Immunity
Article

Crosstalk between Keratinocytes and Adaptive Immune Cells in an IκBα Protein-Mediated Inflammatory Disease of the Skin

Bernd Rebholz,[1] Ingo Haase,[2,*] Birgit Eckelt,[1] Stephan Paxian,[3] Michael J. Flaig,[1] Kamran Ghoreschi,[4] Sergei A. Nedospasov,[5] Reinhard Mailhammer,[6] Svenja Debey-Pascher,[7] Joachim L. Schultze,[7] Günther Weindl,[4] Irmgard Förster,[8] Ralf Huss,[9] Athanasios Stratis,[2] Thomas Ruzicka,[1] Martin Röcken,[4] Klaus Pfeffer,[10] Roland M. Schmid,[3] and Rudolf A. Rupec[1,*]

[1]Department of Dermatology and Allergology, Ludwig-Maximilians-University Munich, Frauenlobstrasse 9-11, D-80337 Munich, Germany
[2]Department of Dermatology and Centre for Molecular Medicine, University of Cologne, Joseph-Stelzmann-Strasse 9, D-50924 Cologne, Germany
[3]Department of Internal Medicine II, Technical University of Munich, Ismaningerstrasse 22, D-81675 Munich, Germany
[4]Department of Dermatology, Eberhard Karls University Tübingen, Liebermeisterstr. 25, D-72076 Tübingen, Germany
[5]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilova Street 32, 119991 Moscow, Russia
[6]GSF-National Research Centre for Environment and Health, Institute of Clinical Molecular Biology and Tumour Genetics, Marchioninistr. 25, D-81377 Munich, Germany
[7]Molecular Tumor Biology and Tumor Immunology, Department of Internal Medicine I, University of Cologne, Kerpener Str. 52, D-50924, Cologne, Germany
[8]Institut für Umweltmedizinische Forschung at the Heinrich-Heine-University Düsseldorf gGmbH, Auf'm Hennekamp 50, D-40225 Düsseldorf, Germany
[9]Institute of Pathology, Ludwig-Maximilians-University Munich, Thalkirchnerstrasse 36, D-80337 Munich, Germany
[10]Institute of Medical Microbiology, Heinrich-Heine-University Düsseldorf, Universitätsstraße 1, D-40225 Düsseldorf, Germany
*Correspondence: ingo.haase@uni-koeln.de (I.H.), rudolf.rupec@med.uni-muenchen.de (R.A.R.)
DOI 10.1016/j.immuni.2007.05.024

SUMMARY

Inflammatory diseases at epithelial borders develop from aberrant interactions between resident cells of the tissue and invading immunocytes. Here, we unraveled basic functions of epithelial cells and immune cells and the sequence of their interactions in an inflammatory skin disease. Ubiquitous deficiency of the IκBα protein (*Ikba*$^{\Delta/\Delta}$) as well as concomitant deletion of *Ikba* specifically in keratinocytes and T cells (*Ikba*$^{K5\Delta/K5\Delta\ lck\Delta/lck\Delta}$) resulted in an inflammatory skin phenotype that involved the epithelial compartment and depended on the presence of lymphocytes as well as tumor necrosis factor and lymphotoxin signaling. In contrast, mice with selective ablation of *Ikba* in keratinocytes or lymphocytes showed inflammation limited to the dermal compartment or a normal skin phenotype, respectively. Targeted deletion of RelA from epidermal keratinocytes completely rescued the inflammatory skin phenotype of *Ikba*$^{\Delta/\Delta}$ mice. This finding emphasizes the important role of aberrant NF-κB activation in both keratinocytes and lymphocytes in the development of the observed inflammatory skin changes.

INTRODUCTION

The pathogenesis of inflammatory diseases at the epithelial border such as psoriasis and inflammatory bowel disease is still unexplained. In psoriasis pathogenesis, there is an intensive discussion concerning the functional role of the epithelium and inflammatory cells. Recent evidence indicates that $CD4^+$ T cells are essential for the initiation and maintenance of the disease and that cross-primed $CD8^+$ T cells respond to antigens derived by keratinocytes (Gudjonsson et al., 2004). In addition, overlapping susceptibility loci associated with autoimmunity define psoriasis as a putative autoimmune disease (Bowcock and Krueger, 2005). Yet, data from transgenic mice, such as the rat insulin promoter-glycoprotein (RIP-GP) mice (Lang et al., 2005), suggest that selective activation of immune cells alone is not sufficient for initiation of an autoimmune disease. These findings can be combined in a model, in which development of an autoimmune disease requires simultaneous aberrant signaling in the immune system and the target organ. Psoriasis is a disease of the skin with a prevalence of approximately 2% in Caucasian populations. Phenotyping of skin-infiltrating cells in psoriasis demonstrates the prevalence of Th1 cells (Ghoreschi et al., 2003). Data from adoptive T cell-transfer experiments (Wrone-Smith and Nickoloff, 1996) demonstrate that injection of autologous blood-derived immunocytes, especially $CD4^+$ T cells, into nonlesional human skin transplanted onto severe combined immunodeficient

mice results in the development of psoriasis in the xenograft. Il-4 therapy of patients with psoriasis improved the disease and skewed the Th1 response into an anti-inflammatory Th2 response (Ghoreschi et al., 2003) suggesting that aberrant activation of adaptive immunity is critical for the development of psoriasis. In contrast, the high therapeutic efficacy of topical treatment with anthralin (Lawrence and Shuster, 1992), which is not known to affect T cell function, as well as genetic data (Bowcock, 2005), which do not necessarily link psoriasis-associated gene regions to "immune loci," strongly suggests that psoriasis also relies on abnormal signaling events in keratinocytes showing a hyperproliferative capacity. The data available at present can be embraced in a model in which psoriatic inflammatory skin disease depends on simultaneous abnormal signaling in both compartments, the keratinocytes and the immune cells.

The transcription factor NF-κB is a complex formed by homodimerization and heterodimerization of the NF-κB family members p50 (NF-κB1), p52 (NF-κB2), RelA (p65), RelB, and c-Rel. Stimuli that are capable of activating IκB kinase (IKK) result in serine phosphorylation, ubiquitination, and subsequent degradation of IκBα, which allows translocation of NF-κB to the nucleus (Hayden and Ghosh, 2004; Karin and Ben-Neriah, 2000; Li and Verma, 2002). In addition to degradation of IκBα, phosphorylation of the transactivating subunit RelA is an essential regulatory step for transcriptional activity (Viatour et al., 2005). NF-κB, most often composed of p50 and RelA, is a crucial mediator of inflammatory processes (Karin and Greten, 2005). To determine whether NF-κB overactivation might be relevant for the induction of an inflammatory skin disease, we generated mice with conditional disruption of its cytoplasmic receptor, *Ikba*. We found that mice with ubiquitous deletion of *Ikba* developed a severe inflammatory skin phenotype that involved the epithelial compartment. Neither keratinocyte- nor T cell-specific deletion of *Ikba* recapitulated this phenotype. Exclusively concomitant deletion of *Ikba* in keratinocytes and T cells resulted in development of the inflammatory phenotype observed in mice with ubiquitous deletion. Finally, this phenotype could be rescued by additional keratinocyte-specific deletion of *Rela*, demonstrating the importance of the NF-κB pathway in keratinocyte-T cell crosstalk in skin inflammation.

RESULTS

Aberrant Keratinization, Microabscesses, and Infiltrating Immune Cells in the Skin of *Ikba*$^{\Delta/\Delta}$ Mice

