C-fos and c-jun Proto-Oncogene Expression Is Decreased in Psoriasis: an In Situ Quantitative Analysis

Nicole Basset-Séguin, Chantal Escot, Jean Pierre Molès, Jean Marie Blanchard, Cécile Kerai, and Jean Jacques Guilhou
Laboratoire de Recherche Dermatologique (NB-S, JPM, CK, JJG), INSERM U128 (CE), and Laboratoire de Biologie Moléculaire URA-CNRS 1191 (JMB), Montpellier, France

Psoriasis is a common, sometimes severe, non-malignant skin disease characterized by hyperproliferation and abnormal differentiation of keratinocytes. Because proto-oncogenes are implicated in both cell proliferation and differentiation, their expression could be modified in skin diseases such as psoriasis. The c-fos and c-jun proto-oncogenes, whose products associate to form a heterodimeric transcription factor, are among the first genes to be expressed when certain cells are stimulated to either proliferate or differentiate. Recent studies in our laboratory have shown that the c-fos proto-oncogene is highly expressed in normal human adult skin. In the present study, we used in situ hybridization with RNA to compare the expression and localization of c-fos and c-jun transcripts in 15 lesional and non-lesional psoriatic skin samples. Two clinical variants of psoriasis were studied: the most severe and chronic form or plaque-type psoriasis (N = 10) and rapidly resolutive guttate-type psoriasis (N = 5). Quantitative analysis was performed using a semi-automated image analyzer and the “Starwise grain” software program. Our control samples included 10 normal skins and eight specimens from other benign hyperproliferative non-psoriatic skin diseases, consisting of three with inflammation (seborrhic dermatitis and atopic dermatitis), and 5 without inflammation (seborrhic keratoses). Control genes we used for in situ hybridization and RNA integrity were keratin 14, which is expressed in the epidermis and was normally expressed in all tissue analyzed, and ribosomal RNA. Our data showed that c-fos and c-jun were expressed to an equivalent extent, both spatially and quantitatively, in all specimens tested. Expression was significantly decreased in plaque-type but not in guttate-type psoriasis. It was also decreased in the three other benign inflammatory cutaneous hyperproliferative disorders, but not in the five non-inflammatory cases. These results were surprising because hyperproliferation was here associated with a decrease in proto-oncogene expression, thus suggesting that c-fos and c-jun do not play a crucial role in the control of keratinocyte proliferation in vivo. However, their reduced expression in some abnormally differentiated skins indicates that both c-fos and c-jun proto-oncogenes may play a key role in keratinocyte differentiation. Their altered expression correlated with severity of the disease and the presence of an inflammatory infiltrate. These data offer a new insight into the role and regulation of these proto-oncogenes in vivo in humans. J Invest Dermatol 97:672–678, 1991

Proto-oncogenes are recognized genes that play a key role in cellular proliferation. Many have been implicated in cell transformation both in vivo and in vitro, after activation by various carcinogens, and this phenomenon is still under extensive study [1–5]. Moreover, increasing efforts are being made to define their role, if any, in benign human disorders and in normal tissues. The c-fos and c-jun proto-oncogenes encode nuclear proteins forming a complex (AP-1) that recognizes a specific DNA sequence within the promoter region of several cellular genes [6–8]. The DNA binding activity of c-fos/c-jun heterodimers leads to both positive and negative transcriptional control of target genes [9–14]. Induction of c-fos and c-jun expression is an immediate-early event that occurs in response to a wide variety of stimuli in many human cell types [15–23]. This early-gene induction has been shown to play a role in cell proliferation and/or differentiation and the importance of this role varies according to the cell type studies [24–34].

We recently reported that the c-fos proto-oncogene is highly expressed in normal human adult skin [35]. In order to further understand the role of c-fos and c-jun in the pathophysiology of human skin, we compared their expression in normal skin, psoriatic skin, and other benign hyperproliferative skin disorders. Psoriasis is

NOR: normal skin
LES: lesional
NLES: non-lesional
PT: plaque-type
GT: guttate-type
ISH: in situ hybridization
SK: seborrheic keratoses
TPA: tetradecanoyl-phorbol-acetate
35S UTP: uridine 5' (α35S) thiotriphosphate
GCH: glucocorticoid hormone

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Reprint requests to: Pr. J.J. Guilhou, Service de Dermatologie-Phlébologie, Hôpital Saint-Charles, 34059 Montpellier cedex France.

