

Immunohistochemical Expression of Nerve Growth Factor in Psoriasis, Alopecia Areata, and Vitiligo Before and After PUVA Therapy

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Abstract

Nerve growth factor (NGF) belongs to a family of neurotrophic proteins termed neurotrophins. It is synthesized and released by human keratinocytes. It mediates its effect by binding to two kinds of transmembrane protein receptors; a high affinity tyrosine kinase receptor (TrK-A) and low affinity p75 receptor. In addition to its role for the development and maintenance of cutaneous innervation, it plays an important role in cutaneous homeostasis, control of hair follicle development and cycling, and melanogenesis. Numerous indications suggest that neurotrophins play an important role in the pathogenesis of a variety of autoimmune diseases. This study aimed to study the NGF expression in cutaneous lesions of psoriasis, alopecia areata and vitiligo before and after PUVA therapy in a trial to investigate its role in their pathogenesis. This study included 30 patients (10 psoriasis, 10 alopecia areata, 10 vitiligo) as well as 10 healthy control subjects. From each patient, two biopsies were taken before and after PUVA therapy. The specimens were examined for NGF expression using immunoperoxidase staining on cryosections.

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NGF was detected in the cytoplasm of keratinocytes in a diffuse granular pattern. Most of the control subjects 60% showed negative expression while 40% showed mild degree of NGF expression in basal cell layer. In psoriatic patients, 80% showed marked expression and 20% showed moderate expression of NGF. In alopecia areata, 80% of patients showed moderate expression and 20% of them showed mild expression of NGF. While in vitiligo all patients showed negative expression of NGF. After PUVA therapy, NGF staining pattern within the keratinocytes was significantly decreased in psoriatic and alopecia areata patients. This study indicated that NGF may have an important role in the pathogenesis of these cutaneous disorders and PUVA therapy was found to be beneficial in reducing NGF expression in the skin.

Introduction

Nerve growth factor (NGF) is a member of a family encoding structurally and functionally related proteins called neurotrophins. The neurotrophins consist of four proteins: NGF, brain derived neurotrophic factor, neurotrophin-3 and neurotrophin-4⁽¹⁾. NGF was the first critical

member of NTs family to be discovered. It was discovered in 1954 and isolated in 1960. Initially, NGF has been described as a growth and differentiation factor for sympathetic and sensory neurons, but this factor also has biologic activities outside the nervous system⁽²⁾. It mediates its effect by binding to two kinds of transmembrane protein receptors, a high affinity tyrosine kinase receptor of 140 KDs (P140) corresponds to the TrK-A protooncogene and a low affinity receptor of 75 KDs (P75) which is a member of TNF- α receptor superfamily and has no tyrosine kinase activity⁽³⁾.

NGF is synthesized and released in skin by proliferating keratinocytes, melanocytes mast cells, macrophages, fibroblasts, Merckel's cells and T-lymphocytes⁽¹⁾. Human keratinocytes synthesize P75 and TrKA receptors which are expressed in basal layer of epidermis⁽⁴⁾. It has a critical role for the development and maintenance of cutaneous innervation. It also, fulfills multiple non neurotrophic functions in skin including regulation of epidermal proliferation and apoptosis, control of hair follicle development and cycling, and melanogenesis^(1,5). Numerous indications suggest that neurotrophins play an important role in the pathogenesis of a variety of autoimmune diseases⁽¹⁻³⁾. However, little is known about their role in autoimmune dermatoses.

Therefore, this study aimed to study the expression of NGF in some autoimmune dermatoses (psoriasis, alopecia areata and vitiligo) before and after PUVA therapy in a trial to explain its role in the pathogenesis of these dermatoses, as well as to study the effect of PUVA on NGF expression.

Materials and Method

This study comprised a total of 30 patients with different autoimmune diseases in addition to 10 healthy control subjects. The studied subjects were divided into:

Group I: Included 10 patients with chronic generalized psoriasis.

Group II: Included 10 patients with alopecia areata.(multiple patches & alopecia totalis).

Group III: included 10 patients with progressive vitiligo.

Group IV: Included 10 healthy subjects served as control.

All the patients had received no treatment for 3 weeks prior to the study. From all the patients, 2 biopsies were taken, one before PUVA therapy and the other after 8 weeks of PUVA therapy (3 sessions/week at a dose ranging from 5 to 12 Joules/cm² according to the skin type and minimal erythema dose of each patient).