To study the consequences of ubiquitous activation of NF-κB for skin homeostasis, we crossed mice carrying a LoxP-site-containing (floxed) *Ikba* allele with Deleter-Cre mice (Schwenk et al., 1995). We used this loss-of-function approach because it mimics the natural events of NF-κB activation observed after natural stimulation more closely than transgenic overexpression of NF-κB. Importantly, transgenic overexpression does not allow mimicking of the quantitative aspects of NF-κB activation and, in addition, may produce misleading results because of gene-insertion artifacts. *Ikba*$^{\Delta/\Delta}$ mice were born with Mendelian frequencies, and the *Ikba* allele was properly and ubiquitously deleted (*Ikba*$^{\Delta/\Delta}$) (Rupec et al., 2005). Skin from newborn *Ikba*$^{\Delta/\Delta}$ mice was inconspicuous compared to skin from wild-type mice (Figure S1 in the Supplemental Data available online). Three days after birth, 100% of mutant mice started to develop generalized skin inflammation, characterized by erythematous thickening and scaling. The severity of the disease increased until day 7 or 8 after birth when *Ikba*$^{\Delta/\Delta}$ mice died. Histological analysis of skin samples from 6-day-old *Ikba*$^{\Delta/\Delta}$ mice revealed epidermal hyperplasia (acanthosis), a thickened cornified layer (hyperkeratosis), foci of nucleus-containing keratinocytes (parakeratosis), loss of the granular layer (hypogranulosis), T cell infiltration, and the formation of microabscesses, all hallmarks of the epidermal changes that characterize psoriasis. Dermal blood vessels were dilated, and infiltrating mononuclear and polymorphonuclear cells accumulated in the upper dermis (Figure 1A). Increased Ki67 expression indicated a large number of proliferating keratinocytes in comparison with mice bearing floxed *Ikba* alleles (*Ikba*$^{f/f}$ mice; Figure 1B). Immunohistochemistry with markers of keratinocyte proliferation and differentiation revealed increased expression of keratin 6 (K6) in the entire epidermis, reduction of loricrin, and broadening of the keratin 5 (K5) and keratin 10 (K10) expressing keratinocyte layers in the epidermal compartment of *Ikba*$^{\Delta/\Delta}$ mice (Figure 1C). Assessment of skin permeability with the toluidine blue and acidic X-Gal staining showed no increase in dye penetration in *Ikba*$^{\Delta/\Delta}$ mice, suggesting no disturbance of the "outside-in" epidermal barrier (Figure S2). All these findings seem to be a direct consequence of *Ikba* deletion in *Ikba*$^{\Delta/\Delta}$ mice because RelA and p50 containing NF-κB dimers were constitutively active in primary keratinocytes (Figures 1D and 1E). In addition, CD54, a typical target gene induced by NF-κB activation, was strongly expressed in the epidermis of *Ikba*$^{\Delta/\Delta}$ mice (Figure 1F). Other NF-κB regulated genes that were induced in the IκBα-deficient epidermis are *Cxcl2*, *Ccl20*, *Il1b*, and *Mmp9*, *Ccl20*, *Ccl5*, and *Il1b* in the IκBα-deficient dermis (Figure S3). Importantly, aberrant NF-κB activation did not result in altered in vitro proliferation of keratinocytes, indicating that the hyperproliferation observed in vivo was not cell autonomous in IκBα-deficient keratinocytes (Figure 1G).

Epidermis-Specific *Ikba* Deletion Causes Selective Keratinocyte Proliferation

To analyze whether epidermis-specific activation of NF-κB is sufficient for induction of the psoriasis-like skin disease, we crossed the *Ikba*$^{f/f}$ mice with keratin5-*cre*2 mice to generate mice with epidermis-specific deletion of *Ikba* (*Ikba*$^{K5\Delta/K5\Delta}$) (Figure S4). *Ikba*$^{K5\Delta/K5\Delta}$ pups were born at the expected Mendelian ratios and were macroscopically indistinguishable from wild-type littermates until day 3. Similar to *Ikba*$^{\Delta/\Delta}$ mice, *Ikba*$^{K5\Delta/K5\Delta}$ mice were growth retarded. On day 3, 100% of *Ikba*$^{K5\Delta/K5\Delta}$ mice developed a skin disease that predominantly improved by week 3.

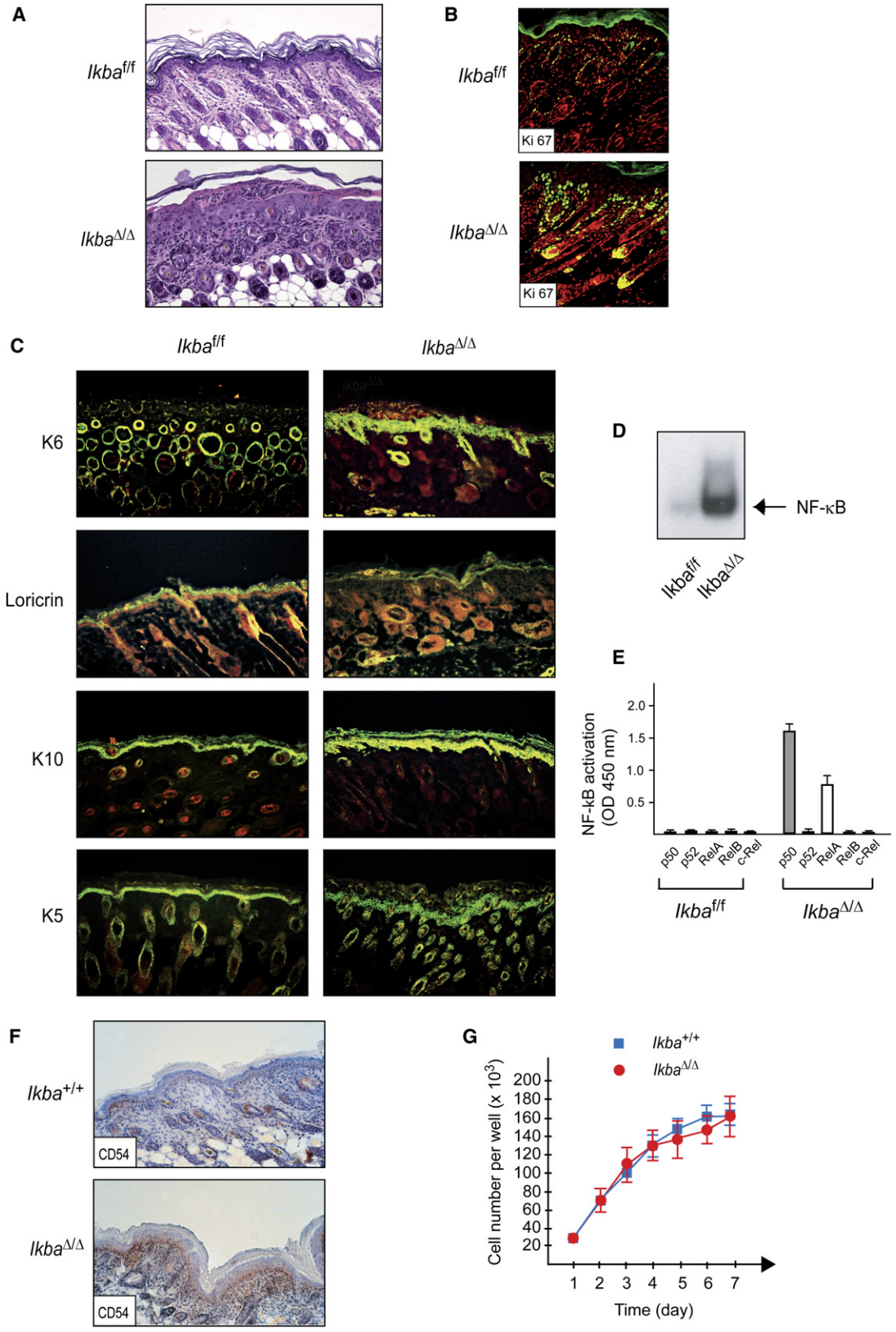


Figure 1. Inflammatory Skin Phenotype of *Ikba*$^{\Delta/\Delta}$ Mice

(A) Skin sections from 6-day-old *Ikba*$^{\Delta/\Delta}$ mice and *Ikba*$^{f/f}$ mice were stained with hematoxylin and eosin (×20).

(B) Analysis of cell proliferation by staining skin sections from 6-day-old *Ikba*$^{\Delta/\Delta}$ mice and *Ikba*$^{f/f}$ mice for Ki67 (green); red staining shows nuclei.

Histopathology of day-6 skin sections revealed a picture different from that of *Ikba*$^{\Delta/\Delta}$ mice. As expected, the epidermis of *Ikba*$^{K5\Delta/K5\Delta}$ mice was thickened with acanthosis, hyperkeratosis, and enhanced Ki67 expression (Figures 2A and 2B). Yet, in sharp contrast to *Ikba*$^{\Delta/\Delta}$ mice, *Ikba*$^{K5\Delta/K5\Delta}$ mice had an intact granular layer, no parakeratosis, and no epidermal infiltration of immune cells, and also had no microabscesses (Figure 2A). Constitutive activation of NF-κB in *Ikba*$^{\Delta/\Delta}$ and *Ikba*$^{K5\Delta/K5\Delta}$ mice was also demonstrable by phosphorylation of the transactivating subunit RelA at Ser536 (Figure 2C). Integrin β1 was strongly upregulated in the skin of *Ikba*$^{\Delta/\Delta}$ mice compared to *Ikba*$^{K5\Delta/K5\Delta}$ and wild-type mice (Figure 2D). Importantly, the differentiation marker K6 was absent from the interfollicular epidermis of *Ikba*$^{K5\Delta/K5\Delta}$ mice (Figure 2E). Because the K6 promoter contains elements responsive to IL-1β (Komine et al., 2001), which plays a putative role in the pathogenesis of psoriasis, we analyzed IL-1β expression by real-time PCR. Although the keratinocyte-derived chemokines macrophage inflammatory protein-2 (MIP-2) and the keratinocyte-derived chemokine (KC) were similarly expressed in both *Ikba*$^{\Delta/\Delta}$ and *Ikba*$^{K5\Delta/K5\Delta}$ mice, the K6-inducing cytokine IL-1β as well as IFN-γ, a cytokine putatively involved in the pathogenesis of psoriasis (Austin et al., 1999; Nestle et al., 1994; Robert and Kupper, 1999), were strongly induced in the dermis of *Ikba*$^{\Delta/\Delta}$ mice but at background amounts in *Ikba*$^{K5\Delta/K5\Delta}$ mice (Figures 2F and 2G). We found no significant difference in the expression of IL-1α and TNF when we compared *Ikba*$^{\Delta/\Delta}$ and *Ikba*$^{K5\Delta/K5\Delta}$ mice (data not shown). In addition, targeted deletion of TNF in an IκBα-deficient background revealed no normalization of K6 expression. This implicates that TNF is not involved in K6 regulation (Figure S10). These results demonstrate that selective deletion of *Ikba* in keratinocytes can induce abnormal proliferation of keratinocytes in vivo without inducing epidermal inflammation and contrasts with the *Ikba*$^{\Delta/\Delta}$ mice where granulocytes and T cells were present in the epidermis.

