Expression and Function of Neurotrophins and Their Receptors in Cultured Human Keratinocytes

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Whereas nerve growth factor has been extensively studied in human keratinocytes, little is known on the role of other members of the neurotrophin family. We investigated the expression and function of neurotrophins and neurotrophin receptors in cultured human keratinocytes. We demonstrated by reverse transcription–polymerase chain reaction that keratinocytes synthesize neurotrophin-3, brain-derived neurotrophic factor, and neurotrophin-4/5. These cells also express tyrosinase kinase A and C, the nerve growth factor and neurotrophin-3 high-affinity receptors, respectively. On the other hand, only the truncated extracellular isoform of tyrosinase kinase B, the high-affinity brain-derived neurotrophic factor and neurotrophin-4/5 receptor, is detected in keratinocytes. Moreover, neurotrophin-3, brain-derived neurotrophic factor, and neurotrophin-4/5 proteins are secreted by human keratinocytes at low levels. Keratinocyte stem cells synthesize the highest amounts of nerve growth factor, while they secrete higher levels of nerve growth factor as compared with transit amplifying cells. Neurotrophin-3 stimulates keratinocyte proliferation, where brain-derived neurotrophic factor or neurotrophin-4/5 does not exert any effect on keratinocyte proliferation. Addition of neurotrophin-3 slightly upregulates the secretion of nerve growth factor, whereas nerve growth factor strongly augments neurotrophin-3 release. Ultraviolet B irradiation downregulates nerve growth factor, whereas it augments neurotrophin-3 and neurotrophin-4/5 protein levels. Ultraviolet A irradiation increases the level of neurotrophin-3, whereas it does not exert any effect on the other neurotrophins. Finally, neurotrophins other than nerve growth factor fail to protect human keratinocytes from ultraviolet B-induced apoptosis. This work delineates a functional neurotrophin network, which may contribute to epidermal homeostasis. ***Key words: keratinocyte/neurotrophin/tyrosine kinase receptor. J Invest Dermatol 121:1515–1521, 2003***

The neurotrophins (NT) consist of a family of four related polypeptide growth factors: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5) (Lewin and Barde, 1996). These structurally and functionally related molecules exert their effects by binding two different classes of transmembrane receptors. The p75 NT receptor binds all NT with low affinity (Chao *et al*, 1986) and modulates signaling initiated by the second class of NT receptors, the trk family of 140 kDa high-affinity tyrosine kinase receptors (Barbacid, 1994). TrkA, trkB, and trkC selectively bind NGF, BDNF, and NT-3, respectively, and trkB can also be activated by NT-4/5 (Dechant, 2001).

NT play a prominent role in the development of the vertebrate nervous system, by influencing differentiation, survival, and cell death of neurons (Snider, 1994). NT can also act on non-neuronal tissues. It has been shown that NGF activates mast cells (Kawamoto *et al*, 2002) and prevents their apoptosis (Kanbe *et al*, 2000). Moreover, NGF stimulates the proliferation of human microvascular endothelial cells (Raychaudhuri *et al*, 2001), whereas it is an autocrine survival factor for lymphocytes (Torcia *et al*, 1996).

Normal human keratinocytes synthesize and release high amounts of biologically active NGF. In addition, human keratinocytes express both the low-affinity (p75) and the high-affinity (trkA) NGF receptors (Pincelli *et al*, 1994). NGF is the key player of an autocrine loop, acting as a mitogen and as a survival factor for human keratinocytes (Di Marco *et al*, 1993; Pincelli *et al*, 1997). In particular, autocrine NGF protects human keratinocytes from ultraviolet (UV)-induced apoptosis (Marconi *et al*, 1999). Moreover, NGF released from keratinocytes exerts paracrine functions on human melanocytes by stimulating their dendricity (Yaar *et al*, 1991) and protecting them from cell death (Pincelli and Yaar, 1997). Whereas NGF is a crucial neurotrophic molecule for skin innervation (Albers *et al*, 1994), the above findings strongly indicate that NGF is to be regarded as an important growth factor in human epidermis as well (Pincelli and Marconi, 2000a). In this respect, NGF could be supported by other members of the NT family, although little is known about these molecules in human skin (Yaar *et al*, 1994; Grewe *et al*, 2000).

This study reports a comprehensive analysis of the expression of all NT family members as well as of their receptors in normal

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Abbreviations: NGF, nerve growth factor; NT, neurotrophin; NT-3, neurotrophin-3; BDNF, brain-derived neurotrophic factor; NT-4/5, neurotrophin-4/5; Trk, tyrosine kinase; KSC, keratinocyte stem cell.

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human keratinocytes. In particular, we provide evidence of the mitogenic role for autocrine NT-3 in these cells and report the different sensitivity of NT to UV light. Finally, we describe an inter-regulation of NT among each other.

MATERIALS AND METHODS

Human (***in vivo***) studies and all animal studies were approved by the Institutional Review Board.

