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Interleukin-13 Receptor in Psoriatic Keratinocytes: Overexpression of the mRNA and Underexpression of the Protein

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Although several cytokines and their receptors have been involved in the development of psoriasis, the etiology is still unknown. In this study we looked for genes possibly involved in the disease by the reverse transcription–polymerase chain reaction differential display technique in lesional and nonlesional skin biopsies from psoriatic patients. We found the mRNA of the α1 chain of the interleukin-13 receptor expressed differentially in psoriatic biopsies. By reverse transcription–polymerase chain reaction, we confirmed an overexpression of the α1 chain of the IL-13 receptor and α chain of the interleukin-4 receptor mRNA in lesional skin psoriatic biopsies, when compared with skin biopsies from healthy subjects ($p < 0.01$). The nonlesional skin obtained from a region close to a lesional zone in psoriatic patients presented also an overexpression of these mRNA in 50% of the samples. Interleukin-13 and interleukin-4 were not detected either as mRNA or as the proteins in any of the biopsies from psoriatic patients or healthy subjects. A monoclonal antibody to the α1 chain of the interleukin-13 receptor detected the receptor in the epidermal keratinocytes of psoriatic patients and of healthy subjects; however, the positive antibody reaction was stronger in skin tissue from healthy subjects than in psoriatic lesional skin tissue ($p < 0.01$), although the mRNA was overexpressed. As interleukin-13 is a pleiotropic immunoregulatory cytokine with a variety of effects on different cell types, including monocytes, B lymphocytes, mast cells, and keratinocytes, we suggest, based on our results, that the interleukin-13 receptor possibly plays an important part in the early inflammatory process of psoriasis; however, its function is lost in the psoriatic keratinocytes.* ***Key words: Interleukin-13/receptor psoriasis/keratinocytes/IL-13 J Invest Dermatol 119:1114–1120, 2002***

Psoriasis is a chronic skin disease characterized by a hyperproliferation of keratinocytes and by an immunologic cellular infiltrate, including activated T lymphocytes, producing multiple cytokines that can influence the epidermal keratinocytes. The specific mechanism through which the keratinocytes attain their hyperproliferative state in psoriasis is unknown. The proliferation and the differentiation of keratinocytes is controlled by growth factors and cytokines, such as the epidermal growth factor, keratinocyte growth factor, transforming growth factor-α, granulocyte-macrophage colony-stimulating factor, interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor-α (Kupper *et al*, 1986; O'Keefe *et al*, 1988; Kamalati *et al*, 1989; Brown and Halper, 1990; Kock *et al*, 1990; Luger and Schwarz, 1990) some of which are produced by the keratinocytes themselves. The expression of some of these cytokines is upregulated in inflammatory skin diseases, and these cytokines are mediators of the leukocyte chemotaxis and the inflammatory and immune responses (Nickoloff, 1991; Nickoloff, 1999).

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Abbreviations: IL-13Rα1, α1 chain of IL-13 receptor; IL-4Rα, α chain of IL-4 receptor.

At the molecular level, the overexpression of growth factors and pro-inflammatory cytokines and their receptors has been described in psoriatic patients. The IL-12 produced by antigen-presenting cells very early during infection *in vivo* has an important pro-inflammatory function. This cytokine plays a part in the differentiation of naive T cells into interferon (IFN)-γ-producing T helper 1 cells (Trinchieri, 1998). IL-12 has already been found overexpressed in free nerve endings of the epidermis, dermal nerve fibers, and in lesional psoriatic skin (Turka *et al*, 1995; Yawalkar *et al*, 1998). Schon *et al* (1997) presented evidence that a particular splenic T cell subset ($CD4^+CD45Rb^{high}$) the same T cell subset that induces colitis in *scid/scid* mice, induces psoriasis-like lesions when transferred into minor haplotype mismatched *scid/scid* mice. Hong *et al* (1999); reported that the IL-12, independently of IFN-γ, plays a crucial part in the pathogenesis of the murine psoriasis-like skin disorder. On the other hand, IL-17 is produced by activated $CD4^+$ T cells and can stimulate epithelial cells. IL-17 together with IFN-γ enhances the mRNA and protein production of pro-inflammatory cytokines IL-6 and IL-8 as well as IL-15. It also enhanced the production of intercellular adhesion molecule-1 and HLA-DR in normal human keratinocytes. The majority of the $CD4^+$ and $CD8^+$ T cell clones derived from lesional psoriatic skin expressed IL-17 mRNA, suggesting that skin-infiltrating T cells can produce this cytokine. In addition, IL-17 mRNA is detectable in biopsies from lesional psoriatic skin, but not in nonlesional control biopsies (Teunissen *et al*, 1998). Epidermal keratinocytes from psoriatic skin lesions

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have shown an increase of the expression of pro-inflammatory IL-8 and its receptor (IL-8R) (Schulz *et al*, 1993; Lamster *et al*, 1995). It has also been demonstrated that the combination of IL-8 and IFN-γ induces the expression of inducible nitric oxide-specific mRNA and of the functional protein in cultured human keratinocytes (Kolb-Bachofen *et al*, 1994). These results suggest an important role for inducible nitric oxide in conjunction with IL-8 and its receptor during the early formation of psoriatic lesions (Bruch-Gerharz *et al*, 1996). IL-15 and its receptor inhibit apoptosis of keratinocytes and have been found to be overexpressed in lesional psoriatic epidermis (Rückert *et al*, 2000), possibly contributing in the formation of the psoriatic lesion. Recently, Blumberg *et al* (2001) reported that overexpression of IL-20 in transgenic mice provokes neonatal lethality, with skin abnormalities, including aberrant epidermal differentiation. Histologic analysis of the skin of the IL-20 transgenic mice showed characteristics similar to that observed in human psoriatic skin; however, immune infiltrates were not observed in the IL-20 transgenic mouse skin. In the biopsies of psoriatic patients there was overexpression of IL-20Rα and IL-20Rβ.

