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Interferon-γ-dependent stimulation of human involucrin gene expression: STAT1 (signal transduction and activators of transcription 1) protein activates involucrin promoter activity

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Involucrin is one of the precursor proteins of the cornified cell envelope of keratinocytes, and is expressed during the later stages of keratinocyte differentiation. Interferon-γ (IFN-γ), a pleiotropic cytokine with anti-proliferative and immunomodulatory activities, is also a potent inducer of squamous differentiation. Using cultured normal human keratinocytes (NHK cells) and simian virus 40-transformed human keratinocytes (SVHK cells), we investigated the effects of IFN-γ on involucrin gene expression. Expression of involucrin was increased by about 3-fold after treating NHK cells with IFN-γ (100 units/ml). Northern blot analyses revealed that IFN-γ increased the expression of involucrin mRNA. The fragment +42 to −2463 in the 5′-flanking region of the human involucrin gene was subcloned into a luciferase reporter vector and the construct (p2463Luc) was transfected into SVHK cells. p2463Luc produced a 3-fold increase in luciferase activity after IFN-γ treatment. Sequence analysis detected two putative IFN-γ-responsive regions [G1 (positions −883 to −874) and G2 (−784 to −775)]. Deletion analyses of the p2463Luc vector revealed that the G1 region is critical for the IFN-γ-dependent up-regulation of the involucrin gene. Gel-shift analyses revealed that STAT1 (signal transduction and activators of transcription 1) protein bound to the G1 region and that involucrin promoter activity was augmented by transfection of a STAT1 expression vector in the presence of IFN-γ. In contrast, transfection of a STAT1 dominant-negative expression vector suppressed the IFN-γ-dependent up-regulation of involucrin promoter activity. These results indicate that IFN-γ stimulates expression of the human involucrin gene via the G1 (−883 to −874) region of the involucrin gene promoter.

Key words: cornified cell envelope, gene expression, gene regulation, keratinocyte, transfection.

INTRODUCTION

The cornified cell envelope (CE) is a highly insoluble structure formed beneath the plasma membrane of keratinizing epidermal cells [1–3]. The CE is 15–20 nm thick and is stabilized by cross-linkage of various precursor proteins by *N*-(γ-glutamyl)-lysine isopeptide bonds and disulphide bonds, catalysed by transglutaminase(s) and sulphydryl oxidase respectively [2,4]. The CE is composed of various precursor proteins, including involucrin [5], loricrin [6], cystatin A [7], small proline-rich protein (SPRR/cornifin) [8], elafin [9] and envoplakin [10]. Recent evidence suggests that involucrin is an early component of the CE and provides a scaffold over which other precursor proteins are deposited [9,11].

The human involucrin gene consists of two exons and one intron. The second exon contains the coding region of involucrin, and consists of an ancestral segment and a modern segment [5]. The modern segment is composed of 39 repeats of a consensus 10-amino-acid sequence. The repeating structure of the modern segment is highly conserved in all higher primates [5].

Immunohistochemical analyses have revealed that involucrin is expressed in squamous tissue, and is detected in the upper spinous layer and the granular layer of normal human epidermis [12]. In contrast, psoriatic hyperproliferative epidermis shows high expression of involucrin. Involucrin expression is observed in the suprabasal layer and the infracornified cell layer [13]. Although there is a considerable amount of information regarding the function and evolution of the protein involucrin [14], less is known about the regulation of its gene expression. So far several nuclear factors, including AP-1 (activator protein-1), TEF-1 (transcriptional enhancer factor-1), POU factors and YY-1 (Yin and Yang 1), are known to regulate transcription of the human involucrin gene [15–21].

Interferon-γ (IFN-γ), a cytokine that is produced by activated T cells and natural killer cells, induces various effects, such as anti-viral responses, cell growth and differentiation [22]. Binding of IFN-γ to its receptor induces dimerization of the receptor, resulting in activation of the receptor-associated Janus tyrosine kinases JAK1 and JAK2. Activated JAK1 and JAK2 subsequently activate a latent cytoplasmic protein, STAT1, a member of the family of **s**ignal **t**ransducers and **a**ctivators of **t**ranscription. Finally, activated STAT1 is translocated to the nucleus, where it activates transcription of IFN-γ-responsive genes by binding to a specific DNA element, IFN-γ-activated sequence (GAS), in the promoter of various target genes [23]. In cultured keratinocytes, IFN-γ also causes irreversible growth arrest accompanied by induction of terminal differentiation [24,25].

