

## An Intimate Relationship Between Intralesional Depigmentation and Peripheral Nervous System in Lichen Simplex Chronicus

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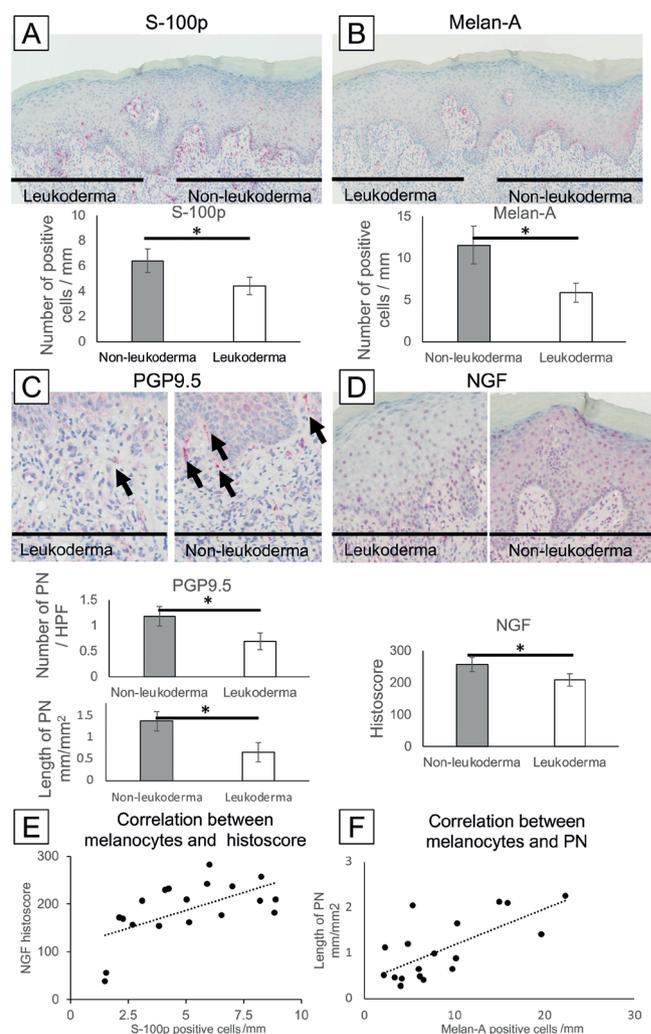
Lichen simplex chronicus (LSC) is a skin disorder characterized by lichenification of the skin as a result of primary excessive scratching (1). Clinically, leukoderma foci are often observed in chronic long-term lesions of LSC. Pruritus is a predominant symptom in LSC. A relationship has been suggested between neural tissue and inflammatory mediators in the perception of itch, which subsequently leads to LSC (2). The concept of a neuroimmune connection, in which close interactions between the nervous and immune systems regulate peripheral inflammation and link psychosocial stress with chronic disease, is now becoming accepted (3). Meanwhile, peripheral nerves have been shown to significantly influence melanocytes and melanogenesis. Both melanocytes and nerve fibres are derived from the neural crest. Nerve growth factor (NGF) is implicated in melanocyte survival, migration and dendricity (4). The number and distribution of nerve fibres, including those that secrete neuropeptides, are altered in lesional skin in vitiligo (5).

The mechanism of leukodermatous alteration in LSC remains unclear. Since pruritus is sometimes provoked by peripheral nerve fibres, and since melanocytes are maintained by NGF, we have focused on nerve fibres as the critical factor connecting pruritus and leukoderma appearing in LSC lesions. In the context of a neuroimmune connection, this study compared melanocytes and peripheral nerves in leukoderma areas with those in non-leukoderma areas in LSC.

### METHODS AND RESULTS

Eleven tissue samples from patients with biopsy-proven LSC who had visited the outpatient dermatology clinic in Tottori University Hospital, Tottori, Japan, were studied. The study protocol was approved by the ethics committee of Tottori University (approval number 18A078). Each specimen contained both leukoderma areas and non-leukoderma areas. LSC lesions were biopsied from 11 patients (3 men and 8 women) age range 21–77 years (mean 49.3 years). Two cases were biopsied from the neck, 1 from the dorsum of the hand, 3 from a lower extremity, 1 from the scrotum and 3 from the vulva. The lesions had existed for a mean  $\pm$  standard deviation (SD) of  $5.84 \pm 3.84$  years. The samples were fixed in 10% formalin and embedded in paraffin for processing. Sections were cut and stained with haematoxylin and eosin. In order to identify leukoderma and non-leukoderma lesions for further immunohistochemical analysis, both areas were stained using Fontana-Masson. Immunohistochemistry was performed using standard ABC methods after optimal antigen retrieval. The number of melanocytes was determined and melanogenesis was demonstrated by performing immunostaining with S-100 protein

(polyclonal Rabbit anti-S-100 protein, dilution: 1:1000; Dako, Glostrup, Denmark), which stains positively for both melanocytes and dendritic cells, and Melan-A (monoclonal mouse antihuman, dilution: 1:10; Dako, Glostrup, Denmark), reflecting the presence of melanosomes, where melanin synthesis occurs. The number of melanocytes was calculated per 1 mm of basal layer length using quantifying NanoZoomer Digital Pathology Images software



**Fig. 1. Immunohistochemical staining** for (A, B) S-100 protein and Melan-A, (C) pan-neuronal marker PGP 9.5, and (D) nerve growth factor (NGF). (A, B) The numbers of S-100 protein-positive cells and Melan-A-positive cells are significantly decreased in leukoderma areas compared with non-leukoderma areas. (C) The number and length of nerve fibers are significantly decreased in leukoderma areas. (D) The NGF expression calculated by histoscore is significantly lower in leukoderma areas. (E, F). There was strong correlation between number of melanocytes and NGF histoscore (correlation function:  $r=0.60$ ) and strong correlation between number of melanocytes and number of peripheral neurons ( $r=0.69$ ).

