Regulation of Epidermal Expression of Keratin K17 in Inflammatory Skin Diseases

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Keratin K17, the myoepithelial keratin, is expressed in psoriasis but is not present in healthy skin. Psoriasis is associated with production of γ interferon (IFNγ), which induces the expression of keratin K17 by activating transcription factor STAT1. Our hypothesis states that the induction of K17 is specific for the inflammatory reactions associated with high levels of IFNγ and activation of STAT1. One of the corollaries of the hypothesis is that the STAT1-activating cytokines should induce the expression of keratin K17, whereas those cytokines that work through other mechanisms should not. Furthermore, because the STAT activation pathway is dependent upon protein phosphorylation events, phosphorylation inhibitors should attenuate the induction of keratin K17, whereas protein phosphatase inhibitors should augment it. To test this hypothesis, we analyzed lesional samples of inflammatory diseases using immunofluorescence, transfected keratinocytes with K17 gene promoter DNAs in the presence of various cytokines, and followed nuclear translocation of STAT1 in keratinocytes using specific antibodies. Confirming the hypothesis, we found that K17 is induced in psoriasis and dermatitis caused by delayed type hypersensitivity, which are associated with high levels of IFNγ, but not in samples of atopic dermatitis, which is not. Two cytokines, interleukin-6 and leukemia inhibitory factor, which can induce phosphorylation of STAT1, can also induce K17 expression, whereas interleukin-3, interleukin-4, interleukin-10, and granulocyte macrophage colony stimulating factor have no effect on K17 expression. As expected, staurosporine and genistein inhibited, whereas okadaic acid augmented, the induction of K17 by IFNγ. Our data indicate that in inflammatory skin diseases, lymphocytes, through the cytokines they produce, differently regulate not only each other, but also keratin gene expression in epidermis, one of their target tissues. *Key words: cytokines/DNA/interferon/promoter. J Invest Dermatol 107:569–575, 1996*

Inflammatory reactions result in an influx of inflammatory cells into the site of tissue damage (Ollier, 1992). The types of lymphocytes in inflammatory infiltrates differ according to the cause of inflammation (Ferrick *et al*, 1995). Murine CD4+ T lymphocytes can be divided into two subclasses, based on the pattern of cytokines released after antigenic stimulation (Mosmann and Coffman, 1989; Rivas and Ullrich, 1992). Th-1 cells secrete interferonγ (IFNγ), lymphotoxin, and interleukin-2 (IL-2), whereas Th-2 cells produce IL-4, IL-5, and IL-10. Consequently, Th-1 cells are more active in providing help for cellular immune reactions, whereas Th-2 cells are more efficient in supporting humoral immune reactions. In addition, these two types inhibit each other during the immune response (Rivas and Ullrich, 1992; Kamogawa *et al*, 1993). Similar subsets of CD4+ T cells have been described in the human immune system, although most human T lymphocytes show a Th-O–type profile, with characteristics of both Th-1 and Th-2 cell types (Romagnani, 1991).

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Abbreviation: LIF, leukemia-inhibiting factor.

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The pathogenesis of psoriasis vulgaris is associated with IFNγ secreting Th-1–like lymphocytes (Schlaak *et al*, 1994). IFNγ enhances inflammatory processes and has been implicated in the pathogenesis of other types of dermatitis, including delayed type hypersensitivity and lichen planus (Barker and MacDonald, 1992; Chu *et al*, 1992; Groves *et al*, 1993). In contrast to psoriasis, atopic dermatitis is reported to involve Th-2–like lymphocytes secreting IL-4 and IL-10. Accordingly, although atopic dermatitis is a rather heterogeneous disorder, therapeutic use of IFNγ seems to be quite effective in its treatment (Hanifin *et al*, 1993; Reinhold *et al*, 1993; Hamid *et al*, 1994; Viac *et al*, 1994).

Perhaps the best studied paradigm for a signal transduction pathway is that of IFNγ (reviewed by Shuai, 1994). Upon binding to the ligand, the IFNγ receptor associates with cell membrane-bound tyrosine kinases of the Jak/Tyk family, which cause phosphorylation of STAT transcription factors, which are thus activated and translocate to the nucleus. In the nucleus, STATs recognize and bind to specific DNA sequences and interact with the transcription machinery to regulate transcription of specific genes (Darnell *et al*, 1994). The regulatory specificity of the cytokine signal at the cell surface is mirrored in the nucleus by the activation of a specific member of the STAT family. For example, STAT1 is activated by IFNγ, epidermal growth factor (EGF), and IL-6; STAT2 (p113) by IFNα; STAT3 by EGF and IL-6; STAT5 by prolactin; and STAT6 by IL-4 (Fu and Zhang, 1993; Gouilleux *et al*, 1994; Hou *et al*, 1994; Schindler *et al*, 1994; Wakao *et al*, 1994).

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We have shown previously that delayed type hypersensitivity reactions result in activation and nuclear translocation of STAT1 in human keratinocytes *in vivo* and in subsequent induction of transcription of keratin K17 (Jiang *et al*, 1993). Within the promoter of the K17 gene, we have identified and characterized two sites that confer the responsiveness to IFNγ, one of which binds STAT1. Normal adult epidermis, however, does not contain K17 (de Jong *et al*, 1991). Keratin K17 is expressed in myoepithelial cells, in basal layers of transitional and pseudostratified epithelia of respiratory and urinary tracts, and during early developmental stages of stratified epithelia (Troyanovsky *et al*, 1992).