In the present study, we analysed the effects of IFN-γ on involucrin expression by cultured normal human keratinocytes (NHK cells). We also identified the regulatory site of IFN-γ-dependent promoter activity using various luciferase reporter

Abbreviations used: CE, cornified cell envelope; GAS, interferon-γ-activated sequence; HA, haemagglutinin; IFN-γ, interferon-γ; JAK, Janus kinase; NF-κB, nuclear factor-κB; NHK cells, cultured normal human keratinocytes; SPRR, small proline-rich protein; STAT, signal transduction and activators of transcription; SVHK cells, simian virus 40-transformed human keratinocytes; TRE, PMA ('TPA')-responsive element.

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vectors, which were connected to the 5′-flanking region of the human involucrin gene.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium was purchased from Gibco (Grand Island, NY, U.S.A.). Penicillin and streptomycin were obtained from M. A. Bioproducts (Walkersville, MD, U.S.A.). [α-^{32}P]dCTP and [γ-^{32}P]ATP were purchased from Amersham (Tokyo, Japan). Lipofectin was obtained from BRM (Bethesda, MD, U.S.A.). Antibodies specific for STAT1α and nuclear factor-κB (NF-κB) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.).

Cell culture

NHK cells were cultured in keratinocyte growth medium containing epidermal growth factor (10 ng/ml), insulin (5 μg/ml) and bovine pituitary extract (50 μg/ml) at 37 °C in a 5% CO_2 atmosphere. Simian virus 40-transformed human keratinocytes (SVHK cells) were generously donated by Dr. M. L. Steinberg (Department of Chemistry, City College, City University of New York, NY, U.S.A.) [26]. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 5% (v/v) fetal calf serum, 100 units/ml penicillin and 100 mg/ml streptomycin at 37 °C in 5% CO_2 in air. The SVHK and NHK cells were maintained in a subconfluent state by subculturing every 4–5 days. Cells were seeded into 60-mm-diam. plastic dishes at densities of 4×10^5 cells/5 ml.

Western blotting

Cell extracts were prepared by the method of Etoh et al. [27]. The cells were washed three times with isotonic phosphate buffer (pH 7.5) containing 30 mM EDTA, and then harvested by scraping with a rubber policeman. The cells were then disrupted with 50 strokes of a Dounce-type glass homogenizer and the homogenate was clarified by centrifugation at 100000 ***g*** for 30 min at 4 °C. Cytosolic extract (30 μg of protein) was electophoresed on SDS/6%-PAGE and electroblotted on to nitrocellulose for 1 h in a buffer containing 25 mM Tris/HCl (pH 8.3), 192 mM glycine and 20% (v/v) methanol. The blots were blocked with 5% (w/v) non-fat dried milk in PBS for 1 h at room temperature, and were then incubated at 4 °C overnight with anti-(human involucrin) antibody (Biomedical Technologies Inc., Stoughton, MA, U.S.A.) that had been diluted 200-fold in Tris-buffered saline (pH 7.6). After washing with 0.1% (v/v) Tween-20 in Tris-buffered saline at room temperature, immunodetection was performed using a blotting detection kit for rabbit antibody (Amersham, Tokyo, Japan).

Northern blot analyses

NHK cells (5×10^6) were homogenized in 6 M guanidine isothiocyanate solution, and total RNA was extracted using the caesium/guanidine method [28]. Total RNA (30 μg) was electrophoresed in 1% (w/v) agarose/formaldehyde gels and transferred to nylon filters. The human involucrin gene was labelled with [^{32}P]dCTP by the random primer method. Hybridization was performed as described previously [15].

