Psoriatic Keratinocytes Express High Levels of Nerve Growth Factor

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Many investigators have reported proliferation of terminal cutaneous nerves and upregulation of various neuropeptides (substance P, vasoactive intestinal polypeptide, calcitonin gene-related peptide) in psoriatic lesions. Nerve growth factor promotes growth of nerves and causes upregulation of neuropeptides like substance P and calcitonin gene-related peptide. In this study we investigated the expression of nerve growth factor in psoriatic lesions, non-lesional psoriatic skin, lichen planus and normal control skin. Immunoperoxidase staining was applied on cryosections prepared from snap-frozen biopsy specimens. The primary antibody used was a polyclonal anti-NGF-β antibody. Nerve growth factor was detected only in the keratinocytes. In psoriatic tissue the number of keratinocytes per square millimeter of epidermis positive for nerve growth factor was 84.7 ± 46.3 compared to 44.8 ± 29.9, 18.9 ± 11.8 and 7.5 ± 16.9, respectively, in non-lesional psoriatic skin, normal skin and lichen planus. Increased expression of nerve growth factor substantiates larger numbers of terminal cutaneous nerves and upregulations of substance P and calcitonin gene-related peptide in psoriatic lesions. In addition, nerve growth factor is mitogenic to keratinocytes, activates T-lymphocytes and can induce migration of inflammatory cellular infiltrates, histological features characteristic of psoriasis. Key words: neurogenic inflammation; NGF; pathogenesis; psoriasis.

(Material and Methods)

Tissue preparation
Biopsies were obtained from chronic psoriasis plaques (n = 8), non-lesional psoriatic skin (n = 5), skin of healthy individuals (n = 5) and lesional lichen planus skin (n = 5). The samples were snap-frozen with liquid nitrogen and stored in the refrigerator under −70°C. The frozen samples were cut into 8-μm cryosections. The sections were mounted on the glass slides and dried for 4 h at room temperature. Then the sections were immersed in 0.05 M, pH 3.0, citric acid buffer for 10 min and subsequently fixed with 4% formalin solution. The sections were washed and then sequentially blocked for endogenous peroxidase activity and non-specific antibody binding sites with 3% hydrogen peroxide and 1.5% normal goat serum (Vector Laboratories, Burlingame, CA) and for endogenous biotin binding using the Vector blocking kit (Vector Laboratories, Burlingame, CA) placed in the eye-piece. The sections were examined for the presence of positively stained cells in which staining could be appreciated without the help of a reticle/grid (10 × 10 mm with 1 mm boxes; Microscopics, Inc., Milford, MI) placed in the eye-piece. The number of cells positive for NGF in per square mm of epidermis was calculated by dividing the total number of cells in the psoriatic skin have not been sufficiently addressed. As in other tissues, nerve growth factor (NGF) in the skin plays a role in regulating innervation (12). NGF also causes upregulation of neuropeptides (13). In this study, in order to understand the cause for the upregulation of neuropeptides and the hyperproliferation of terminal cutaneous nerves in psoriasis, we have investigated the expression of NGF in psoriatic plaques, non-lesional psoriatic skin, lichen planus and normal skin.

RESULTS
Positive staining was observed in the tissues stained only with the polyclonal antibody. Sections stained with the normal rabbit serum and the sections which were stained with the NGF antibody preabsorbed with NGF did not show any positive staining for NGF. All sections were examined by one investigator (WYJ), and independent confirmation of the observations we proposed a role for the neuropeptides in the pathogenesis of psoriasis, suggesting that neurogenic inflammation triggered by exogenous and endogenous stimuli may be an underlying mechanism (4). Various investigators have explored this relatively new field of cutaneous neuroimmunology, and with the exceptions of a few reports (6, 7) others have reported an increased number of terminal cutaneous nerves along with upregulation of one or more of the neuropeptides such as substance P (SP), vasoactive intestinal polypeptide (VIP) and calcitonin gene-related peptide (CGRP) in psoriatic lesions (8–11).

