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K252a, a High-Affinity Nerve Growth Factor Receptor Blocker, Improves Psoriasis: An *In Vivo* Study Using the Severe Combined Immunodeficient Mouse–Human Skin Model

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The peripheral nervous system, in addition to its sensory and motor functions, can induce a local inflammatory response known as neurogenic inflammation. This phenomenon plays a critical role in several inflammatory diseases, e.g., asthma, atopy, rheumatoid arthritis, psoriasis, and ulcerative colitis. Neurogenic inflammation and the role of nerve growth factor (NGF) have been extensively studied in psoriasis. There are increased levels of NGF in the keratinocytes and upregulation of NGF receptor (NGF-R) in the cutaneous nerves of psoriatic plaques. NGF can influence all the salient pathologic events noticed in psoriasis such as proliferation of keratinocytes, angiogenesis, T cell activation, expression of adhesion molecules, proliferation of cutaneous nerves, and upregulation of neuropeptides. In this double-blinded, placebo-controlled study, we addressed the role of NGF/NGF-R in psoriasis in an *in vivo* system using the severe combined immunodeficient (SCID) mouse–human skin model of psoriasis. The transplanted psoriatic plaques on the SCID mice ($n = 12$) were treated with K252a, a high-affinity NGF receptor blocker. Psoriasis significantly improved following 2 wk of therapy. The length of the rete pegs changed from 308.57 ± 98.72 to 164.64 ± 46.78 μm ($p < 0.01$, Student's *t* test). A similar improvement of psoriasis was observed by directly inhibiting NGF with NGF-neutralizing antibody. NGF-neutralizing antibody in normal saline at 10 ng ($n = 4$) and 20 ng ($n = 4$) per kilogram of body weight doses were used. Both doses of NGF-neutralizing antibody reduced rete peg lengths significantly, e.g., from 298.5 ± 42.69 to 150.52 ± 32.93 μm ($p < 0.05$, Student's *t* test). This study provides evidence for the role of NGF and its high-affinity receptor in the pathogenesis of psoriasis and insights to develop novel therapeutic modalities.

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A role of neurogenic inflammation in the pathogenesis of psoriasis is substantiated by a number of observations: exacerbations during periods of stress, marked proliferation of terminal cutaneous nerves, and upregulation of neuropeptides (SP, VIP, CGRP) in the psoriatic plaques, therapeutic response to neuropeptide-modulating agents such as capsaicin, somatostatin, and peptide T, and clearance of active plaques of psoriasis at the sites of anesthesia following traumatic denervation of cutaneous nerves (Bernstein *et al*, 1986; Farber *et al*, 1986, 1991; Wallengren *et al*, 1987; Naukkarinen *et al*, 1989; Camisa *et al*, 1990; Leeman *et al*, 1991; Raychaudhuri and Farber, 1993; Al'Abadie *et al*, 1995). The unique features of resolution of psoriasis at sites of anesthesia, upregulation of neuropeptides and a marked proliferation of terminal cutaneous nerves in psoriatic plaques, encouraged us to search for the mechanism of neural influence. Because nerve growth factor (NGF) augments tissue innervation (Wyatt *et al*, 1990) and plays a critical role in regulating certain neuropeptides such as SP and CGRP (Schwartz *et al*, 1982; Lindsay and Harmar, 1989), we investigated the role of NGF in psoriasis. Along with other investigators we observed that keratinocytes in lesional and nonlesional psoriatic tissue express high levels of NGF compared to the controls (Fantini *et al*, 1995; Raychaudhuri *et al*, 1998), and there is a marked upregulation of NGF receptor (NGF-R) in the terminal cutaneous nerves of psoriatic lesions (Raychaudhuri *et al*, 2000a, b).

Although clinical and laboratory studies suggest a critical role of NGF and its receptor (NGF-R) system in the inflammatory process of psoriasis, it has not yet been substantiated by any direct evidence. The biologic functions of the neurotrophins are mediated through two classes of cell surface receptors, the trk family of tyrosine kinases and the p75 neurotrophin receptor. NGF, the best-characterized member of the neurotrophin family, mediates cellular responses by a high-affinity receptor (trkA) and a low-affinity receptor (p75). Although the Trk receptors are responsible for most of the survival and growth properties of the neurotrophins (Cordon-Cardo *et al*, 1991; Allsopp *et al*, 1993; Majdan *et al*, 2001), the actions of p75 neurotrophin receptor fall into two categories (Hempstead *et al*, 1991; Ross *et al*, 1996; Maliartchouk and Saragovi, 1997; Majdan *et al*, 2001). First, p75 neurotrophin receptor

Abbreviations: NGF, nerve growth factor; NGF-R, nerve growth factor receptor; SCID, severe combined immunodeficient

This work is dedicated in memory of Dr Eugene M. Farber, President, Psoriasis Research Institute, Palo Alto, CA.

