because IκBζ was demonstrated to be vital for the development of psoriasis-like skin inflammation, we next investigated whether local abrogation of IκBζ function in the skin affected disease development in the imiquimod-induced psoriasis-like skin inflammation model. IκBζ-specific siRNAs or control siRNAs were injected intradermally into the dorsal skin of the mice 1 d before the first application of imiquimod. Macroscopically, local knockdown of IκBζ in the skin clearly reduced the imiquimod-induced inflammation, showing no redness, no infiltration, and less desquamation in the area injected with IκBζ-specific siRNA compared with control siRNA-injected skin (Fig. 5*A*). In addition, mRNA expression of the inflammatory markers *Il17c*, *Il19*, and *Il22* was significantly lower in IκBζ siRNA-treated skin than in control siRNA-treated skin (Fig. 5*B*). In addition, H&E staining of skin sections from the mice revealed that knockdown of IκBζ by siRNA markedly reduced imiquimod-induced epidermal thickening and dermal cell infiltration (Fig. 5*C*). Likewise, immunofluorescence analysis showed infiltration of T cells in skin sections from imiquimod-treated mice injected with control siRNA, whereas these cells were not observed in skin sections from imiquimod-treated mice injected with IκBζ-specific siRNA (Fig. 5*D*). Together, these data demonstrate that IκBζ is strictly required for the imiquimod-induced psoriasis-like skin inflammation to develop and identify IκBζ as a potential therapeutic target in psoriasis.

Characterization of IL-17A–Induced IκBζ Expression. Knowing that IκBζ plays a key role both in vitro and in vivo in the regulation of specific psoriasis-associated genes, we next studied the underlying molecular mechanism. Some of the effects of IL-17A on gene expression have been demonstrated to be mediated through mRNA stabilization. In addition, IL-17A–induced *NFKBIZ* expression has been reported to be controlled at the level of mRNA stability in different cell types (16, 25). To characterize the IL-17A–mediated induction of *NFKBIZ* mRNA in human keratinocytes, we first stimulated cells with IL-1β for 1 h to increase the transcript of *NFKBIZ* (Fig. S4). Then, actinomycin D was added to the cells for 1 h before stimulation with vehicle or IL-17A. As shown in Fig. 6*A*, the decay in *NFKBIZ* mRNA was similar in IL-17A–treated and vehicle-treated cells. This demonstrates that in human keratinocytes, IL-17A stimulation does not increase the stability of *NFKBIZ* mRNA (Fig. 6*A*). To exclude the possibility that IL-17A–induced proteins secondarily regulate *NFKBIZ* mRNA, we repeated this experiment using IL-17A for both initial stimulation and restimulation. However, the stability of *NFKBIZ* mRNA remained unaltered between IL-17A treatment and vehicle treatment (Fig. 6*B*). As control, human keratinocytes were incubated with actinomycin D for 1 h before stimulation with IL-17A for 1.5 h (Fig. 6*C*). In this setup, preincubation with actinomycin D before treatment with IL-17A down-regulated *NFKBIZ* mRNA levels to 0.40-fold compared