Plasmid constructs

The deletion vectors, p2463Luc, pr2463Luc (reverse-orientation p2463Luc), p2093Luc, p1611Luc, p824Luc and p705Luc, were generated from a genomic involucrin clone (HI-1) which contains 5 kb upstream of the promoter region [7,21] using a PCR procedure with primers HI-V1–HI-V6: HI-V1, 5′-ATGTGTCA-TGGGATATAGCT-3′ (−2463 to −2444); HI-V2, 5′-CAG-ATACTGAGCCCTGCTAA-3′ (−2093 to −2074); HI-V3, 5′-TTTTGGATTAGATTGTAAAA-3′ (−1611 to −1592); HI-V4, 5′-CTGATCTTTATGCCTTCATG (−824 to −805); HI-V5, 5′-GTGGGCCGTGCTTTGGAGTT-3′ (−705 to −686); HI-V6, 5′-CCAGACTCACAGTAAGGCTG-3′ (+23 to +42). In order to construct the p2463Luc and pr2463Luc expression vectors, we performed PCR amplification using the HI-V1 and HI-V6 oligomers. The PCR product was subcloned into the pCR2.1 vector (Invitrogen, Carlsbad, CA, U.S.A.). The inserts were isolated with *Hin*dIII and *Xho*I and inserted into the *Hin*dIII and *Xho*I sites of the pGL-Basic vector (Promega, Madison, WI, U.S.A.). Vectors p2093Luc, p1611Luc, p824Luc and p705Luc were constructed using HI-V2/HI-V6, HI-V3/HI-V6, HI-V4/HI-V6 and HI-V5/HI-V6 repectively.

Each STAT1-binding-site-deleted fragment was generated by an 'overlap extension' method [29] using PCR with the following oligonucleotides: HI-V7, 5′-AGCTTACAGAGTGGGGAT-TTG-3′ [−898 to −868 with deleted G1 region (−883 to −874)] (antisense oligomer); HI-V8, 5′-CCACTCTGTAAGCTATTT-CTAGA-3′ [−891 to −859 with deleted G1 region (−883 to −874)] (sense oligomer); HI-V9, 5′-GACAAACCCTTAAGT-ACTTTTTT-3′ [−799 to −767 with deleted G2 region (−784 to −775)] (antisense oligomer); HI-V10, 5′-ACTTAAGGGTTTG-TCATTATAAAAAG-3′ [−791 to −756 with deleted G2 region (−784 to −775)] (sense oligomer). To construct the p1606-ΔG1Luc vector, we performed two PCR amplifications using the HI-V3/HI-V7 oligomers or the HI-V8/HI-V6 oligomers with the p2463Luc vector as the template. Next we performed a PCR with the HI-V3/HI-V6 oligomers using mixed products derived from the initial PCR. The second PCR product was subcloned into the pCR2.1 vector. Using the T7 promoter oligomer, we performed sequence analyses to confirm both the orientation of the promoter and the absence of the deleted portion. The deletion fragments, isolated by digestion with *Hin*dIII and *Xho*I, were inserted into the *Hin*dIII and *Xho*I sites of pGL-Basic vector. The p1606-ΔG2Luc vector was constructed using the HI-V3, HI-V6, HI-V9 and HI-V10 oligonucleotides. Using the p1606ΔG1Luc vector, we performed PCR with the HI-V3, HI-V6, HI-V9 and HI-V10 oligomers and constructed the p1606ΔG12Luc vector.

Plasmids expressing haemagglutinin peptide (HA)-tagged wild-type (HA-STAT1) and mutant (Tyr-701 replaced by Phe; HA-STAT1F) STAT1 were kindly provided by Dr. T. Hirano (Osaka University Medical School, Suita, Osaka, Japan) [30]. The β-galactosidase expression vector was kindly supplied by Dr. T. Watanabe (Medical Institute of Bioregulation, Kyushu University, Japan).

Transfection and luciferase assay

Transfection of plasmid DNA into the cells was performed by the liposome method (using lipofectin) [31]. Typically, 5 μg of reporter plasmid and 2 μg of β-galactosidase plasmid were transfected into 1×10^5 SVHK cells. The β-galactosidase plasmid was used as the internal standard to normalize transfection efficiency. After 48 h, the cells were washed twice with PBS, dissolved in 250 μl of cell lysis reagent (Promega) and harvested by scraping. Luciferase assay was performed using a Berthold

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luminometer and Promega luciferase assay kits. Cell extract (20 μl) and luciferase (100 μl) were mixed and the luciferase activity was measured. The β-galactosidase activity in the transfected cell extracts was measured spectrophotometrically [28].