Epidermal Involvement in Cutaneous Inflammation Requires IκBα-Deficiency in Keratinocytes and T Cells

Immunostaining of immune cells in skin from *Ikba*$^{\Delta/\Delta}$ mice and *Ikba*$^{K5\Delta/K5\Delta}$ mice demonstrated increased numbers of CD4$^+$ and CD8$^+$ T cells, Gr-1$^+$ neutrophils, and macrophages in the dermal compartment of both *Ikba*$^{\Delta/\Delta}$ and *Ikba*$^{K5\Delta/K5\Delta}$ mice (Figure 3A). Comparing *Ikba*$^{\Delta/\Delta}$ to *Ikba*$^{K5\Delta/K5\Delta}$ mice clearly revealed that immune cells did not infiltrate the epidermis and remained restricted to the dermal compartment in *Ikba*$^{K5\Delta/K5\Delta}$ mice, where CD8$^+$ T cells and Gr-1$^+$ neutrophils infiltrated the epidermis of *Ikba*$^{\Delta/\Delta}$ mice. CD4$^+$ cells infiltrated the skin of *Ikba*$^{\Delta/\Delta}$ mice with an average of 57/1.6 μm^2 and infiltrated the skin of *Ikba*$^{K5\Delta/K5\Delta}$ mice with an average of 97/1.6 μm^2. CD8$^+$ cells infiltrated the skin of wild-type mice with an average of 7/1.6 μm^2, the skin of *Ikba*$^{\Delta/\Delta}$ mice with an average of 122/1.6 μm^2, and the skin of *Ikba*$^{K5\Delta/K5\Delta}$ mice with an average of 160/1.6 μm^2. F4/80 positive cells infiltrated the skin of wild-type mice with an average of 29/1.6 μm^2, the skin of *Ikba*$^{\Delta/\Delta}$ mice with an average of 75/1.6 μm^2, and the skin of *Ikba*$^{K5\Delta/K5\Delta}$ mice with an average of 75/1.6 μm^2. Gr-1-positive cells infiltrated the skin of *Ikba*$^{\Delta/\Delta}$ mice with an average of 47/1.6 μm^2 and the skin of *Ikba*$^{K5\Delta/K5\Delta}$ mice with an average of 35/1.6 μm^2. Because cytokines released within the dermis strongly affect proliferation and differentiation of keratinocytes within the epidermal compartment, we crossed *Ikba*$^{\Delta/\Delta}$ mice with recombination-activating gene 2 (Rag2)-deficient mice (*Rag2*$^{-/-}$ mice). *Ikba*$^{\Delta/\Delta}$ *Rag2*$^{-/-}$ mice remained macroscopically and histopathologically normal (Figure 3B and Figure S5; data not shown), showing that activated T and B cells are required for the enhanced keratinocyte proliferation observed in *Ikba*$^{\Delta/\Delta}$ and *Ikba*$^{K5\Delta/K5\Delta}$ mice. To investigate whether lymphocytes are also necessary for the maintenance of the disease, we grafted skin from 6-day-old *Ikba*$^{\Delta/\Delta}$, *Ikba*$^{K5\Delta/K5\Delta}$, or control mice onto *Rag2*$^{-/-}$ mice. Four weeks later, grafts were analyzed microscopically. No difference was detected among *Ikba*$^{\Delta/\Delta}$, *Ikba*$^{K5\Delta/K5\Delta}$, and control grafts, suggesting that IκBα-deficient T and B cells were essential for disease maintenance (Figure 3C).

Because skin from *Ikba*$^{\Delta/\Delta}$ mice remained healthy when transplanted onto *Rag2*$^{-/-}$ mice, we next crossed *Ikba*$^{f/f}$ mice with lck-*cre* mice to generate *Ikba*$^{lck\Delta/lck\Delta}$ mice (Hennet et al., 1995). This resulted in complete deletion of *Ikba* in T cells only (Figure S6). Skin of 6-day-old *Ikba*$^{lck\Delta/lck\Delta}$ mice was normal and did not reveal any difference as compared to that of control (*Ikba*$^{f/f}$) mice (Figure 3D; Figures S7 and S8). Similarly, *Ikba*$^{my\Delta/my\Delta}$ mice with IκBα deletion that is in neutrophils and macrophages and that results from crossing of *Ikba*$^{\Delta/\Delta}$ with LysM-*cre* mice (Clausen et al., 1999), and IκBα-deficient fetal liver cell chimeras (*Ikba*$^{FL\Delta/FL\Delta}$) (Rupec et al., 2005), had a macroscopically and histologically normal skin phenotype (data not shown). We found no alterations in the expression of epidermal differentiation markers in *Ikba*$^{lck\Delta/lck\Delta}$, *Ikba*$^{my\Delta/my\Delta}$, and *Ikba*$^{FL\Delta/FL\Delta}$ (data not shown). In sharp contrast to the IκBα mutations affecting immune cells only, concomitant deletion of *Ikba* specifically in keratinocytes and T cells (*Ikba*$^{K5\Delta/K5\Delta\ lck\Delta/lck\Delta}$) resulted in a phenotype that was undistinguishable from that observed in *Ikba*$^{\Delta/\Delta}$ mice (Figures 2D, 3E, and 3F).

(C) Immunostaining of skin sections from 6-day-old *Ikba*$^{\Delta/\Delta}$ mice and *Ikba*$^{f/f}$ mice for the indicated epidermal proliferation markers (green staining).
(D and E) NF-κB binding activity from primary keratinocytes was measured by gel mobility-shift analysis (D) and specified for NF-κB subunits by ELISA (E). Bar graphs show mean ± SD, n = 4.
(F) Immunostaining of skin sections from 6-day-old *Ikba*$^{\Delta/\Delta}$ and control mice with a CD54 antibody for detection of ICAM-1 expression.
(G) Growth curves of primary keratinocytes in culture. A total of 30,000 cells isolated from *Ikba*$^{\Delta/\Delta}$ (red circles) and wild-type mice (blue squares) were seeded per well, cultured for 7 days, and collected at different time points. Results are the mean (±SD) of cell numbers determined in cultures from three mice.