Abbreviations:
K14: keratin 14
SCBC: small cell basal compartment
LCUC: large cell upper compartment
mRNA: messenger RNA
a common (2% of the general population) dermatosis characterized by an approximately tenfold increase in keratinocyte growth kinetics and transit through the epidermis. Its origin is still unknown and numerous potential pathogenesis pathways have been studied [36–41]. Moreover, psoriasis is a good model for studying the mechanisms underlying hyperproliferation of the epidermis. The prime role played by cellular oncogenes in cell proliferation prompted us to study whether these genes are important in the hyperproliferative state of psoriatic keratinocytes. We present here a comparative quantitative analysis of the expression of c-fos and c-jun in normal, psoriatic, and other hyperproliferative human skins.

MATERIAL AND METHODS

Probes and Tissues The human c-fos probe has been previously described [35]. The murine c-jun probe was a generous gift from R. Bravo (Hind III–EcoRI, 1.8-kb insert cloned in pBS). The human keratin 14 probe was kindly provided by D. Roop (Houston, Texas) and corresponded to a PstI–PstI, 200-bp insert cloned in pGEM. The filaggrin probe (BamH1–BamH1 1.2-kb insert) was a generous gift from P. Steinert (Bethesda, Maryland). The murine 28S rRNA probe was a 1.3-kb EcoRI–BamH1 insert cloned in pBS. Anti-sense RNA probes were synthesized from linearized plasmids using an SP6/T7 in vitro RNA synthesis kit (Amersham) in the presence of 35S UTP (specific activity 800 Ci/mmoles).

Tissue samples were obtained from normal healthy volunteers (NOR) undergoing plastic surgery (N = 10). Lesional (LES) and non-lesional (NLES) skin of plaque-type (N = 10) and guttate-type (N = 5) psoriatic patients, as well as other benign cutaneous lesions with hyperacanthosis (five clinically and histologically non-inflammatory seborrheic keratoses, two atopic, and one seborrheic dermatitis) were obtained after informed consent. Immediately after removal, tissue samples were embedded in OCT (Miles France), snap frozen in liquid nitrogen, and stored at −70°C until use.

In Situ Hybridization Experiments In situ hybridization (ISH) was performed as described previously [3–35]. All hybridized sections were exposed in Ilford K5 gel emulsion. Exposure was 1 week for c-fos, c-jun, and control probes (sense K14 probe and RNA transcribed from the SP6 vector alone) and 4 d for K14 anti-sense hybridized sections. Slides were developed in Kodak Dektol D19 for 150 seconds at 22°C and then stained with hematoxylin and eosin. RNA integrity was checked by hybridization of sample sections of the tissue with a murine antisense 28S RNA probe (exposed for 2 d); all tissues studied showed normal ribosomal RNA expression.

Quantification of In Situ Hybridization Hybridized signals for c-fos and c-jun probes on autoradiographed sections was quantified by semi-automatic counting of silver grains (Imstar image analyzer, Starwise grain software program, France) as previously described [42].

Cell sizes used in this study were determined as the mean surface of 100 individual cells in each sample of hematoxylin and eosin stained normal and pathological skin sections (three different specimens of each pathologic tissue were studied). Two cell compartments were defined: the small cell basal compartment (SCBC) and the large cell upper compartment (LCUC) as shown in Fig 1. Cells in both compartments were larger in hyperacanthotic tissues than in normal skin. These differences were taken into account when calculating the number of grains per cell for all tissue specimens tested.

