Clinical, histological, and immunohistochemical analysis in search for the cause of epidermal thickening in prurigo nodularis

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Howard Chu

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

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(Directed by Professor Kwang Hoon Lee)

Prurigo nodularis (PN) is a chronic pruritic skin disorder characterized by multiple hyperkeratotic nodules. Although several hypotheses have been suggested, the exact mechanisms of the pathogenesis have not been elucidated. Also, PN is known to be associated with atopic dermatitis (AD), yet their associations remain largely unknown.

In this study, patients of PN and PN with AD (PN-AD) were compared with normal subjects clinically, histologically and through immunohistochemical studies.

The results showed that PN-AD patients had significantly higher total IgE levels and eosinophil counts than that of PN group. Histologic assessment revealed increased epidermal thicknesses and dermal inflammatory cell counts of PN and PN-AD groups when compared to

normal subjects. Immunohistochemical analyses showed that uptake of SDF 1- α, TSLP, IFN- γ, IL-4, IL-13, IL-18, IL-31, and IL-33 were increased in the tissues of PN and PN-AD groups, in which the staining intensities of SDF 1- α, TSLP, IL-4, and IL-13 of PN-AD group were higher than that of PN group. In the contrary, the staining intensities of IL-18, IFN- γ, and IL-33 were statistically higher in the PN-AD group than PN group.

Through the findings of this study, the pathogenesis of PN without AD may be different from that of PN with AD, in which IL-18, IFN- γ, and IL-33 may be associated, suggesting that epidermal injury may be the initial cause that induces IL-18 and IL-33, which then enhances IFN- γ, resulting in the inflammatory process of PN.

Key words: prurigo nodularis, atopic dermatitis, immunohistochemistry, cytokines

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I. INTRODUCTION

Prurigo nodularis (PN) is a dermatologic condition characterized by multiple, hyperkeratotic nodules accompanied by severe pruritus[1]. Its histological features are hyperkeratosis, irregular acanthosis, and a predominant lymphocytic inflammatory infiltrate and vertically oriented collagen bundles in the papillary dermis[2]. PN has been reported to be associated with various dermatological, psychosocial and systemic diseases, in which an underlying disease may be associated in up to 87% of the patients[1]. Associated dermatologic conditions are atopic dermatitis (AD), nummular eczema, and mycobacterial infections, and other systemic diseases include diabetes mellitus, renal failure, and thyroid diseases[3,4]. However, the mechanisms of the pathogenesis of the underlying pruritus that leads to the pruritic nodules, especially the thickening of the epidermis, in PN is unknown.

AD is a well-known skin disorder related to PN, in which a study of 108 patients has found 18.5% of PN patients with underlying AD[5]. Past studies have found PN accompanied by AD to have sensitization to environmental allergens, similar to that of AD, and an earlier onset than those without AD, thus should be regarded as a different entity from PN without AD[6]. Only few studies have investigated on the relationship of AD and PN, and the differences in the pathogenesis of PN with and without AD have not been studied.

Previous studies have elucidated the involvement of cutaneous neuropathies and the inflammatory processes related to PN. The characteristic neurological changes observed include hypertrophy and proliferation of dermal nerves[7]. Other studies, however, have found decreased intra-epidermal nerve fiber density[8], suggesting the variability of neuropathies in PN. In addition, nerve growth factor, calcitonin gene-related peptide and substance P immunoreactive nerves have been found to be increased in the lesions[9,10], henceforth, cutaneous neuropathy has been considered a feature of PN.

Regarding the inflammatory processes, the involvement of various cells and cytokines has been studied. Eosinophilic granules, T lymphocytes and mast cells have been found to be involved in the inflammation of PN[11]. The expressions of STAT6 and STAT3 have been found to be activated in the epidermis of PN[12]. Also, increased expression of mRNAs of interleukin (IL)-4, 17, 22, and 31 have been found in the lesions[13], suggesting the predominant involvement of Th2 cytokines in the pathogenesis. A recent study comparing

the lesions and non-lesions of patients with PN found that inflammatory cell infiltrate mainly consists of T lymphocytes, particularly CD8+ cells, CD15+ neutrophils, and CD68+ macrophages[14]. Despite these findings, the treatment of PN remains difficult is many cases, thus further elucidation of the pathogenesis of needed to develop more effective therapeutic methods.