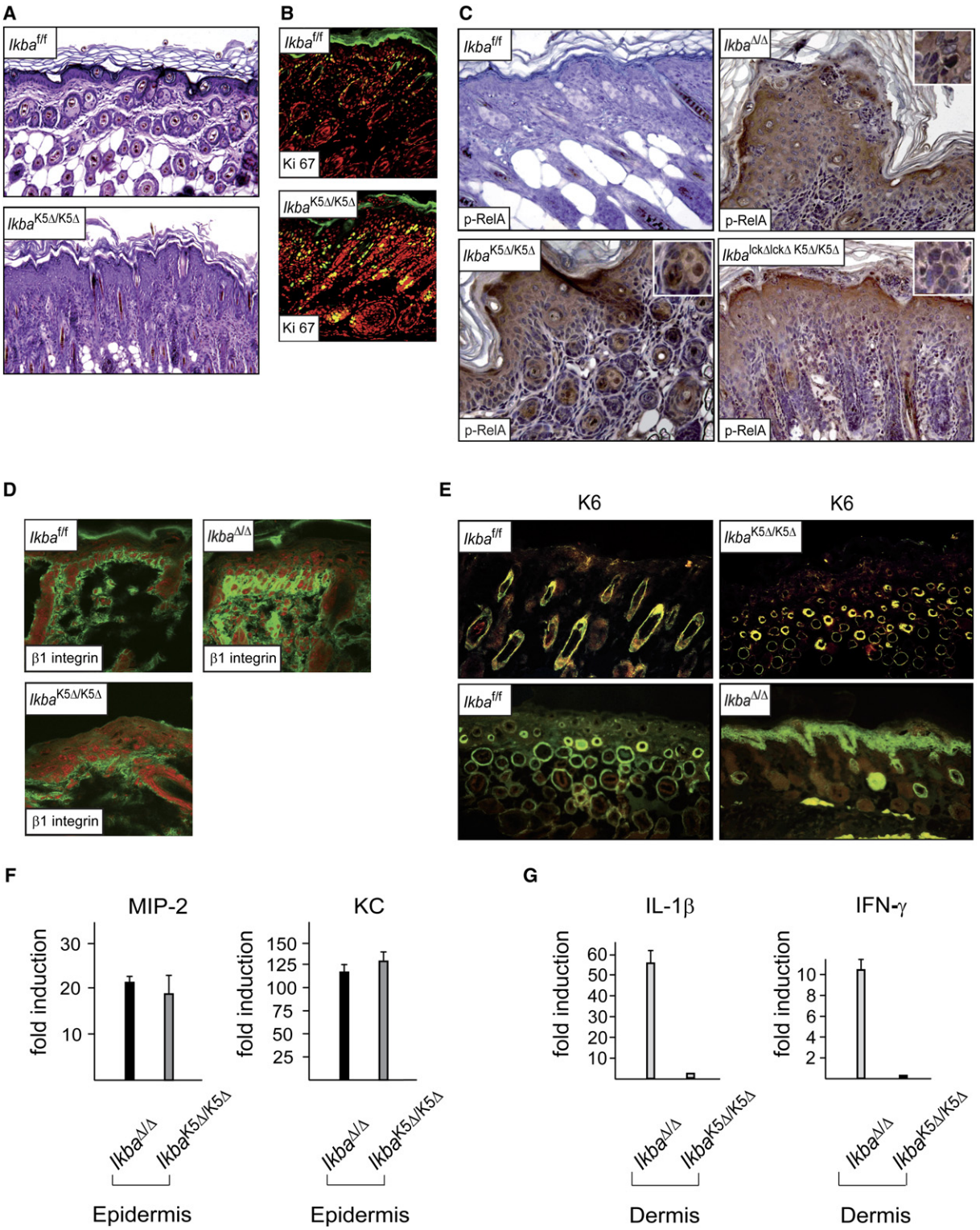


Figure 2. Epidermis-Specific Knockout of IκBα

(A) Skin sections from 6-day-old *Ikba*$^{K5\Delta/K5\Delta}$ mice and *Ikba*$^{f/f}$ mice were stained with hematoxylin and eosin (×20).

(B) Analysis of cell proliferation by staining skin sections from 6-day-old *Ikba*$^{K5\Delta/K5\Delta}$ mice and *Ikba*$^{f/f}$ mice for Ki67 (green); red staining shows nuclei.

(C) Immunostaining of skin section from 6-day-old *Ikba*$^{\Delta/\Delta}$, *Ikba*$^{K5\Delta K5\Delta}$, and *Ikba*$^{K5\Delta/K5\Delta\ lck\Delta/lck\Delta}$ mice and control mice with a specific antibody against RelA phosphorylated at Ser536. Nuclear staining is shown in the insets.

(D) Immunostaining of skin sections from 6-day-old *Ikba*$^{\Delta/\Delta}$, *Ikba*$^{K5\Delta K5\Delta}$, and control mice with a specific antibody against integrin β1. Red counterstaining shows nuclei.

Thus, although the presence of both lymphocytes and polymorphonuclear leukocytes is required for the inflammatory skin disease, loss of IκBα from immune cells alone is not sufficient for disease initiation. Together, the data strongly suggest that simultaneous IκBα deficiency in both the epidermal compartment and the immune system is needed for initiation and maintenance of this inflammatory disease of the skin.

Skin Inflammation Depends on TNF and LT

Inflamed tissue is characterized by a local milieu maintained by the production of proinflammatory cytokines. Tumor necrosis factor (TNF), lymphotoxin (LT)α, and LTβ are NF-κB-regulated key mediators of inflammation (Aggarwal, 2003). Serum TNF was under a detectable amount in control mice and strongly increased in *Ikba*$^{\Delta/\Delta}$ mice (Figure 4A). We inhibited TNF signaling by crossing *Ikba*$^{\Delta/\Delta}$ mice with either TNF receptor 1 (TNFR1)-deficient (Pfeffer et al., 1993) (*Ikba*$^{\Delta/\Delta}$ *Tnfrsf1a*$^{-/-}$) or TNF-deficient (Pasparakis et al., 1996) (*Ikba*$^{\Delta/\Delta}$ *Tnf*$^{-/-}$) mice. We inhibited LTα signaling by crossing *Ikba*$^{\Delta/\Delta}$ mice with LTα-deficient (De Togni et al., 1994) (*Ikba*$^{\Delta/\Delta}$ *Lta*$^{-/-}$) mice. In all three double-deficient mice, inflammation was reduced but still clearly detectable, pointing to the involvement of TNF-independent proinflammatory pathways (Figure 4 C and Figures S9 and S10; data not shown). Next, we crossed *Ikba*$^{\Delta/\Delta}$ mice with TNF-LTΔ3 mice, which are deficient for TNF, LTα, and LTβ (Kuprash et al., 2002) (*Ikba*$^{\Delta/\Delta}$ TNF-LTΔ3 mice). The skin of these animals showed no signs of inflammation and had an entirely normal phenotype (Figure 4C and Figure S10), demonstrating that the inflammatory disease resembling psoriasis was dependent on TNF and LT signaling.

Epidermal RelA Coordinates Epidermal-Dermal Cell Interactions

The transactivating NF-κB subunit identified in *Ikba*$^{\Delta/\Delta}$ and *Ikba*$^{K5\Delta/K5\Delta}$ keratinocytes was RelA. To directly analyze the contribution of RelA to the development of the inflammatory skin phenotype in *Ikba*$^{\Delta/\Delta}$ and *Ikba*$^{K5\Delta/K5\Delta}$ mice, we generated mice carrying LoxP site-containing *RelA* alleles (*Rela*$^{f/f}$). These were crossed with keratin5-*cre*2 mice for obtaining mice with epidermis-specific deletion of RelA (Figure S12). Mice with epidermis-specific deletion of *Rela* (*Rela*$^{K5\Delta/K5\Delta}$) were phenotypically inconspicuous. Neither skin sections from 5-day-old nor skin sections from 4-month-old *RelA*$^{K5\Delta/K5\Delta}$ mice showed any signs of abnormal epidermis differentiation or proliferation, when compared to *Rela*$^{f/f}$ or wild-type controls (Figure 5A). As a consequence, the expression pattern of skin differentiation markers was identical to that of control skin (Figure S13). When backcrossed to *Ikba*$^{\Delta/\Delta}$ mice, *Ikba*$^{\Delta/\Delta}$ *Rela*$^{K5\Delta/K5\Delta}$ double-mutant mice, with ubiquitous deletion of *Ikba* and epidermis-specific deletion of *Rela*, did not develop the inflammatory skin disease (Figures 5B and 5C). These mice exhibited no differences in the expression of epidermal differentiation markers compared to wild-type mice (Figure S13). Interestingly, deletion of only one *Rela* allele strongly improved but did not abolish the phenotype of *Ikba*$^{\Delta/\Delta}$ mice. Mice deficient for IκBα and heterozygous for the epidermis-specific *Rela* mutation, *Ikba*$^{\Delta/\Delta}$ *Rela*$^{+/K5\Delta}$, showed hyperkeratotic, scaly, and inflamed skin only at sites exposed to mechanical friction (data not shown), similarly to Koebner's phenomenon in psoriatic individuals. Thus, epidermal RelA expression was crucial for initiation and maintenance of the inflammatory skin disease in IκBα-deficient mice.