is a Trk coreceptor that can enhance or suppress neurotrophin-mediated Trk receptor activity. Second, p75 neurotrophin receptor autonomously activates signaling cascades that result in the induction of apoptosis or in the promotion of survival. To determine the significance of NGF/NGF-R system in the inflammatory process of psoriasis, we evaluated therapeutic efficacies of K252a, a high-affinity NGF receptor inhibitor (Koizumi *et al*, 1988; Lazarovici *et al*, 1989; Berg *et al*, 1992), and NGF-neutralizing antibody. In this study the transplanted psoriatic plaques on the severe combined immunodeficient (SCID) mouse–human skin model were treated with intralesional injections of K252a and NGF-neutralizing antibody.

Results

K252a inhibits nerve regeneration in psoriatic plaques In each mouse, the acceptance of the human skin graft was confirmed by histologic and immunohistochemical staining. Compared to normal skin, increased levels of NGF in the keratinocytes were maintained in the transplanted psoriatic plaque (Fig 1*a,b*).

It is anticipated that marked upregulation of NGF should augment proliferation of nerves in the transplanted plaques. Indeed we observed complete regeneration of cutaneous nerves in the transplanted psoriatic plaques within 4 wk of transplantation. Nerves stained positively with PGP9.5, MAP-2, and p75 antibody. We used trkA antibody from several sources but did not get a good staining of the nerves. Nevertheless, with the p75 antibody the nerve staining was best. It showed more nerves in the psoriatic tissue compared to PGP9.5 and MAP-2. Also p75-stained nerves were more prominent in respect to the degree of fluorescence and linear measurement. Because the nerves stained much better with the p75 antibody and also upregulation of p75 receptor is a marker of an *in vivo* effect of NGF, for quantification of nerve regeneration we used the data from p75 antibody staining. The numbers of regenerated terminal cutaneous nerves positive for NGF-R were significantly higher in the transplanted psoriatic plaques compared to the normal human skin grafts as seen in Fig 2(*a,c*). Grafts took 3 to 4 wk for wound healing; the earliest biopsies were available on the 4th week of transplantation. In transplanted normal skin, no nerves could be stained in the biopsies collected on the 4th week (Fig 2*c*); few scattered fine nerves in mid-dermis started appearing in the 12th week, whereas in transplanted psoriatic plaques in the 4th week, distinct multiple large nerves traversing from dermis to the epidermis were identified (Fig 2*a*). This suggests the *in vivo* effect of increased levels of NGF from the keratinocytes of psoriatic plaques. The same plaque (Fig 2*a*) following 2 wk of treatment with K252a does not demonstrate distinct NGF-R-positive nerves (Fig 2*b*).

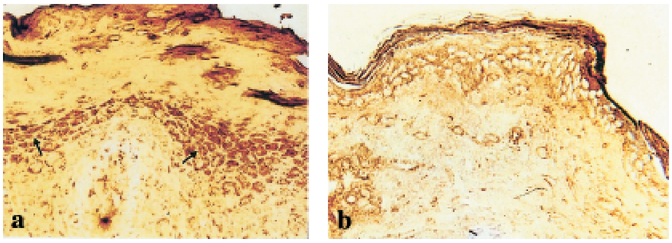

Figure 1
Histologic section of a transplanted psoriatic plaque demonstrates marked expression of NGF in the keratinocytes (*a*) compared to the normal skin (*b*). Magnification × 400.

K252a improves clinical, histologic, and immunologic features of psoriasis Transplanted plaques treated with K252a had significant clinical and histologic improvement compared to the controls. After 2 wk of treatment with K252a, transplanted psoriatic plaques had reduced scales, erythema, and infiltration. The histologic improvement in the treated plaques was evidenced by the significant reduction of hyperkeratosis, acanthosis, and lymphomononuclear cellular infiltrates (Fig 3*a–c*; Table I). In Fig 3(*a*), marked dermal infiltrates are noticed before treatment, whereas 2 wk after treatment (Fig 3*c*) with K252a there is barely any infiltrate in the dermis. In the K252a-treated plaques, there was significant thinning of the epidermis, the length of the