Keratins have been used as markers of phenotype of epithelial cells (O'Guin *et al*, 1987). Basal cells produce keratins K5 and K14, differentiating nonkeratinizing epithelia K4 and K13, cornea K3 and K12, epidermis K1 and K10, palmoplantar keratinocytes K2 and K9, and simple epithelia K8 and K18, whereas hair and nails contain their own, "hard" keratins. In pathologic conditions, such as inflammation, neoplasms, and wound healing, additional keratins are expressed, such as K6, K16, and K17. Whereas K6 and K16 are associated with activated, migratory keratinocytes, K17 is found in contractile epithelia. If the function of keratin cytoskeletal network is to provide mechanical strength, then K6 and K16 keratins may modify the cytoskeleton enabling the cell to migrate, whereas K17 allows it to become contractile. The contractility in wound healing is important, but its role in inflammatory processes is not clear.

Our hypothesis states that the induction of K17 proceeds via activation of STAT1 transcription factor. Consequently, all STAT1-activating cytokines should induce the expression of keratin K17, and those cytokines that work through other mechanisms should not.

Our hypothesis is supported by the evidence presented here that K17 is induced strongly and specifically by IFNγ, a product of Th-1 lymphocytes. In contrast, IL-4 and IL-10 neither regulate K17 transcription by themselves nor affect the induction by IFNγ. Furthermore, inhibitors of tyrosine kinases, such as genistein and staurosporine, inhibit K17 induction by IFNγ, whereas inhibitors of protein kinase C and inhibitors of the Ras/mitogen activated protein kinase (MAPK) C pathway do not, which indicates that K17 induction by IFNγ does not involve protein kinase C or Ras/MAPK.

We also show that the expression of K17 in human inflammatory diseases correlates with the cytokine profiles in these diseases. For example, the lesional skin from psoriasis or delayed type hypersensitivity does contain K17, whereas the lesional epidermis from atopic dermatitis patients does not. Our data describe at the molecular level the mechanisms that regulate and change K17 gene expression in human epidermal keratinocytes *in vitro* as well as in human epidermal diseases *in vivo*.

MATERIALS AND METHODS

DNA Constructs The plasmids containing keratin promoters and the control plasmids pRSVZ have been described previously (Jiang *et al*, 1990, 1991, 1993). The plasmid containing the intracellular adhesion molecule 1 (ICAM-1) promoter was a gift from S. W. Caughman (Emory University, Atlanta, GA). The plasmid expressing RasDN was obtained from E. Skolnick (New York University Medical Center, New York). DNA primers were synthesized on a Gene-Plus synthesizer (Pharmacia, Piscataway, NJ) according to their promoter sequences. All DNAs used in transfections were purified using the Magic Megapreps DNA purification system (Promega, Madison, WI).

Cells and Transfection Normal human foreskin epidermal keratinocytes were a generous gift from Dr. M. Simon (SUNY Stony Brook, Stony Brook, NY). The cultures were initiated using 3T3 feeder layers as described previously (Randolph and Simon, 1994) and then frozen in liquid N_2 until used. Once thawed, the keratinocytes were grown without feeder cells in defined serum-free keratinocyte medium supplemented with epidermal growth factor and bovine pituitary extract (keratinocyte–serum-free medium, GIBCO, Gaithersburg, MD). Cells were expanded through two 1:4 passages before transfection and transfected at 80–100% confluency.

Transfections using polybrene with dimethyl sulfoxide shock were performed as previously described (Jiang *et al*, 1991). Each transfection contained 15 μg K17CAT per dish and 3 μg RSVZ per dish. The construct expressing Ras dominant negative mutant (15 μg per dish) was cotransfected with the K17CAT construct in the experiments involving Ras/MAPK pathway. The cells were then incubated with or without IFNγ in combination with granulocyte macrophage colony stimulating factor (GM-CSF), IL-3, IL-6, IL-10, leukemia-inhibiting factor (LIF), and various kinase and phosphatase inhibitors as indicated. IFNγ, IL-10, and LIF were from R&D Systems (Minneapolis, MN); GM-CSF, IL-3, and IL-6 were from U.B.I. (Lake Placid, NY); cAMP, staurosporine, and genistein were from Sigma (St. Louis, MO); bisindolylmaleimide was from Calbiochem (San Diego, CA); and okadaic acid was from GIBCO BRL (Gaithersburg, MD). Thirty-six to 48 h after transfection, cells were washed twice with phosphate-buffered saline and harvested by scraping. The cell disruption by repeated freeze–thaw cycles, and chloramphenicol acetyl transferase (CAT) and β-galactosidase assays have also been described previously (Jiang *et al*, 1990, 1993). All CAT values were normalized for transfection efficiency by calculating the ratio of CAT activity to β-galactosidase in each transfected plate. Each transfection experiment was separately performed three or more times, with each data point resulting from duplicate or triplicate transfections.