Therefore, we aimed to review and identify the key histologic features of PN and investigate the involvement of various cytokines through immunohistochemical studies, and investigate the differences between PN with and without AD.

II. MATERIALS AND METHODS

1. Patient Selection

16 patients clinically and histologically diagnosed with PN were recruited, in which 8 patients were diagnosed with AD (PN-AD) according to the diagnostic features of Hanifin and Rajka[15] and the remaining 8 patients did not have AD. According to the protocol approved by the Yonsei University College of Medicine, paraffin-embedded tissues of the patients were obtained for histological and immunohistochemical analyses. A group of 8 patients who had no pathological findings in their biopsy specimen were set as the control group.

3 additional patients were recruited prospectively and blood samples (20 cc) were obtained for FACS (fluorescence-activated cell sorting) analysis.

2. Clinical Assessment

Through the review of the subjects' medical charts, the information of their age, sex, and comorbid diseases were obtained. Laboratory findings of complete blood count (CBC), routine chemistry, and total IgE levels were reviewed to evaluate the presence of eosinophilia and any other abnormal findings.

3. Histological Assessment

Through Hematoxylin and Eosin (H&E) staining of the tissues, the thicknesses of the epidermis were evaluated by separately measuring the longest distance

from the corneal layer to the basal layer and from the sub-corneal level to the basal layer. The dermal thickness was assessed by measuring the longest distance from the sub-epidermal level to the subcutaneous layer. The measurements were compared between the subjects of PN-AD, those without AD, and the control. Metamorph® Microscopy Automation & Image Analysis Software (Molecular Devices, LLC., Sunnyvale, CA, USA, 2006) was used for the measurements. The number of infiltrated inflammatory cells counted at 400x magnification in the papillary dermis where the cells are most concentrated, and an average number of the count in three different fields were obtained. The number of vasculatures were counted in the dermis of within 100 μm distance from the epidermal-dermal junction. The numbers of the inflammatory cells and vasculatures were compared among the three groups.

4. Immunohistochemical Assessment

Immunohistochemistry was performed in 10% formalin-fixed, paraffin-embedded tissues of PN and normal control groups. Dissected tissues were washed several times with distilled water, and residual fixative was removed by treatment with 1% sodium borohydride for 1 hour. Tissues were pretreated with 3% hydrogen peroxide solution for 10 minutes, washed with distilled water, and incubated 5 minutes with 1x TBST (Tris-buffered saline + 0.1% Tween 20). Tissues were treated with normal goat serum (Vector Laboratories, Burlingame, CA, USA) for 1 hour at room temperature to prevent

nonspecific reactions. Tissues were then incubated overnight with the following antibodies: stromal derived factor (SDF)-1 α, galectin 10, thymic stromal lymphopoietin (TSLP), interferon (IFN)- γ, IL-4, -13, -18, -22, -31, -33. The findings through the analysis were compared between the group of PN without AD, with AD, and the control group.

5. FACS analysis

From the PBMC (peripheral blood mononuclear cell) of the 3 additional patients and 3 normal subjects recruited, cells were washed with phosphate-buffered saline (PBS) and stained with a fixable viability dye. After washing, cells were labeled at 4°C for 30 minutes with anti-CD4, -CD8, -IL-17, -IL-22, -IFN-γ, and -IL-4 antibodies conjugated with fluorescent dye (eBioscience, San Diego, CA, USA). Cells were quantified using a BD FACSVerse flow cytometer, and the data were analyzed using FlowJo Software (BD Bioscience, San Jose, CA, USA).