Two other cytokines that are gaining increased importance as negative modulators of inflammatory processes are IL-10 and IL-11. Expression of IL-10 and its receptor have been shown to be decreased in psoriatic skin. These molecules are down-modulated by the pro-inflammatory cytokine IL-8, and are induced by anti-psoriatic glucocorticosteroids in normal cultured keratinocytes (Günter *et al*, 1997). Treatment of psoriatic patients with recombinant human IL-11 caused a reduction in lesion severity that correlated with a polarization toward a T helper 2 phenotype in lesional tissue, as measured by a reduction in IFN-γ mRNA and an elevation of IL-4 mRNA, compared with the pretreatment levels (Trepicchio *et al*, 1999). In a cell culture model of murine T cell differentiation, recombinant human IL-11 acts by inhibiting IL-12-induced IFN-γ production and enhances IL-10 production. The expression of the IL-11Rα and the cytokine were detected in human and murine $CD4^+$ and $CD8^+$ lymphocytes, which suggests that recombinant human IL-11 can directly interact with T cells (Bozza *et al*, 2001).

In order to find other molecules that could be involved in psoriasis, in this study we used the reverse transcription–polymerase chain reaction–(reverse transcription–PCR) differential display technology, in lesional and nonlesional skin from psoriatic patients. We found overexpression of IL-13 receptor α1 (IL-13Rα1) mRNA in lesional skin, but a low expression of the protein.

MATERIALS AND METHODS

Human skin samples For the reverse transcription–PCR differential display assay 4 mm skin biopsies were taken from lesional and nonlesional skin from three patients with untreated psoriasis. For the gene expression analysis (reverse transcription–PCR) and the immunohistochemical studies, biopsies from lesional and nonlesional skin were taken from another 10 patients with psoriasis. Biopsies of lesional skin were taken from one patient each with atopic dermatitis, polymorphous light eruption, pemphigus vulgaris, lichen planus, seborrheic keratoses, and dermatofibroma. Biopsies of normal skin were also taken from 10 healthy subjects, which were used as a control group. Every fresh biopsy was cut into two parts. One part was immediately frozen in liquid nitrogen and was used for RNA extraction and the other part was fixed in 4% p-formaldehyde and used for immunohistochemical analysis. Written consent was obtained from each patient and healthy controls according to the ethics protocol registered at the Hospital General de México.

Differentiated primary keratinocyte cultures and IL-13 treatment Normal human keratinocytes were prepared from neonatal foreskins according to Keratinocyte-Serum Free Medium (K-SFM) instructions (Gibco-BRL, Life Technologies, Rockville, MD). The cells were grown until 65% of confluence in a humidified atmosphere with 5% CO_2 at 37°C in K-SFM supplemented with 20 μg per ml bovine pituitary extract and 0.1 ng per ml epidermal growth factor (Gibco-BRL, Life Technologies). Subcultures were done in p60 mm petri dishes and the differentiation of cells was induced by incubation in supplemented K-SFM containing 10% fetal bovine serum. On day 3 fresh medium was added to each dish. After 5 d, the differentiated keratinocytes were treated with IL-13 (25 ng per ml) in fresh supplemented K-SFM with fetal bovine serum during 18 h. The total RNA was obtained with Trizol reagent (Gibco BRL Life Technologies).

RNA isolation To minimize RNA degradation, frozen skin biopsies were homogenized in Trizol reagent (Gibco BRL) with a tissue homogenizer (Pro Scientific, Inc. Oxford, CT). RNA was obtained by the Trizol reagent method. Contaminating DNA was removed by treating the RNA with deoxyribonuclease I (Gibco BRL, Life Technologies). The RNA pellet was dissolved in RNase-free water and the amount of RNA was determined by spectrophotometry at 260 nm. The integrity of RNA was analyzed in a 0.8% agarose gel electrophoresis.

Reverse transcription–PCR differential display The reverse transcription–PCR differential display technique was performed by the method of Liang and Pardee (1992). cDNA were then synthesized in 20 μl RNase-free reaction containing 3 μg of deoxyribonuclease-treated total RNA, 500 μM deoxynucleoside triphosphates (Pharmacia, Piscataway, NJ), 10 mM dithiothreitol (Gibco BRL, Life Technologies), 1 U of RNase inhibitor (Promega, Madison, WI), 2.5 μM of one of three oligo $dT_{11}M$ degenerate primers (where M = G, A, or C), 1 × first-strand buffer and 200 U of SuperScript II RNase H^- Reverse Transcriptase (Gibco BRL, Life Technologies). The reactions were incubated for 1 h at 42°C. The cDNA was amplified by PCR in a 25 μl reaction containing the same dT11M primer (2.5 μM), 0.5 μM of one of 20 arbitrary 10 mer primer (decamer) of random sequence (AP1-20; Gen Hunter, Nashville, TN), 1 μl of the cDNA reaction (diluted 1 : 10), 1 × PCR buffer, and additional 2 mM $MgCl_2$ and 1 U of *Taq* DNA polymerase (Gibco BRL, Life Technologies). The reaction was incubated in a Techne thermocycler (Cambridge) at 92°C for 3 min followed by 45 cycles at 92°C for 40 s; at 42°C for 1 min; at 72°C for 1 min. Finally, it was kept at 72°C for 5 min and then held at 4°C. Amplified fragments were separated in a 6% polyacrylamide gel (Cleangel DNA Analysis Kit; Pharmacia) for 4 h. Gels were stained with a silver stain kit (Bio-Rad, Hercules, CA). The differential bands were excised from the gel, heated in 100 μl of water for 10 min, cooled to room temperature, and briefly spun in a microfuge (ICE, Micromax RF, Needham Heights, MA). The supernatant was transferred to a fresh microfuge tube and the fragment precipitated in the presence of 10 mM glycogen, 0.3 M sodium acetate, and 2 volumes of ethanol (EtOH) at −20°C for 30 min The DNA pellet was washed with 70% EtOH, dried, and resuspended in 10 μl of sterile water. The excised bands were re-amplified in a 40 μl reaction with the same set of primers used to generate it. Reactions containing the correct size band were cloned into the pCRII vector as recommended by the supplier (Invitrogen, San Diego, CA). Recombinant plasmids were run through electrophoresis in a 1% agarose gel and purified with the Qiagen kit (Qiagen, Chatsworth, CA) for DNA sequencing.