Nuclear extraction and gel-retardation analyses

Nuclear extraction and gel-retardation analyses were performed using the previously described methods [7]. The oligonucleotide probe corresponded to positions −893 to −864, which includes the IFN-γ-responsive site.

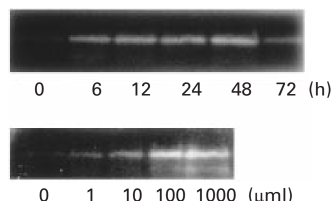

Figure 1 Effects of IFN-γ on involucrin protein expression

Upper panel: NHK cells were incubated with IFN-γ (100 units/ml) for up to 72 h, and cytosolic extracts (30 μg of protein) were used to determine involucrin protein expression. Lower panel: NHK cells were incubated with various concentrations of IFN-γ for 48 h, and cytosolic extracts (30 μg of protein) were used to determine the involucrin protein expression.

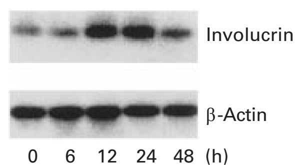

Figure 2 Northern blot analysis of involucrin mRNA in IFN-γ-treated NHK cells

NHK cells were incubated with IFN-γ (100 units/ml) for up to 48 h. Portions of 30 μg of total RNA were used to determine the expression of involucrin mRNA.

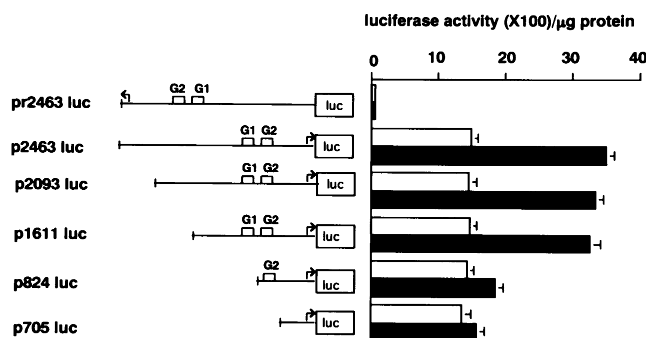

Figure 3 Effect of IFN-γ on involucrin promoter activity

Various luciferase expression vectors were transfected into SVHK cells, followed by incubation for 24 h. The transfected cells were then cultured in the presence (■) or absence (□) of IFN-γ (100 units/ml) for 24 h, and luciferase activity was measured. Average activities were obtained from at least three independent experiments. G1 and G2 indicate the putative IFN-γ-responsive sites.

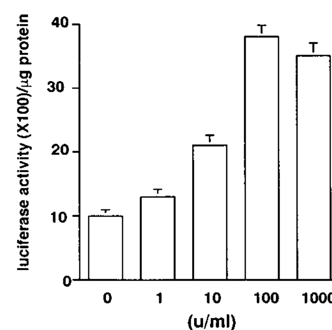

Figure 4 Effects of various concentrations of IFN-γ on involucrin promoter activity

p2463Luc vector was transfected into SVHK cells, followed by incubation for 24 h. The transfected cells were then cultured in the presence of various concentrations of IFN-γ (units/ml) for 24 h, and luciferase activity was measured. Average activities were obtained from at least three independent experiments.

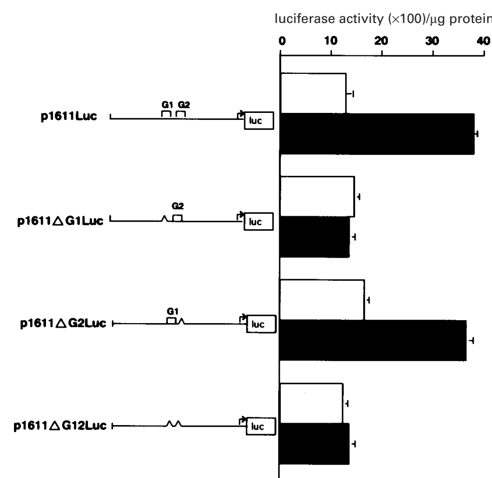

Figure 5 Effects of IFN-γ on luciferase activity following transfection of various IFN-γ-responsive-site deletion p1611Luc vectors into SVHK cells