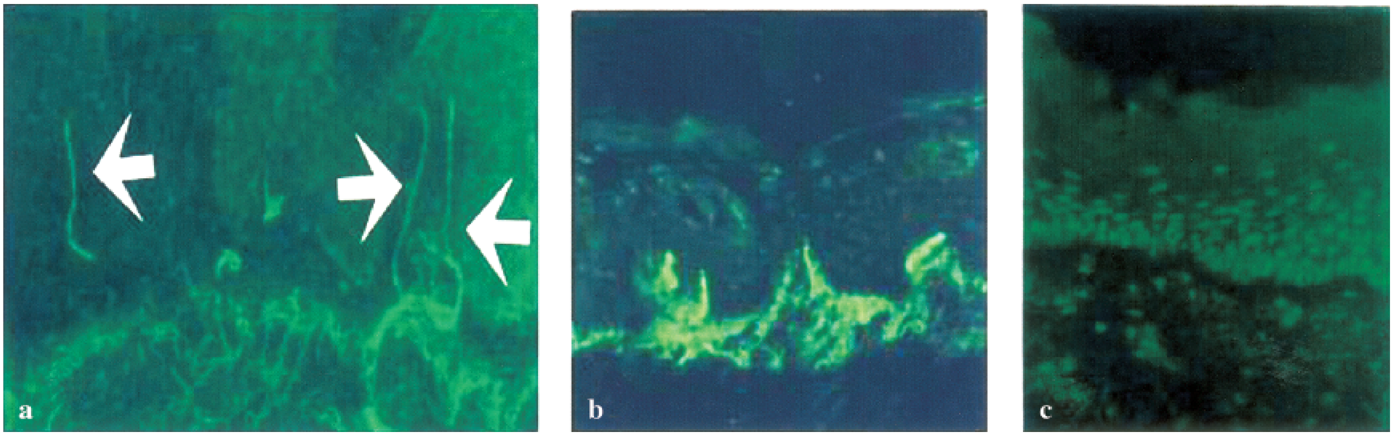

Figure 2
Transplanted psoriatic plaque demonstrates marked regeneration of NGF-R-positive nerve fibers. (*a*). The transplanted psoriatic plaque at the fourth week of transplantation shows several long-terminal cutaneous nerves positive for NGF-R. (*b*) The same plaque (*a*) following 2 wk of treatment with K252a shows reduced thickness of the epidermis and with only rudimentary NGF-R-positive nerve fibers. *Arrows*, nerve fiber. Magnification (*a,b*) × 160. (*c*) No regeneration of nerves can be seen in the transplanted skin from a normal subject at the fourth week of transplantation. Magnification × 200.

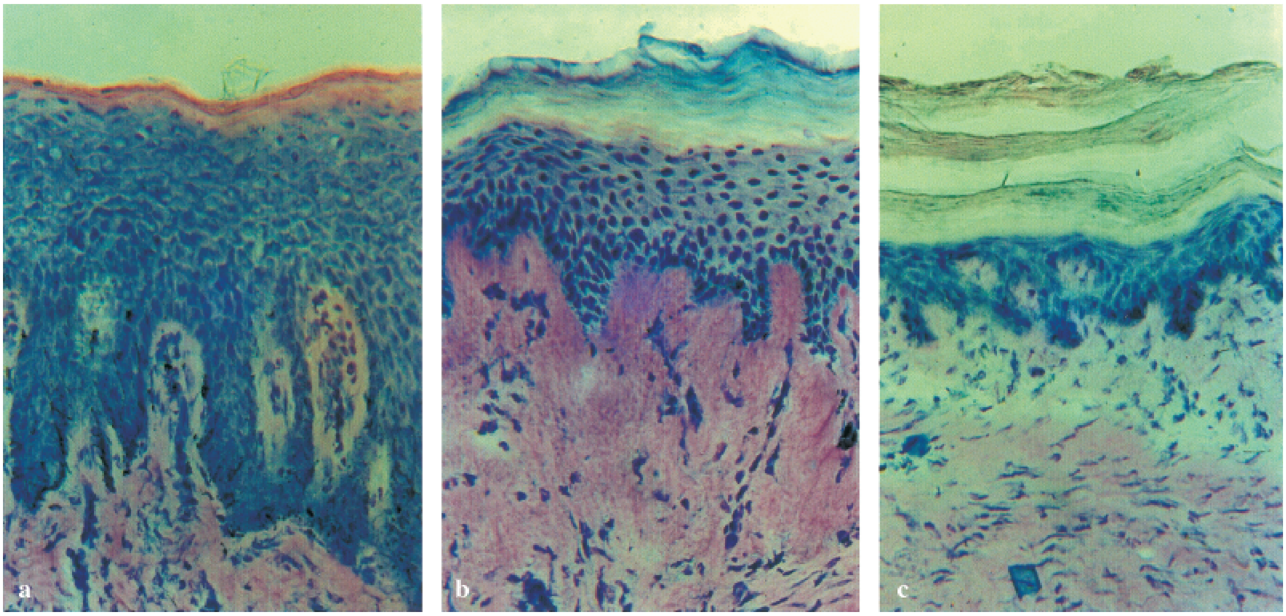

Figure 3
Complete resolution of the histologic features of psoriasis following treatment with K252a therapy. (*a*) Histology of the plaque before treatment demonstrates the characteristic morphologic features of psoriasis. (*b*) The lesion 1 wk after completion of intralesional treatment with K252a demonstrates significant reduction of acanthosis and reduced amount of infiltrates. (*c*) At the end of 2 wk after treatment complete resolution of hyperkeratosis, acanthosis and barely any dermal infiltrate can be noticed. Magnification × 160.