Gel Retardation Experiments Keratinocytes were grown to confluency and then treated with 1000 U IFNγ per ml or 100 ng EGF per ml, or left untreated. After 45 min, cells from two 100-mm dishes were harvested by scraping, collected by precipitation, washed with phosphate-buffered saline, and resuspended in 100 μl of buffer containing 20 mM *N*-hydroxyethylpiperazine–*N'*-2-ethanesulfonic acid (pH 7.8), 450 mM NaCl, 0.4 mM ethylenediamine tetraacetic acid, 0.5 mM dithiothreitol, 25% glycerol, 0.5 mM phenylmethylsulfonyl fluoride. The suspension was freeze-thawed four times and spun at 4°C to remove debris. Whole cell extracts, approximately 5 μg of protein, were first incubated 15 min on ice with or without a 200-fold molar excess of double-stranded DNAs used as unlabeled competitors in 25 μl of binding buffer containing 20 mM Tris-HCl, pH 8, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM ethylenediamine tetraacetic acid, 4 mM spermidine, 10% glycerol, 2% polyvinyl alcohol, and 2 μg of poly(dI-dC).

The probe was end-labeled using the Klenow fragment and [α-^{32}P]dCTP, 50 μCi per reaction, and purified by gel filtration using Sephadex G50 columns (Chroma-Spin, Clontech, Palo Alto, CA). Oligonucleotide probe (80,000 cpm), between 1 and 10 pg, was then added and incubated for additional 30 min on ice. Probe bound to nuclear protein was resolved from free probe through a 5% polyacrylamide gel (acrylamide:bis-acrylamide = 29:1), at 120 V for 150 min. After drying, gels were autoradiographed overnight at −70°C on XAR 5 film (Kodak, Rochester, NY) with screen intensifiers.

Immunohistology All biopsies were taken after obtaining informed consent from patients and laboratory volunteers according to the protocols authorized by the New York University (NYU) Medical Center Institutional Review Board. Four-millimeter punch biopsies were taken from lesional skin of three adult atopic dermatitis patients, one of whom was an acute erythrodermic male patient; the other two were females with recurrent eczematous lesions on the flexor surface of their arms. Four male and four female psoriatic patients gave biopsies from their lesional skin. The forearms of healthy volunteers were injected with a tuberculosis allergen purified protein derivative skin test. After 48 h, punch biopsies were taken from the tuberculosis allergen purified protein derivative injection site and from an uninjected adjacent site on each volunteer. Biopsies were embedded in Tissue Tek OCT compound (Miles Scientific, Elkhart, IN), frozen in liquid nitrogen, and stored at −70°C. Frozen sections were cut from 4 to 6 μm, collected onto gelatin-coated slides, and stained according to standard immunofluorescent staining protocol (O'Guin *et al*, 1987). The primary antibody used was CK17 mouse monoclonal antibody (IBL Research Products, Cambridge, MA). The secondary antibody used was anti-mouse IgG rhodamine conjugate preabsorbed with human serum proteins (Sigma Immunochemicals, St. Louis, MO).

Human epidermal keratinocytes were grown to fourth passage and plated on glass coverslips. These were grown for 72 h in serum-free medium (GIBCO), after which the medium was changed to keratinocyte basal medium and the cells were incubated for another 24 h. The keratinocytes were then treated with IFNγ (1000 U per ml) for varying lengths of time ranging from 1 min to 1 h and with IL-3 (300 ng per ml), IL-6 (1000 ng per ml), GM-CSF (500 ng per ml), or LIF (100 ng per ml) for 30 min. The cells were washed twice with phosphate-buffered saline fixed with methanol:acetone (1:1) for 5 min, dried, and stored at −20°C. The coverslips were stained with the STAT1-specific antibody (gift from Dr. J. Darnell, Rockefeller University Medical Center, New York) and anti-rabbit IgG fluorescein isothiocyanate-conjugated secondary antibody (Sigma Immunochemicals).