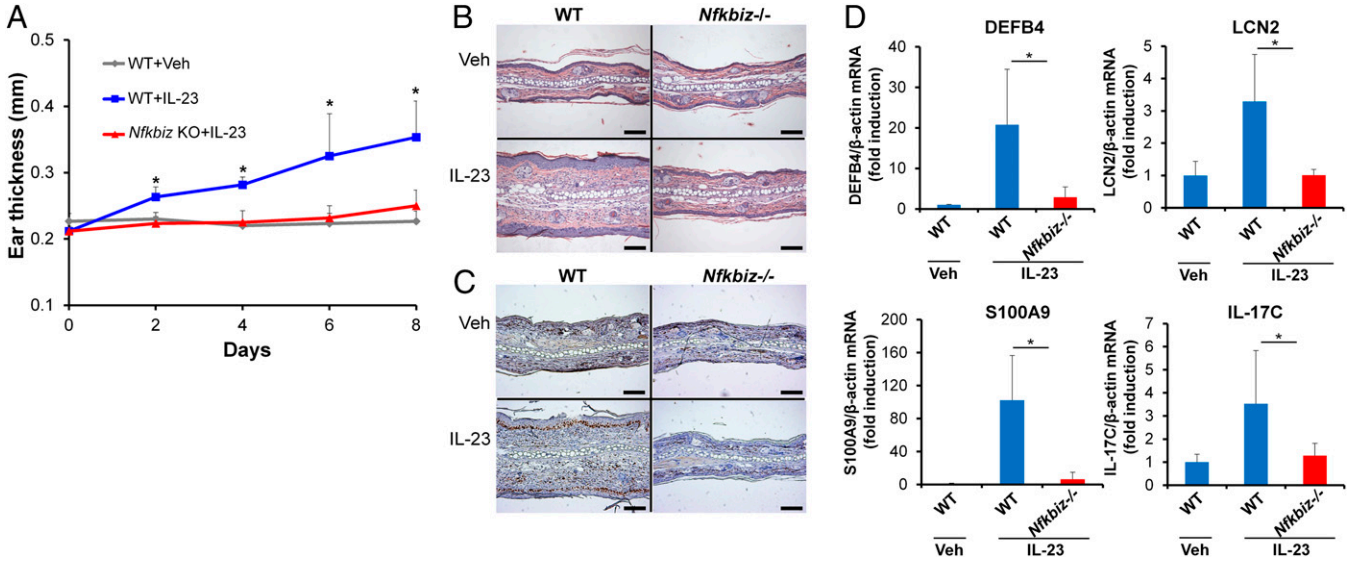

Fig. 4. IL-23-induced psoriasis in mice is dependent on IκBζ. (*A*) Ear thickness of WT and *Nfkbiz* KO mice injected intradermally with IL-23 or vehicle (Veh) every other day for 8 d. Data points represent the mean of six mice ± SD **P* < 0.01 compared with IL-23–treated *Nfkbiz* KO mice, one-way repeated measures analysis of variance followed by a Holm–Sidak test. (*B* and *C*) Sections of ears recovered from IL-23–treated WT and *Nfkbiz* KO mice were stained with (*B*) H&E or (*C*) for Ki67. (Scale bars, 100 μm.) (*D*) mRNA expression of *Defb4*, *Lcn2*, *S100a9*, and *Il17c* was analyzed by qPCR in ear biopsies from IL-23–treated WT and *Nfkbiz* KO mice. *β-Actin* was used for normalization. Results are expressed as mean ± SD from five mice. **P* < 0.05, Student's *t* test.