Keratinocyte cultures Normal human keratinocytes were obtained from foreskin and cultured as described (Pincelli *et al*, 1997) after informed consent. Briefly, keratinocytes were plated on mytomycin C-treated 3T3 cells (2.4×10^4 per cm^2, ATCC, Rockville, Maryland) and cultivated in Dulbecco's modified Eagle's medium and Ham's F12 (DMEM/F12, 3:1 ratio) media (Seromed-Biochrom, Berlin, Germany). Subconfluent secondary cultures were trypsinized with 0.05% trypsin/0.02% ethylenediamine tetraacetic acid (Seromed) and replated for the experiments in DMEM/F12 on 3T3 cells or in defined serum-free medium (1×10^4 per cm^2; keratinocyte growth medium (KGM), Clonetics Corp., San Diego, California) without hydrocortisone. In order to enrich cell population in keratinocyte stem cells (KSC), keratinocytes were divided into three populations and cultured in serum-free medium. They were first allowed to adhere to type IV collagen (Sigma, St Louis, Missouri) for 5 min (population 1, KSC), and the nonadherent cells were then transferred to fresh collagen-coated dishes and allowed to attach overnight (population 2, young transit amplifying cells, TA). Finally, keratinocytes not yet attached after one night were plated on to type IV collagen to obtain a third population (population 3, TA cells). Populations were cultured for 5 d up to subconfluency. The three subpopulations were characterized according to the β1 integrin expression and colony-forming efficiency as described (Tiberio *et al*, 2002).

UV radiation UV radiation was delivered with a battery of TL 20W/12 RS lamps for UVB and TL 20W/09N for UVA (Philips Medical Brakel, The Netherlands). Before irradiation, keratinocytes were washed once with phosphate-buffered saline and irradiated in the presence of phosphate-buffered saline. Controls were sham irradiated for identical periods. Doses were measured by Goldilux Smart Meter (Oriel Instruments, Stratford, Connecticut) right before irradiation.

Reverse transcription and polymerase chain reaction (reverse transcription–PCR) Total cellular RNA was extracted from cultures using TRI Reagent method performed as described by Sigma. One microgram of total cellular RNA extracted was reverse-transcribed and amplified as described (Pincelli *et al*, 1994) with a DNA Thermal Cycler (Perkin-Elmer, Norwalk, Connecticut). In order to perform a semiquantitative evaluation of mRNA, β-actin was used as a house-keeping gene. The relative intensity of bands on autoradiograms was quantitated by scanning laser densitometry. The linearity of PCR reaction was obtained by plotting values from densitometric analysis in each band *versus* the cDNA concentration. Nucleotide sequences of the oligomers (MWG Biotech, Ebersberg, Germany) used were as follows: NGF: 5′-TCATCATCCCATCCCATCTT-3′ 5′-CTTGACAAAGGTGTGAGTCG-3′ (nucleotides: 533–547 and 777–796; fragment: 264 bp; 28 cycles); BDNF: 5′-AGCCTCCTCTGCTCTTTCTGCTGGA-3′, 5′-CTTTTGT-CTATGCCCCTGCAGCCTT-3′ (nucleotides: 870–894 and 1143–1167; fragment: 298 bp); NT-3: 5′-TTTCTCGCTTATCTCCGTGGCATCC-3′, 5′-GGCAGGGTGCTCTGGTAATTTTCCT-3′ (nucleotides 100–124 and 243–266; fragment: 167 bp); NT-4/5: 5′-ATGCTCCCTCTCCCC-TCAT-3′, 5′-GCATGGGTCTCAGGCCCG3′ (nucleotides 475–493 and 1099–1116; fragment: 642 bp); trkA: 5′-GGCTCCTCGGGACTGCGATG-3′, 5′-CAGGAGAGAGACTCCAGAGCG-3′ (nucleotides 214–233 and 459–479; fragment: 266 bp); trkB: 5′-CCGCTAGGATTTGGTGTACT-GAGCCTTCT-3′, 5′ CCACTGTCATCAGATGAAATGTTCGTTATC-CT-3′ (nucleotides 630–658 and 1262–1293; fragment: 664 bp), intracellular trkB: 5′-TCGCAGATGCTGCATATAGC-3′ 5′-ATCAGCTCATA-CACCTCCTC-3 (nucleotides 2481–2500 and 2868–2887; fragment: 407 bp); extracellular truncated trkB: 5′-GACACTCAGGATTTGTACTGCC-3′, 5′-TCCGTGTGATTGGTAACATGTATT-3′ (nucleotides 990–1011 and 1481–1504; fragment: 515 bp); trkC: 5′-GGAAAGGTCTTCCTGGCCG-AGTGC-3′, 5′-GCTTTCCATAGGTGAAGATCTCCC-3′ (nucleotides 1803–1826 and 2395–2418; fragment 616 bp); β-actin: 5′-TGGAT GATG-ATATCGCCGCGCTCG-3′, 5′-CACATAGGAATCCTTCTGACCCA-3′ (nucleotides 75–98 and 213–235; fragment 161 bp; 25 cycles). The PCR was carried out at least three times for each sample. No reverse-transcribed mRNA and buffer without template were used as controls.

Enzyme-linked immunosorbent assay (ELISA) assay Keratinocytes were cultivated both in KGM and on a 3T3 feeder layer. In the latter case, the feeder layer was removed prior to keratinocyte lysis. NGF, BDNF, NT-3, and NT-4/5 quantitation was performed by a two-site enzyme immunoassay (Quantikine, Promega Madison WI) according to manufacturer's instructions. The samples concentration was determined by absorbance at 540 nm against recombinant human NT standard protein.