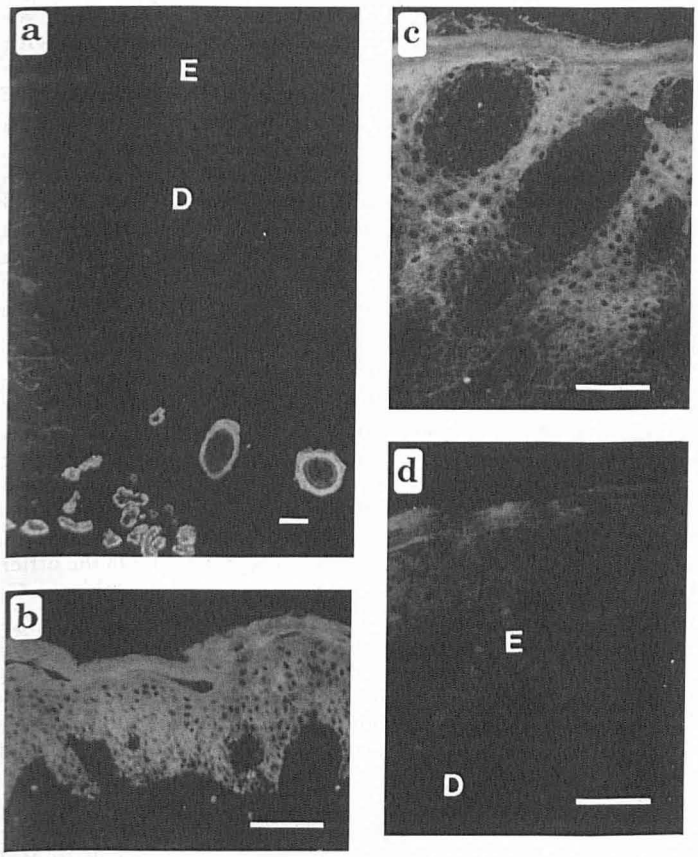

Figure 1. Keratin K17 is not expressed in epidermis from patients with atopic dermatitis, but is expressed in psoriatic epidermis and delayed type hypersensitivity reaction sites. Biopsies were sectioned and stained with K17 specific antibody. The positions of epidermis *(E)* and dermis *(D)* are marked. *Scale bars*, 15 μm. *(a)* Healthy skin. *(b)* Delayed type hypersensitivity. *(c)* Psoriatic lesional skin. *(d)* Atopic dermatitis. The findings presented are representative of eight psoriatic samples, three with delayed type hypersensitivity and three with atopic dermatitis.

RESULTS

Keratin K17 Is expressed in Psoriatic Epidermis but Not in Atopic Epidermis Healthy human interfollicular epidermis does not contain K17 keratin, although in the contractile epithelia of sweat glands and outer root sheet of hair follicles copious amounts of K17 are found **(Fig 1*A*)**. To determine whether K17 is expressed in inflammatory dermatoses, we examined biopsies from three adult patients with atopic dermatitis and three controls. Eight biopsies from psoriasis patients and three with tuberculosis allergen purified protein derivative-induced delayed type hypersensitivity were also obtained. These specimens were sectioned and stained with anti-K17 antibody.

In psoriatic epidermis, K17 was found throughout the cytoplasm of suprabasal cells (de Jong *et al*, 1991; Schlaak *et al*, 1994). The basal layers were completely spared and even the first suprabasal layers express much less K17 keratin than the layers above them **(Fig 1*C*)**. In delayed type hypersensitivity, the staining was also exclusively suprabasal but more patchy, not as homogeneous as in psoriasis **(Fig 1*B*)**. In contrast, atopic dermatitis was completely devoid of K17 keratin **(Fig 1*D*)**. Although the epidermis was as hypertrophic as in psoriasis, and the basal layer just as convoluted, in atopic skin, parakeratosis and keratin K17 are absent.

Th-2–Type Cytokines, IL-4 and IL-10, Did Not Affect the K17 Expression Induced by IFNγ Because we did not detect keratin K17 in lesional skin of atopic dermatitis, where Th-2–type cytokines predominate, we questioned whether those cytokines

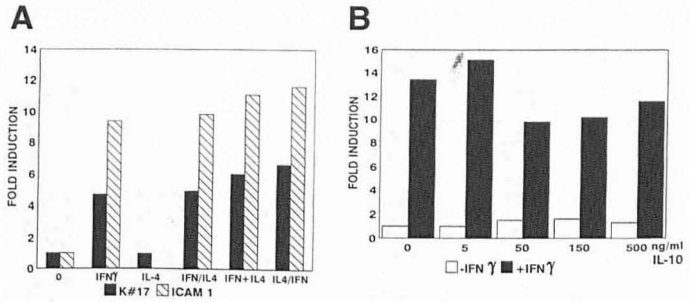

Figure 2. IL-4 and IL-10 have no effect on the induction of K17 by IFNγ. *(A)* Keratinocytes were transfected with K17CAT or ICAM-CAT constructs and incubated with or without IFNγ. IL-4 was added 2 h prior to (*IL4/IFN*), simultaneously with (*IFN+IL4*), or 2 h after addition of IFNγ (*IFN/IL4*). *(B)* Keratinocytes were transfected with K17CAT and incubated with or without IFNγ. IL-10 was added 30 min prior to addition of IFNγ.

interfere with the induction of K17 by IFNγ. Therefore, we transfected plasmid K17CAT into primary cultures of human epidermal keratinocytes, which were then cultured in the presence or absence of IFNγ, in combination with IL-4 or IL-10. Whereas IFNγ strongly induced transcription from the K17 gene promoter, IL-4 by itself did not increase or decrease the transcription of K17, nor did it affect the induction of K17 by IFNγ **(Fig 2*A*)**.

We examined the effect of the time of addition of the two cytokines: we added IL-4 2 h prior to, simultaneously with, or 2 h after addition of IFNγ. Both K17 and ICAM gene promoters were examined, and the results show that the time of addition of IL-4 has no effect on either promoter **(Fig 2*A*)**.