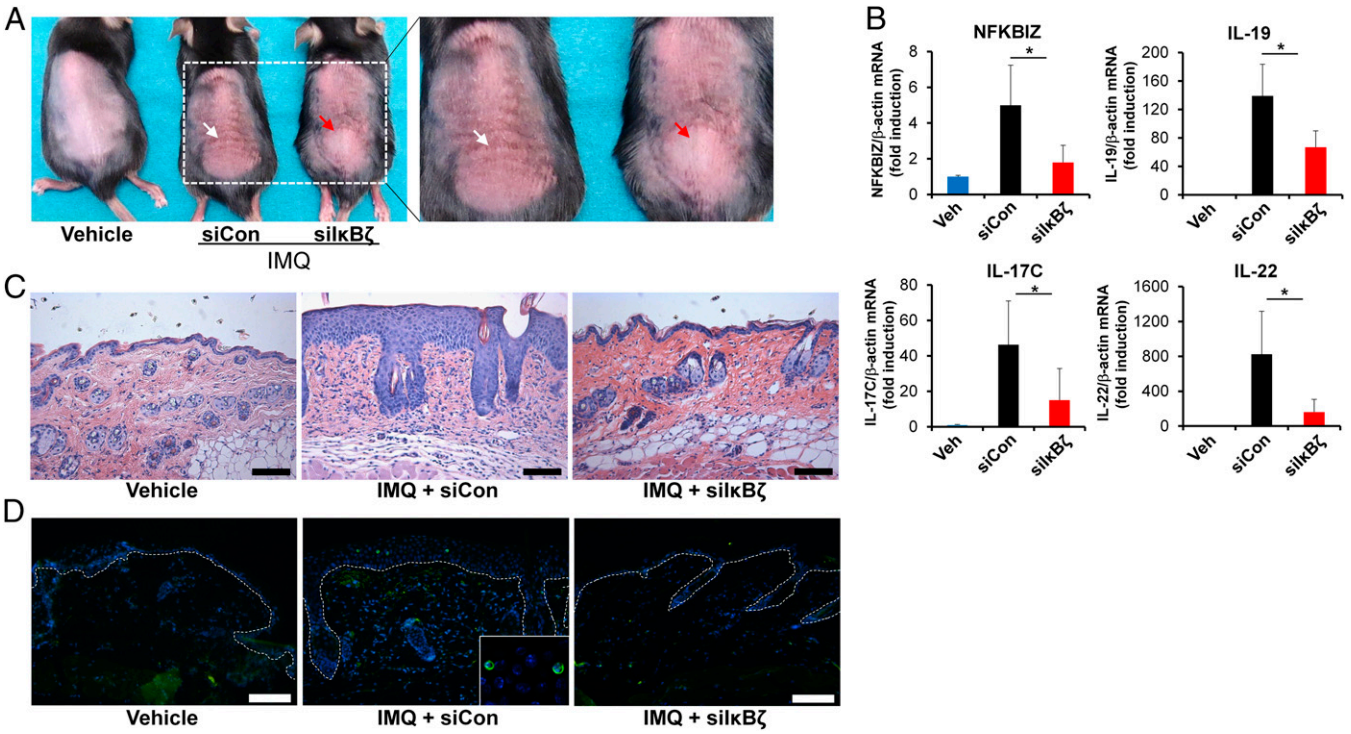

Fig. 5. Local inhibition of IκBζ by siRNA reduces imiquimod-induced psoriasis-like skin inflammation. (*A*) Mice were treated intradermally with IκBζ siRNA (siIκBζ) or nonspecific control siRNA (siCon) 1 d before start of imiquimod (IMQ) or vehicle treatment daily for 3 d. Red and white arrows indicate IκBζ siRNA and nonspecific control siRNA-treated areas, respectively. (*B–D*) Mice were treated as in *A*. At day 3, skin biopsies were taken from vehicle-treated and imiquimod-treated mice within the control siRNA-treated and IκBζ siRNA-treated area. (*B*) mRNA expression of the indicated proteins was analyzed by qPCR and is expressed as the mean ± SD from five mice. **P* < 0.05, Student's *t* test. *β-Actin* was used for normalization. (*C*) H&E staining of skin section from the mice. (*D*) Skin section from the mice was analyzed for T cells (CD3) by immunofluorescence staining. (Scale bars, 100 μm.)

with 20.4-fold in IL-17A–stimulated cells not preincubated with actinomycin D. Thus, as transcription was blocked before IL-17A stimulation, *NFKBIZ* mRNA rapidly decayed, reflecting a short half-life of *NFKBIZ* mRNA in cultured human keratinocytes.

In vitro, we have demonstrated that IL-17A is a key inducer of IκBζ. To further substantiate these findings in vivo, tissue samples from imiquimod-treated IL-17A– and TNFα-deficient mice were analyzed. *Nfkbiz* mRNA expression was significantly reduced in both imiquimod-treated IL-17A– and TNFα-deficient mice compared with wild-type mice, and no significant difference in *Nfkbiz* mRNA expression between IL-17A– and TNFα-deficient mice was observed (Fig. S5). These data indicate that mechanisms other than the IL-17A pathway are also involved in the regulation of IκBζ. Moreover, the expression level of *Nfkbiz* seen in imiquimod-treated IL-17A– and TNFα-deficient mice reflects the degree of inflammation seen on ear thickness, and it supports the important role of IκBζ in psoriasis-like skin inflammation.

Discussion

Although several inflammatory factors, including TNFα and IL-17A, are known to play a major role in the pathogenesis of psoriasis (26, 27), our understanding of the underlying molecular