Various IFN-γ-responsive-site deletion p1611Luc vectors were transfected into SVHK cells, and the cells were treated with IFN-γ (500 units/ml) for 24 h. Vectors: p1611ΔG1Luc, p1611Luc vector with deletion of the IFN-γ-responsive site −883 to −874 (G1); p1611ΔG2Luc, vector with deletion of the IFN-γ-responsive site −784 to −775 (G2); p1611ΔG12Luc, vector with deletion of both IFN-γ-responsive sites (−883 to −874 and −784 to −775). The average luciferase activities were obtained from at least three independent experiments. ■, IFN-γ-treated; □, control.

RESULTS

IFN-γ increases involucrin protein and mRNA levels in NHK cells

To determine the effects of IFN-γ on involucrin expression, we performed Western blot and Northern blot analyses using NHK cells. IFN-γ increased the expression of involucrin protein by about 3-fold. The effect was time- and concentration-dependent. It was detected by 6 h, and the maximal effect was observed at 48 h (Figure 1). An increase in involucrin expression was detected at 10 units/ml IFN-γ, and the maximal effect was observed at 100 units/ml (Figure 1). Northern blot analyses revealed that IFN-γ also increased involucrin mRNA levels, with the maximal effect being observed at 12–24 h (Figure 2). These results indicate

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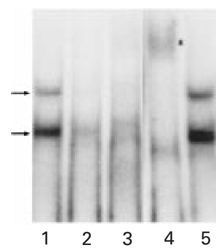

Figure 6 Gel-shift analysis of nuclear extracts from IFN-γ-treated SVHK cells

Nuclear extracts from IFN-γ-treated SVHK cells were treated with the synthesized oligomer containing the IFN-γ-responsive site (-883 to -874). Lane 1, nuclear extract from IFN-γ-treated SVHK cells; lane 2, nuclear extract from IFN-γ-treated SVHK cells in the presence of a 100-fold excess of unlabelled probe; lane 3, nuclear extract from IFN-γ-treated SVHK cells in the presence of a 100-fold excess of unlabelled consensus probe; lane 4, nuclear extract from IFN-γ-treated SVHK cells in the presence of anti-STAT1 antibody; lane 5, nuclear extract from IFN-γ-treated SVHK cells in the presence of anti-NF-κB antibody. Arrows indicate the DNA–nuclear-protein complex. The asterisk indicates the supershifted band.

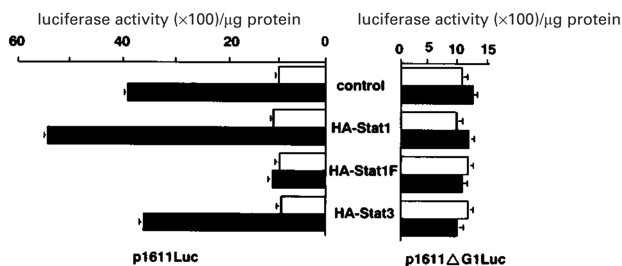

Figure 7 Effects of STAT1 and STAT3 proteins on involucrin promoter activity

STAT1 and STAT3 expression vectors were transfected into SVHK cells with p1611Luc or p1611ΔG1Luc, followed by incubation for 48 h. HA-STAT1 and HA-STAT3 are the STAT1 and STAT3 expression vectors respectively; HA-STAT1F is the dominant-negative STAT1 expression vector. ■, IFN-γ-treated; □, control.

that the increased involucrin protein expression is associated with increased levels of involucrin mRNA.

IFN-γ increases transcription of the involucrin gene

To determine the effect of IFN-γ on involucrin promoter activity, the construct containing the $+42$ to -2463 fragment was attached to the luciferase gene (p2463Luc) and transfected into SVHK cells. p2463Luc resulted in expressed luciferase activity levels 20 times higher than did the reverse-orientation construct (pr2463Luc) (Figure 3) or a construct lacking the flanking region (results not shown). Luciferase activity in p2463Luc-transfected SVHK cells was increased 2–3-fold by IFN-γ (Figures 3 and 4). The effect was observed at 10 units/ml IFN-γ, and the maximal effect occured at 100 units/ml (Figure 4).