DNA sequencing PCR fragments cloned into the pCRII vector were sequenced via the BigDye™ terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA). The sequences were compared with those stored in the DNA sequence database via the BLASTN and FASTA programs of the NIH server (Altschul *et al*, 1990).

Northern analysis Fifteen micrograms of total RNA of lesional and nonlesional skin biopsies from a psoriatic patient were run through electrophoresis in 1% agarose-formaldehyde-gels as performed by Thomas (1980). The gel was soaked in 20 × SSPE buffer (3M NaCl, 0.2 $MNaH_2PO_4$-H_2O, 25 mMEDTA; pH7.4 for 1 h, blotted on to a Hybond-N^+ nylon membrane (Amersham Pharmacia Biotech, Piscataway, NJ) and hybridized with ^{32}P-labeled cDNA probes at 55°C overnight. The membranes were washed at 62°C in 0.1 × sodium citrate/chloride buffer and 1% sodium dodecyl sulfate for 15 min and autoradiographed on Kodak film.

Reverse transcription–PCR analysis First strand cDNA was prepared from 3 μg of RNA and 0.5 μg of oligo $(dT)_{12-18}$ primers using Moloney Murine Leukemia Virus Reverse Transcriptase (Gibco BRL, Life Technology) under the conditions suggested by the supplier. After the reverse transcription, amplification was carried out by PCR using *Taq* DNA polymerase (Gibco BRL, Life Technology) with 1 mM $MgCl_2$ in a Techne thermocycler (Cambridge). PCR was performed with the following primers: for IL-13Rα1 (5′-CACAGTGTCCAAATAATGGTC-AAGG-3′ and 5′-TCAAAGTATCAGGAAGAACACCAGG-3′), yielding a 320 bp product; for IL-13Rα2 (5′-GGGACCTATTCCAGCAAGGT-

G-3′ and 5′-TGAGACTCATATTGAACATTTGGCC-3′), yielding a 433 bp product; for IL-4Rα (5′-GTCTGCAGATGAGGACTAGGGC-3′ and 5′-TACTCTCATGGGATGTGGCG-3′) yielding a 200 bp product; for IL-13 (5′-TGGAGCATCAACCTGACAGC-3′ and 5′-TCCTTTACAAA-CTGGGCCACC-3′) yielding a 189 bp product; for IL-4 (5′-CCGAGTT-GACCGTAACAGACATC-3′ and 5′-TGGTTGGCTTTTCACAGG-3′) yielding a 241 bp product; for β-actin (5′-GTGGGGCGCCCCAGGC-ACCA-3′ and 5′-CTCCTTAATGTCACGCACGATTTC-3′) yielding a 550 bp product. Cycle times were 40 s at 92°C, 50 s at 55°C, and 30 s at 72°C for 30 cycles. Products were separated on agarose gels and stained with ethidium bromide. A mock PCR (without cDNA) was included to exclude contamination in all experiments. To evaluate mRNA expression semiquantitatively, β-actin message expression was used to normalize the cDNA amount to be used. The intensity of the amplified band was measured in an Imager Ready Apparatus (Alpha Innotech Corporation, San Leandro, CA) to obtain the IL-13R or IL-4R/β-actin relation.

Immunohistochemical analysis The immunohistochemical analysis was developed in 7 μM p-formalized-paraffin sections of biopsies. Mouse monoclonal antibody (MoAb) to human IL-13Rα1 (Diaclone, Besançon, Bourgogne, France), rabbit polyclonal IgG to IL-4Rα (Santa Cruz Biotechnology), mouse MoAb to human IL-13 (Pharmingen, San Diego, CA) and to human IL-4 (Pharmingen) were used to assess expression of the proteins, followed by secondary biotinylated anti-mouse or anti-rabbit, amplified with immunoperoxidase (DAKO EnVision™ System Peroxidase, Carpinteria, CA), and visualized with diaminobenzidine as chromogen. For negative controls, the first MoAb was either omitted or replaced by an irrelevant isotype-matched reagent. Staining intensity was scored with a digital Imager Ready Apparatus (Alpha Innotech Corporation).

RESULTS

Differential expression of mRNA of IL-13Rα1 in lesional skin from patients with psoriasis Our criterion for differentially regulated PCR fragments was to select those bands present in at least two of the three psoriatic biopsies and absent from any control samples (or vice-versa). We found this to be the best way to prevent isolation of false positives due to natural genetic variability among people and/or endogenous PCR variability. When we developed a reverse transcription–PCR differential display reaction with $T_{11}G$ and AP4 primer, we observed a 580 bp band of cDNA that was expressed in lesional skin but not in the nonlesional skin of the three psoriasis patients studied (**Fig 1*A***). This re-amplified and ^{32}P-labeled band only hybridized with the RNA of lesional skin and not with the RNA of nonlesional skin of a psoriatic patient (**Fig 1*B***). The probe for β-actin used as control hybridized with identical intensity with both RNA (**Fig 1*C***). When the band of 580 bp was cloned and the nucleotide sequence determined, we found that this sequence corresponds to the mRNA of IL-13Rα1.