6. Statistical Analysis

Data are presented as mean ± standard deviation. Results were analyzed using paired Student t tests and one-way analysis of variance. Deviations were considered statistically significant when $p < 0.05$. SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

III. RESULTS

1. Clinical analysis of AD patients with and without AD

16 patients with PN, of which 8 patients with AD and the remaining 8 without AD, were analyzed (Table 1). The mean age of the patients were 32.81 years old with a male to female ratio of 1:1.29. The mean age of the patients with AD was 28.00 years old, which was lower than those without AD (37.63 years old), although the difference was not statistically significant (p=0.16). The mean total IgE level of the patients was 874.14 kU/L, and those with AD had a significantly higher level of 1568.13 than those without AD, which was 180.15 kU/L ($p < 0.05$). The mean eosinophil count of the patients was 0.44 x 10^3/μL, in which those AD had a significantly higher value than those without AD (0.65 and 0.23, $p < 0.05$). Regarding the comorbidities, of the PN patients with AD, except for one patient who had allergic contact dermatitis, none of the patients had other atopic diseases or comorbidities. Of the patients without AD, diabetes mellitus, hypertension, hyperthyroidism and chronic kidney disease were found to be accompanied (Table 2).

Table 1. Comparison between PN and PN-AD

Characteristics	PN	PN-AD	*p*-value
No. subjects	8	8	
Age, yrs (range)	37.63	28.00	0.16
Sex (M:F)	1:1.25	1:1	
Eosinophil count (x10^3/μL)	0.23	0.65	<0.05
Total IgE, kU/L	180.15	1568.13	<0.05

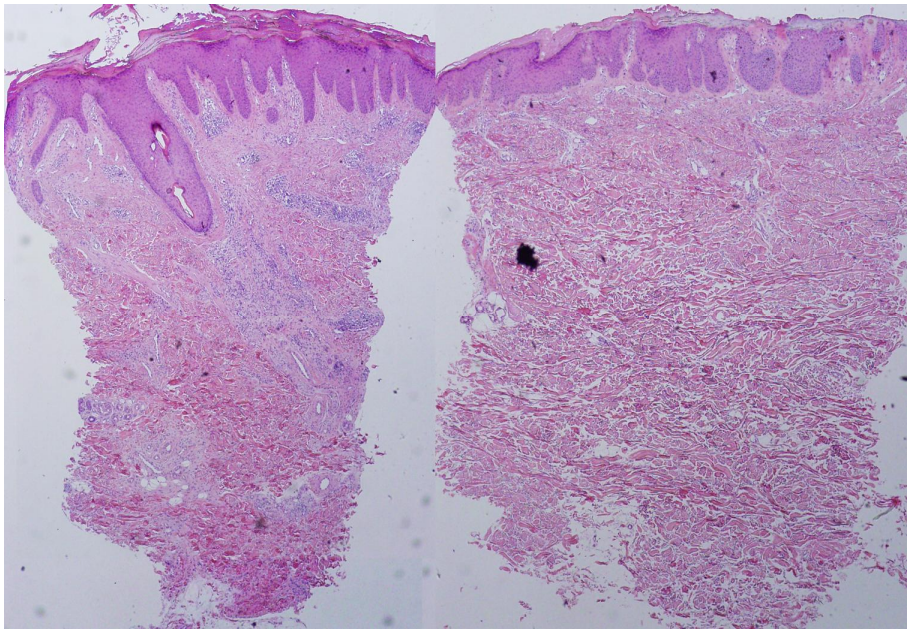
Table 2. Number of comorbid diseases accompanied by PN patients without AD.

Comorbid disease	Number of Patients
Diabetes mellitus	2
Hypertension	2
Chronic kidney disease	1
Hyperthyroidism	1

2. Histological assessment shows increased epidermal thickness, but no difference in the dermal thickness in patients with PN and PN-AD when compared with normal

The epidermal thicknesses and dermal thicknesses of the tissues of the patients of PN, PN- AD, and normal groups were measured on H&E sections (Fig. 1A and 1B). The mean epidermal thickness excluding the corneal layer of PN group was 267.88 μm and that of PN-AD group was 247.21 μm, which both were significantly increased than that of NL group (46.03 μm), (Fig. 1C). When the epidermis was measured including the stratum corneum, the thicknesses were also significantly increased in the PN and PN-AD group when compared with NL group (Fig. 1D). The dermal thicknesses were also measured, and unlike the epidermis, the dermal thicknesses did not show any differences among the three groups (Fig. 1E). The ratio of the thicknesses was determined (Table 3), in which the epidermis: dermis ratio of the PN and PN-AD groups were 0.15:1 and 0.13:1 and no significant difference was found between the two groups. When compared with the ratio of the normal group (0.02:1), the differences were significantly different ($p < 0.05$), suggesting a definite increase in the thickness of epidermis in PN and PN-AD.