The counting procedure is summarized in Fig 1. Background values (BG) were evaluated in dermal areas with few cells (Fig 1B).
The number of grains per cell was calculated for both the SCBC (Fig 1C) and the LCUC (Fig 1D). Each biopsy was analyzed in two independent experiments and hybridization signals were quantified in at least 300 epidermal cells per experiment. The variance in successive assays was negligible, in agreement with the results of previous studies [3,42]. No significant difference was observed when measuring at least 40 tissues in two separate experiments (Student t test, p > 0.5%). It was thus possible to assess differences between cell compartments and tissue samples relative to the distribution of probes of the same approximate length exposed during the same period of time. Differences due to probe types and sequences could not be avoided. In this study, we performed a comparative and quantitative analysis for the c-fos and c-jun probes. Negative control slides hybridized with the K14 sense probe or transcripts derived from the plasmid alone gave the same signals as the background [3,35].

### RESULTS

#### Hybridization and Quantification of Skin Sections with Image Analyzer

In situ hybridization is a unique method for localizing RNA expression on heterogeneous tissue sections. As shown in Fig 2, the localization of transcripts in various skin compartments varied according to the gene studied. For instance, in normal human skin, c-fos transcripts were found throughout the epidermis, but with a significant increase in the number of grains per cell in basal keratinocytes. Keratin 14 expression was localized in basal and suprabasal keratinocytes with a consistent and strong intensity, whereas filagrin expression was restricted to the granular epidermal layer (Fig 2). The presence and distribution of all of these transcripts were correlated with the presence of the corresponding proteins, as confirmed by immunofluorescence (data not shown).

#### Variations in c-fos and c-jun Proto-Oncogene Expression Were Parallel in all Tissue Samples Studied

Generally, in the various tissue types, levels of expression of c-jun were very close to those of c-fos (Table I). These two proto-oncogenes were also co-localized in all instances. Indeed, c-fos and c-jun transcripts were found throughout the normal and pathologic epidermis but a significantly higher number of grains per cell was found in the basal compartment than in the upper compartment (Fig 3A, B, and C).

#### The Expression of c-fos and c-jun is Significantly Decreased in Chronic Plaque-Type Psoriasis

Comparative analysis of grain counts per cell obtained after ISH of the c-fos and c-jun probes

<table>
<thead>
<tr>
<th></th>
<th>SCBC</th>
<th>LCUC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c-fos(SE)</td>
<td>c-jun(SE)</td>
</tr>
<tr>
<td>Normal skin (n = 10)</td>
<td>28.2 (1.9)</td>
<td>31 (2.6)</td>
</tr>
<tr>
<td>Lesional plaque-type psoriatic skin (n = 10)</td>
<td>8.3 (0.9)</td>
<td>10 (1.6)</td>
</tr>
<tr>
<td>Lesional guttate-type psoriatic skin (n = 5)</td>
<td>21.2 (4.7)</td>
<td>28 (4.4)</td>
</tr>
<tr>
<td>Nonlesional plaque-type psoriatic skin (n = 10)</td>
<td>25 (2)</td>
<td>33.5 (1.9)</td>
</tr>
<tr>
<td>Nonlesional guttate-type psoriatic skin (n = 5)</td>
<td>30.2 (3.9)</td>
<td>28.9 (3)</td>
</tr>
<tr>
<td>Inflammatory hypertrophic skin diseases (n = 3)</td>
<td>8 (3.1)</td>
<td>10.3 (3.6)</td>
</tr>
<tr>
<td>Seborrheic keratoses (n = 5)</td>
<td>26.6 (2.2)</td>
<td>26.4 (4.9)</td>
</tr>
</tbody>
</table>

*Results are given in number of grains per cell.*

![Figure 2](image.png)

**Figure 2.** Gene expression can be localized by ISH in heterogeneous tissues. Levels and localization of c-fos (A), keratin 14 (B), and filagrin (C) transcripts are shown in normal skin (NOR) and psoriatic skin (LES).
Figure 3. ISH of normal skin (NOR), lesional (LES), and non-lesional (NLES) plaque-type psoriatic skin with c-fos (A), c-jun (B), and keratin 14 (C) probes.
with various tissues revealed a significant ($p < 0.001$) and similar decrease in the expression of both oncogenes in lesional (LES) plaque-type (PT) psoriatic skin, when compared to the expression observed in normal (NOR) or non-lesional psoriatic skin (NLES) (Fig 3A, B). This decrease was seen in both the basal and upper compartments (Table I). Conversely, the K14 probe showed a normal pattern of expression in all of these tissue samples (Fig 3C).