The underlying causes for increased nerves and neuropeptides in the psoriatic skin have not been sufficiently addressed. As in other tissues, nerve growth factor (NGF) in the skin plays a role in regulating innervation (12). NGF also causes upregulation of neuropeptides (13). In this study, in order to understand the cause for the upregulation of neuropeptides and the hyperproliferation of terminal cutaneous nerves in psoriasis, we have investigated the expression of NGF in psoriatic plaques, non-lesional psoriatic skin, lichen planus and normal skin.
NGF-positive cells with the surface area. The data are described in Table I. In psoriatic tissues upper and mid epidermic keratinocytes expressed high levels of NGF (Fig. 1). The number of keratinocytes in per millimeter of epidermis stained for NGF was 12.11 ± 7.15 in psoriatic tissues compared to 2.55 ± 1.71, 0.64 ± 0.40 and 0.59 ± 1.31, respectively, in non-lesional, normal skin and lichen planus (p < 0.01). The differences in the NGF-positive keratinocyte numbers were more significant when keratinocytes/mm² were compared instead of keratinocytes/mm (Table I). NGF expression in both lesional (p < 0.01) and non-lesional (p < 0.05) psoriatic keratinocytes was significantly higher compared to the normal control skin and the lichen planus skin. The stratum corneum stained positively in both psoriasis and control skin. Positive staining of the stratum corneum was considered as a non-specific reaction.

DISCUSSION
NGF is produced by the keratinocytes during embryonic development and during other circumstances which are not fully understood. In this study we have observed higher levels of NGF in the keratinocytes of the mid and upper epidermis of psoriatic tissues, compared to the controls (Fig. 1 A–C ). As we know, psoriasis is a maturation disorder of the keratinocytes and therefore it is possible that immature keratinocytes at a certain phase of their cell cycle produce more NGF. Overexpression of NGF is known to induce nerve growth factor receptor (NGF-R) on the nerves (12). In a separate study we have observed a marked upregulation of NGF-R in psoriatic lesions (14). Expression of larger amounts of NGF-R (14), along with an increased number of nerves (8), further substantiates an increased activity of NGF in psoriatic lesions.

It is worth noting that the expression of NGF is significantly higher in non-lesional psoriatic keratinocytes as well. In our study where we investigated for NGF-R expression we found similar results. We did not observe an upregulation of NGF in the keratinocytes of lichen planus cases. In our earlier study we did not observe an increased expression of NGF-R in lichen planus (14). This suggests that the increased expression of NGF in the keratinocytes of lesional and non-lesional psoriatic tissue may not be a secondary event due to an inflammatory reaction.

NGF is mitogenic to keratinocytes (15, 16). NGF recruits mast cells and promotes their degranulation, both of which are early events in a developing lesion of psoriasis (17, 18). In addition NGF activates T lymphocytes and can recruit inflammatory cellular infiltrates (19–21). Thus, it is possible that expression of NGF is required before the influx of mast cells and lymphocytes, which in turn would initiate the inflammatory process of psoriasis.

Fantini et al. have earlier reported that NGF levels are higher in the tissue extracts from psoriatic lesions (22). This is the first direct evidence to show that the lesional psoriatic keratinocytes express high levels of NGF. Observations in this study that psoriatic keratinocytes produce a larger amount of

Table I. Expression of NGF in the keratinocytes of lesional and non-lesional psoriatic skin, lichen planus and normal skin

<table>
<thead>
<tr>
<th>Biopsies</th>
<th>Numbers</th>
<th>No. of NGF-positive keratinocytes (KC) (x ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NGF+ KC/mm²</td>
</tr>
<tr>
<td>Psoriatic skin</td>
<td>8</td>
<td>12.11 ± 7.15</td>
</tr>
<tr>
<td>Non-lesional skin</td>
<td>8</td>
<td>2.55 ± 1.71</td>
</tr>
<tr>
<td>Normal skin</td>
<td>5</td>
<td>0.64 ± 0.40</td>
</tr>
<tr>
<td>Lichen planus</td>
<td>5</td>
<td>0.59 ± 1.31</td>
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Fig. 1. Histological section of psoriatic plaque (A), demonstrating large amounts of NGF in the upper and mid epidermic keratinocytes compared to normal skin (B) and lichen planus (C). Arrows indicate the NGF-positive keratinocytes (x 400).
NGF compared to the controls constitute a significant finding. These observations further substantiate a role for the neurogenic inflammation in the pathogenesis of psoriasis and provide explanations for the following unanswered features of psoriasis: hyperproliferation of terminal cutaneous nerves, upregulation of neuropeptides (SP, CGRP) and disappearance of active psoriatic plaques at sites of anesthesia.

REFERENCES