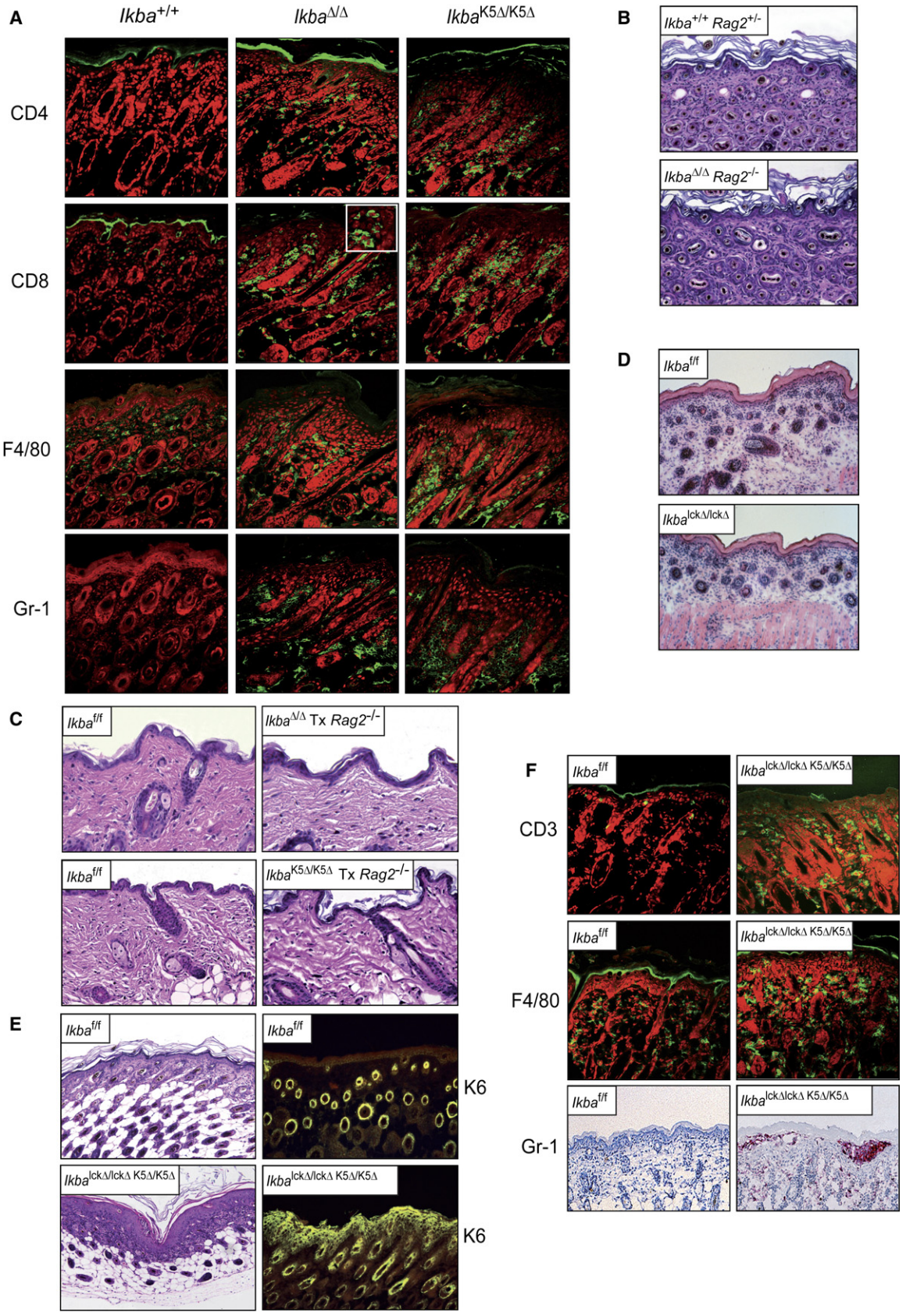
DISCUSSION

The data presented here show that simultaneous activation of NF-κB in immune cells and in keratinocytes is sufficient for the induction of a persistent skin inflammation that shares many similarities with human psoriasis. NF-κB activation by selective deletion of IκBα either in the epidermis or in immune cells alone did not give rise to the full inflammatory skin phenotype. The skin disease required concomitant IκBα deletion in keratinocytes and lymphocytes and was strictly dependent on signaling through cytokines of the TNF superfamily, a feature shared with autoimmune diseases such as psoriasis or rheumatoid arthritis. Importantly, targeted deletion of RelA in keratinocytes of double-transgenic *Ikba*$^{\Delta/\Delta}$ *Rela*$^{K5\Delta/K5\Delta}$ mice prevented disease development and demonstrated that aberrant signaling through RelA within the epidermis was essential for the development of the inflammatory skin disease.

Various transgenic and knockout mice have been generated that mimic aspects of inflammatory diseases at body surfaces such as the skin (Cheng et al., 1992), IFN-γ (Carroll et al., 1997), integrins (β1, α2β1, α5β1) (Carroll et al., 1995), Stat3 (Sano et al., 2005), and others (Nickoloff and Wrone-Smith, 1997; Schön, 1999). Many of these mutations directly or indirectly modulate NF-κB activity. In most models, investigations into disease mechanisms have been restricted to either the skin or the immune system. Here, we asked whether a disturbed crosstalk between keratinocytes and immunocytes is causal for the inflammatory process already at its initiation.

By evaluating contributions of various tissue compartments to disease development, we show that epidermis-specific targeting of *Ikba* results in keratinocyte hyperproliferation, but without inflammation in the epidermal compartment. The infiltration of the epidermis by inflammatory cells might involve integrin β1 because we found

(E) Immunostaining of skin sections from 6-day-old *Ikba*$^{K5\Delta/K5\Delta}$ mice and *Ikba*$^{f/f}$ mice for the indicated epidermal differentiation marker (green staining). (F and G) Induction of the indicated genes in the skin of 6-day-old *Ikba*$^{\Delta/\Delta}$ and *Ikba*$^{K5\Delta/K5\Delta}$ mice was measured by real-time RT-PCR with RNA samples prepared from six different *Ikba*$^{\Delta/\Delta}$ and *Ikba*$^{K5\Delta/K5\Delta}$ mice. Triplicate determination was performed for each mRNA sample. Gene-expression levels were normalized with tubulin for each mRNA preparation, and the n-fold increase in *Ikba*$^{\Delta/\Delta}$ and *Ikba*$^{K5\Delta/K5\Delta}$ mice was calculated by comparison with the result obtained in wild-type mice. Results are the mean (±SD) of seven mice.


A
Ikba+/+
IkbaΔ/Δ
IkbaK5Δ/K5Δ
CD4
CD8
F4/80
Gr-1
B
Ikba+/+ Rag2+/-
IkbaΔ/Δ Rag2-/-
D
Ikbaf/f
IkbaIckΔ/IckΔ
C
Ikbaf/f
IkbaΔ/Δ Tx Rag2-/-
Ikbaf/f
IkbaK5Δ/K5Δ Tx Rag2-/-
E
Ikbaf/f
Ikbaf/f
K6
IkbaIckΔ/IckΔ K5Δ/K5Δ
IkbaIckΔ/IckΔ K5Δ/K5Δ
K6
F
Ikbaf/f
IkbaIckΔ/IckΔ K5Δ/K5Δ
CD3
Ikbaf/f
IkbaIckΔ/IckΔ K5Δ/K5Δ
F4/80
Ikbaf/f
IkbaIckΔIckΔ K5Δ/K5Δ
Gr-1

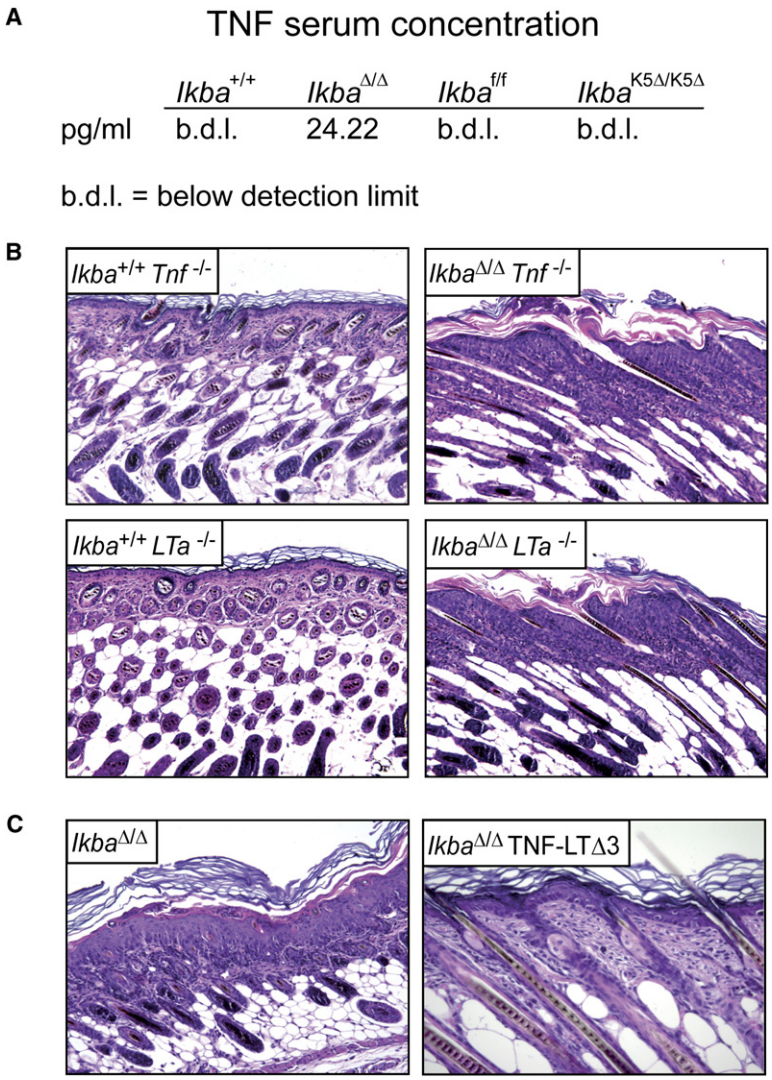


Figure 4. Requirement of TNF and LT for the Development of the Skin Phenotype in *Ikba*$^{\Delta/\Delta}$ Mice

(A) Analysis of the TNF serum concentration in 6-day-old *Ikba*$^{\Delta/\Delta}$, *Ikba*$^{K5\Delta/K5\Delta}$, and control mice. ELISA data of one representative experiment (of three repeated experiments) are presented.

(B) Skin sections from 7-day-old *Ikba*$^{\Delta/\Delta}$ *Tnf*$^{-/-}$, *Ikba*$^{\Delta/\Delta}$ *Lta*$^{-/-}$, and *Ikba*$^{+/+}$ *Tnf*$^{-/-}$ mice.