Table I. Effect of K252a treatment on the rete peg length (μm) of the transplanted psoriatic plaques (n = 20)

Group	Before treatment	After treatment
K252a treatment: 50 μg BID for 14 days (n = 12)	308.57 ± 98.72	164.64 ± 46.78
Control: normal saline (n = 8)	269.37 ± 57.78	209.37 ± 74.00

rete pegs changed from 308.57 ± 98.72 to 164.64 ± 46.78 μm 1 wk after completion of treatment ($p < 0.01$, Student's *t* test; Table I). In the control group, the before- and after-therapy rete peg lengths were 269.37 ± 57.78 and 209.37 ± 74.00 μm ($p > 0.1$, Student's *t* test) (Table I). Comparing the K252a arm to the control arm, the changes in rete peg lengths from before to after therapy were statistically different from each other ($p < 0.01$, Wilcoxon rank sum test). HLA-DR-positive lymphocytic infiltrates and intraepidermal CD8+ lymphocytes were significantly reduced in the K252a-treated plaques. We particularly focused on these two phenotypes of lymphocytes because intraepidermal localization of CD8+ lymphocytes is a unique immunopathologic features of psoriasis and HLA-DR-positive lymphocytes identifies the activated CD4+ infiltrates. To demonstrate the infiltrates, we have taken microphotographs of the histologic sections from before and after treated transplanted psoriasis plaques. We have observed that the inflammatory infiltrates (hematoxylin and eosin staining), HLA-DR-positive lymphocytic infiltrates and intraepidermal CD8+ lymphocytes were significantly reduced in the K252a-treated plaques. In pre- and post-treated tissues intraepidermal CD8+ lymphocytes were calculated by using a reticule as described earlier (Raychaudhuri *et al*, 1998, 1999). In the K252a-treated plaques, the number of CD8+ lymphocytes per square millimeter of epidermis reduced from 58 ± 10.8 to 12 ± 8.5 1 wk after completion of treatment ($p < 0.01$, Student's *t* test). In the control group, the numbers of CD8+ lymphocytes per square millimeter of epidermis before and after treatment were 60 ± 12.8 and 53 ± 15 ($p > 0.1$, Student's *t* test).

In 12 K252a-treated transplanted plaques, there was a significant decrease in NGF-R-positive dermal papillary nerves; the numbers of pre- and post-treatment NGF-R-positive nerve fiber numbers were 9.8 ± 2.3 and 3.9 ± 1.4 ($p < 0.002$, Student's *t* test, Table II). In the control group of 8 transplanted plaques the pre- and post-therapy NGF-R-positive nerve fiber numbers were 7.9 ± 2.1 and 7.9 ± 1.6 ($p = 0.97$, Student's *t* test, Table II). In the untreated group of 4 transplanted plaques, the numbers of NGF-R-positive nerve fibers were 10 ± 2.6 at 4 wk after transplantation and 10.3 ± 3.9 at 7 wk after transplantation ($p = 0.95$, Student's *t* test). All parameters were examined at synchronized time points among all groups.

We have taken three serial biopsies at different time points on several untreated transplanted plaques. We did not observe any significant exacerbation or improvement of the histologic features of psoriasis as a result of the trauma induced by the biopsy procedure. The transplanted psoriasis plaques are expected to lose the characteristic features of psoriasis with time. To address this variation we kept untreated plaques and plaques treated with saline as controls. We have observed that immunologic and histologic features of psoriasis were maintained in the transplanted plaques for 4 mo. Other investigators have found similar findings as well (Gilhar *et al*, 1997; Sugai *et al*,

Table II. Effect of K252a on NGF-R expression of dermal papillary nerves in the transplanted psoriatic plaques

Treatment	No. of psoriatic plaques	No. of NGF-R-positive nerve fibers/2-mm punch biopsy ($\pm$ SD)		
		Week 0[a]	Week 3	p value
K252a	12	9.8 $\pm$ 2.3	3.9 $\pm$ 1.4	$<$0.002
Control (normal saline)	8	7.9 $\pm$ 2.1	7.9 $\pm$ 1.6	0.97
Untreated	4	10.0 $\pm$ 2.6	10.3 $\pm$ 3.9	0.95

[a]Week 0 = 4 wk after transplantation.

1998; Nickoloff *et al*, 1995). In this study we completed all experimental works in each mouse within 8 wk of transplantation. The control plaques treated with saline for the same duration did not show any significant improvement, further suggesting that the histologic improvements were specifically due to therapeutic efficacy of K252a.

