Cutting Edge: NF-κB p65 and c-Rel Control Epidermal Development and Immune Homeostasis in the Skin

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Psoriasis is an inflammatory skin disease in which activated immune cells and the proinflammatory cytokine TNF are well-known mediators of pathogenesis. The transcription factor NF-κB is a key regulator of TNF production and TNF-induced proinflammatory gene expression, and both the psoriatic transcriptome and genetic susceptibility further implicate NF-κB in psoriasis etiopathology. However, the role of NF-κB in psoriasis remains controversial. We analyzed the function of canonical NF-κB in the epidermis using CRE-mediated deletion of p65 and c-Rel in keratinocytes. In contrast to animals lacking p65 or c-Rel alone, mice lacking both subunits developed severe dermatitis after birth. Consistent with its partial histological similarity to human psoriasis, this condition could be prevented by anti-TNF treatment. Moreover, regulatory T cells in lesional skin played an important role in disease remission. Our results demonstrate that canonical NF-κB in keratinocytes is essential for the maintenance of skin immune homeostasis and is protective against spontaneous dermatitis. The Journal of Immunology, 2015, 194: 2472–2476.

Materials and Methods

Animals

Keratin 14 (K14)ΔκB, RelAΔκB, and c-RelΔκB mice (18–20) were kept in specific pathogen–free conditions in the animal care facility at Columbia University. Experiments were conducted under Institutional Animal Care and Use Committee approval.

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Abbreviations used in this article: DKO, double knockout; K14, keratin 14; LN, lymph node; qRT-PCR, quantitative RT-PCR; Treg, regulatory T cell; WT, wild-type.

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Flow cytometry

Spleen and lymph node (LN) cells were isolated by mechanical desegregation in PBS + 3% FBS. Whole skin was minced, digested for 3 h at 37°C in DMEM (Life Technologies) with 1 mg/ml Collagenase IV and 1 mg/ml DNase I (both from Sigma), and strained. Cell suspensions were stained using the following Abs from eBioscience: TCR-β (H57), CD4 (RM4.5), CD8 (53-6.7), CD19 (Ebio1D3), CD90.2 (53-2.1), CD45 (30F11), CD44 (IM7), NK1.1 (PK136), TCR-γδ (EbioGL3), CD11c (N418), CD11b (M1/70), IL-33R (ST2), CD25 (PC61, 7D4), and Foxp3 (FJK16s). Foxp3 staining was performed using the eBioscience kit. Cells were acquired on a LSR II (BD Biosciences) and analyzed with FlowJo software.

mRNA expression

For quantitative RT-PCR (qRT-PCR), frozen tissues were dissociated using Lysing Matrix D tubes (MP). Total RNA was isolated using TRIzol reagent and reverse transcribed with Superscript III (Life Technologies). qRT-PCR with SYBR Green (Quanta Biosciences) was performed on a CFX96 or 384 (Bio-Rad); all values are relative to GAPDH. Primers sequences are available upon request.

Histology

Ear or skin specimens were fixed with 4% neutral-buffered formalin for 4 d, transferred to 70% ethanol, and embedded in paraffin. Five-micrometer sections were cut, deparaffinized, stained with H&E or TUNEL, imaged using an Axio M2 (Zeiss) microscope, and processed using AxioVision and ImageJ software. Epidermal thickness was measured on ≥15 random fields/specimen; mean thickness is shown.

Results and Discussion

The role of NF-κB in skin biology and pathophysiology remains ambiguous. Although mice lacking p65, cRel, and TNF exhibit defects in epidermal differentiation (21), it is not clear whether this is the result of a keratinocyte-intrinsic requirement for NF-κB. Therefore, we crossed mice carrying either floxed Rela or c-Rel alleles to a transgenic mouse expressing Cre under the control of the K14 promoter. K14\textsuperscript{ΔRela}\textsuperscript{cKO} (K14\textsuperscript{ΔRela}) and K14\textsuperscript{Δc-Rel}\textsuperscript{cKO} (K14\textsuperscript{Δc-Rel}) pups were born at Mendelian ratios and displayed reduced expression of Rela and c-Rel mRNA, respectively, in the epidermis (Supplemental Fig. 1A). Histological analyses showed normal epidermal thickness and keratinocyte differentiation (data not shown), indicating that deletion of Rela or c-Rel did not affect skin development. Next, we used 2,4-dinitrofluorobenzene to induce contact hypersensitivity. K14\textsuperscript{ΔRela} and K14\textsuperscript{Δc-Rel} mice exhibited increased ear swelling and TNF and IFN-γ expression compared with littermate controls (Supplemental Fig. 1C–F). Consistent with a recent study using mice lacking RelA in the epidermis (22), these data...
sugest that RelA and c-Rel have a nonredundant, keratinocyte-intrinsic, immunoregulatory role in skin.