Reverse transcription–PCR expression of IL-13Rα1 mRNA and IL-4R mRNA in skin biopsies from psoriatic patients and control subjects IL-13Rα1 expression was analyzed by reverse transcription–PCR in 10 lesional and nonlesional biopsies of psoriatic patients. We observed that IL-13Rα1 mRNA was overexpressed (320 bp band) in a 100% of the lesional skin from psoriatic patients (**Fig 2*A***) as compared with the lower expression in the skin of healthy subjects (**Fig 2*B***), of which only one subject (10%) overexpressed this molecule but to a lesser degree. Five of the 10 patients (50%) also overexpressed the IL-13Rα1 mRNA in the nonlesional skin (**Fig 2*A***, lanes 6–10), which was taken from a healthy region, about 1 cm from a psoriatic plaque. In the biopsies from patients with other dermatologic diseases (**Fig 2*A***, lanes 11–16) and in the nondifferentiated and differentiated keratinocytes obtained from primary culture of keratinocytes (**Fig 2*B***, lanes 13 and 14), the expression of the IL-13Rα1 mRNA was low, similar to that from the skin of the 10 healthy subjects studied. The differentiated keratinocytes stimulated with IL-13 did not overexpress the IL-13Rα1 mRNA (**Fig 2*B***, lane 15) but did express the IL-13Rα2 mRNA (**Fig 2*B***, lane 17). A lesional and nonlesional skin biopsy from one patient did not express the IL-13Rα2 mRNA (data not shown). When we semiquantified the intensity of the RNA expression and determined the IL-13Rα1/β-actin ratio, we found a significant difference in the mean intensity of expression of the IL-13Rα1 mRNA between the psoriatic lesional skin and the skin from healthy subjects ($p < 0.01$). Even though 50% of nonlesional skin of the patients with psoriasis overexpressed the mRNA of IL-13R there was a significant difference between lesional skin and nonlesional skin of the psoriatic patients ($p < 0.05$, and between the nonlesional psoriatic skin and the skin of healthy subjects ($p < 0.05$) (**Fig 3*A***).

As human IL-13 and IL-4 share the same receptor, we studied the expression of IL-4Rα in the samples and found also a differential overexpression of IL-4Rα (200 bp band) similar to that observed for the IL-13Rα1 in the psoriatic patients (**Fig 2*C***). The 10 skin biopsies from healthy subjects also expressed the molecule but with lower intensity than the lesional skin of patients with psoriasis (**Fig 2*D***). When we determined the relation IL-4R/β-actin we only found a significant differential expression between the lesional skin from patients with psoriasis and the skin from healthy subjects ($p < 0.01$) and between the lesional and nonlesional skin of the patients with psoriasis ($p < 0.05$). (**Fig 3*B***).

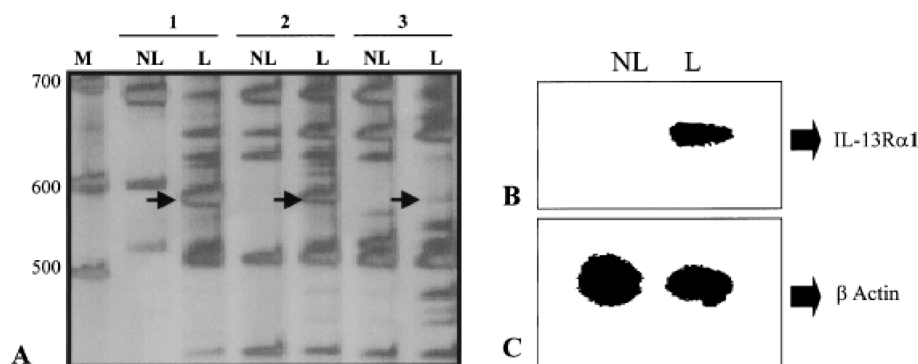

Figure 1. Differential expression of IL-13Rα1 mRNA in lesional and nonlesional skin from patients with psoriasis. *Panel A:* 6% PAGE stained with silver, of reverse transcription–PCR differential display analysis of nonlesional (NL) and lesional (L) skin from three patients with psoriasis. Reverse transcription–PCR differential display was developed with $T_{11}G$ and Ap4 primers. The *arrows* indicate the differential band which corresponded to mRNA to IL-13Rα1. *Lane M* contains a DNA size marker. *Panels B and C*: northern blot analysis with total RNA of nonlesional (NL) and lesional (L) skin from one patient with psoriasis. The cDNA of 580 bp obtained from the reverse transcription–PCR differential display was used as probe (^{32}P-labeled) to detect the IL-13Rα1 mRNA (*panel B*). The paper was washed and incubated another one with ^{32}P-labeled probe to β-actin, which was obtained by reverse transcription–PCR.