IL-10 has been reported to activate STAT1 in certain cell types and to induce FcγRI expression as IFNγ does. On the other hand, IL-10 is also known to work as an inhibitor of immune reaction in various situations. This prompted us to investigate whether IL-10 can induce K17 as IFNγ does or inhibit the induction of K17 by IFNγ. Experiments in **Fig 2*B*** show that IL-10, like IL-4, neither regulates the expression of K17 by itself nor inhibits the effect of IFNγ. Identical results were obtained when HeLa cells were transfected: neither IL-4 nor IL-10 affected K17 expression in the presence or absence of IFNγ (data not shown). In keratinocytes IL-10 did not cause nuclear translocation of STAT1 (A. Semát and

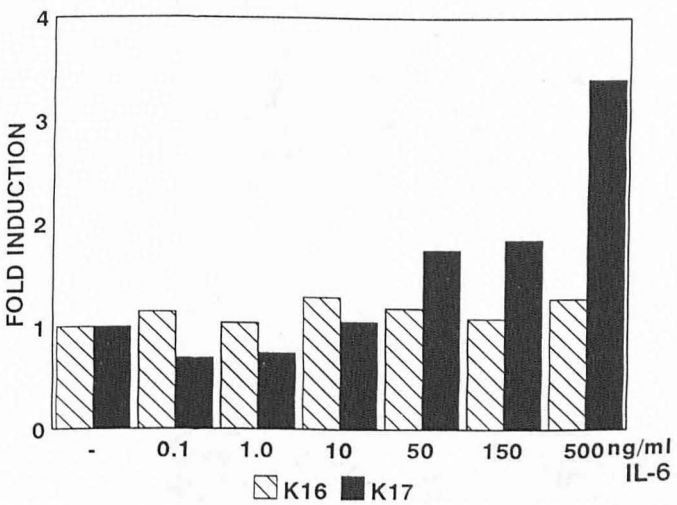

Figure 3. High concentration of IL-6 can induce K17 promoter. Keratinocytes were transfected with K17CAT or K16CAT and then incubated with various concentrations of IL-6. The experiment was performed with two different batches of keratinocytes, both times with duplicate transfections.

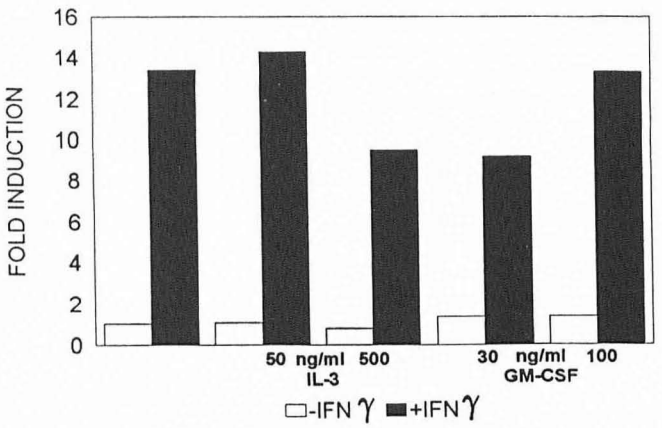

Figure 4. GM-CSF and IL-3 have no effect on the induction of K17. GM-CSF or IL-3 was added to keratinocytes transfected with K17CAT alone or 30 min prior to addition of IFNγ.

M. Blumenberg, unpublished observations). At present we do not know whether the lack of effect by IL-4 and IL-10 in keratinocytes is due to the lack of corresponding receptors, components of signal transduction cascades, or other causes.

IL-6 and LIF Can Induce Transcription of the K17 Gene Promotor The cytokines that share gp130 as the β component of their receptors, namely IL-6, IL-11, LIF, oncostatin M, and ciliary neurotrophic factor, can activate STAT1 in their signal transduction (Gearing *et al*, 1992; Taga *et al*, 1992). Specifically, a high concentration of IL-6 has been reported to induce the binding of STAT1 to the SIE sequence in HepG2 cells (Harroch *et al*, 1994; Yuan *et al*, 1994; Zhong *et al*, 1994). We therefore investigated whether such cytokines can also induce K17 expression in keratinocytes. At high concentration (more than 150 ng per ml), IL-6 was able to induce K17 **(Fig 3)**. As a negative control we transfected a construct containing K16 keratin gene promoter, which responds to EGF but not to IFNγ. The very high concentration of IL-6 required is probably because of an insufficient amount of the α-subunit of the IL-6 receptor. LIF was not able to induce K17 in keratinocytes, which probably do not express LIF receptor in culture. LIF strongly induced K17 expression in HeLa cells (data not shown). These results confirm that those cytokines that can activate the transcription factor STAT1 can induce the expression of keratin K17.

GM-CSF, IL-3, IFNα, or IFNβ Has No Effect on Regulation of K17 Gene GM-CSF and IL-3 belong to the same cytokine family because they share the same β-component of their receptors, p120, distinct from the gp130 described above (Kitamura *et al*, 1991). They have been reported to induce a DNA-binding protein of 80 kDa that binds to the same GRR site as STAT1 and to inhibit the induction of FcγRI by IFNγ (Larner *et al*, 1993). When we tested GM-CSF and IL-3 to determine whether they interfere with IFNγ in induction of K17, we found that these cytokines neither induced K17 expression by themselves nor inhibited the effect of IFNγ **(Fig 4)**. We conclude that signal transduction via p120 has no effect on induction of K17 expression.

Through their receptors and Jak/Tyk kinases, all three interferons activate related transcription factors; namely, IFNα and IFNβ induce ISGF-3, whereas IFNγ induces STAT1, one of the subunits of ISGF-3 (Fu *et al*, 1992; Loh *et al*, 1992). IFNα and IFNβ share a common receptor and signaling pathway. We found that neither IFNα nor IFNβ induce K17 (data not shown), which indicates that the induction of K17 by interferons is specific for IFNγ.