To substantiate this observation, we further investigated whether similar improvement of psoriasis could be induced by blocking the NGF. Anti-human monoclonal NGF-neutralizing antibody (Wako) was injected intralesionally two times in a week for 2 wk in eight mice. Doses of NGF-neutralizing antibody were determined from our earlier *in vitro* studies (Raychaudhuri *et al*, 2001a, b) where we observed that NGF-neutralizing antibody inhibited NGF-induced T cell activation, endothelial cell proliferation, and induction of intercellular adhesion molecule (ICAM-1) on endothelial cells. In this study, NGF-neutralizing antibody was dissolved in normal saline and used at the dose of 10 ng (n = 4) and 20 ng (n = 4) per kg of body weight. Four control mice were treated with isotype antibody (IgG2) dissolved in normal saline. We observed significant improvement of psoriasis in the NGF-neutralizing antibody-treated transplanted plaques compared to the plaques treated with placebo (Fig 4*a,b*).

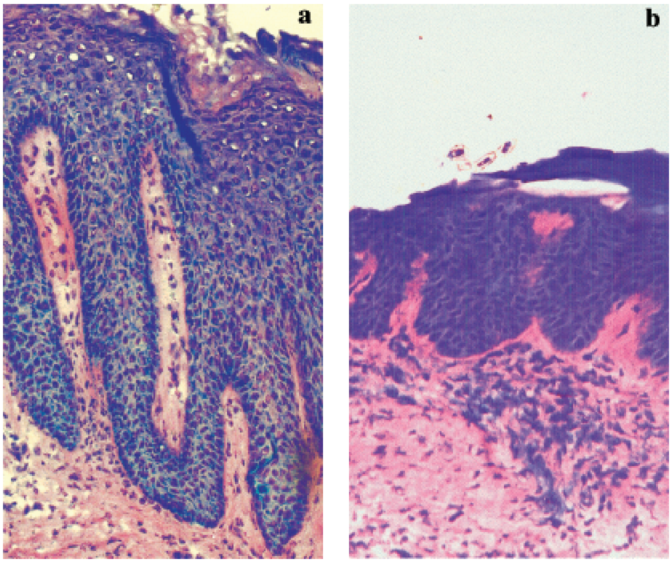

Figure 4
Histologic improvement of the psoriasis graft treated with NGF-neutralizing antibody (10 ng per kg of body weight) is evidenced by thinning of the epidermis and reduction of the infiltrates in the papillary dermis. (*a*) Before therapy and (*b*) after therapy. Magnification $\times$ 160.

Rete peg lengths before therapy with 10 and 20 ng per kg of body weight of NGF antibody were 298.5 $\pm$ 42.69 and 306.78 $\pm$ 66.96 μm and on the third week following treatment were 150.52 $\pm$ 32.93 and 162.02 $\pm$ 38.62 μm, respectively. In both doses of NGF-neutralizing antibody reduction of rete peg lengths was statistically significant ($p < 0.05$, Student's *t* test), whereas in mice treated with the placebo, rete peg length before and after treatment were 292.58 $\pm$ 68.78 and 310 $\pm$ 62.68 μm (Table III).

Discussion

NGF, the best-characterized member of the neurotrophin family, exerts its effects by binding two classes of transmembrane receptors, a low-affinity receptor of $\sim$75 kDa (p75) (Johnson *et al*, 1986) and a high-affinity tyrosine kinase receptor of $\sim$140 kDa (TrkA) (Kaplan *et al*, 1991). The high-affinity binding site requires expression of the TrkA proto-oncogene (Bothwell, 1991). TrkA can mediate NGF-induced effects in the absence of p75 (Klein *et al*, 1991; Barbacid, 1993), the functional significance of this low-affinity receptor in NGF signal transduction is currently under investigation (Davies, 1997). It has been reported that p75 can influence the function of trk receptors by augmenting the affinity of TrkA for NGF (Hempstead *et al*, 1991). Also it has been shown that p75 forms a complex with TrkA (Ross *et al*, 1996) and modulates TrkA trophic signals (Maliartchouk and Saragovi, 1997).

Both p75 and TrkA are expressed in human keratinocytes. Similar to neuronal cells/PC12 cells, in human keratinocytes NGF also stimulates TrkA phosphorylation.[1] Although in human keratinocytes, p75 mRNA and protein expression are increased during their exponential growth phase (Di Marco *et al*, 1993), K252, a potent inhibitor of TrkA phosphorylation, but not anti-p75, abrogates NGF-induced keratinocyte proliferation (Pincelli *et al*, 1994). This suggests that TrkA is the functional NGF receptor in human keratinocytes; the role of p75 in respect to effect of NGF on keratinocyte biology remains to be clarified.