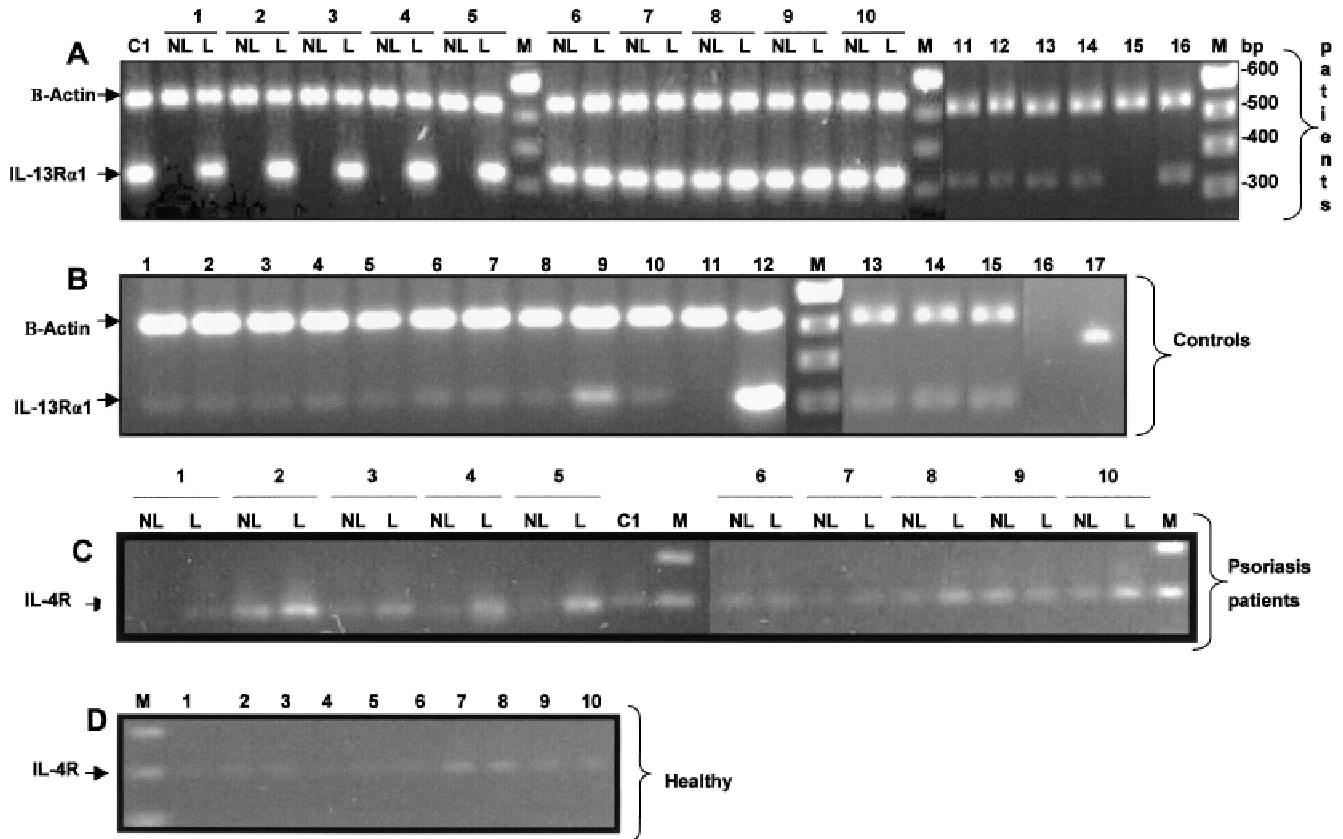

Figure 2. Reverse transcription–PCR expression analysis of IL-13Rα1 mRNA and of IL-4Rα mRNA in skin samples of patients and controls. *Panel A:* reverse transcription–PCR for IL-13Rα1 in nonlesional (NL) and lesional (L) skin biopsies from 10 patients with psoriasis (*lanes 1–10*). Reverse transcription–PCR of IL-13Rα1 in lesional skin biopsies from patients with other dermatologic diseases such as: atopic dermatitis (*lane 11*), polymorphous light eruption (*lane 12*), pemphigus vulgaris (*lane 13*), seborrheic keratoses (lane 14), lichen planus (*lane 15*), and dermatofibroma (*lane 16*). M = DNA size marker, C1 = Cos-7 cells (used as positive control of expression of IL-13Rα1 and IL-4Rα). *Panel B:* reverse transcription–PCR of IL-13Rα1 in skin biopsies from 10 healthy subjects (*lanes 1–10*). Reverse transcription–PCR of IL-13Rα1 in nonlesional (*lane 11*) and lesional (*lane 12*) skin biopsies from psoriatic patient 1. Reverse transcription–PCR of IL-13Rα1 in nondifferentiated (*lane 13*), differentiated keratinocytes (*lane 14*), differentiated keratinocytes treated with IL-13 (*lane 15*). Reverse transcription–PCR of IL-13Rα2 in differentiated keratinocytes nontreated (*lane 16*) and treated with IL-13 (*lane 17*). *Panel C:* reverse transcription–PCR of IL-4Rα in nonlesional (NL) and lesional (L) skin biopsies from 10 patients with psoriasis (*lanes 1–10*). C1 = Cos-7 cells. *Panel D:* reverse transcription–PCR of IL-4Rα in skin biopsies from 10 healthy subjects (*lanes 1–10*). *Lane M* contains a DNA size marker.

Reverse transcription–PCR expression of IL-13 mRNA and IL-4 mRNA in skin biopsies from psoriatic patients and control subjects When we studied the expression of the cytokines IL-13 (189 bp band) and IL-4 (241 bp band) in the skins of patients and healthy subjects, we did not find expression of the two cytokines in any of the studied samples. We used phytohemagglutinin-activated mononuclear cells obtained from bronchial lavage of a patient with asthma as positive expression of IL-13 and IL-4 (data no shown).

Immunohistochemical detection of IL-13Rα1, IL-4Rα, IL-13, and IL-4 in the biopsies of the patients with psoriasis The histopathologic analysis of the lesional skin biopsies was characteristic of a psoriatic skin, with an intense leukocyte infiltrate, whereas all the nonlesional biopsies were identical to the biopsies of healthy subjects.