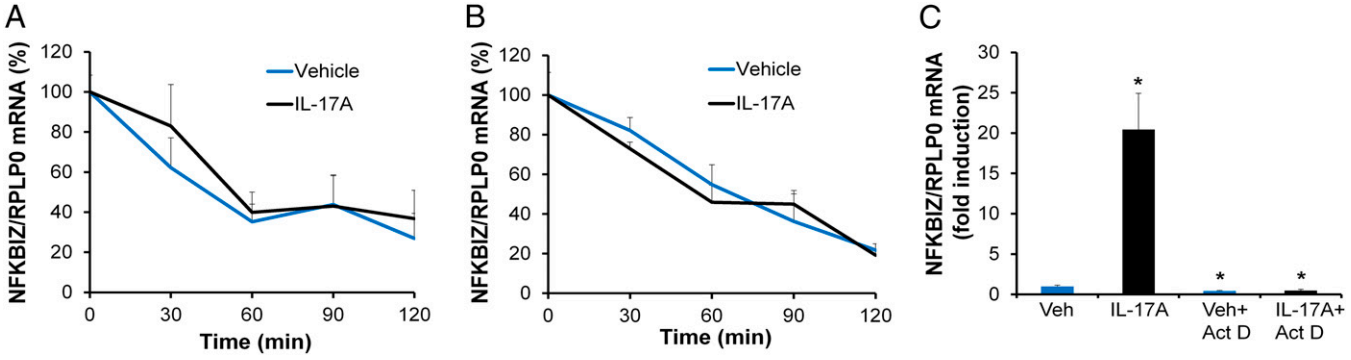

Fig. 6. Characterization of IL-17A–induced IκBζ expression. (*A* and *B*) Cultured human keratinocytes were prestimulated with (*A*) IL-1β for 1 h or (*B*) IL-17A for 6 h before actinomycin D was added for 1 h. Then, cells were stimulated with IL-17A and harvested at 30-min intervals. *NFKBIZ* mRNA expression was analyzed by qPCR. *RPLP0* expression was used for normalization. Points indicate mean ± SD ($n = 3$). (*C*) Human keratinocytes were preincubated with or without actinomycin D for 1 h before stimulation with vehicle or IL-17A for another 1.5 h. qPCR was used to determine the mRNA expression of *NFKBIZ*. *RPLP0* expression was used for normalization. Results are expressed as mean ± SD ($n = 3$). **P* < 0.05 compared with vehicle control, Student's *t* test.

mechanisms remains limited. Intracellular signaling pathways and their role in psoriasis have recently attracted much interest, and signaling pathways such as the NF-κB, JAK/STAT, and p38 MAPK pathway have been demonstrated to be altered in psoriatic skin (11, 28–30). Here, we identify for the first time to our knowledge IκBζ as a key regulator in the development of psoriasis and as an important transcriptional coactivator mediating downstream effects of IL-17A.

IκBζ has previously been demonstrated to be highly expressed in Th17 cells, dendritic cells, and macrophages upon TLR stimulation, whereas IκBζ is barely detectable in resting cells (18, 31). In agreement with a previous study (10), we found that IκBζ was highly expressed in human keratinocytes upon IL-17A stimulation, whereas TNFα stimulation had only a minor effect on IκBζ expression. Interestingly, we identified IκBζ as a key regulator of a number of psoriasis-associated genes, including IL-17A downstream genes such as *DEFB4*, *CCL20*, *S100A7*, and *LCN2*, indicating that IκBζ is a transcriptional regulator of IL-17A–driven effects. Moreover, our ChIP analyses revealed that IκBζ was recruited to the promoter region of *DEFB4*, *CCL20*, *IL-17C*, and *LCN2*, which was associated with increased histone H3K4 trimethylation, indicating that IκBζ directly regulates transcription of these genes.

Because IκBζ was found to be critical for gene transcription of several cytokines and antimicrobial peptides playing pathogenic roles in psoriasis, we explored the role of IκBζ in a psoriasis-like skin inflammation model in mice induced by the TLR7/8 ligand, imiquimod. Although IκBζ-deficient mice develop periocular inflammation that is restricted to the facial surface and caused by defective tear secretion (14, 32, 33) (Fig. S6), they could be used in this model, as inflammation was never observed on the dorsal skin or on the ears and symptom-free mice were used. Clinically and histologically, IκBζ-deficient mice were completely resistant to imiquimod-induced psoriasis-like skin inflammation. Moreover, expression of IL-17 signature genes such as *S100a7*, *Lcn2*, *S100a9*, and *Defb4* was significantly reduced in IκBζ-deficient mice compared with wild-type mice. This finding demonstrates that IκBζ plays a crucial role in psoriasis development. It should be noted that, as previously reported (32), 80–90% of the IκBζ-deficient mice die in utero due to so far unknown reasons. It can therefore not be fully excluded that the remaining viable mice have additional genetic or epigenetic alterations that might interfere with the development of psoriasis-like skin lesions. However, we consider this possibility unlikely, as intradermal injection of IκBζ-specific siRNA into wild-type mice resulted in a similar absence of psoriasis-like skin inflammation.