The Levels of c-fos and c-jun Transcripts Differ in the Gut-tate-Type Psoriasis from Those of Plaque-Type Psoriasis Lesional and non-lesional skin from five patients with acute guttate-type (GT) psoriasis were analyzed for c-fos and c-jun expression and compared to chronic PT psoriasis. In all of these GT psoriasis cases, hyperkeratosis (an increased number of keratinocyte layers) was less pronounced than in the PT cases. In contrast to PT psoriasis, we observed no significant decrease in the number of grains per cell in LES skin of GT psoriasis when compared to NOR or NLES GT psoriatic skin (Table I).

Decrease in c-fos and c-jun Transcript Levels Is a Common Feature of Benign Hyperproliferative Cutaneous Disorders

Eight other benign hyperproliferative skin diseases were analyzed for c-fos and c-jun expression. These studies showed that a similar and significant ($p < 0.001$) decrease in the expression of both oncogenes was present in disorders involving morphologic changes of the skin that resemble changes that occur in psoriasis, such as atopic or seborrheic dermatitis. These disorders were similar with respect to the presence of an intense inflammatory infiltrate and a hyperkeratotic and hyperproliferative epidermis (Fig 4). Conversely, no decrease in the expression of these proto-oncogenes was found in seborrheic keratoses, a non-inflammatory benign cutaneous tumor (Table I, Fig 4). Nevertheless the proliferative and hyperkinetic capacity of keratinocytes in seborrheic keratoses is not clearly demonstrated. This may explain the normal expression of c-fos and c-jun proto-oncogenes in that pathology.

DISCUSSION

In situ hybridization and quantification with a semi-automatic image analyzer were used to compare c-fos and c-jun proto-oncogenes transcripts in pathologic versus normal human skin. In the present study, we show that c-fos expression varied concurrently with that of c-jun in all tissue specimens studied. Moreover, expression of both proto-oncogenes was significantly decreased in plaque-type psoriatic skin, but not in guttate-type psoriatic skin. This decrease was not specific to psoriasis but was common to all dermatosis cases characterized by hyperproliferation and inflammation.

We chose to study c-fos and c-jun expression by ISH for several reasons: 1) results of ISH on normal human skin with the c-fos probe had been previously confirmed at the RNA level by Northern blot and at the protein level by immunofluorescence and Western blot [35]. 2) ISH requires only limited amounts of biologic material, thus is easily obtainable from patients. 3) ISH allows localization of cells expressing the gene to be studied. This is important when studying heterogeneous tissues like human skin. 4) Quantification, which was previously considered as being difficult with ISH, has been facilitated with the development of specific software and image analyzers.

Increased expression of some cellular oncogenes has been found in hyperproliferative tissues and cells [1]. Accordingly, a number of studies have shown that the c-fos and c-jun proto-oncogenes play a crucial role in cell proliferation [24,25]. We recently reported that the c-fos proto-oncogene was highly expressed in normal human skin and that most transcripts could be found in the basal epidermal compartment that contains proliferative skin cells. However, contradictory results were obtained in recent studies concerning c-fos ex-