Biopsies were immediately frozen in liquid nitrogen and stored at -70 °C. The 3-5 micron thick section slides were fixed in cold acetone and incubated for 5-10 min in 0.1 hydrogen peroxide in PBS for 30 min. for immunoperoxidase staining (Santa - Cruz Biotechnology Inc ABC staining system)⁽⁶⁾. The slides were incubated with anti-NGF antibody, biotin, conjugated secondary antibody and streptavidin peroxidase complex, each for 30 min at room temperature then washed by PBS for 5 min. Then they were incubated in peroxidase substrate for 30 seconds and washed by PBS for 10 min. The sections were washed by water 1-2 drops of haematoxylin were put, and then dehydrated through alcohol and xylene. The slides were examined under light microscope.

The intensity of staining was designated as:

- (0):** (negative staining); no positive cells.
- (+):** (mild intensity of staining); less than 25% of positive cells.
- (++):** (moderate intensity of staining); 25-50% of cells are positive.
- (+++):** (marked intensity of staining); more than 50% of cells are positive.

Results

In positive cases : keratinocytes stained positively for NGF. The stain was present in the cytoplasm in a diffuse granular pattern especially along the dermoepidermal junction. Positive staining was also found in the inflammatory infiltrate in the dermis. Stratum corneum stained

positively was considered as a non specific reaction.

Most of the control subjects (60%) showed negative expression while in (40%) of control subjects showed mild degree of NGF expression in the basal cell layer (Fig. 1,2).

Before PUVA (Table 1):

- In psoriatic patients, 80% of them showed marked expression while 20% showed moderate expression of NGF (Fig. 3,4,5).
- In alopecia areata patients, 20% of them showed mild expression , while 80% showed moderate expression of NGF (Fig. 6,7,8).
- In vitiligo, all patients showed negative expression of NGF (100%), (Fig. 9,10).
- After PUVA therapy (table 2) it was observed that NGF staining pattern within keratinocytes throughout the epidermis was substantially decreased in the basal cell layer up to the mid-spinous layer, where staining of the upper epidermal cell layer was unchanged, (Fig. 11,12).

As comparing the intensity of NGF expression in patients before and after PUVA, it was found that there was significant decrease in NGF expression after PUVA in psoriatic and alopecia areata patients, (Table 2).

Discussion

Cellular responses to NGF are mediated by two classes of receptors; TrKs (A and B) and P75. TrKA can mediate NGF effects in the absence of P75, while P75 may modulate or enhance TrKA trophic signals to NGF⁽⁷⁾. Only recently, it was suggested that NGF binding to P75 in cells that do not express TrKs results in increased intracellularly ceramide activation, transcription of Nuclear Factor Kappa B and finally apoptosis⁽⁸⁾.

In the present study, NGF expression was significantly elevated in psoriatic patients as compared to control. This is consistent with the previous reports, which found increased NGF expression in keratinocytes in both lesional and non lesional psoriasis^(9,10). They supposed that,

NGF is mitogenic to keratinocytes. It stimulates keratinocytes proliferation and protects them from apoptosis through binding to high affinity receptor (Trk-A) resulting in an autocrine loop for NGF in keratinocytes⁽¹¹⁾. Moreover, it was found that there was increased NGF level in the epidermis of psoriasis when measured by two site ELISA method⁽¹²⁾. In addition, there was increased expression of NGF receptors in psoriasis, whereas neither NGF nor its receptors were found to be increased in lichen planus, suggesting that NGF plays an important role in the pathogenesis of psoriasis⁽¹³⁾.

NGF can exert a number of effects on immune-inflammatory cells; it recruits mast cells and promotes their degranulation, it activates T-lymphocytes and protects neutrophils from apoptosis. Thus, it is possible that expression of NGF is required to initiate the inflammatory process in psoriasis^(14,15). Another possible role for NGF is that it stimulates neuropeptides synthesis which have a role in the inflammatory and proliferative process in psoriasis⁽¹⁶⁾.

As regard alopecia areata there was mild to moderate expression of NGF which decreased after PUVA therapy. This is consistent with previous reports that showed high levels of neurotrophins in other tissues affected by autoimmune disorders^(17,18). Palikina et al., (2002)⁽¹⁹⁾ found that in alopecia areata affected mice, NGF level in anagen hair follicle, was elevated in the inner root sheath and all Trk receptors were downregulated in the outer root sheath. In contrast, the apoptotic receptor P75 was upregulated in the outer root sheath and was ectopically expressed in the dermal papillae. Therefore it is suggested that NGF may be responsible for inducing apoptosis in hair follicles in alopecia areata^(19,20).