To analyze the distribution of IL-13Rα1, immunohistologic staining was performed on skin sections derived from lesional and nonlesional skin of psoriasis patients, as well as skin sections from normal healthy donors. Staining of lesional psoriatic tissues with the MoAb IL-13Rα1, revealed the expression of the protein in the epidermal keratinocytes (**Fig 4*C*,*F***). Also, positive results were observed, in nonlesional skin of psoriatic patients and in the skin of healthy subjects (**Fig 4*B*,*E*** and ***A*,*D***, respectively). In most samples, however, the intensity of the positive reaction was higher in the skin of healthy subjects and in nonlesional skin than in lesional skin from psoriatic patients. We observed that as the psoriatic lesion increases in size, the intensity of the reaction decreases. The mean score of intensity in healthy skin was approximately twice as high as that in psoriatic skin (52.93 in healthy skin *vs* 28.02 in psoriatic skin, $p < 0.01$). The mean score of nonlesional skin was 41.67 ($p < 0.05$ lesional *vs* nonlesional skin and $p > 0.05$, nonlesional *vs* healthy skin).

When we analyzed by immunohistochemistry the expression of IL-4Rα, we observed a positive staining in the epidermal keratinocytes, with a similar score of intensity in all three types of tissue assayed ($p > 0.05$) (**Fig 4*J*–*L***). Hodgkin lymphoma tissue sections were used as a positive control for IL-13Rα1 and IL-4Rα (data not shown).

To corroborate the expression of the IL-13Rα1 in keratinocytes we used nondifferentiated and differentiated keratinocytes from the primary culture and we observed that these cells were also stained with the MoAb IL-13Rα1 (**Fig 4*G*** and **4*H***, respectively).

Staining of the healthy skin and psoriatic tissues with the MoAb to IL-13 and IL-4 was negative. Mononuclear peripheral blood cells from an asthma patient were used as a positive control for these cytokines (data not shown).

DISCUSSION

In an effort to identify and clone psoriasis-specific genes, Rivas *et al* (1997) using the reverse transcription–PCR differential

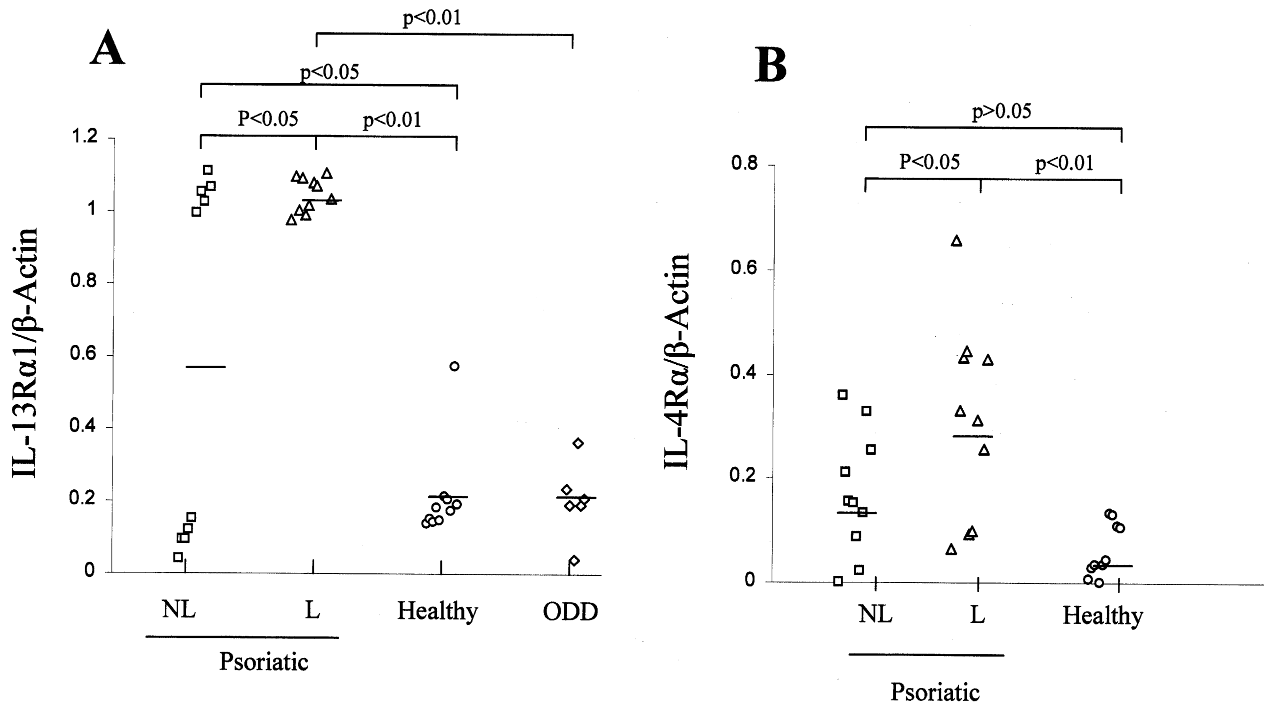

Figure 3. Comparison of the expression of IL-13Rα1 mRNA (*panel A*) and IL-4Rα mRNA (*panel B*) in nonlesional (NL) and lesional (L) skin from psoriatic patients, skin from healthy subjects, and skin from patients with other dermatologic diseases (ODD). The intensity of each band was measured in the Imager Ready Apparatus (Alpha Innotech Corporation) and normalized against that of β-actin. Bars indicate the mean of intensity. Variance analysis was done by the Duncan method.

display technique in biopsies from psoriatic patients, found a differential expression of four genes not previously described in psoriasis: connexin 26 (gap junction protein), squamous cell carcinoma antigen-1 (a serine protease inhibitor), and mitochondrial NAD subunits 5 and 6. Later, Di Sepio *et al* (1998) used the same technique in keratinocyte lines treated *in vitro* with tazarotene, and identified a tumor suppressor gene (TIG-3), which was found later in biopsies from psoriatic patients treated with tazarotene.