(Hamamatsu Photonics, Hamamatsu, Japan). In order to identify nerve fibres in the papillary dermis, immunohistostaining was performed with the pan-neuronal marker PGP 9.5 (mouse monoclonal antibody, dilution: 1:750, Abcam, Cambridge, UK). The number and length of nerve fibres in the papillary dermis were then calculated per high-power field (HPF) (number) and per mm<sup>2</sup> (length), respectively. In order to evaluate the amount of NGF produced by keratinocytes, immunostaining for NGF was performed (E-12, mouse monoclonal antibody, dilution: 1:20; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). Staining intensity was scored as 0, 1, 2 or 3, corresponding to the presence of negative, weak, intermediate, and strong cytoplasmic staining, respectively. An NGF histoscore was calculated as (3×percentage of cells with strong staining) + (2×percentage of cells with intermediate staining) + percentage of cells with weak staining, giving a histoscore range of 0–300. Statistical analysis was performed using the *t*-test to compare the numbers of melanocytes, the numbers and lengths of nerve fibres in the papillary dermis and NGF histoscores in leukoderma areas and non-leukoderma areas. The data were considered significant if the *p*-value was <0.01.

The mean ± numbers of S-100 protein-positive cells per 1 mm of basal layer were 4.25 ± 2.31/mm in leukoderma areas and 6.41 ± 3.13/mm in non-leukoderma areas (*n* = 11, *p* < 0.01) (Fig. 1A). The mean ± SD numbers of Melan-A-positive cells/1 mm of the basal layer were 5.87 ± 3.40/mm in leukoderma areas and 11.56 ± 6.78/mm in non-leukoderma areas (*n* = 9, *p* < 0.01) (Fig. 1B). The numbers of nerve fibres in the papillary dermis were 0.69 ± 0.54/HPF in leukoderma areas and 1.18 ± 0.63/HPF in non-leukoderma areas (*n* = 11, *p* < 0.01) (Fig. 1C). The mean ± SD lengths of nerve fibres were 0.661 ± 0.421 mm/mm<sup>2</sup> in leukoderma areas and 1.377 ± 0.74 mm/mm<sup>2</sup> (*n* = 11, *p* < 0.01) in non-leukoderma areas (Fig. 1C). NGF histoscores were 163 ± 64 in leukoderma areas and 200 ± 74 in non-leukoderma areas (*n* = 11, *p* < 0.01) (Fig. 1D). There were strong correlations between the number of S-100 protein-positive cells and NGF histoscore (correlation function: *r* = 0.60) (Fig. 1E) and between the number of Melan-A-positive cells and the length of peripheral neurones (*r* = 0.69) (Fig. 1F).

## DISCUSSION

These results show that the number of melanocytes, the length and number of peripheral nerve fibres, and the expression level of NGF in the epidermis of leukoderma areas are reduced compared with those in the epidermis of non-leukoderma areas in LSC. In addition, the results reveal a strong correlation among each of these factors, suggesting an intimate relationship between a reduction in peripheral nerves and the formation of leukoderma in LSC lesions.

NGF is a neurotropic polypeptide that is necessary for the survival and differentiation of sensory and sympathetic neurones (6). Human keratinocytes synthesize and secrete biologically active NGF. In human skin, NGF is released in increasing amounts by proliferating keratinocytes, whereas secretion ceases in more differentiated keratinocytes (4). In many skin diseases marked by chronic inflammation, such as atopic dermatitis, psoriasis and prurigo, it has been reported that NGF concentration is elevated in lesions (7–9). In fact, the number of peripheral nerve fibres and the concentration of NGF in LSC lesions have been reported to be increased (10).

Melanocytes express tyrosine kinase receptors that mediate the effects of NGF (4). The expression of these receptors is upregulated by various stimuli, such as UVB

irradiation (4). When melanocytes are maintained in a growth factor-depleted medium, the addition of NGF promotes survival of melanocytes (11). There is an intimate physical connection of the intraepidermal nerve endings to human epidermal melanocytes via neuropeptides (12). Therefore, we speculate that NGF plays a significant role in the interactions between the peripheral nervous system and melanocytes. Although further studies are needed to explore this hypothesis, our finding of a reduction in NGF expression in leukoderma areas compared with non-leukoderma areas in LSC suggests that decreased production of NGF in keratinocytes could trigger melanocyte reduction.

The current study has some limitations. Although the study focused on the relationship between melanocytes and peripheral nerves, many other factors, including keratinocytes, fibroblasts, immune cells and external stimuli, can influence both melanocytes and melanogenesis. Each factor stimulates the discharge of several paracrine hormones that regulate melanocytes, making melanogenesis a complex biological process (12). It is likely that the decrease in peripheral nerves is only one result of many pathways for downregulation of melanogenesis. Studies on other factors may answer the question as to why and how peripheral nerves are diminished.

Further research is needed to elucidate the complex mechanism of leukoderma formation in LSC.

*The authors have no conflicts of interest to declare.*

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