The Effect of Inhibitors of Protein Kinases and Phosphatases on the Induction of K17 by IFNγ To evaluate the involvement of protein kinases in the induction of K17 by IFNγ, after the transfection but before adding IFNγ, we treated the keratinocytes with various kinase inhibitors. Staurosporine, known to inhibit most protein serine, threonine, and tyrosine kinases, as well as genistein, a specific tyrosine kinase inhibitor (Yuan *et al*, 1994), prevented K17 induction by IFNγ **(Fig 5*A,B*)**. On the other hand, bisindolylmaleimide, a specific inhibitor of protein kinase C, did not inhibit induction of K17 expression by IFNγ **(Fig 5*C*)**. Equivalent results were obtained in HeLa cells, and we also tested the effects of genistein on the induction of ICAM1 and found that it can inhibit ICAM1 induction as well (data not shown), confirming that protein phosphorylation, and more specifically tyrosine phosphorylation, is an essential component of induction of K17 and ICAM-1 expression by IFNγ.

Many signal transduction pathways converge at the Ras protooncoprotein (Ullrich and Schlessinger, 1990). Ras transmits, for example, EGF and platelet-derived growth factor signals to Raf protein kinases, which conduct them further to the nucleus. Furthermore, under certain conditions EGF can activate STAT1 (Fu and Zhang, 1993; Ruff-Jamison *et al*, 1993). Although Jak/Tyk signaling in the case of STAT proteins does not directly involve the Ras/Raf pathway, it is possible that the two pathways interact and modulate the regulation of K17 gene transcription. Therefore, we co-transfected a construct expressing a Ras dominant negative mutant protein with K17CAT and found that this mutant had no effect on induction by IFNγ, although it did inhibit induction of K16 by EGF (data not shown). In addition, because cAMP is known to interfere with the Ras/MAPK pathway by phosphorylating and inhibiting protein kinase Raf1 (Wu *et al*, 1993), we raised the intracellular cAMP levels in transfected keratinocytes before adding IFNγ. The results showed that raising the level of intracellular cAMP, while preventing the induction of K16 by EGF, did not interfere with the induction of K17 by IFNγ (data not shown).

We also examined the effects of okadaic acid, a protein phosphatase inhibitor, which is known to induce, *inter alia*, MAPK activation by preventing dephosphorylation of signaling cascade proteins. Okadaic acid alone induced K17 expression and had an

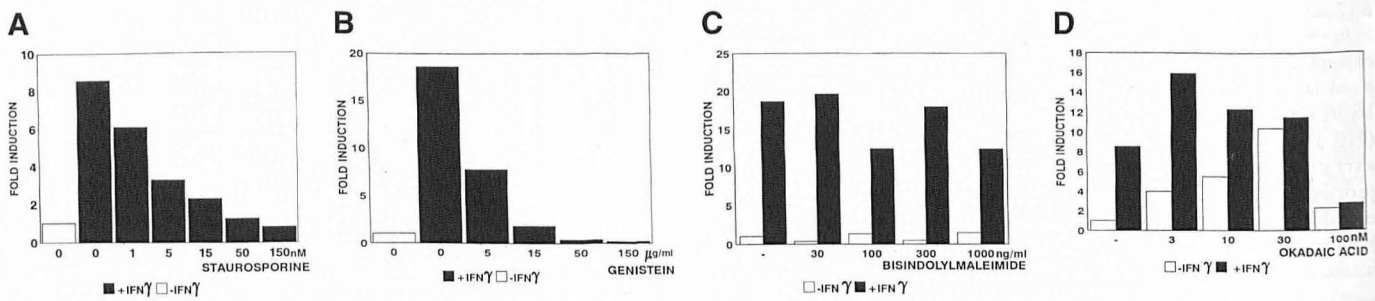

Figure 5. Tyrosine phosphorylation but not protein kinase C is an essential component of induction of K17 by IFNγ. Keratinocytes were transfected with K17CAT construct and incubated with staurosporine *(A)*, genistein *(B)*, bisindolylmaleimide *(C)*, or okadaic acid *(D)* either alone, or in combination with IFNγ. At high concentration (100 nM) okadaic acid inhibits transcription of transfected DNA.

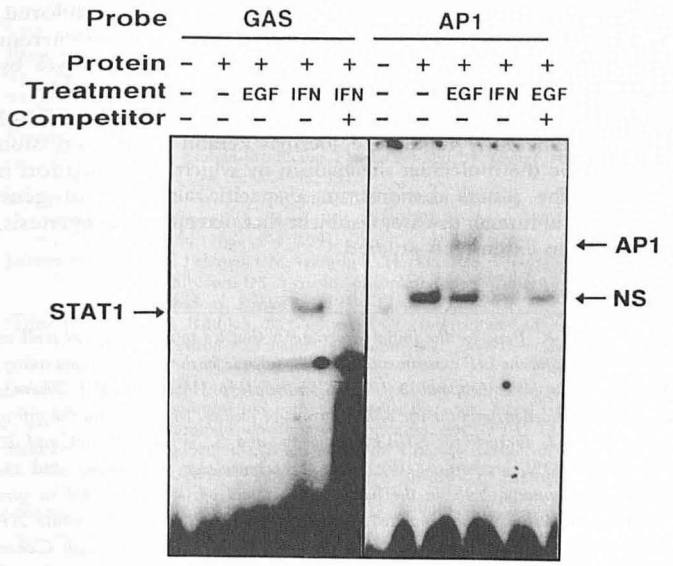

Figure 6. IFNγ strongly and specifically activated protein binding to the STAT1 binding site GAS. Gel-shift analysis of STAT1 activation. *Arrows* point to the specific shifted bands.