In this study, with the same technique we detected a differential expression of mRNA of the IL-13Rα1 in three biopsies from psoriatic patients. Afterwards we confirmed this differential overexpression between the lesional skin of 10 more psoriasis patients and the skin of 10 healthy subjects ($p < 0.01$). It is important to point out that the mRNA for IL-13Rα1 was also overexpressed in 50% of the nonlesional skin of the patients studied, which can be explained because biopsies were taken from an area very near to the lesional tissue and, although they were histologically healthy, the results indicate differences at the molecular level. The overexpression of IL-13Rα1 was specific of psoriasis. The control patients with atopic dermatitis and polymorphous light eruption, which are mediated by T helper 2 lymphocytes, presented lower expression of this receptor, as did the control patients with other dermatologic diseases.

As the IL-13R complex is composed of two different chains, IL-13Rα1 or IL-13Rα2, and IL-4Rα (Hilton *et al*, 1996), we looked for the expression of the IL-4Rα mRNA in the skin of patients with psoriasis and we found a similar differential overexpression between the lesional skin from psoriatic patients and skin from healthy subjects ($p < 0.01$), which confirms the overexpression of the IL-13R complex. Our results are as those reported by Prens *et al* (1996) by *in situ* hybridization; they detected an increased expression of IL-4R mRNA in the psoriatic cells, but they did not study the expression of IL-13Rα1 mRNA.

Four structural models of IL-13R involving different combinations of IL-13Rα1, IL-13Rα2, or IL-4Rα have been proposed (Murata *et al*, 1997). IL-13Rα1 binds IL-13 with low affinity by itself, but when paired with IL-4Rα, it binds IL-13 with high affinity and forms a functional IL-13R. IL-13Rα1 chain presents the tyrosine residues that mediate the activation of signal transduction by means of STAT3 and STAT6. In contrast, IL-13Rα2, by itself, binds IL-13 with high affinity, but does not cause activation of its signaling pathway (Osawa *et al*, 2000; Umeshita-Suyama *et al*, 2000; Kawakami *et al*, 2001). We observed that IL-13 induces the expression of IL-13Rα2 mRNA but not of IL-13Rα1 mRNA in differentiated keratinocytes of a primary culture. Similar results were obtained by David *et al* (2001), although not in primary culture, but in a human keratinocyte cell line (HaCaT) treated with both IL-13 and IL-4; furthermore, they found that these two cytokines did not induce the overexpression of IL-4Rα. In a different model, endothelial cells treated with tumor necrosis factor-α, Lugli *et al* (1997) found overexpression of IL-4Rα but not of IL-13R mRNA. In our study, we detected an overexpression in the psoriatic lesions of both IL-13Rα1 and IL-4Rα mRNA, which could be playing an important part in psoriasis; however, we do not know what that role could be, nor which the inducer is. All these results indicate the differences in modulation of receptor expression, in different target cells, by different inducers.

The immunohistochemical assay with MoAb IL-13Rα1 revealed that the epidermal keratinocytes of the nonlesional and lesional biopsies of patients with psoriasis and of skin biopsies from healthy subjects were expressing the IL-13R. This result correlated with the differentiated and nondifferentiated keratinocytes from primary cultures, used as control, which also expressed this receptor. Akaiwa *et al* (2001), demonstrated, by immunohistochemistry, the expression of IL-13R in keratinocytes, hair follicles, and sebaceous and sweat glands of the skin from healthy subjects; however, we observed that the intensity of the reaction with the specific MoAb decreases as the histologic severity of psoriatic lesions increases, and there was no correlation with overexpression of mRNA. These results suggest that the IL-13R is normally present in the skin of all subjects, but when the psoriatic process is triggered, the mRNA to IL-13Rα1 is overexpressed, producing its receptor. Then, IL-13 could have an important effect in the normal keratinocyte, but the expression of the protein is stopped by an unknown mechanism as the lesion advances. We observed a similar phenomenon with IL-13RJ as reported for IL-10 in psoriatic patients, human keratinocytes do not produce IL-