Following injury to the cutaneous nerves, denervated skin is reinnervated by two mechanisms: axonal regeneration and collateral reinnervation (Devor *et al*, 1979). NGF has a regulating role on both of these processes (Taniuchi *et al*,

[1]Zhai S, Pincelli C, Yaar M, Gonsalves J, Gilchrest BA: The role of nerve growth factor in preventing keratinocyte apoptosis [abstract]. J Invest Dermatol 104:572, 1995

Table III. Effect of NGF-neutralizing antibody therapy on the rete peg length (μm) of the transplanted psoriatic plaques (n = 12)

Group	Before treatment	After treatment
NGF-neutralizing antibody		
10 ng/kg, 2 × per week for 2 wk (n = 4)	298.50 ± 42.69	150.52 ± 32.93
20 ng/kg, 2 × per week for 2 wk (n = 4)	306.78 ± 66.96	162.02 ± 38.62
Control (isotype antibody): 2 × per week for 2 wk (n = 4)	292.58 ± 68.78	310.00 ± 62.68

1986; Owen *et al*, 1989). Further, it has been claimed that keratinocytes are an important source of NGF for wound healing, and topical administration of NGF significantly accelerates regeneration of nerve fibers (Matsuda *et al*, 1998). An increased level of NGF in keratinocytes of psoriatic plaques is an established phenomenon (Fantini *et al*, 1995; Raychaudhuri *et al*, 1998). Because mouse and human NGF are 90% homologous it is likely that mouse nerves will promptly proliferate into the transplanted plaques on a SCID mouse. Upregulation of the p75 receptor is a unique *in vivo* effect of NGF (Wyatt and Davies, 1993). Accordingly, we observed a marked proliferation of NGF-R (p75)-positive nerve fibers in the transplanted psoriatic plaque compared to the transplanted normal human skin (Fig 2*a,c*). These observations substantiate the *in vivo* effect of NGF released from the keratinocytes of psoriatic plaques. Following treatment with K252a we observed that the number of NGF-R-positive cutaneous nerves in the treated plaques was markedly reduced (Fig 2*b*). In K252a-treated transplanted plaques, there was a significant decrease in NGF-R-positive dermal papillary nerves, pre- and post-treatment NGF-R-positive nerve fiber numbers were 9.8 ± 2.3 and 3.9 ± 1.4 ($p < 0.002$, Table II). This provides further evidence that the lesional NGF in transplanted plaques is functionally active.

K252a, an alkaloid toxin isolated from *Nocardiopsis,* was originally characterized as an inhibitor of protein kinase C and cyclic nucleotide-dependent kinases (Kase *et al*, 1987). Subsequently, K252a in nanomolar quantities has been shown to be a specific inhibitor for NGF-induced neuritic outgrowth in PC12 cell (Koizumi *et al*, 1988; Lazarovici *et al*, 1989). The inhibition of cellular effects of NGF by K252a is mediated by blocking trk proto-oncogene tyrosine phosphorylation and kinase activities (Berg *et al*, 1992). The functional marker of trk phosphorylation is the activation of c-*fos* oncogene transcription (Ehrhard *et al*, 1993, 1994). K252a inhibits the trk activity which in turn decreases c-*fos* oncogene transcription, increases intracellular calcium, and stimulates the phosphorylation cascade produced by NGF in PC12 cells (Lazarovici *et al*, 1989).

The role of NGF is particularly relevant in the pathogenesis of psoriasis. NGF is mitogenic to keratinocytes (Pincelli *et al*, 1994; Wilkinson *et al*, 1994). NGF recruits mast cells and promotes their degranulation (Aloe and Levi-Mantalcini, 1977; Pearce and Thompson, 1986), both of which are early events in a developing lesion of psoriasis. In addition, NGF activates T lymphocytes, recruits inflammatory cellular infiltrates (Thorpe *et al*, 1987; Bischoff and Dahinden, 1992; Lambiase *et al*, 1997), is mitogenic to endothelial cells, and induces intercellular adhesion molecule on endothelial cells (Raychaudhuri *et al*, 2001a, b). NGF is also known to upregulate the expression of substance p (Lindsay and Harmar, 1989). Thus, K252a being a potent inhibitor of the NGF/NGF-R signal transduction system, it is expected to antagonize these critical events essential for the inflammatory and proliferative processes of psoriasis. In this study we observed that treatment with K252a influenced several salient pathologic features of psoriasis. Following 2 wk of therapy with K252a, the thickness of the epidermis/acanthosis reduced from 308.57 ± 98.72 to 164.64 ± 46.78 μm (Table I). In addition downregulation of HLA-DR expression and marked reduction of dermal CD4+ and intraepidermal CD8+ T cell infiltrates were noticed.