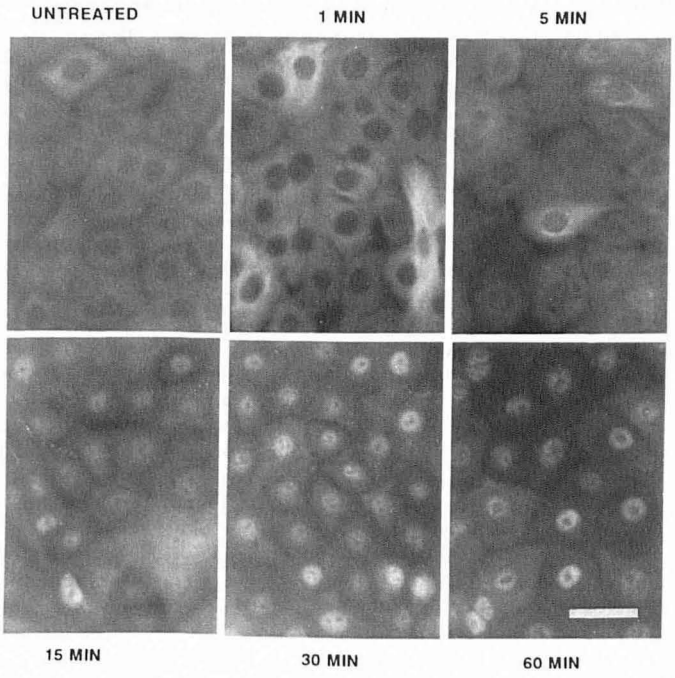

Figure 7. STAT1 translocates into the nucleus in a time-dependent manner after stimulation by IFNγ. Keratinocytes grown on coverslips were exposed to IFNγ and stained with STAT1-specific antibody. *Scale bar*, 15 μm.

additive effect on K17 induction by IFNγ, i.e., caused superinduction **(Fig 5*D*)**. Apparently, dephosphorylation attenuates the IFNγ signal; therefore, blocking the dephosphorylation can augment the induction of K17 expression by IFNγ.

Taken together, these results suggest that the induction of K17 expression proceeds directly via phosphorylation and activation of the STAT1 transcription factor and does not necessarily involve another signal transduction cascade.

Activation of STAT1 in Human Epidermal Keratinocytes

To demonstrate activation of STAT1 in human epidermal keratinocytes by IFNγ, we used gel shift assays. Whole cell extracts were prepared from untreated keratinocytes and keratinocytes treated with IFNγ or EGF. The extracts were added to radiolabeled DNA oligonucleotides that contain either a strong STAT1 binding sequence or the AP1 consensus binding sequence. EGF strongly and specifically activated proteins binding to the AP1 consensus, whereas IFNγ activated binding to the STAT1 binding site **(Fig 6)**. This experiment demonstrates the specificity in activation of the signal-transducing transcription factors.

Upon stimulation by IFNγ, STAT1 is translocated to the nucleus in various types of cells. Because keratinocytes are an important target of IFNγ, and because it seems that IFNγ induces K17 expression through the STAT1 pathway, we determined the time course for translocation of STAT1 into the nucleus **(Fig 7)**. We observed a very weak and diffuse background staining before stimulation. After 1 min of treatment with IFNγ, the STAT1 epitope was found mainly in cytoplasm. After 5 min, it started to translocate into the nucleus. The intensity of the signal in the nucleus increased for 30 min and then remained in the nucleus at least until the 60- min timepoint. The strong cytoplasmic staining 1 min after stimulation was probably due to the activation of STAT1 protein by phosphorylation, which is subsequently followed by translocation to the nucleus.

We examined next whether IL-6, LIF, GM-CSF, or IL-3 causes STAT1 translocation to the nucleus **(Fig 8)**. The cells grown on coverslips were exposed to those cytokines for 30 min. The STAT1 epitope showed weak staining and nuclear translocation with IL-6 and LIF, but IL-3 and GM-CSF had no effect. These results correlate very well with the functional results from transfection experiments.

DISCUSSION

It has been suggested that the majority of infiltrating T lymphocytes in psoriasis and delayed type hypersensitivity are Th-1–like, whereas those in atopic dermatitis are Th-2–like (Hamid *et al*, 1994; Schlaak *et al*, 1994). Using immunofluorescent techniques, we have

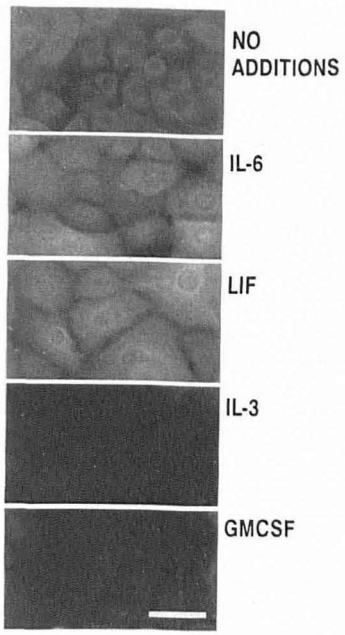

Figure 8. The effects of IL-6, LIF, IL-3, and GM-CSF on nuclear translocation of STAT1. The cells grown on coverslips were exposed to the cytokines for 30 min and then stained with STAT1-specific antibody. *Scale bar*, 15 μm.

shown that keratin K17 is expressed in lesional psoriatic skin and in delayed type hypersensitivity but not in atopic dermatitis.