The essential role of IκBζ in psoriasis was not restricted to a TLR7/8-induced psoriasis-like skin inflammation, as similar results were obtained using the IL-23–induced psoriasis-like skin inflammation model, which is highly dependent on IL-17A signaling (24). Because the expression of IκBζ was found also to be elevated in lesional skin from patients with psoriasis, these data strongly suggest that IκBζ is a key player in the pathogenesis of psoriasis. In addition to the data obtained in the two mouse models where IκBζ was systemically depleted by gene knockout, we found that local knockdown of IκBζ in the skin clearly reduced imiquimod-induced psoriasis-like skin inflammation as well as the expression of important psoriasis-related genes. Thus, based on these findings, it is tempting to speculate that local inhibition of IκBζ could be a potential future treatment strategy in psoriasis.

IL-22 is a cytokine produced by Th17 and Th22 cells known to play an important role in psoriasis pathogenesis (3, 34, 35). Overexpression of IL-22 results in psoriasis-like skin alterations in mice (36), and IL-22 deficiency protects mice from imiquimod-induced psoriasis-like skin inflammation (37). Here, we show that IL-22 is highly reduced in IκBζ-deficient mice receiving imiquimod, whereas IL-22 expression remains unaltered in IL-17A–deficient mice treated with imiquimod. Because IL-22 is known to act on keratinocytes by stimulating their proliferation (38), these results could explain the lack of keratinocyte proliferation (Ki67 staining) observed in IκBζ-deficient mice after imiquimod treatment, which was not observed in IL-17A–deficient mice.

Emerging evidence suggests that IL-17A plays a crucial role in the pathogenesis of psoriasis. For example, recent clinical trials have demonstrated a remarkable improvement of disease severity when IL-17A– and IL-17RA-targeting therapeutics are used (5, 7, 39–41); and in January 2015, the first drug targeting IL-17A (secukinumab) was approved in the European Union and the United States for psoriasis treatment (8). In the imiquimod model, the expression of IL-17 signature genes such as *S100a7a*, *S100a9*, and *Defb4* was only partially reduced in IL-17A–deficient mice, but highly reduced in IκBζ-deficient mice, also reflecting the inflammatory response seen in these mice as measured by ear thickness. IL-17F is another IL-17 family member produced by Th17 cells (27). IL-17F is known to be elevated in lesional psoriatic skin (27, 42) as well as in mice skin treated with imiquimod (23). Because IL-17F and IL-17A signal through the same receptor complex, namely IL-17RA and IL-17RC, functional redundancy of IL-17F could explain the rather limited reduction in the expression level of these IL-17 signature genes observed in imiquimod-treated IL-17A–deficient mice. Interestingly, we found that the inflammatory phenotype was blocked to a greater extent in IκBζ-deficient mice than in IL-17A– or TNFα-deficient mice. The IL-17A and TNFα pathways are known to be crucial for the induction of psoriasis, which can also been seen by the remarkable efficacy of targeting these cytokines in the treatment of psoriasis (4, 7). Because our data showed that IL-17A and TNFα both are capable of inducing IκBζ (Fig. 1 *B* and *C*) an inhibition of IκBζ blocks both these crucial pathways. However, our results do not exclude that IκBζ is involved in other inflammatory pathways playing a role in psoriasis.

Although IL-17A is known to increase the expression of IκBζ, the molecular mechanisms involved are not fully understood. Previous reports suggest that IL-17A regulates IκBζ expression through stabilization of its mRNA as demonstrated in the two cell lines NIH 3T3 and A549 (16, 25). However, we showed here that in primary human keratinocytes, IL-17A–induced expression of IκBζ was mediated at a transcriptional level and not through mRNA stabilization. Thus, the molecular machinery underlying the regulation of IκBζ could be cell-type specific. The mechanism by which imiquimod induces psoriasis-like skin inflammation is not fully understood, although IL-17 cytokines like IL-17A, IL-17C, and IL-17F are believed to play important roles (23, 43). The adaptor protein Act1 is one of the key components in the IL-17 signaling pathway (44), and recently Act1-mediated signaling was demonstrated to play an essential role in imiquimod-induced psoriasis-like skin inflammation (45). Although IL-17 has been demonstrated to regulate IκBζ through an Act1-dependent mechanism in mice (46, 47) and humans (19), the exact signaling mechanism involved needs still to be determined.