(A)



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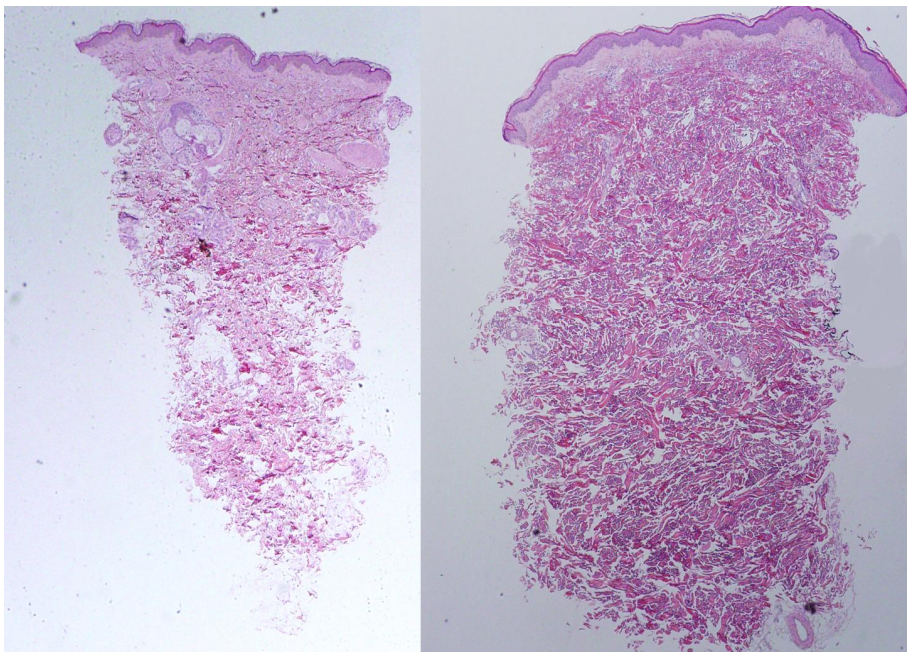

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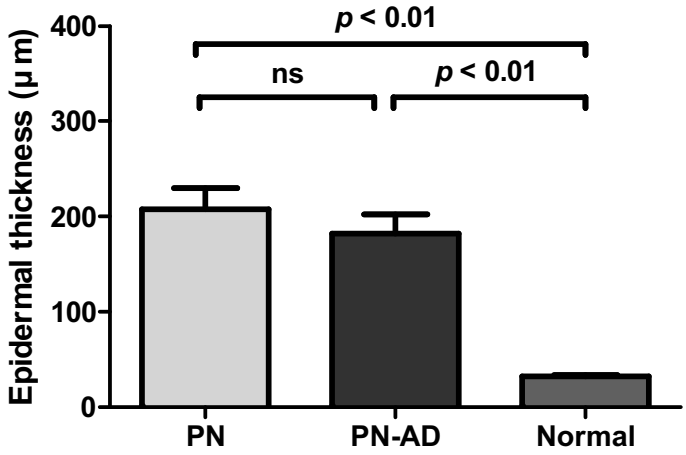
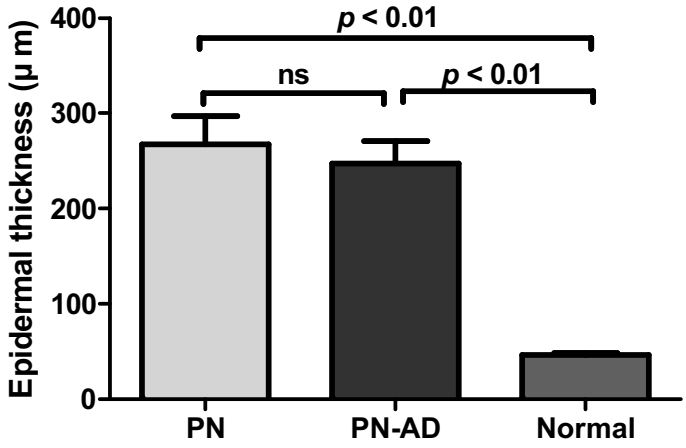