We hypothesized that the presence of K17 in psoriasis and delayed type hypersensitivity is due to the high levels of IFNγ in the lesional skin and that its absence in atopic dermatitis is either due to low levels of IFNγ or to the inhibitory effects of Th-2–type cytokines in the lesional skin. Thus, we investigated whether IL-4 and IL-10, Th-2–type cytokines, could affect the induction of K17 by IFNγ or suppress the expression of K17 by themselves. We found that IL-4 and IL-10 neither regulate the expression of K17 nor inhibit the effect of IFNγ. This implies that the absence of K17 in atopic dermatitis is due to low levels of IFNγ, rather than to the inhibitory effects of IL-4 and IL-10.

The molecular mechanism of induction by IFNγ depends on activation of STAT1 transcription factor, which is sufficient for expression of K17 keratin. Groups of cytokines and growth factors often act through a common signal transduction mechanism, and we have tested two such groups, one represented by IL-3 and GM-CSF, which act through p120, the other by IL-6 and LIF, which act through gp130 and activate STAT1. Cytokines that activate STAT1, such as IL-6, also induce K17, which may be important, e.g., in ultraviolet-induced damage that is associated with IL-6 release. The presence of IL-6 has been demonstrated in psoriasis (Grossman *et al*, 1989), although IL-6 is produced by many cell types, and indeed skin is a major site of IL-6 catabolism (Castell *et al*, 1990). We show that both IL-6 and IFNγ can be inducers of keratin K17 *in vivo*. We note, however, that LIF activated K17 only in HeLa cells, and not in keratinocytes, which means that the selectivity derives, *inter alia*, from the repertoire of the receptors and signaling molecules. On the other hand, it is clear that the EGF pathway, suggested also to be able to activate STAT1 (Ruff-Jamison *et al*, 1993), does not induce K17 (Jiang *et al*, 1993). Indeed, blocking the EGF signal transduction cascade at Ras and Raf proteins had no effect on K17 promoter transcription. Furthermore, IL-3 and GM-CSF, two cytokines produced by activated keratinocytes, as well as the other two members of the interferon family, IFNα and IFNβ, had no effect on K17 expression. This implies that the induction of expression of K17 is a paracrine effect of lymphocytes and not a result of autocrine stimulation by keratinocytes.

The induction of K17 expression parallels activation of STAT1. Inhibitors of protein phosphorylation, particularly tyrosine phosphorylation, inhibit induction, whereas inhibitors of dephosphorylation augment it. Furthermore, cytokines that promote strong nuclear translocation of STAT1 strongly induce K17, those that cause weak translocation induce weakly, and those that do not cause translocation induce not at all.

Although *in vitro* induction of ICAM-1 seems stronger than induction of K17 **(Fig 2)**, copious amounts of K17 keratin are present in psoriasis *in vivo* **(Fig 1)**. This may reflect a very high stability and slow turnover rates of keratin proteins relative to cell surface markers. Furthermore, although IFNγ causes the expression of ICAM1 and HLA-DR markers in all cell layers (Terui *et al*, 1987; Lisby *et al*, 1989; Singer *et al*, 1989; Esgleyes-Ribot *et al*, 1994), the expression of K17 is strictly suprabasal **(Fig 1)**. This reflects rigorous regulation of keratin gene expression: keratins K5 and K14 are strictly basal, whereas the expression of differentiation markers K1 and K10, activation markers K6 and K16, and K17 is strictly suprabasal. These keratins are excluded from the basal layer.

In addition to STAT1, several transcription factors bind to the promoter of K17 keratin gene (V. Milisavljevic *et al*, 1996). An interferon-regulatory factor consensus element exists upstream from the STAT1 binding site, but the roles of additional elements as independent or accessory transcription factors are not yet clear. For example, K17 keratin protein is a normal constituent of contractile epithelia, where IFNγ is not ordinarily present. These additional transcriptional elements may also be responsible for restricting the expression of K17 protein to the suprabasal cells of the epidermis.

We do not know which cytokines, in addition to IFNγ, induce K17 keratin *in vivo*. The post-transcriptional events, such as those that affect mRNA and protein stability, also remain unexplored. Perhaps the most intriguing question, and a subject of our current efforts, is why IFNγ changes the cytoskeleton of keratinocyte by inducing synthesis of contractile protein in inflamed epidermis.

Nevertheless, our results clearly demonstrate that Th-1 and Th-2 cytokines differently regulate epidermal keratin gene expression and describe the molecular mechanism by which the regulation is effected. The results demonstrate a specific alteration of gene expression in human disease, a subject that, except in oncogenesis, has not been extensively studied.

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