The G1 region (-883 to -874) is critical for up-regulation of the involucrin gene by IFN-γ

To identify the regulatory site for the IFN-γ-dependent stimulation of involucrin gene expression, six deletion constructs were transfected into SVHK cells and the cells were cultured in the presence or absence of IFN-γ. The construct containing fragment $+42$ to -1611 responded to IFN-γ (Figure 3). There are two putative IFN-γ responsive sites (G1, AAAGATTTCT; G2, AAAGTAGTTT) within the $+42$ to -1611 region of the involucrin gene (Figure 3). Three IFN-γ regulatory site deletion constructs were transfected into SVHK cells. Deletion of the G1 region (-883 to -874) or the G1 plus G2 (-784 to -775) regions completely abolished IFN-γ-dependent promoter activity (Figure 5). On the other hand, deletion of G2 alone resulted in no loss in IFN-γ responsiveness. These results indicate that the G1 sequence (-883 to -874) is critical for the IFN-γ-dependent promoter activity.

STAT1 binds to the IFN-γ-responsive site (-883 to -874) and increases involucrin promoter activity

The IFN-γ receptor has been shown to activate cytosolic STAT1 protein, which forms a homodimer, is translocated to the nucleus and binds to the GAS on activation [23]. To determine the binding protein(s) in the IFN-γ-responsive site (-883 to -874), a 30 bp synthetic oligonucleotide representing the IFN-γ-responsive site (-883 to -874) was evaluated using a DNA gel-shift assay. Incubation of the oligonucleotide with a nuclear extract from IFN-γ-treated SVHK cells yielded two DNA–protein binding complexes (Figure 6, lane 1). The specificity of the complexes was confirmed by a competition assay using an excess amount of the same unlabelled probe (Figure 6, lane 2) and a STAT1 consensus binding site oligonucleotide probe (Figure 6, lane 3). Furthermore, the addition of anti-STAT1 antibody decreased the specific bands, while a supershifted band appeared near the top of the lane (Figure 6, lane 4). The supershifted band was not detected by the addition of anti-NF-κB antibody (Figure 6, lane 5).

To determine the role of STAT1 protein in transcription of the involucrin gene, wild-type (HA-STAT1) and mutant (HA-STAT1F) STAT1 expression vectors were transfected into SVHK cells. Co-transfection of p1611Luc vector with the wild-type STAT1 expression vector, HA-STAT1, augmented the luciferase activity of IFN-γ-treated SVHK cells (Figure 7). In contrast, the transfection of the dominant-negative vector, HA-STAT1F, abolished the promoter activity of IFN-γ-treated SVHK cells. A wild-type STAT3 expression vector, HA-STAT3, did not affect promoter activity. The increase in promoter activity resulting from transfection of HA-STAT1 was not observed on transfection of p1611ΔG1Luc, which lacks the G1 region (Figure 7).

DISCUSSION

IFN-γ is a multifunctional cytokine that shows anti-viral, anti-tumour, immunoregulatory and pro-inflammatory activities [23]. In keratinocytes, IFN-γ induces the expression of markers of squamous differentiation, such as transglutaminase 1 and SPRR/cornifin, which is accompanied by irreversible cell growth arrest [24]. Ultrastructural analysis has revealed that IFN-γ induces squamous cell differentiation of NHK cells [32]. In the present study we have demonstrated that IFN-γ also induces the expression of involucrin at both the mRNA and protein levels in NHK cells. The difference between the maximal expression levels of mRNA and protein might indicate the time lag between transcription and translation (Figures 1 and 2). The transfection of STAT1 expression vectors and gel-shift assays revealed that the increased expression of involucrin mRNA is mediated by interaction of the STAT1 protein and the GAS present in the 5′ region upstream of the involucrin gene (positions -883 to -874). Since no GAS has been detected in the transglutaminase

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1 or SPRR/cornifin genes, other mechanisms, such as a protein kinase C-dependent pathway, may be involved in the activation of these genes.