Positive staining for NGF in the inflammatory infiltrate in dermis is also consistent with others who found that both dermal dendritic cells and CD8+ cells in the inflammatory infiltrate expressed P75, implicating involvement of neurotrophins in the control of apoptosis in CD8+ lymphocytes⁽¹⁹⁾.

Interestingly, NGF administration into alopecia areata affected skin was found to be associated with a reduction in the number of CD8+ cells, which coexpressed P75. Conversely,

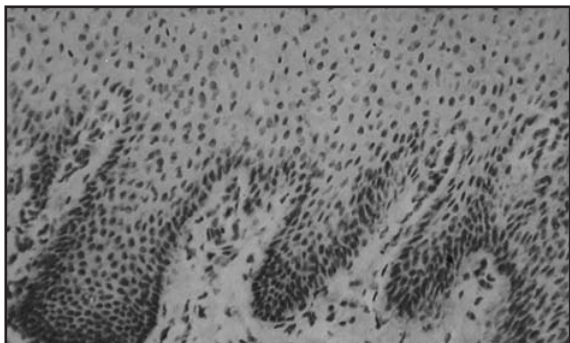


FIG .1. Normal skin showing negative expression of NGF (PAPx 200).

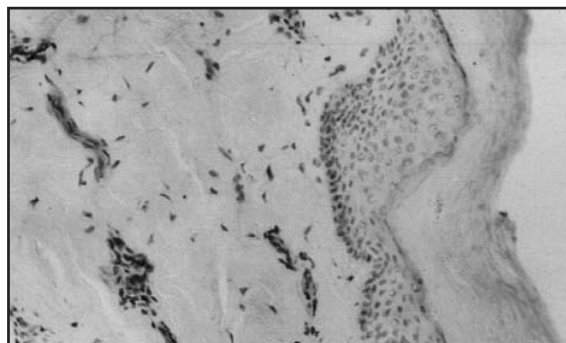


FIG .2. Normal skin showing mild expression of NGF in the basal cell layer of the epidermis (PAPx 200).

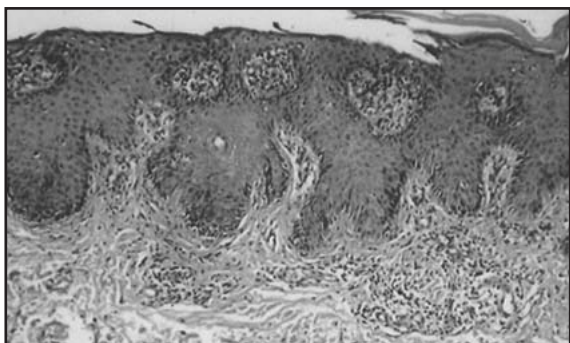


FIG .3. A case of psoriasis (H&E x120).

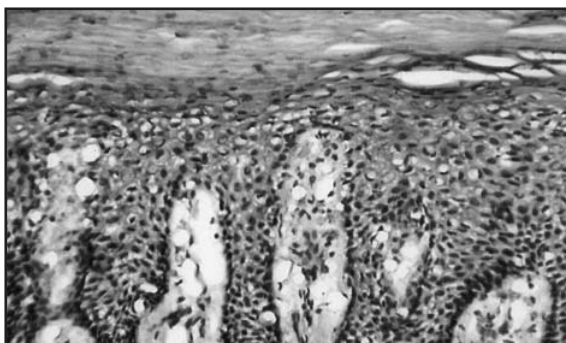


FIG .4. Marked epidermal expression of NGF in psoriasis case (PAP x 200).

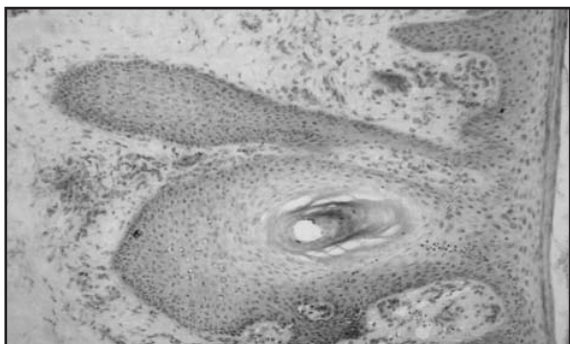


FIG .5. Moderate epidermal expression of NGF in psoriasis case (PAP x 120).