(D)



(E)

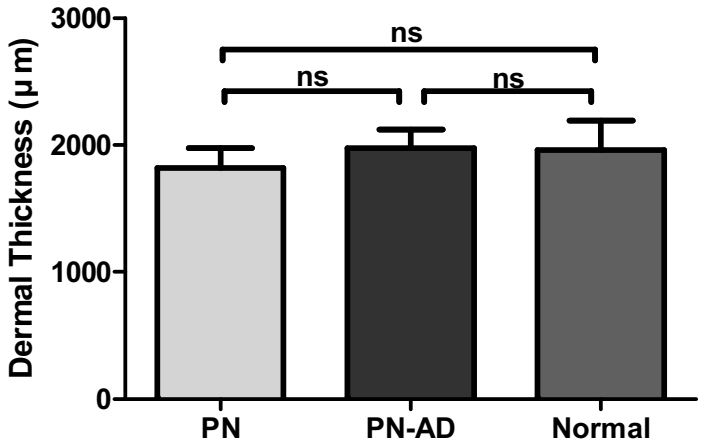


Figure 1. Tissues of (A) PN (left), PN-AD (right) and (B) normal were analyzed and compared on H&E sections. (C) The epidermal thicknesses excluding the stratum corneum of the three groups, PN, PN-AD, and normal, were compared. The average epidermal thickness of PN (267.88 μm) and PN-AD (247.21 μm) were significantly increased compared to that of normal group (46.03 μm), (p<0.01). (D) The epidermal thicknesses were also measured while including the stratum corneum, in which the thicknesses of PN and PN-AD groups were also significantly increased when compared to that of normal group (p < 0.01). (E) The dermal thicknesses of the three groups did not show significant differences, in which the average thicknesses of PN, PN-AD, and normal groups were 1820.21, 1977.27, and 1960.11 μm, respectively.

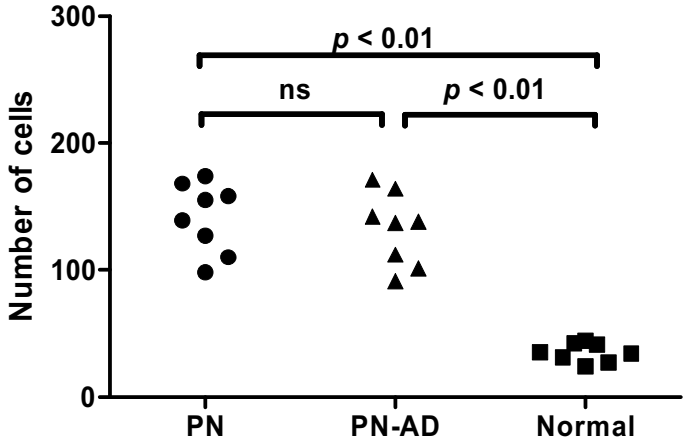
Table 3. The ratio of the thicknesses epidermis to dermis of PN, PN-AD, and normal groups

	PN	PN-AD	Normal
Mean epidermal thickness (μm)	267.88	247.21	46.03
Mean dermal thickness (μm)	1820.21	1977.27	1960.11
Epidermis: Dermis	0.15:1	0.13:1	0.02:1

3. Inflammatory cellular infiltration and the number of vessels in the dermis were found to be increased in patients with PN and PN-AD

The number of inflammatory cells infiltrated in the dermis were counted on a x400 section on H&E slides of the tissues of PN, PN-AD and normal groups (Fig. 2A). The number of infiltrated cells were significantly higher in the PN and PN-AD groups than the normal group. The number of cells of PN and PN-AD groups did not show any difference. The average number of vasculatures were also evaluated on a x100 magnification within 100 μm distance from the epidermal-dermal junction (Fig. 2B). The number of dermal vessels between PN (17.13) and PN-AD groups (19.25) did not show any differences, but they were significantly higher when compared with the normal group (6.38) ($p < 0.001$).