Cell proliferation assay Keratinocytes (6000 cells per well) were grown on 96-well plates in KGM. Forty-eight hours after seeding medium was changed and NT (Sigma) or anti-NT-3 neutralizing antibody (Bertollini *et al*, 1997) were added to the cultures. Forty-eight hours after addition of NT, ^3H thymidine (1 mCi per well, Amersham-Pharmacia Biotech, Rainham, UK) incorporation was performed and cells collected 12 h later. The incorporated radioactivity was determined by β-counter.

TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling) staining Keratinocytes were seeded at a density of 1×10^4 per cm^2. Forty-eight hours later, cells were preincubated with NT (100 ng per mL), 48 h before UVB (50 mJ per cm^2) or sham irradiation. UVB radiation was delivered with a battery of lamps (TL20W/12 RS UVB Philips Medical). Keratinocytes were directly stained on chamber slides by the "*In situ* cell death detection kit" (Roche Diagnostics, Mannheim, Germany), as described (Pincelli *et al*, 1997). Negative control was obtained by replacing the primary incubation with a nucleotide mixture without TdT. Fluorescent specimens were analyzed by a Confocal Scanning Laser Microscopy (Leica TCS4D) in conjunction with a conventional optical microscope (Leica DM IRBE).

RESULTS

Expression of NT and their receptors in human keratinocytes As NGF levels are highest in proliferating keratinocytes and tend to disappear with keratinocyte differentiation (Di Marco *et al*, 1993; Pincelli *et al*, 1994), we evaluated NT expression in preconfluent keratinocytes. Preconfluent keratinocytes express NT-3, BDNF, and NT-4/5 mRNA (**Fig 1**).

We also evaluated trk expression in secondary cultures prior to divisions into subpopulations. Preconfluent keratinocytes express trkA and trkC, but do not express the full-length trkB receptor (**Fig 2*a***). By contrast, keratinocytes express the truncated isoform of trkB, lacking the intracellular domain (**Fig 2*b***).

Human keratinocytes secrete all NT Keratinocytes release NGF, NT-3, NT-4/5, and BDNF, even though in low amounts. NGF levels are 5-fold more elevated than the other NT in supernatants and tend to be significantly higher in supernatants than in cell lysates (**Fig 3**).

KSC express highest levels of NGF NGF mRNA is highest in KSC, whereas they tend to decrease in young TA cells and they almost disappear in TA cells (**Fig 4*a***). ELISA assay confirms that NGF is released in higher amounts from KSC as compared with young TA and TA cells. On the other hand, whereas all NT are almost undetectable in TA cells, there is no difference in NT-3, NT-4/5, and BDNF between KSC and young TA cells (**Fig 4*b***).

NT-3, but not NT-4/5 or BDNF stimulates keratinocyte proliferation We first tested the toxic effect of NT in human keratinocytes. **Table I** shows that NT do not exert toxic effects neither affect cell viability at 24 and 72 h. In order to analyze the functions of NT other than NGF in human keratinocytes, we first treated preconfluent keratinocytes with increasing concentrations of NT. Our results show that NT-3 stimulates keratinocyte proliferation. The effect is statistically significant only with 100 ng per mL. This effect is specifically blocked by addition of decreasing dilutions of anti-NT-3 neutralizing antibody, in the absence of exogenous NT-3. Indeed, anti-NT-3 treatment inhibits keratinocyte proliferation, indicating that autocrine NT-3 promotes keratinocyte proliferation. By contrast, BDNF and NT-4/5 do not exert any effect on keratinocyte proliferation (**Fig 5**).

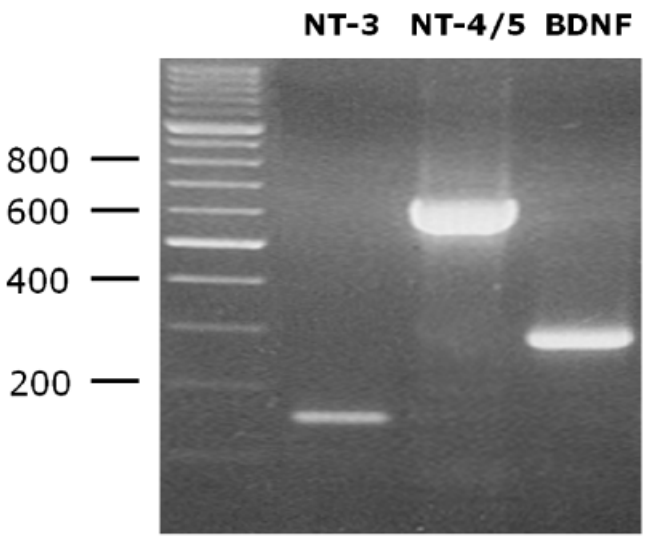

Figure 1. Expression of NT mRNA in human keratinocytes. Total cellular RNA was extracted from preconfluent keratinocytes and reverse-transcribed. NT-3, NT-4/5, and BDNF mRNA were evaluated by PCR and ethidium bromide staining.