(C) Skin sections from 5-day-old *Ikba*$^{\Delta/\Delta}$ and *Ikba*$^{\Delta/\Delta}$ TNF-LTΔ3 mice were stained with hematoxylin and eosin.

upregulated expression of this adhesion molecule in *Ikba*$^{\Delta/\Delta}$ mice in contrast to *Ikba*$^{K5\Delta/K5\Delta}$ and wild-type mice. Hyperproliferation of keratinocytes in vivo depends on the presence of lymphocytes in the skin, is due to the inflammatory tissue milieu, and is not cell autonomous because an ex vivo proliferation assay revealed no difference in the growth of *Ikba*$^{\Delta/\Delta}$ keratinocytes compared to wild-type keratinocytes.

Cell-cycle regulation in keratinocytes seems to be independent from intrinsic RelA activity because wild-type and *Ikba*$^{\Delta/\Delta}$ keratinocytes showed identical proliferative behavior in vitro, although NFκB signaling was constitutively active in the latter. This is further supported by the normal skin phenotype of mice with epidermis-specific deletion of *Rela*. The discrepancy in comparison to previously published results (Zhang et al., 2004), demonstrating hyperproliferation in RelA deficient skin, may be due to the different experimental approach.

The central role of RelA signaling in keratinocytes for disease induction was most strikingly demonstrated by

Figure 3. Inflammation in the Skin of *Ikba*$^{\Delta/\Delta}$ and *Ikba*$^{K5\Delta/K5\Delta}$ Mice and *Ikba*$^{K5\Delta/K5\Delta\ lck\Delta/lck\Delta}$ Mice

(A) Skin sections from 6-day-old *Ikba*$^{\Delta/\Delta}$, *Ikba*$^{K5\Delta/K5\Delta}$, and control mice were immunostained with antibodies recognizing T cells (CD4 and CD8), macrophages (F4/80) and neutrophilic granulocytes (Gr-1) (green signal). The inset in the CD8 staining of skin from *Ikba*$^{\Delta/\Delta}$ mice shows positive cells in the epidermal layer. Red counterstaining shows nuclei.

(B–D) Skin sections from 6-day-old *Ikba*$^{\Delta/\Delta}$ *Rag2*$^{-/-}$ mice, *Ikba*$^{\Delta/\Delta}$ skin transplanted onto *Rag2*$^{-/-}$ mice (*Ikba*$^{\Delta/\Delta}$ Tx *Rag2*$^{-/-}$), *Ikba*$^{K5\Delta/K5\Delta}$ skin transplanted onto *Rag2*$^{-/-}$ mice (*Ikba*$^{K5\Delta/K5\Delta}$ Tx *Rag2*$^{-/-}$), 6-day-old *Ikba*$^{lck\Delta/lck\Delta}$ mice, and control mice were stained with hematoxylin and eosin.

(E) Skin sections from 7-day-old *Ikba*$^{K5\Delta/K5\Delta\ lck\Delta/lck\Delta}$ and control mice were stained with hematoxylin and eosin and immunostained for the indicated epidermal differentiation marker (green staining).

(F) Skin sections from 7-day-old *Ikba*$^{K5\Delta/K5\Delta\ lck\Delta/lck\Delta}$ and control mice were immunostained with antibodies recognizing T cells (CD3), macrophages (F4/80), and neutrophilic granulocytes (Gr-1) (green signal).

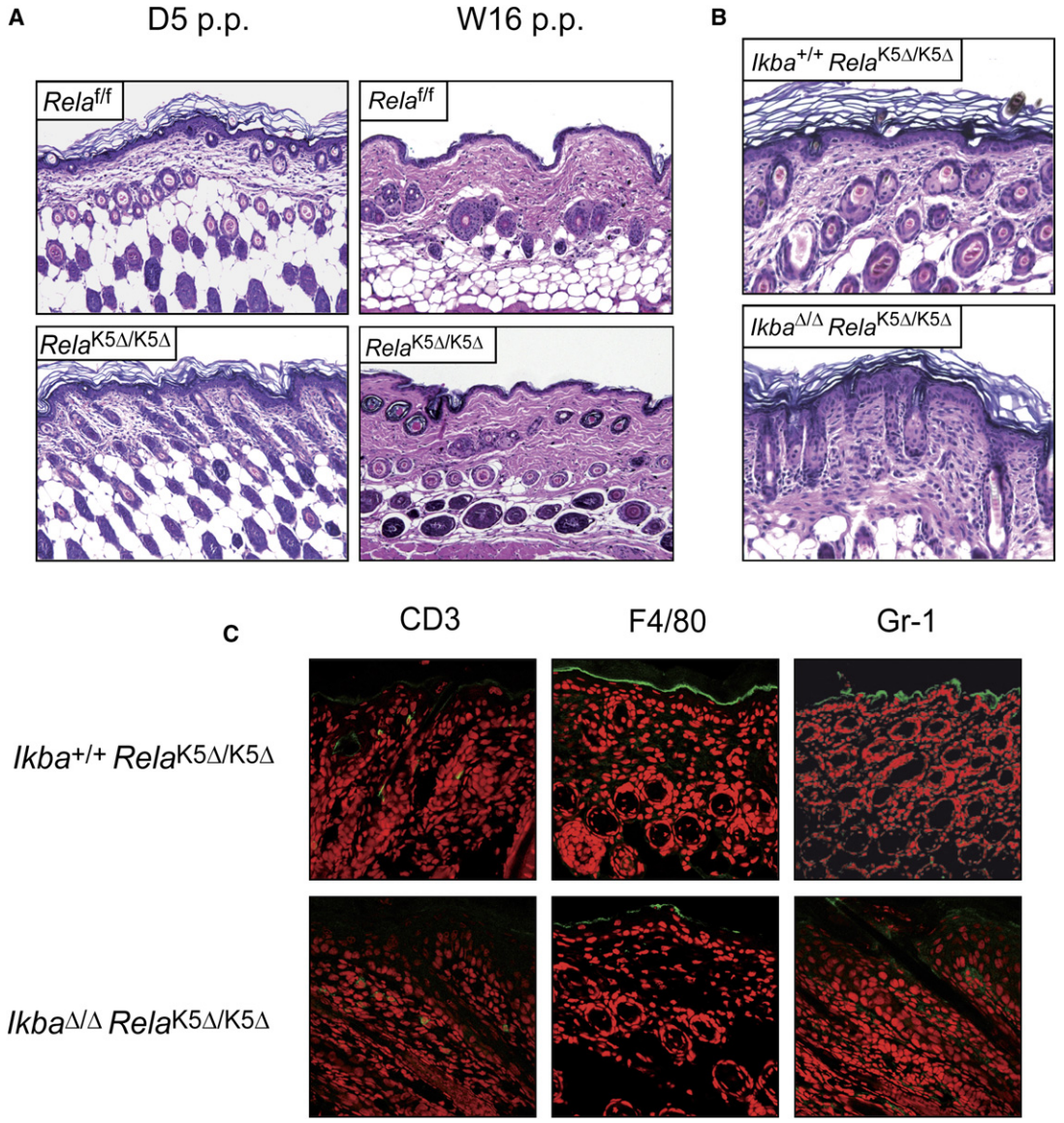


Figure 5. The Skin Phenotype in *Ikba*$^{\Delta/\Delta}$ Mice Depends on Epidermal RelA Expression

(A) Skin sections from 5-day-old and 16-week-old *Rela*$^{K5\Delta/K5\Delta}$ and control mice were stained with hematoxylin and eosin.

(B and C) Staining of skin sections from 6-day-old *Ikba*$^{\Delta/\Delta}$ *Rela*$^{K5\Delta/K5\Delta}$ and control mice were stained with hematoxylin and eosin (B) or antibodies against the indicated immune cell markers (C). Red counterstaining shows nuclei.

double knockout animals that have ubiquitous deletion of IκBα and epidermis-specific deletion of RelA and that showed a normal phenotype. This is in line with the observation that skin inflammation is absent in mice with specific deletion of IκBα in immune cells only (*Ikba*$^{lck\Delta/lck\Delta}$, *Ikba*$^{my\Delta/my\Delta}$, and *Ikba*$^{FL\Delta/FL\Delta}$) and highlights the important pathogenic role of the epithelium. By using several different approaches, we here demonstrate a functional, pathogenic cooperation that is mediated by RelA between keratinocytes and immune cells. The exact pathogenic steps that link RelA activation in keratinocytes to T cell infiltration still remain open, although production of chemotactic factors such as S100A8, S100A9, KC, and MIP-2 and increased expression of integrinβ1 are likely to be of relevance.

TNF blockers have recently been introduced into the treatment of inflammatory diseases such as psoriasis (Koo and Khera, 2005). We provide genetic evidence that, in addition to TNF and TNFR1 signaling, LTs may be important mediators in the pathogenesis of psoriasis. LTα forms homotrimers with signaling through TNFR1 and TNFR2 and forms heterotrimers with LTβ signaling through LTβR (Pfeffer, 2003). Only in a triple-knockout deficient for *Tnf*, *Lta*, and *Ltb* was the development of the skin phenotype efficiently inhibited, whereas it was significantly attenuated in single knockouts for either *Tnfrsf1a* or

Tnf or Lta. Our data therefore support a therapeutic approach in which LTs are inhibited in addition to TNF.