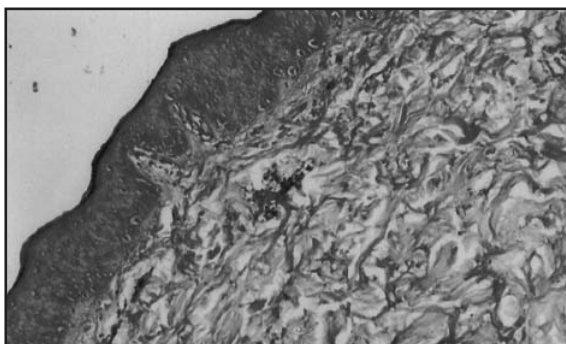


FIG .6. A case of alopecia areata (H & E x 120).

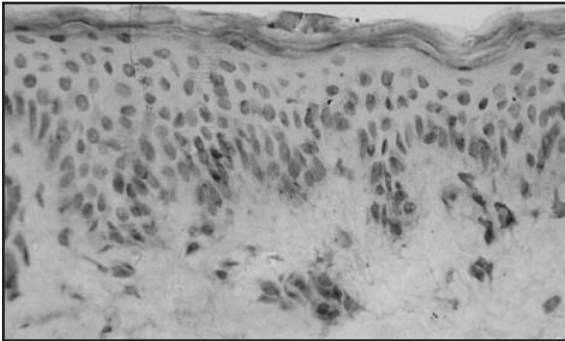


FIG .7. NGF mild expression in the basal cell layer of the epidermis in a case of alopecia areata (PAP x 200).

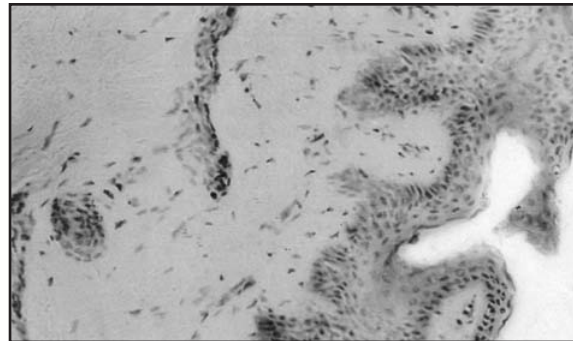


FIG .8. NGF moderate expression in the epidermis in a case of alopecia areata (PAPx 200).

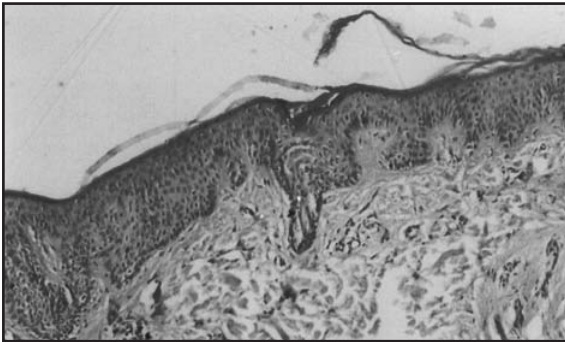


FIG .9. A case of vitiligo (H&E x120).

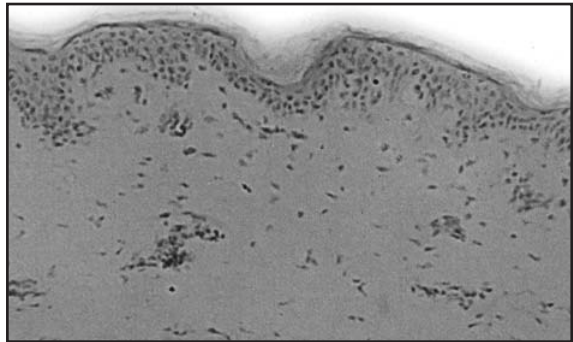


FIG .10. A case of vitiligo showing negative expression of NGF (PAPx 100).