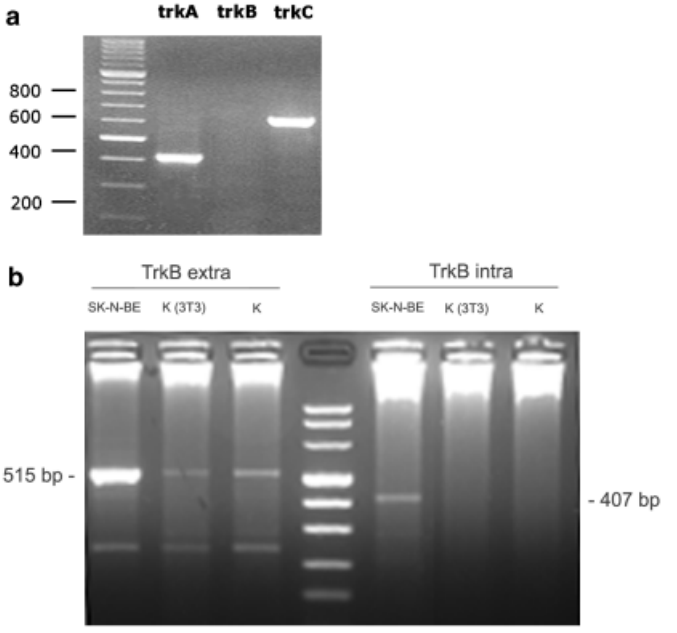

Figure 2. Expression of NT receptors in human keratinocytes. Total cellular RNA was extracted from subconfluent keratinocytes and reverse-transcribed. TrkA, trkB, and trkC were evaluated by PCR and ethidium bromide staining (*a*). Total cellular RNA was extracted from the neuroblastoma cell line SK-N-BE (positive control) and from keratinocytes cultivated either on 3T3 or in KGM. Full-length and truncated (see *Materials and Methods*) form of TrkB was evaluated by reverse transcription–PCR (*b*).

Reciprocal influence of NT Whereas NT-4/5 and BDNF do not affect other NT release, NGF strikingly upregulates NT-3 release. NT-3 in turn slightly increases NGF secretion (**Fig 6**).

Effect of UVB and UVA irradiation on NT secretion We have previously reported that UVB strikingly downregulates the expression of NGF mRNA and the secretion of NGF in keratinocytes. In order to evaluate the effect of UV light on NT secretion, keratinocytes were exposed to UVB and UVA irradiation and NT were measured. As expected, UVB diminishes NGF secretion in both cell lysates and cultured supernatants in a concentration-dependent manner. By contrast, UVB significantly augments NT-3 and NT-4/5 expression and release, whereas BDNF is not affected (**Fig 7*a,b***). UVA irradiation increases NT-3 but not NGF release in both cell lysates and cultured supernatants. Whereas BDNF is slightly increased by UVA irradiation, NT-4/5 is minimally augmented with 3 J per cm^2 only in cell lysates. (**Fig 7*c,d***).

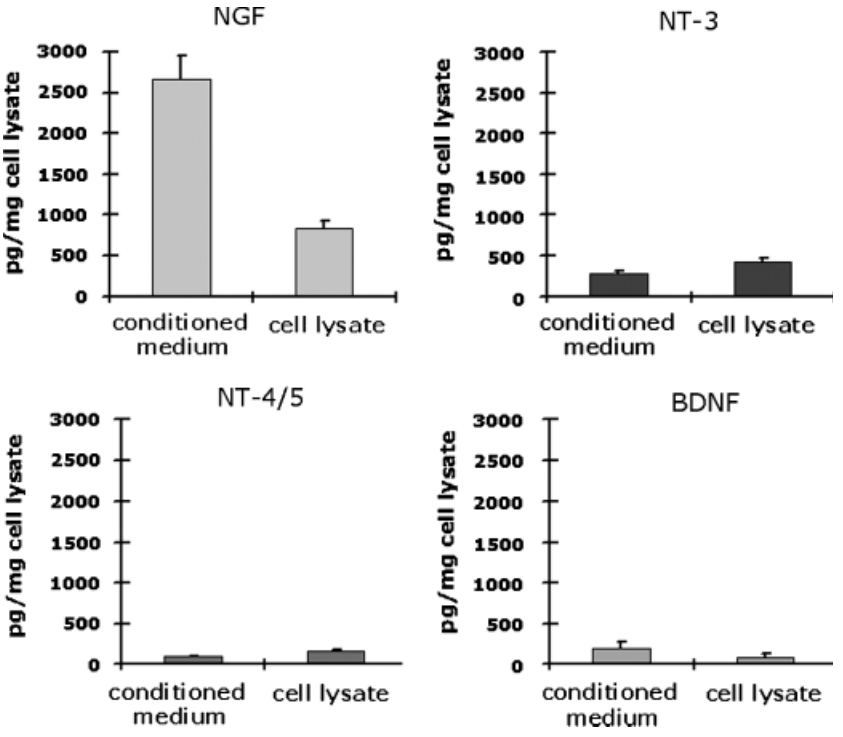

Figure 3. NT protein expression in human keratinocytes. Keratinocyte were cultivated in DMEM/F12 on 3T3 cells up to preconfluency and NT proteins were measured by ELISA in cell lysates and in conditioned medium, as described in *Materials and Methods*. NT protein levels are given in pg per mg of cell lysate and results are expressed as mean ± SEM of triplicate from three different experiments.