It is essential to understand the NGF inhibitory functions of K252a. In the past 15 years, a series of experiments has been performed by several investigators to demonstrate that K252a can block NGF-induced neurite outgrowth in PC12 cells, proliferation of keratinocytes, and activation of T cells (Koizumi *et al*, 1988; Lazarovici *et al*, 1989; Pincelli *et al*, 1994). Recently we reported the effects of NGF on endothelial cell biology. We have observed that NGF is mitogenic to endothelial cells and induces intercellular adhesion molecule on endothelial cells (Raychaudhuri *et al*, 2001a, b). These effects of NGF on endothelial cells are inhibited by K252a and NGF-neutralizing antibody. Activated T cells play a significant role in the pathogenesis of psoriasis. Using SCID–human skin chimeras we have reported that intralesional injection of autologous peripheral blood monocytes activated with NGF converts transplanted nonlesional psoriatic skin to active psoriasis plaques (Raychaudhuri *et al*, 2000a, b, 2001a, b). This conversion does not occur if the autologous peripheral blood monocytes are activated in the presence of a NGF antibody or K252a.

Keratinocyte proliferation is the most characteristic histologic feature of psoriasis. NGF by its autocrine action induces proliferation of keratinocytes; it has been shown that K252a abrogates NGF-induced keratinocyte proliferation (Pincelli *et al*, 1994). It is also reported that autocrine NGF protects human keratinocytes from apoptosis through its high-affinity receptor trkA, and keratinocytes treated with K252a or anti-NGF underwent apoptosis (Pincelli *et al*, 1997). Increased levels of NGF have been demonstrated in the keratinocytes of lesional and nonlesional psoriatic skin (Fantini *et al*, 1995; Raychaudhuri *et al*, 1998). Recently, we carried out a study to investigate whether psoriatic keratinocytes produce higher amounts of NGF by culturing keratinocytes from nonlesional psoriatic skin biopsies (data unpublished). A significant observation was that the keratinocytes from psoriatic patients synthesized higher levels of NGF compared to keratinocytes from normal subjects. We further noticed that K252a and monoclonal NGF-R antibody (trk A) inhibited production of NGF and proliferation of these keratinocytes (Raychaudhuri *et al*, 2001a, b; S.P. Raychaudhuri, submitted for publication).

Elucidation of the molecular and cellular mechanisms responsible for the pathogenesis of psoriasis had been significantly handicapped owing to lack of an ideal animal model. Recent establishment of the SCID–human skin chimeras with transplanted psoriasis plaques has opened new vistas to study the molecular complexities involved in psoriasis (Nickoloff *et al*, 1995). Histologic and immunologic features of psoriasis can be maintained in the transplanted plaques for more than 3 mo (Gilhar *et al*, 1997; Sugai *et al*, 1998) and our experience is that these features are maintained for more than 6 mo. In this study, we have demonstrated that K252a, an inhibitor of signal transductions induced by NGF/NGF-R interaction, is therapeutically effective in psoriasis. Efficacy is evidenced by decreased thickness of the rete pegs, reduced infiltrates, and normalization of the stratum corneum, whereas the control group treated with normal saline did not improve (Table I). A role of NGF in the pathogenesis of psoriasis is further substantiated by our observation that K252a (a NGF receptor antagonist) not only improved psoriasis, but a similar improvement could be reproduced by directly inhibiting NGF with a NGF-neutralizing antibody. Clinical and histologic improvement of psoriasis observed in this study in response to NGF-neutralizing antibody and a NGF-R-blocking agent demonstrates a novel therapeutic approach for psoriasis.

Materials and Methods

Transplantation of psoriasis plaque on to the SCID mouse Patients for this study were recruited from the psoriasis clinic of the Psoriasis Research Institute (Palo Alto, CA). The enrolled patients had generalized plaque psoriasis, involving 5% to 10% of the total skin. These patients did not receive any systemic treatment for psoriasis or phototherapy in the past 6 mo and did not receive any topical preparations other than emollients in past 6 wk. Shave biopsies (2.5 × 2.5 cm) were obtained from active plaques located on the thigh or arm of 12 psoriatic patients. Each piece of biopsy was divided into four equal parts of approximately 1 cm^2 size. Thus, 48 grafts on SCID mice were made from 12 shave biopsies obtained. Twelve grafts were treated with K252a, 8 grafts were treated with normal physiologic saline, and 4 grafts were kept as untreated controls. To avoid the individual response variability, K252a-treated transplanted plaques and the corresponding saline controls were used from the same patients. The experiments were conducted after approval by the Institutional Review Board of Santa Clara Medical Center for human material research. Informed consent was obtained from each volunteer according to Helsinki Principles.