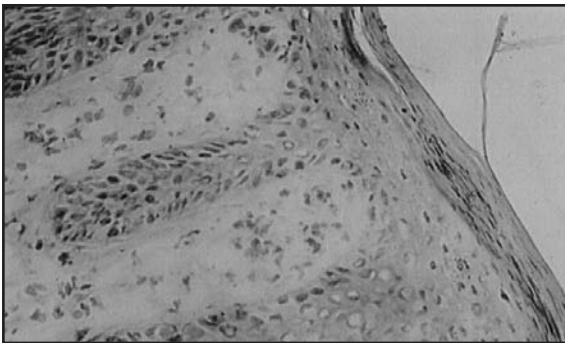


FIG .11. A case of psoriasis after PUVA therapy revealed decreased expression of NGF (PAPx 200).

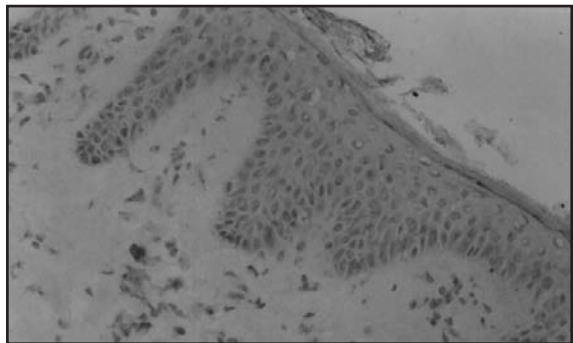


FIG .12. A case of alopecia areata after PUVA therapy revealed negative expression of NGF (PAPx 120).

treatment of alopecia areata affected mice with a cyclic peptide that blocks P75 prominently increased the number of CD8+ cells in the skin. These data raised a possibility of exploring stimulators of neurotrophin signaling through P75 for the treatment of autoimmune hair loss⁽⁵⁾.

In vitiligo, NGF expression was negative in all cases. This may be consistent with the hypothesis that melanocytes can synthesize NGF since they are derived from the neural crest and share many signaling molecules with neurones⁽²¹⁾. However, others supposed that NGF detected in melanocytes may result from transfer to melanocytes after its synthesis in the neighboring cells, such as basal keratinocytes or even nerve endings. NGF acts in a paracrine fashion on human melanocytes which express the low and high affinity receptors and display increased dendricity upon its stimulation⁽⁴⁾.

Recently it is supposed that keratinocytes in vitiligo produce lower level of growth factors which regulate melanocyte growth and survival. Therefore the decreased production of NGF by keratinocytes in vitiligo may be a factor contributing to melanocytes damage since NGF is important for melanocytes survival after injury⁽²²⁾.

As regard the effect of PUVA on NGF, the present study demonstrated that PUVA can reduce the elevated NGF in the skin of psoriasis and alopecia areata. The exact mechanism is unknown. However Stefanato et al⁽⁴⁾ showed that NGF immunostaining was markedly reduced in the lower epidermal layers 72h after UV radiation. This may be due to the temporary cessation of NGF synthesis by basal cell layer keratinocytes due to UV-induced down-regulation of gene transcription and /or an accelerated transfer of NGF following UV radiation⁽²³⁾.

It could be concluded that NGF may have an important role in the pathogenesis of psoriasis, alopecia areata and vitiligo. Moreover, PUVA therapy is effective in reducing NGF level in the skin. Because this study investigated the staining patterns of NGF and not protein levels, further studies would be required to corroborate these findings. The progress in this area of research will lead to the development of multiple uses for neurotrophins and their agonists-antagonists in different autoimmune diseases.

Table (1): Comparison between the intensity of NGF expression in patients group and control.

Group Intensity	Control		Psoriasis		Alopecia areata		Vitiligo	
	No.	%	No.	%	No.	%	No.	%
0	6	60	-	-	-	-	10	100
+	4	40	-	-	2	20	-	-
++	-	-	2	20	8	80	-	-
+++	-	-	8	80	-	-	-	-
Chi-Square			89.63		47.523		10.85	
P			< 0.001		<0.05		>0.05	

Table (2): Comparison between intensity of NGF expression in patients groups before and after PUVA.

Group Intensity	Psoriasis		Alopecia areata		Vitiligo	
	Before	After	Before	After	Before	After
0	-	-	-	-	10	10
+	-	4	2	10	-	-
++	2	6	8	-	-	-
+++	8	0	-	-	-	-
Chi-Square	53.25		69.235		-	
P	< 0.05		< 0.05		-	

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