(A)



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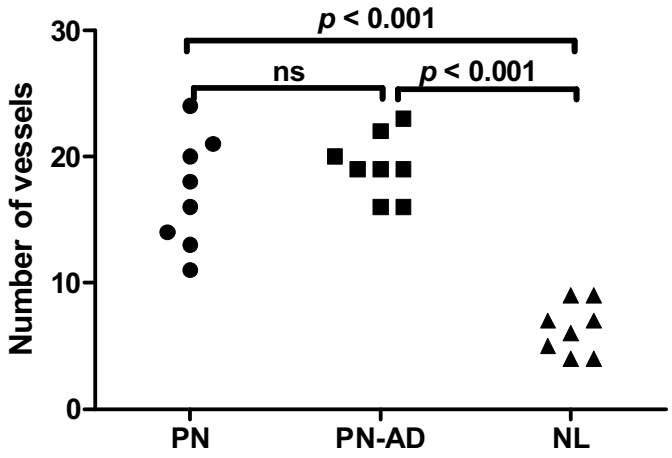
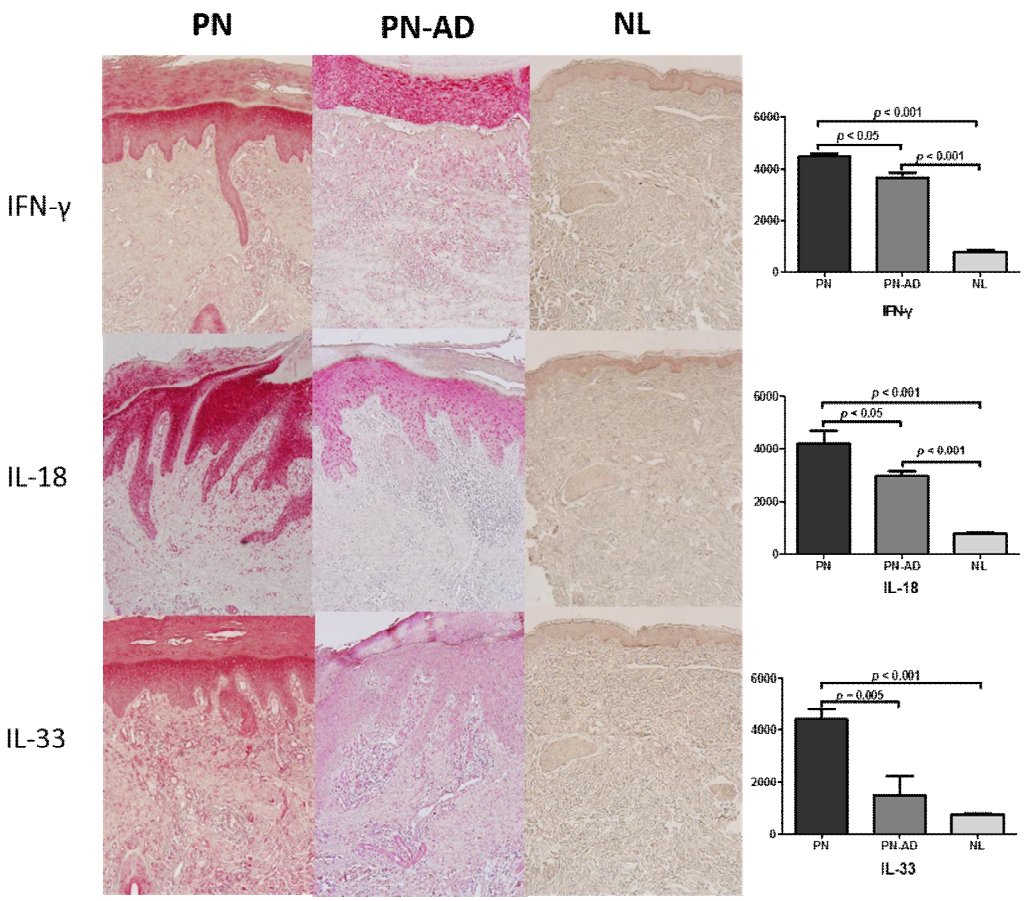