It is known that involucrin expression is also increased by PMA, a potent activator of protein kinase C [15–17]. Previously we showed that the α and η isoforms of protein kinase C increased the expression of involucrin, through the interaction between activator protein-1 and the PMA ('TPA')-responsive element (TRE) present in the 5′ region upstream of the involucrin gene (positions −119 to −113) [15]. No increase in protein kinase C activity was detected in IFN-γ-treated SVHK cells (results not shown). The transfection of p1611ΔG1Luc vector, which lacks GAS but contains a TRE, into SVHK cells resulted in increased luciferase activity on addition of PMA (results not shown), but not of IFN-γ. Furthermore, the IFN-γ-dependent increase in involucrin promoter activity was not affected by pretreatment of the cells with the protein kinase C inhibitor H-7 (results not shown). These results suggest that IFN-γ increases involucrin expression through the STAT1 protein, without a significant contribution by protein kinase C.

When IFN-γ binds to its membrane receptor, two members of the Janus tyrosine kinase family, JAK1 and JAK2, are activated, followed by tyrosine phosphorylation and activation of STAT1. Activated STAT1 forms a homodimer that binds to the GAS present in various IFN-γ-dependent genes. It is known that STAT1 is composed of two isoforms, STAT1α and STAT1β, due to alternative splicing. The homodimer of STAT1α activates transcription of GAS-containing genes. In contrast, the homodimer of STAT1β can bind GAS, but does not induce transcription [33]. In addition to STAT1α and STAT1β, a distinct STAT1α-like protein has been described [34]. STAT1α-like protein binds to IFN-γ-responsive element on the intercellular adhesion molecule-1 gene in cultured human keratinocytes [34]. Our results indicate that transfection of the STAT1 expression vector, which is derived from STAT1α cDNA, increased involucrin promoter activity in the presence of IFN-γ. Conversely, a dominant-negative STAT1 expression vector abrogated IFN-γ-dependent promoter activity. Furthermore, two specific DNA–nuclear-protein complexes were totally supershifted by anti-STAT1α antibody. These results indicate that STAT1α protein, but not STAT1α-like or other STAT protein(s), regulates involucrin gene transcription in keratinocytes.

Involucrin expression is markedly increased in the psoriatic hyperproliferative epidermis. Interestingly, infiltrating lymphocytes in psoriasis show a Th1 or Th1-like cytokine profile, secreting IFN-γ [35,36]. Takematsu and Tagami [37] demonstrated increased IFN-γ activity in suction blister fluid from psoriatic skin. This increased activity was due to the elevated levels of IFN-γ protein and mRNA [38]. These findings suggest that IFN-γ plays a significant role in the up-regulation of involucrin expression in the psoriatic epidermis.

In contrast, the expression of the IFN-γ receptor in psoriatic skin has been controversial [39,40]. In normal epidermis, the IFN-γ receptor is observed throughout the epidermis except the cornified cell layer, while it was only detected in the lower part of the psoriatic epidermis [39]. van den Oord et al. [40] reported that IFN-γ receptors were restricted to the basal cell layer in normal epidermis, while in psoriatic lesions additional suprabasal immunoreactive foci or diffuse immunoreactivity of the entire epidermis was observed. The discrepancy might be due to the different antibodies used for the detection of the IFN-γ receptor, or to the fact that psoriatic epidermis can be subdivided into two different types, with and without a granular layer, which have different turnover times [41]. It should be noted, however, that involucrin expression in psoriasis occurs much earlier than normal, close to the basal cell layer where IFN-γ receptors are available. This is in line with the notion that IFN-γ has more marked biological effects in less well-differentiated keratinocytes [42].

Recent evidence indicates that the IFN-γ/STAT pathway inhibits transforming growth factor-β/SMAD signalling through the induction of anti-SMAD, Smad7 [43]. In the present study we have identified transcriptional regulation of the involucrin gene by IFN-γ via the STAT1-dependent pathway. Saunders and Jetten [24] described similar IFN-γ-dependent induction of transglutaminase 1 and SPRR/cornifin. Thus IFN-γ appears to affect keratinocyte proliferation and differentiation via the STAT-mediated pathway, the characterization of which will clarify the nature of the regulatory mechanisms in keratinocyte biology.

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