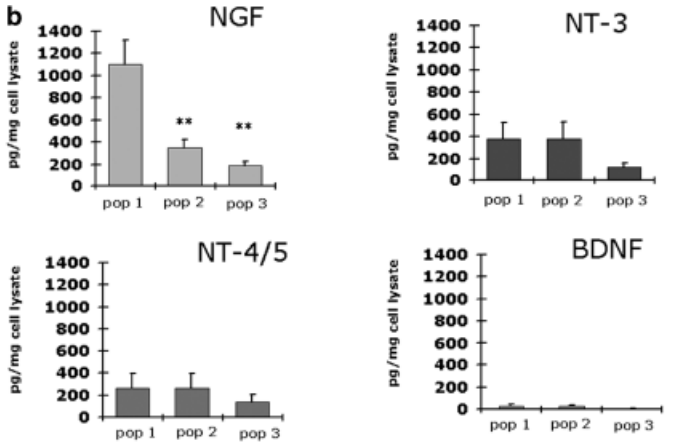

Figure 4. NT expression in KSC. Total cellular RNA was extracted from keratinocytes enriched for KSC (pop 1), young TA (pop 2) cells and TA cells (pop 3) (see *Materials and Methods*) and reverse transcribed. NGF mRNA was evaluated by PCR and ethidium bromide staining. Relative intensity of bands on autoradiograms was quantified by scanning laser densitometry. Values are expressed as fold variations compared with control cells (*a*). Keratinocyte were cultivated in KGM up to preconfluency and NT were measured by ELISA in supernatants. NGF protein levels are given in pg per mg of cell lysate and results are expressed as mean ± SEM of triplicate from three different experiments (*b*). $^{**}p < 0.01$.

Table I

	Trypan blue 24h % + SD	Neutral red 24h % + SD	Trypan blue 72h % + SD	Neutral red 72h % + SD
cntrl	95.4 + 4.5	0.41 + 0.04	93.2 + 5.5	0.69 + 0.07
NGF	97.6 + 3.7	0.42 + 0.06	94.6 + 3.2	0.71 + 0.06
NT3	96.2 + 4.1	0.40 + 0.09	96.0 + 2.6	0.77 + 0.08
NT4	94.6 + 5.1	0.49 + 0.04	90.5 + 4.1	0.75 + 0.03
BDNF	93.7 + 7.5	0.46 + 0.03	91.0 + 4.5	0.68 + 0.05

NT other than NGF fail to protect keratinocytes from UVB-induced apoptosis NT exert different effects on neuronal cell survival (Casaccia-Bonefil *et al*, 1998), whereas NGF over-expression protects human keratinocytes from UVB-induced apoptosis (Marconi *et al*, 1999). We first evaluated the effect of NT on keratinocyte apoptosis. NT do not seem to influence cell death in these cells (**Fig 8*a***). We then analyzed the function of NT in relation to UVB-induced apoptosis. Keratinocytes were sham or UVB irradiated after the addition of NT. Whereas NGF exerts a protective effect against UVB-induced apoptosis, the other NT do not prevent cell death (**Fig 8*b***).

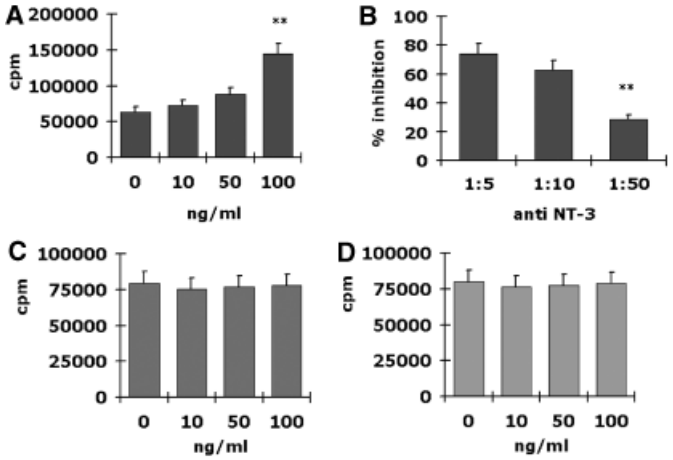

Figure 5. NT effects on keratinocyte proliferation. Keratinocyte were seeded at a density of 6000 per well and cultured in KGM for 48 h. ^{3}H thymidine incorporation was determined with increasing doses of NT-3 (*A*). Increasing concentrations of anti-NT-3 neutralizing antibody were added to keratinocyte cultures, in the absence of exogenous NT-3 (*B*). ^{3}H thymidine incorporation was also determined with increasing doses of NT-4/5 (*C*) and BDNF (*D*). Each point represents the mean ± SEM of separate determinations in six different experiments. Student's t test was used for comparison of the means. $^{**}p < 0.01$.