Our results show that RelA activity in keratinocytes is required for initiation of the inflammatory skin phenotype after ablation of IκBα. At first glance, this may seem surprising because IKK2 deletion that causes an inhibition of NF-κB activation produces a phenotype similar to that observed in *Ikba*$^{\Delta/\Delta}$ mice (Pasparakis et al., 2002). Our analyses revealed, however, that the mechanisms leading to these two skin conditions differ fundamentally in their T cell dependence. This suggests that skin homeostasis requires balanced NF-κB activity: Constitutive activation of NF-κB will lead to a T cell-dependent inflammatory skin disease, whereas inhibition of NF-κB activation results in T cell-independent skin inflammation.

In summary, we have characterized pathogenic interactions between epithelial cells covering a body surface and immune cells. We believe that this model gives novel insight into the pathogenesis of inflammatory skin diseases but could also be of relevance to inflammatory conditions of other body surfaces, such as the gut and the urinary tract.

EXPERIMENTAL PROCEDURES

Generation of *Ikba*$^{\Delta/\Delta}$, *Ikba*$^{K5\Delta/K5\Delta}$, *Ikba*$^{lck\Delta/lck\Delta}$, *Ikba*$^{my\Delta/my\Delta}$, and *Rela*$^{K5\Delta/K5\Delta}$ Mice

Mice carrying a floxed *Ikba* allele (*Ikba*$^{f/f}$) (Rupec et al., 2005) were crossed with Deleter-*cre* mice (Schwenk et al., 1995) (*Ikba*$^{\Delta/\Delta}$) and with transgenic mice expressing Cre recombinase under the control of the keratinocyte-specific keratin5 promoter (Tarutani et al., 1997) (*Ikba*$^{K5\Delta/K5\Delta}$), T cell-specific *lck* promoter (Hennet et al., 1995) (*Ikba*$^{lck\Delta/lck\Delta}$), and neutrophil and macrophage-specific LysM promoter (Clausen et al., 1999) (*Ikba*$^{my\Delta/my\Delta}$), respectively. To obtain *Ikba*$^{\Delta/\Delta}$ *Rela*$^{K5\Delta/K5\Delta}$ mice, we crossed offspring of *Rela*$^{f/f}$ (Algül et al., 2007)-K5-*cre*2 intercross with *Ikba*$^{\Delta/\Delta}$ mice. We generated *Ikba*$^{\Delta/\Delta}$ *Rag2*$^{-/-}$ mice, *Ikba*$^{\Delta/\Delta}$ TNF-LTΔ3 mice, and *Ikba*$^{\Delta/\Delta}$ *Tnfrsf1a*$^{-/-}$and *Ikba*$^{\Delta/\Delta}$ *Tnf*$^{-/-}$ mice by breeding *Ikba*$^{\Delta/\Delta}$ mice into the respective backgrounds. The genetic background was C57BL/6 for *Ikba*$^{f/f}$, *Rela*$^{f/f}$, K5-*cre*2, LysM-*cre*, lck-*cre*, *Rag2*$^{-/-}$, *Tnfrsf1a*$^{-/-}$, *Tnf*$^{-/-}$, *Lta*$^{-/-}$, and TNF-LTΔ3 mice.

PCR

Mice were genotyped by PCR (sequence available upon request); expected sizes of the PCR products are for the *Ikba* gene 340 bp for the floxed allele, 250 bp for the wild-type allele, and 500 bp for the deleted allele. For the *Rela* gene, the expected sizes are 294 bp for the floxed allele, 260 bp for the wild-type allele, and 400 bp for the deleted allele.

Histology and Immunohistochemistry

Skin samples were fixed in 4% formaldehyde for 24 hr and embedded in paraffin, and 5 μm serial sections were stained with hematoxylin and eosin (H and E). Phosphorylated RelA was detected with antiphospho-p65 (Cell Signaling Technology). For frozen sections, skin samples taken from the back were snap frozen in 2-methylbutane (Merck) and embedded in OCT Tissue Tec Media (Miles). Five-micrometer sections were cut with a Cryotom (Leica), taken up in 4% PBS, and rinsed with water. Antibodies against CD31 and CD54 (R & D Systems) were used together with the biotin-streptavidin detection system (Vector Systems). ABC-system block and the Avidin-Biotin-Block (DAKO) were used when appropriate. For visualization of antibody binding, AEC (Sigma) was applied until the desired color intensity developed. After washing in H_2O, sections were counterstained with hematoxylin and mounted with Aquatex (Merck). Staining of inflammatory and proliferating cells was performed as described previously (Pasparakis et al., 2002). The following rat monoclonal antibodies were used on frozen sections: anti-CD3 and -CD8 (both 1:1000; Chemicon), anti-CD4 (1:50; BD Biosciences PharMingen), and Ki-67 (1:50; clone TEC-3, Dako).

Outside-In Barrier Assays

For Toluidine blue staining, newborn mice were killed, bathed in methanol for 5 min, and, after a washing step with PBS, incubated for 15 min in 0.1% Toluidine blue (Sigma). For acidic X-Gal staining, newborn mice were killed, rinsed with water, and incubated in 4 mM $K_3Fe(CN)_6$, 4 mM $K_4Fe(CN)_6$, 2 mM $MgCl_2$, 1 mg/ml 5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside (Sigma), and 0.1 M KH_2PO_4 at pH 4.5, at 30°C for 6 hr.

Electrophoretic Mobility-Shift Assay and NF-κB ELISA

Electrophoretic mobility shift assays were performed as described previously (Rupec et al., 2005). The NF-κB ELISA was performed according to the instructions of the manufacturer (Active Motif).

Isolation and Culture of Mouse Keratinocytes and T Cells

Keratinocytes were isolated from the skin of newborn mice as described (Roper et al., 2001) and cultured in collagen type IV-coated plates in SFM keratinocyte medium (GIBCO) on feeder cells. Cells were resuspended at 1 × 10^6 per 100 μl of PBS containing 1% FCS, pretreated with Fc-block (anti-CD16/anti-CD32, PharMingen), and stained for 30 min at 4°C with the indicated mouse antibodies. Viable cells, as determined by forward and side scatter profiles, were sorted with a MoFlo Sorter (Cytomation).

Skin Grafts

We surgically transplanted skin from 6-day-old *Ikba* $^{\Delta/\Delta}$, *Ikba* $^{K5\Delta/K5\Delta}$, and control mice into the dorsal region of 7- to 8-week-old anaesthetized *Rag2*$^{-/-}$ mice. The average size of donor grafts was 1–1.5 cm diameter. The grafts were dressed with Jelonet* gaze (Smith + Nephew) and Fixomull stretch (BSN medical) bandage. After 10–12 days, the bandages were removed. For *Ikba* $^{\Delta/\Delta}$ and *Ikba* $^{K5\Delta/K5\Delta}$ grafts, the donor origin was verified by PCR with RNA isolated from keratinocytes.

RT-PCR

Total RNA was isolated from cultured keratinocytes or from full skin by the Trizol protocol (GIBCO/BRL). Total RNA was digested with DNase I (Sigma) for removal of DNA contamination and reverse-transcribed with SuperScript II according to the manufacturer's instructions (GIBCO/BRL). PCR amplifications were performed with the primers according to standard methods. Primer sequences are available upon request.

TNF ELISA

For determination of TNF levels in murine serum, an ELISA kit (Quantikine, R & D systems) was used according to the instructions of the manufacturer.

cDNA Libraries and Processing of Microarrays

cRNA preparation was performed with the Illumina TotalPrep RNA Amplification Kit (Ambion Europe) according to the manufacturer's instructions. cRNA (1.5 μg) was hybridized to Sentix Mouse-6 Expression Beadchips (Illumina) and scanned on Illumina BeadStation 500×. For data collection and analysis, Illumina BeadStudio and dChip 1.3 software were used.

Supplemental Data

Thirteen figures are available at http://www.immunity.com/cgi/content/full/27/2/296/DC1/.

ACKNOWLEDGMENTS

We thank D. V. Kuprash for providing the TNF-LTΔ3 mice. We are grateful to J. Minda for maintaining our mouse colonies and Alfred Funteh for technical help with immunostaining. This work was supported by the Deutsche Forschungsgemeinschaft (RU 601/3-2 and Wilhelm Sander-Stiftung to R.A.R, SFB 575, 576 to K.P., and SFB 589 to I.H.) and the Howard Hughes Medical Institute (S.A.N.).

Received: September 14, 2006
Revised: March 30, 2007
Accepted: May 29, 2007
Published online: August 9, 2007

REFERENCES

Aggarwal, B.B. (2003). Signalling pathways of the TNF superfamily: A double-edged sword. Nat. Rev. Immunol. *3*, 745–756.