Figure 4. ISH of seborrheic dermatitis (SD), atopic dermatitis (AD), and seborrheic keratosis (SK) with c-fos (A) and c-jun (B) probes.
pression in full-thickness psoriatic skin. Mordovtsev reported an increase in c-fos expression by dot blot hybridization of total RNA extracts [43], whereas Elder found a slight decrease in c-fos transcripts in total RNA extracts analyzed by Northern blot [44]. Differences in the extraction and hybridization methods could explain these discrepancies. In the present study, we investigated c-fos and c-jun expression in psoriatic and non-psoriatic hyperproliferative skin diseases. We confirm and extend Elder's results by showing a decrease in c-fos and c-jun transcripts, which we localized using ISH in both epidermal compartments of plaque-type psoriatic skin. These results can be interpreted in various ways. We excluded any problem of RNA degradation in psoriatic skin by checking for ribosomal RNA and the K14 gene expression in all tissues tested. Defective cellular pathways leading to c-fos and c-jun expression could be possible. Interestingly, a c-AMP-responsive, a TPA-responsive and a serum-responsive element are present in the promoter region of c-fos and c-jun genes [20–23]. A defect in A and C protein kinases has also been reported in psoriatic skin [38,39,45]. However, TPA-induced c-fos and c-jun expression was previously found to be normal in psoriatic skin [44]. An abnormality in the c-AMP pathway regulating c-fos expression could be possible but has yet to be documented. We performed studies using an organ-culture system in which keratinocytes are allowed to grow around and from the skin biopsy on a sterile coverslip. In that system, c-fos expression, studied by immunohistochemistry procedure and with a specific rabbit anti-human c-fos antibody (as previously described [35]), was identical after serum induction in psoriatic or normal skin-derived keratinocytes. These preliminary results (data not shown) indicate that the serum response element present in the promoter region of the c-fos gene functions normally in psoriatic skin-derived keratinocytes. The decrease in c-fos and c-jun transcript levels could also be explained by a higher instability or a down-modulation of these specific messenger RNA. We did not address these questions directly, but we did note that the c-fos protein, which down-modulates its own transcription [13], was not increased in lesional psoriatic skin (data not shown).

Although it is surprising that these genes were not expressed in hyperproliferative psoriasis tissues, it should be pointed out that 1) their expression in normal skin is not only restricted to proliferating cells, known to be strictly limited within basal keratinocytes [46,2] and decrease in the expression of these proto-oncogenes affects both the basal and the upper epidermal compartments, thus suggesting that this decrease could be more linked to the abnormal phenotype of the cells than to their proliferative capacity. Moreover, a slight but non-significant decrease in c-fos and c-jun expression was observed in non-lesional psoriatic skin, suggesting that this abnormal gene expression is latent in normal-appearing skin of psoriatic patients. In lesional cuttate-type psoriatic skin, no significant decrease in the expression of both proto-oncogenes was observed. Differences in the presentation and clinical course of these two forms of the disease suggest that chronicity, and more pronounced morphologic changes in the epidermis as observed in PT psoriatic skin, are required for any significant decrease in c-fos and c-jun expression. Moreover, these proto-oncogenes are also less expressed in other chronic inflammatory hyperproliferative diseases in which morphologic epidermal changes are very close to changes observed in psoriatic skin. These results suggest that inflammation can influence c-fos and c-jun expression. Nevertheless, inflammation is present in both PT and GT psoriasis and is generally more pronounced in the latter case in which c-fos and c-jun expression is not significantly decreased. Mutual transrepression between the glucocorticoid receptor and AP1 activity was recently demonstrated [47–49]. Low hormone concentrations are required for transrepression of AP1 by the glucocorticoid hormone (GCH). During stress, GCH concentration is high and patients with chronic inflammatory cutaneous disorders are under tension. This may explain the low level of c-fos and c-jun transcripts, reflecting the low AP1 activity in these diseases. It could be speculated that this high GCH level also corresponds to a physiologic “anti-inflammation” reflex to these pathologic states.

If c-fos and c-jun do not play a role in psoriatic keratinocyte proliferation, they could be implicated in cell differentiation [27–34]. Psoriatic keratinocytes have an abnormal differentiation maturation [50]. Because c-fos and c-jun act as transcriptional activators for a number of genes [6–14], the appearance of an abnormal phenotype could be a consequence of their decreased expression, as seen in psoriasis. In vitro studies are required to understand the role of these proto-oncogenes in the physiology of human epidermal cells. Finally, another common feature of skin disorders characterized by decreased expression of c-fos and c-jun is the presence of an inflammatory infiltrate. This finding may be of relevance for regulating the expression of these genes in vivo.

In conclusion, this study shows that keratinocyte hyperproliferation in psoriasis is not associated with overexpression of c-fos and c-jun proto-oncogenes and suggests that it involves a different cellular pathway.

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