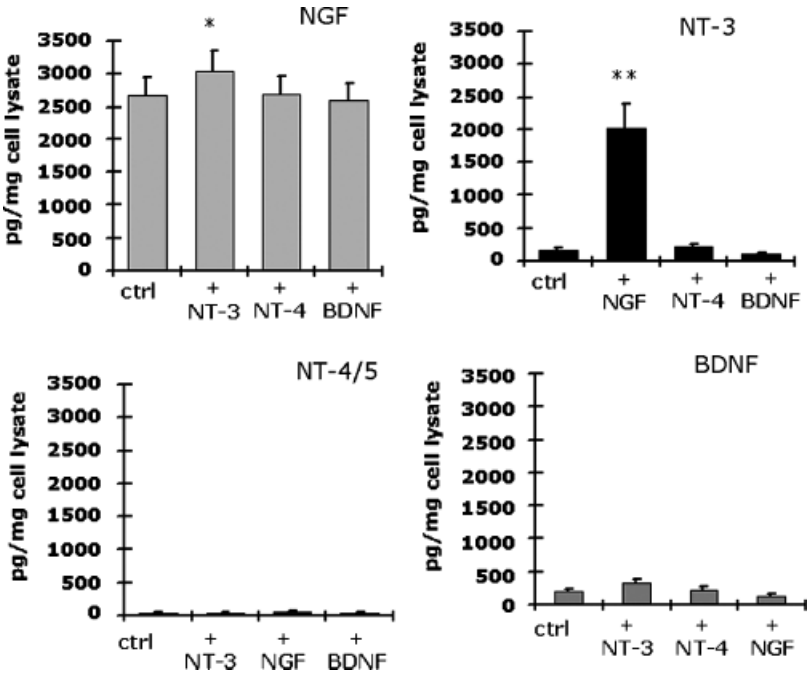

Figure 6. Reciprocal influence of NT. Keratinocytes were cultivated in KGM and stimulated with 100 ng per mL NGF, NT-3, BDNF, or NT-4/5 for 48 h. NT protein levels were measured in cell lysates and results are given in pg per mg of cultured supernatants. Data are expressed as mean ± SEM of triplicate from three different experiments. $^{*}p < 0.05$; $^{**}p < 0.01$.

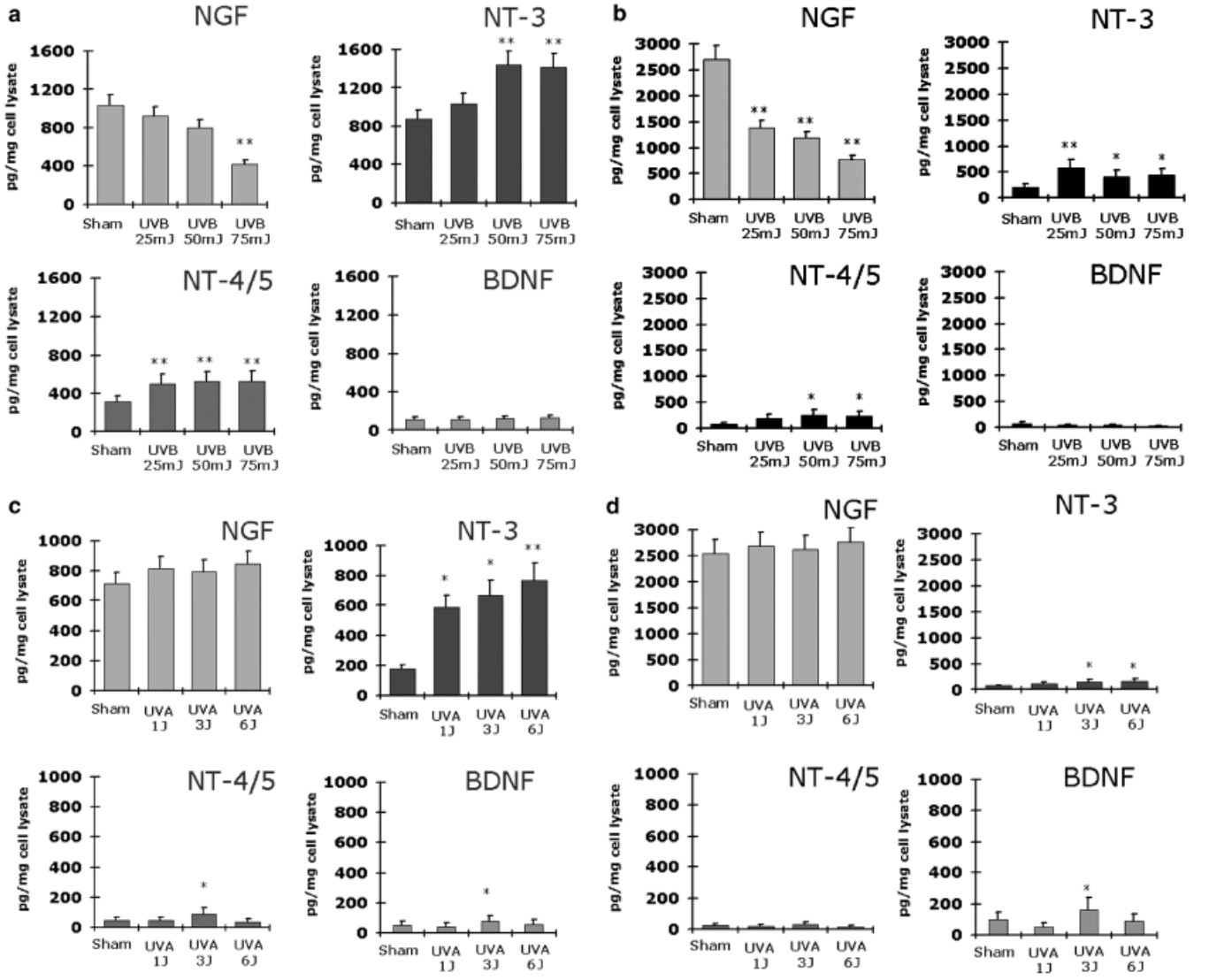

Figure 7. Effect of UV light on NT secretion. Keratinocytes were cultivated in DMEM/F12 up to preconfluency and irradiated with 25, 50, and 75 mJ per cm^2 UVB (*a,b*) and 1, 3, and 6 J per cm^2 UVA (*c,d*). NT were measured by ELISA a and c cell lysates, b and d, conditioned medium. NT protein levels are given in pg per mg of cell lysate and results are expressed as mean ± SEM of triplicate from three different experiments. $^{*}p < 0.05$; $^{**}p < 0.01$.