The CB17 SCID mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed at the Stanford University Research Animal Facility (Palo Alto, CA) in a pathogen-free environment. All experiments were carried out in compliance with the relevant laws and institutional guidelines. The animal experimental protocol review committee of Stanford University has approved the protocol for animal experiments. The protocol to use the plaques of psoriasis was approved by the Medical Review Board, Santa Clara Valley Medical Center (Santa Clara, CA), and each donor signed an informed consent. Under general anesthesia, a graft bed of approximately 1 cm^2 was created on the shaved area of the back of a 7- to 8-wk-old mouse by removing a full-thickness skin sample keeping the vessel plexus intact on the fascia covering the underlying back muscles. The partial thickness human skin obtained by shave biopsy was then orthotopically transferred onto the graft bed. Nexaband liquid, a veterinary bandage (Veterinary Products Laboratories, Phoenix, AZ), was used to attach the human skin to the mouse skin, and an antibiotic ointment (bacitracin) was applied. After 3 tp 4 wk of transplantation, a 2-mm punch biopsy was obtained to confirm the acceptance of the graft.

Treatment of the transplanted psoriatic lesions with K252a A 2-mm punch biopsy was obtained before therapy to determine the psoriatic histology of the graft. Mice of the treatment group received K252a at 50 μg per kg of body weight BID intralesionally for 14 days (Alexis Biochemical Corp., San Diego, CA). Each dose of K252a was dissolved in 150 μL of normal saline. We determined the dose of K252a on the basis of *in vivo* and *in vitro* dose–response experiments carried out by our group and other investigators (Raychaudhuri *et al*, 2001a, b; Pincelli *et al*, 1994; Levine *et al*, 2000). A biologically active and nontoxic dose was chosen to study its effect on psoriasis. In brief, biologic action was determined by assessing the dose of K252a for inhibition of NGF-induced proliferation of keratinocytes, lymphocytes, and endothelial cells. In these experiments, NGF was used at the dose of 25 to 200 ng per mL and K252a was used at the dose of 50 to 200 nM. We used 100-fold higher doses for *in vivo* experiments. K252a was used BID at the dose of 25, 50, and 100 μg in SCID mice to determine its tolerability. All three doses were well tolerated without any side effects. We chose the middle dose that is 50 μg BID for further experiments. Mice of the control group received the same volume of normal saline. One week after the last (28th) injection, biopsies were collected from the transplants of both treatment and control groups. The skin tissues were immediately embedded in optimal cutting temperature (OCT) reagent and snap-frozen in liquid nitrogen. Cryosections of 6 to 8 μm were then prepared for histologic (hematoxylin/eosin) and immunohistochemical staining; sections of 12 to 14 μm thickness were prepared for cutaneous nerve staining.

Psoriatic plaques of eight mice were also treated with 10 and 20 ng per kg of body weight of anti-human monoclonal NGF-neutralizing antibody (Wako, Richmond, VA) with two intralesional injections per week for 2 wk. Isotype control antibody IgG2 (Wako) was injected intralesionally twice a week for 2 wk into psoriatic plaques of four mice. One week after the last injection, biopsies were collected from the transplants of both NGF-neutralizing antibody and control groups for histologic examination.

Immunoperoxidase staining for HLA-ABC, HLA-DR, and NGF The 6- to 8-μm tissue sections were incubated with anti-HLA-ABC monoclonal antibody (1:1000 dilution) and anti-HLA-DR antibody (1:100 dilution) (Immunotech, Westbrook, ME) for 18 h at 4°C. For NGF, a polyclonal anti-NGFβ antibody (Chemicon International Inc., Temecula, CA) was used at the dilution of 1:20. Standard protocol for immunohistochemical staining was followed (Raychaudhuri *et al*, 1998, 1999).

Immunofluoresence staining for cutaneous nerves and CD8 The 12- to 14-μm tissue sections were used to stain the cutaneous nerves. Sections were incubated with following antibodies: Monoclonal anti-MAP2 antibody (1:200, Sigma, St. Louis, MO)), anti-NGF-R (p75) monoclonal antibody (1:40 dilution, Boehringer Mannheim, Germany), and anti-PGP 9.5 monoclonal antibody (1:800, Ultraclone, Cambridge, UK) for 18 h at 4°C. After being washed, the sections were incubated with fluorescein isothiocyanate-conjugated horse anti-mouse IgG at 1:100 dilution (Sigma). For trkA, polyclonal antibodies from two sources (Chemicon International Inc.; Santa Cruz Biotechnology, Santa Cruz, CA) were used in various dilutions. CD8 monoclonal antibody (Boehringer Mannheim, Germany) was used at 1:100 dilution. For both immunoperoxidase and immunofluorescence staining, specificity of the antibodies was confirmed by preabsorbing the primary antibody and using proper positive and negative controls. We used a standardized protocol to study terminal cutaneous nerves in psoriasis plaques (Raychaudhuri *et al*, 2000a, b). In brief, nerve regeneration was identified by counting the total number of NGF-R-positive nerve fibers traversing vertically in the papillary dermis toward the epidermis in the linear length of the 2-mm biopsy specimens.

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