Taken together, this study uncovers a novel crucial regulatory mechanism involved in the development of psoriasis. Moreover, our data suggest that targeting IκBζ, either systemically or topically, may be a possible future strategy in the treatment of psoriasis that could be superior to TNFα and IL-17A antagonists.

Materials and Methods

Biopsies. Four-millimeter punch biopsies were collected from normal, healthy controls. Four-millimeter punch biopsies or keratome biopsies were collected from patients with psoriasis from the center of a chronic plaque and from nonlesional psoriatic skin and were immediately snap frozen in liquid nitrogen. Biopsies from lesional and nonlesional psoriatic skin were taken as paired samples from the same body region.

Mice and Treatments. *Nfkbiz*$^{-/-}$ mice were derived as previously described (32) and wild-type littermates were used as controls. *Il17a*$^{-/-}$ mice were purchased from The Jackson Laboratory. *Tnf*$^{-/-}$ mice were derived as described (48) and backcrossed to the C57BL/6 strain for at least 10 generations. They were viable and fertile and did not display any phenotypic abnormalities. All mice were on a C57BL/6 genetic background and were used at 6–10 wk of age. Both females and males were used in this study. Mice were kept under specific pathogen-free conditions and provided with food and water ad libitum. Mice were treated with a daily dose of 45 mg 5% imiquimod cream (Aldara; 3M Pharmaceuticals) or vehicle cream topically on their shaved back and right ear for 5 d. Ear thickness was assessed daily using a Mitutoyo digimatic indicator. On day 5, ears were collected for histological and qPCR analysis. The back of the mice was scored clinically for psoriatic symptoms such as scaling, erythema, and thickness on a scale from 0 (no alteration) to 4 (very distinct alteration) as previously described (23). Moreover, a cumulative score (scaling plus erythema plus thickening) was calculated (scale 0–12).

PBS (10 μL) or recombinant mIL-23 (0.5 μg per 10 μL; eBioscience) was injected intradermally into the ears of wild-type or *Nfkbiz*$^{-/-}$ mice every other day for 8 d. Ear thickness was measured using a Mitutoyo digimatic indicator. On day 8, ears were collected for histology and qPCR.

Cell Cultures. Normal human keratinocytes were obtained by trypsinization of skin samples from adult patients undergoing plastic surgery as previously described (49). Second-passage keratinocytes were grown in K-SFM (Gibco, Life Technologies) at 37 °C and 5% CO_2. At 24 h before stimulation with TNFα (10 ng/mL) and/or IL-17A (100 ng/mL), the medium was changed to keratinocyte basal medium (KBM, the same as K-SFM, but without growth factors). In some experiments, keratinocytes were pretreated with IL-1β (10 ng/mL) or IL-17A (100 ng/mL) for 1 h, then incubated with actinomycin D (7.5 μg/mL) for 1 h, and subsequently stimulated with IL-17A (100 ng/mL) at different time points.

Western Blotting. Equal protein amounts were separated by SDS/PAGE and blotted onto nitrocellulose membranes. Membranes were incubated with anti-IκBζ (cat. no. 9244; Cell Signaling Technology) or *β-actin* (cat. no. A-1978; Sigma-Aldrich). IκBζ was detected with anti-rabbit IgG-HRP (cat. no. 7074; Cell Signaling Technology) and *β-actin* with anti-mouse IgG-HRP (cat. no. p0447; Dako) in a standard ECL reaction (Amersham Biosciences) according to the manufacturer's instructions.

Statistics. In the time-kinetic experiments statistical analysis was performed using a one-way repeated measures analysis of variance followed by a Holm–Sidak test. Elsewhere, a Student's *t* test was used. A probability test was made to test for normal distribution, and a probability of $P < 0.05$ was regarded as statistically significant.

Study Approval. The study was conducted in compliance with the Declaration of Helsinki, and signed informed consent was obtained from each patient before inclusion in the study. All animal studies were approved by the Danish Animal Experiments Inspectorate (2012-15-2934-00517 and 2014–15-0201-00409). The Regional Ethical Committee of Region Midtjylland, Denmark approved the experiments with patients with psoriasis (M-20090102) and the experiments with cultured human keratinocytes (M-20110027).

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