DISCUSSION

Keratinocytes are both the source and the target of a number of cytokines and growth factors that contribute to epidermal proliferation, differentiation and apoptosis (Nickoloff *et al*, 1995; Bernstein and Vaughan, 1999). NGF is one of these growth factors: blockade of endogenous NGF inhibits keratinocyte proliferation and induces apoptosis, indicating that an important autocrine loop exists in the epidermis, where NGF plays a crucial part (Pincelli and Marconi, 2000b). This study demonstrates that NGF is synthesized and released by human keratinocytes in greater amounts than other NT. Moreover, NGF appears to be expressed and secreted in higher levels by KSC cells as compared with more differentiated cells. This is intriguing in view of the fact that KSC are protected from cell death (Tiberio *et al*, 2002) and one could speculate that autocrine NGF exerts its anti-apoptotic activity mostly on this keratinocyte subpopulation. On the other hand, it is interesting to note that the other NT, which are not elevated in KSC, do not exert anti-apoptotic activity.

We report that other NT family members are expressed in keratinocytes and could participate in a novel "neurotrophin network" within the epidermis. We present evidence that human keratinocytes synthesize and secrete all of the NT. Whereas others have reported that human keratinocytes fail to synthesize NT-3 (Grewe *et al*, 2000), the presence of this NT is confirmed in the current study not only by detection of RNA but also by protein release. Whereas all of the NT are synthesized in human keratinocytes, only low levels of NT protein are detected, suggesting that release in the medium only takes place under certain circumstances and upon various stimuli. NT storage and release is a matter of debate also in the nervous system: it appears that NT undergo regulated secretion in response to depolarization by high potassium (Blochl and Thoenen, 1995; Wang and Poo, 1997; Franke *et al*, 2000) or in response to calcium (Canossa *et al*, 1997; Kang and Schuman, 2000). In preconfluent, still proliferating keratinocytes, used in this study, the K^+ channel modulating Ca^{2+} influx is not yet active (Mauro *et al*, 1997). It is thus feasible that, under these conditions, NT release is not allowed. Moreover, it is possible that NT secretion in keratinocytes occurs only in dener-

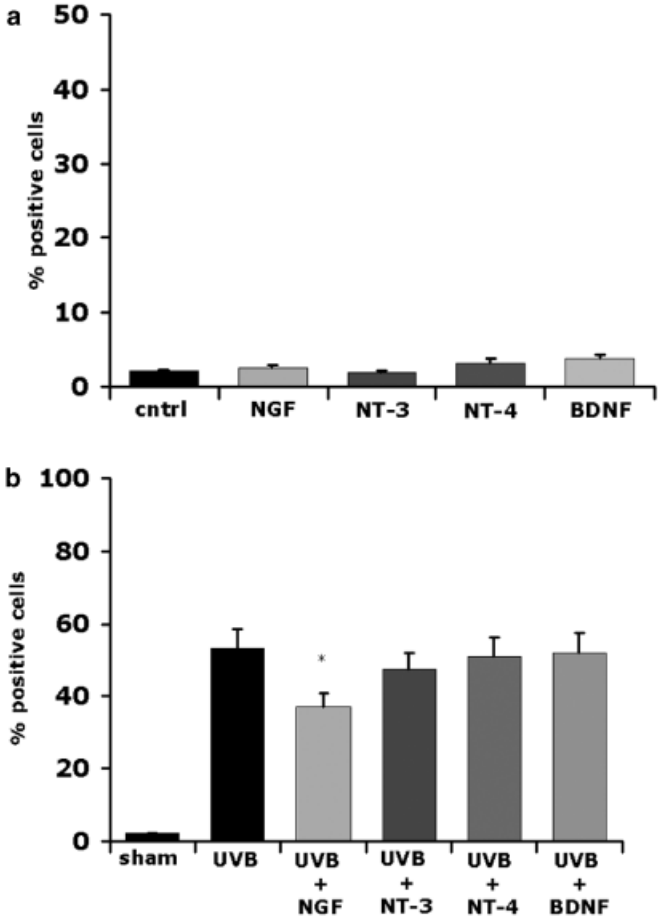

Figure 8. NT effect on keratinocyte apoptosis. Cells were treated for 24 h with all NT and apoptosis was measured by TUNEL staining. About 100 cells were counted in randomly selected fields for each point. Percentages of positive cells are expressed as the mean ± SEM of three experiments. Student's t test was used for comparison of the means (*a*). Cells were preincubated for 48 h with NT and directly stained 24 h after UVB or sham irradiation with the TUNEL method (*b*). Positive cells were counted as in (*a*). $^{*}p < 0.05$.