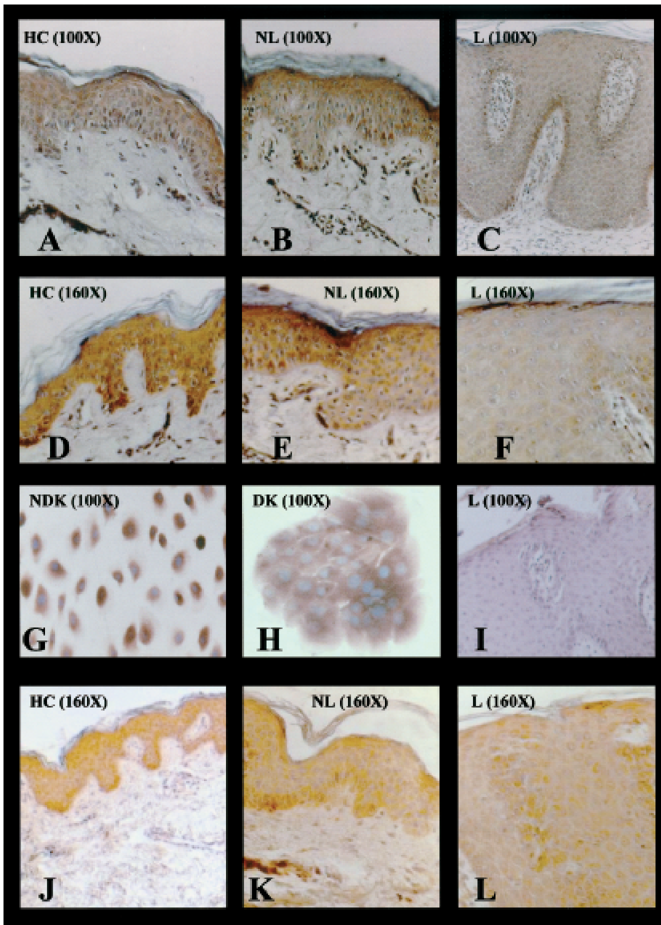

Figure 4. Immunohistochemical detection of IL-13Rα1 and IL-4Rα in nonlesional (NL) and lesional (L) skin biopsies from psoriatic patients and healthy controls (HC) and differentiated (DK) and nondifferentiated keratinocytes (NDK). MoAb IL-13Rα1 staining: *panel A* to *panel H*. Rabbit polyclonal antibody IL-4Rα staining: *panel J* to *panel L*. Negative control with irrelevant isotype-matched reagent: *panel I*.

10 under normal conditions (Teunissen *et al*, 1997) and, although IL-10 was not detected by immunostaining in psoriatic epidermis (Nickoloff *et al*, 1994), IL-10 mRNA has been detected in psoriatic lesions by reverse transcription–PCR (Olaniran *et al*, 1996). Furthermore, a functional receptor for IL-10 has been demonstrated on normal human keratinocytes and its expression is dramatically decreased in acute lesional but not in the nonlesional psoriatic epidermis (Günter *et al*, 1997).

On the other hand, in contrast to the results obtained with mouse MoAb to IL-13Rα1, with the rabbit polyclonal IgG antibody to IL-4Rα we observed a positive reaction in the epidermal keratinocytes with similar intensity in the three tissues assayed. As mentioned above, Prens *et al* (1996), found by immunohistochemistry the IL-4Rα protein overexpressed in psoriatic lower epidermal layers.

IL-13 and IL-4 or their mRNA were not found in any of the assayed tissues. IL-4 mRNA was also undetectable in skin homogenates of psoriatic lesions by other groups (Uyemura *et al*, 1993; Prens *et al*, 1996). These results suggest that the induction of the overexpression of mRNA IL-13Rα1 in the psoriatic keratinocytes is not induced by the IL-13 and IL-4 cytokines. We have confirmed in a primary culture of keratinocytes the results obtained by David *et al* (2001), in a keratinocyte cell line, that the IL-13 does not modulate the overexpression of mRNA IL-13Rα1. Nevertheless, we cannot discard the possibility that cytokines produced by other cells could induce the overexpression of the mRNA of IL-13Rα1 in the psoriatic keratinocyte.

The relevance of the elevated mRNA IL-13R expression observed in psoriatic keratinocytes in this study is not entirely clear, because the effects of IL-13 in the keratinocytes are seldom studied.

It is known that IL-13 is produced predominantly by T helper 2 lymphocytes, and acting together with IL-4, has effects on several immune cells, including B lymphocytes and macrophages, stimulating their proliferation, differentiation, and effector functions. In immune cells, these cytokines inhibit the production of inflammatory cytokines (IL-1, IL-6, IL-8, and tumor necrosis factor-α) (Minty *et al*, 1993; De Waal *et al*, 1993) and the IL-13 and IL-4 could down-modulate, together with IL-10 and IL-11 (Günter *et al*, 1997; Trepicchio *et al*, 1999; Bozza *et al*, 2001), the inflammatory processes of psoriasis.

The overexpression of IL-13R in certain epithelial tumors is associated with a clear growth-inhibitory response to IL-13 by those tumor cells (Serve *et al*, 1996). IL-13 in the psoriatic keratinocytes could be playing a part in the down-modulation of the inflammatory and proliferative processes of psoriasis; however, the effect of IL-13 on the proliferation or in the inhibition of proliferation of normal and psoriatic keratinocytes has not been studied.

In contrast, IL-13 and IL-4 synergistically increase RANTES production by normal keratinocytes treated with IFN-γ (Wakugawa *et al*, 2001), and IL-13 stimulated IL-6 production in normal human keratinocytes (Derocq *et al*, 1994) and human lung fibroblast (Doucet *et al*, 1998). There are reports that the IL-6 production is increased in monocytes and keratinocytes from patients with psoriasis (Neuner *et al*, 1991). IL-8 is another inflammatory cytokine that is modulated by IL-13 in a human colon epithelial cell line (Kolios *et al*, 1996). Expression of adhesion molecules is increased by IL-13 and IL-4 in human endothelial cells (Kotowicz *et al*, 1996) and human lung fibroblasts (Doucet *et al*, 1998). These reports suggested that the IL-13 in keratinocytes of patients with psoriasis could induce the production of adhesion molecules and inflammatory cytokines, which could be markers of the initiation of the inflammatory process of psoriasis.

On the other hand CDw60 is an acetylated form of the GD3 ganglioside that is expressed in keratinocytes and T lymphocytes from psoriatic patients (Skov *et al*, 1997), but also is present in other tissues from normal subjects (Gocht *et al*, 2000). T cell clones from lesional psoriatic skin, activated with anti-CDw60 antibody in the presence of accessory cells and/or phorbol ester, and the lymphokines released by these T cells, were capable of inducing intercellular adhesion molecule-1 and HLA-DR expression on normal keratinocyte cultures (Baadsgaard *et al*, 1990). High expression of CDw60 in normal keratinocytes is induced by IL-13 and IL-4 from activated T cells from psoriatic patients and this high induction is blocked by IFN-γ (Huang *et al*, 2001). CDw60 acts as a costimulator for T cell activation, but its role on the psoriatic keratinocytes is still unknown.

We propose that the altered expression of IL-13R in psoriasis might play an important part in the initiation and in the development of the disease. Much further work is needed to understand fully the involved mechanisms.

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