Algül, H., Treiber, M., Lesina, M., Nakhai, H., Saur, D., Geisler, F., Pfeifer, A., Paxian, S., and Schmid, R.M. (2007). Pancreas-specific RelA/p65 truncation increases susceptibility of acini to inflammation-associated cell death following cerulein pancreatitis. J. Clin. Invest. *117*, 1490–1501.

Austin, L.M., Ozawa, M., Kikuchi, T., Walters, I.B., and Krueger, J.G. (1999). The majority of epidermal T cells in Psoriasis vulgaris lesions can produce type 1 cytokines, interferon-γ, interleukin-2, and tumor necrosis factor-α, defining TC1 (cytotoxic T lymphocyte) and TH1 effector populations: A type 1 differentiation bias is also measured in circulating blood T cells in psoriatic patients. J. Invest. Dermatol. *113*, 752–759.

Bowcock, A.M. (2005). The genetics of psoriasis and autoimmunity. Annu. Rev. Genomics Hum. Genet. *6*, 93–122.

Bowcock, A.M., and Krueger, J.G. (2005). Getting under the skin: The immunogenetics of psoriasis. Nat. Rev. Immunol. *5*, 699–711.

Carroll, J.M., Crompton, T., Seery, J.P., and Watt, F.M. (1997). Transgenic mice expressing IFN-γ in the epidermis have eczema, hair hypopigmentation, and hair loss. J. Invest. Dermatol. *108*, 412–422.

Carroll, J.M., Romero, M.R., and Watt, F.M. (1995). Suprabasal integrin expression in the epidermis of transgenic mice results in developmental defects and a phenotype resembling psoriasis. Cell *83*, 957–968.

Cheng, J., Turksen, K., Yu, Q.C., Schreiber, H., Teng, M., and Fuchs, E. (1992). Cachexia and graft-vs.-host-disease-type skin changes in keratin promoter-driven TNFα transgenic mice. Genes Dev. *6*, 1444–1456.

Clausen, B.E., Burkhardt, C., Reith, W., Renkawitz, R., and Förster, I. (1999). Conditional gene targeting in macrophages and granulocytes using LysMcre mice. Transgenic Res. *8*, 265–277.

De Togni, P., Goellner, J., Ruddle, N.H., Streeter, P.R., Fick, A., Mariathasan, S., Smith, S.C., Carlson, R., Shornick, L.P., Strauss-Schoenberger, J., et al. (1994). Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. Science *264*, 703–707.

Ghoreschi, K., Thomas, P., Breit, S., Dugas, M., Mailhammer, R., van Eden, W., van der Zee, R., Biedermann, T., Prinz, J., Mack, M., et al. (2003). Interleukin-4 therapy of psoriasis induces Th2 responses and improves human autoimmune disease. Nat. Med. *9*, 40–46.

Gudjonsson, J.E., Johnston, A., Sigmundsdottir, H., and Valdimarsson, H. (2004). Immunopathogenic mechanisms in psoriasis. Clin. Exp. Immunol. *135*, 1–8.

Hayden, M.S., and Ghosh, S. (2004). Signaling to NF-κB. Genes Dev. *18*, 2195–2224.

Hennet, T., Hagen, F.K., Tabak, L.A., and Marth, J.D. (1995). T-cell-specific deletion of a polypeptide N-acetylgalactosaminyl- transferase gene by site-directed recombination. Proc. Natl. Acad. Sci. USA *92*, 12070–12074.

Karin, M., and Ben-Neriah, Y. (2000). Phosphorylation meets ubiquitination: The control of NF-κB activity. Annu. Rev. Immunol. *18*, 621–663.

Karin, M., and Greten, F.R. (2005). NF-κB: Linking inflammation and immunity to cancer development and progression. Nat. Rev. Immunol. *5*, 749–759.

Komine, M., Rao, L.S., Freedberg, I.M., Simon, M., Milisavljevic, V., and Blumenberg, M. (2001). Interleukin-1 induces transcription of keratin K6 in human epidermal keratinocytes. J. Invest. Dermatol. *116*, 330–338.

Koo, J., and Khera, P. (2005). Update on the mechanisms and efficacy of biological therapies for psoriasis. J. Dermatol. Sci. *38*, 75–87.

Kuprash, D.V., Alimzhanov, M.B., Tumanov, A.V., Grivennikov, S.I., Shakhov, A.N., Drutskaya, L.N., Marino, M.W., Turetskaya, R.L., Anderson, A.O., Rajewsky, K., et al. (2002). Redundancy in tumor necrosis factor (TNF) and lymphotoxin (LT) signaling in vivo: Mice with inactivation of the entire TNF/LT locus versus single-knockout mice. Mol. Cell. Biol. *22*, 8626–8634.

Lang, K.S., Recher, M., Junt, T., Navarini, A.A., Harris, N.L., Freigang, S., Odermatt, B., Conrad, C., Ittner, L.M., Bauer, S., et al. (2005). Toll-like receptor engagement converts T-cell autoreactivity into overt autoimmune disease. Nat. Med. *11*, 138–145.

Lawrence, C.M., and Shuster, S. (1992). Therapeutic effect of anthralin. J. Am. Acad. Dermatol. *26*, 138–139.

Li, Q., and Verma, I.M. (2002). NF-κB regulation in the immune system. Nat. Rev. Immunol. *2*, 725–734.

Nestle, F.O., Turka, L.A., and Nickoloff, B.J. (1994). Characterization of dermal dendritic cells in psoriasis. Autostimulation of T lymphocytes and induction of Th1 type cytokines. J. Clin. Invest. *94*, 202–209.

Nickoloff, B.J., and Wrone-Smith, T. (1997). Animal models of psoriasis. Nat. Med. *3*, 475–476.

Pasparakis, M., Alexopoulou, L., Episkopou, V., and Kollias, G. (1996). Immune and inflammatory responses in TNFα-deficient mice: A critical requirement for TNFα in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. J. Exp. Med. *184*, 1397–1411.

Pasparakis, M., Courtois, G., Hafner, M., Schmidt-Supprian, M., Nenci, A., Toksoy, A., Krampert, M., Goebeler, M., Gillitzer, R., Israël, A., et al. (2002). TNF-mediated inflammatory skin disease in mice with epidermis-specific deletion of IKK2. Nature *417*, 861–866.

Pfeffer, K. (2003). Biological functions of tumor necrosis factor cytokines and their receptors. Cytokine Growth Factor Rev. *14*, 185–191.

Pfeffer, K., Matsuyama, T., Kundig, T.M., Wakeham, A., Kishihara, K., Shahinian, A., Wiegmann, K., Ohashi, P.S., Kronke, M., and Mak, T.W. (1993). Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to L. monocytogenes infection. Cell *73*, 457–467.

Robert, C., and Kupper, T.S. (1999). Inflammatory skin diseases, T cells, and immune surveillance. N. Engl. J. Med. *341*, 1817–1828.

Roper, E., Weinberg, W., Watt, F.M., and Land, H. (2001). p19ARF-independent induction of p53 and cell cycle arrest by Raf in murine keratinocytes. EMBO Rep. *2*, 145–150.

Rupec, R.A., Jundt, F., Rebholz, B., Eckelt, B., Weindl, G., Herzinger, T., Flaig, M.J., Moosmann, S., Plewig, G., Dörken, B., et al. (2005). Stroma-mediated dysregulation of myelopoiesis in mice lacking IκBα. Immunity *22*, 479–491.

Sano, S., Chan, K.S., Carbajal, S., Clifford, J., Peavey, M., Kiguchi, K., Itami, S., Nickoloff, B.J., and DiGiovanni, J. (2005). Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. Nat. Med. *11*, 43–49.

Schön, M.P. (1999). Animal models of psoriasis - what can we learn from them? J. Invest. Dermatol. *112*, 405–410.

Schwenk, F., Baron, U., and Rajewsky, K. (1995). A cre-transgenic mouse strain for the ubiquitous deletion of *loxP*- flanked gene segments including deletion in germ cells. Nucleic Acids Res. *23*, 5080–5081.

Tarutani, M., Itami, S., Okabe, M., Ikawa, M., Tezuka, T., Yoshikawa, K., Kinoshita, T., and Takeda, J. (1997). Tissue-specific knockout of the mouse Pig-a gene reveals important roles for GPI-anchored proteins in skin development. Proc. Natl. Acad. Sci. USA *94*, 7400–7405.

Viatour, P., Merville, M.P., Bours, V., and Chariot, A. (2005). Phosphorylation of NF-κB and IκB proteins: Implications in cancer and inflammation. Trends Biochem. Sci. *30*, 43–52.

Wrone-Smith, T., and Nickoloff, B.J. (1996). Dermal injection of immunocytes induces psoriasis. J. Clin. Invest. *98*, 1878–1887.

Zhang, J.Y., Green, C.L., Tao, S., and Khavari, P.A. (2004). NF-κB RelA opposes epidermal proliferation driven by TNFR1 and JNK. Genes Dev. *18*, 17–22.