Spontaneous dermatitis in mice lacking RelA and c-Rel in keratinocytes

It was reported previously that RelA has a growth-inhibitory role in keratinocytes (23) and prevents keratinocyte differentiation (24). However, no changes were observed in epidermal differentiation upon keratinocyte-specific deletion of RelA. Therefore, to assess whether they are redundant in epidermal development, we deleted RelA and c-Rel in keratinocytes (double-knockout [DKO] mice), which led to a full ablation of RelA and minimal residual c-Rel in DKO epidermis (Supplemental Fig. 1A, 1B). DKO pups were born at expected Mendelian ratios but exhibited visible skin lesion from day 5, which spread rapidly and covered most of the body by day 12 (Fig. 1A). Early lesions were well-demarcated, scattered, rigid, scaly plaques without edematous or exudative reaction. H&E staining revealed hyperkeratosis and focal parakeratosis, which are features of psoriatic lesions. Epidermal thickening and dermal and epidermal mononuclear infiltrates were observed (Fig. 1B, 1E). DKO mice exhibited apoptotic loci in all layers of the epidermis (Fig. 1F).

No lethality was observed; in >90% of the mice, the skin lesions resolved gradually, and dorsal epidermal thickness returned to normal by day 30 (Fig. 1C–E). Disease recurrence was not observed in any animal; however, the abdominal thickening and dermal and epidermal mononuclear infiltrates were observed (Fig. 1B, 1E). DKO mice exhibited apoptotic loci in all layers of the epidermis (Fig. 1F).

To further explore skin immune homeostasis, we performed flow cytometry on D16 tissues. We observed a 2-fold increase in the proportion of CD45+ leukocytes among total skin cells in DKO mice (Fig. 2A), which correlated with mononuclear cell infiltration (Fig. 1B). TCR-β⁺ T cells were increased in the skin of DKO mice (Fig. 2B). The CD4⁺Foxp3+ Treg proportion and number were dramatically enhanced, accounting for up to 60% of the skin-infiltrating CD4⁺ T cell compartment (Fig. 2C, 2D). We observed a 3–5-fold increase in the numbers of type II innate lymphoid cells, which were implicated in inflammatory responses in the skin (25), in both the LN and skin of DKO mice compared with wild-type (WT) controls (Fig. 2E, 2F). K14 is expressed by medullary thymic epithelial cells (26), which are important for Treg selection and deletion of autoreactive T cells (27). However, no changes were observed in medullary thymic epithelial cells or T cell subsets in the thymus of day-7 DKO mice (Supplemental Fig. 2). Thus, the dermatitis in DKO mice is likely due to a keratinocyte-intrinsic function of NF-κB. Together, these results indicate that canonical NF-κB in keratinocytes is required for their optimal differentiation, as well as maintenance of immune homeostasis in the skin.
TNF contributes to chronic inflammatory diseases, including psoriasis, and anti-TNF treatments are a first-line treatment for moderate to severe psoriasis (28). Therefore, we asked whether TNF mediated the dermatitis of DKO mice. TNF mRNA was increased in the skin of DKO mice 7 d after birth (Fig. 3A). Injection of an anti-TNF mAb prior to the appearance of the symptoms strongly reduced skin lesions, epidermal thickening, leukocyte infiltration, and apoptosis compared with vehicle treatment (Fig. 3B–D, data not shown). These data definitively establish that TNF can drive psoriasis-like dermatitis independent of activation of the canonical NF-kB pathway in keratinocytes.

**Tregs play a protective role in the remission of dermatitis**

In contrast to K14 creIKK2fl/fl mice (10, 13), DKO mice spontaneously begin to recover 3 wk after birth. This led us to explore the mechanism of “remission.” Because we observed a massive Treg expansion in DKO skin prior to remission, we tested whether Tregs contributed to remission by injecting an anti-CD25 Ab. This protocol achieved a 50% reduction in Foxp3+ T cells 2 d after treatment (Fig. 4A). Treg-depleted DKO mice exhibited worsened pathology, with increased skin immune infiltrate (Fig. 4B–D). These results suggest that Tregs were necessary for disease recovery and highlight the role of Tregs in skin immune homeostasis.

It was proposed that the pathogenesis of psoriasis may follow a two-step model. First, environmental and/or genetic factors drive keratinocyte dysfunction and production of chemokines or inflammatory cytokines; in turn, activation of immune cells, such as dendritic cells and macrophages, may trigger a strong T cell–dependent inflammatory response, leading to increased proliferation of epidermal cells and clinical symptoms. In this article, we show that perturbation of the canonical NF-kB in the epidermis can trigger cell death, immune infiltration, and hyperkeratosis. These data indicate that NF-kB supports skin immune homeostasis and may prevent uncontrolled TNF-dependent leukocyte recruitment and activation.

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**Disclosures**

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**References**