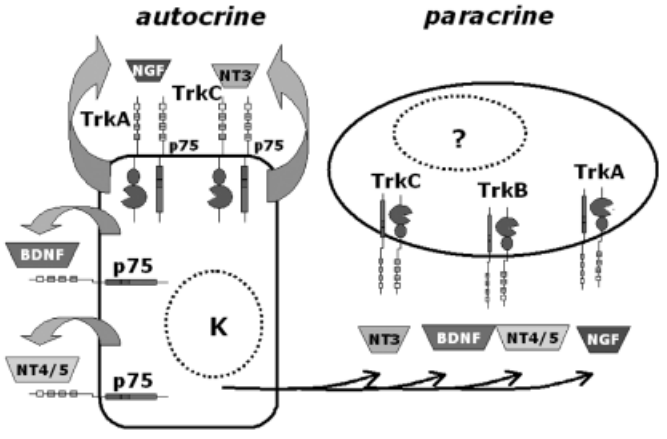

Figure 9. Hypothetical scenario of the NT network in human epidermis. Keratinocyte-derived NGF and NT-3 can act both in an autocrine manner and in a paracrine manner through trkA and trkC, respectively. As functional trkB is not expressed in human keratinocytes, BDNF and NT-4/5 can only act in a paracrine fashion on other skin cells; however, the possibility exists that these NT might also exert some effects on cells that express only the low-affinity NT receptor p75 (see text).

vated areas, such as in diabetic skin, as a compensatory mechanism (Kennedy *et al*, 1998). Finally, NT, secreted either by keratinocytes themselves or possibly by other skin cells, may induce the release of other NT through the regulated secretory pathway, such as in the nervous system (Kruttgen *et al*, 1998). Indeed, in this study, NGF and NT-3 stimulate each other release, possibly contributing to the amplification of the autocrine loop where both NT play a crucial part in keratinocyte proliferation.

The expression of trk receptors in human keratinocytes has been reported previously. There is agreement on the localization of trkA that, with different techniques, has been shown to be expressed only in the basal epidermal layer (Shibayama and Koizumi, 1996; Terenghi *et al*, 1997). This finding has been confirmed in a previous work by reverse transcription–PCR performed on proliferative basal keratinocytes and supports the role of this tyrosine kinase receptor in the autocrine survival loop sustained by NGF in human keratinocytes (Pincelli, 2000). By contrast, whereas trkC mRNA was detected in basal keratinocytes (Terenghi *et al*, 1997), immunohistochemical studies have not confirmed the protein expression in the same location (Shibayama and Koizumi, 1996). The present study provides evidence that proliferating human keratinocytes express trkC at the mRNA level, whereas NT-3 stimulation of keratinocyte proliferation supports the expression of the functional receptor protein. Failure of BDNF or NT-4/5 to exert any activity on keratinocytes confirms the absence of the full-length trkB in these cells. In fact, we show that human keratinocytes only express the truncated isoform of trkB, which has been postulated to act as a dominant inhibitory modulator of trkB signaling (Kryl and Barker, 2000). On the other hand, BDNF might induce cell cycle arrest or apoptosis in human keratinocytes expressing the low-affinity NT receptor p75, which mediates these effects in most cell systems (Bamji *et al*, 1998).

NT and NT receptors seem to be differently expressed in human and mouse skin. The work on NT carried out in mouse skin has shown that p75 is not expressed in basal epidermal keratinocytes (Botchkarev *et al*, 1998), at variance with human epidermis (Fantini and Johansson, 1992). With respect to the trk receptors, murine epidermal keratinocytes express trkB but not trkC. In addition, no NT immunoreactivity is observed in nonhairy murine keratinocytes (Botchkarev *et al*, 1998). It would appear that, in the mouse system, NT are derived mostly from nerve fibers or dermal cells and are involved in the hair cycle control (Botchkarev *et al*, 1999b, 2000). On the contrary, NT seem to play a more important part in the human system as growth factors. Autocrine NT-3, similarly to NGF, stimulates keratinocyte proliferation, most likely through trkC and trkA receptors, suggesting that these NT, also by upregulating each other release, can co-operate in the maintenance of epidermal homeostasis. As trkB is not functioning in human keratinocytes, it remains to be established whether NT-4/5 exerts paracrine activities on other cell types.

With respect to apoptosis, it is interesting to note that only NGF is downregulated by UVB and protects keratinocytes from UVB-induced cell death, as previously reported (Marconi *et al*, 1999). On the other hand, other NT are not reduced by UVB. This confirms that, among NT, NGF only can be considered as "the survival factor" for human keratinocytes. More detailed studies utilizing keratinocytes from NT-3/trkC-knock-out mice are necessary, however, to rule out the possibility that this NT is actually not involved in the protection of keratinocytes from UVB-induced apoptosis.

In conclusion, this study describes a network of NT that, together with their receptors, could act both in an autocrine and in a paracrine manner in human epidermis. Ongoing studies will clarify which cells other than keratinocytes are the target and/or the source of NT in human skin (**Fig 9**). If the observation of a relationship between NGF and keratinocyte-derived cytokines (Pincelli, 2000) will be confirmed for other NT, one could envi-

sage a complex system whereby epidermal NT and cytokines co-operate in the control of skin homeostasis and inflammation.

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