Figure 2. (A) The number of infiltrated inflammatory cells were counted at a high-power field (x400) and an average number was obtained from three different fields. The numbers of the PN (141.13) and PN-AD (132.01) groups were higher than that of normal (34.75), (p<0.01). (B) The number of vessels were counted in the dermis of within 100 μm distance from the epidermal-dermal junction, in which the numbers of PN and PN-AD groups were significantly higher than the numbers of normal group (p < 0.001)

4. Immunohistochemical anaylsis revealed significantly increased staining of IL-18, IL-33, and IFN-γ in PN-AD group

Immunohistochemical stains of IL-1 family members, IL-18 and IL-33, and IFN-γ were evaluated and the intensities were assessed and compared (Fig. 3A). In both PN and PN-AD groups, IL-18, IL-33, and IFN-γ stained positive, whereas the normal group stained negative. The staining intensities of PN and PN-AD groups were significantly higher than that of the normal group ($p < 0.001$). When the intensities of the three cytokines were compared between PN and PN-AD groups, the differences were also significant, in which the intensities were higher in the PN-AD group ($p < 0.05$). IL-31 also stained positive for PN and PN-AD groups, and when the intensities were compared, PN and PN-AD groups had significantly higher staining intensities than normal ($p < 0.01$ and $p < 0.05$, respectively), although the difference between PN and PN-AD groups were not significant (Fig. 3B).

(A)



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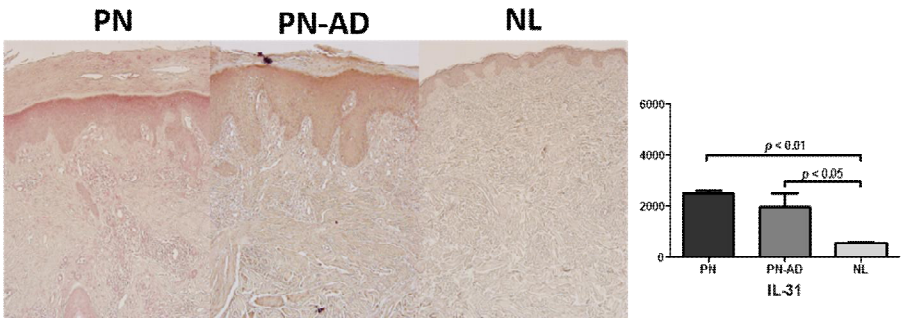


Figure 3. (A) Immunohistochemical analyses of IFN- γ, IL-18, and IL-33 stained positive for PN and PN-AD groups, and the staining intensities of PN group were significantly higher than that of PN-AD and normal groups. (B) IL-31 also stained positive for PN and PN-AD groups, and the intensity was higher for PN group, but the difference of the two groups were not significant.

5. Immunohistochemistry of Th2 cytokines, IL-4, IL-13, and TSLP, and SDF 1-α stained positive for tissues of PN and PN-AD

Immunohistochemical stains of Th2 cytokines were also evaluated and their staining intensities were assessed and compared. IL-4, IL-13, and TSLP stained positive for both PN and PN-AD groups (Fig. 4). The staining intensities of the tissues of PN and PN-AD groups were significantly higher than that of the normal group ($p < 0.001$). Although the staining intensities for PN-AD group were higher than that of PN group, the differences were not statistically significant. For chemokine SDF 1-α, the immunohistochemical stain was also positive in PN and PN-AD groups, and the intensity was higher in PN-AD group than PN group, but the difference was also not significant.

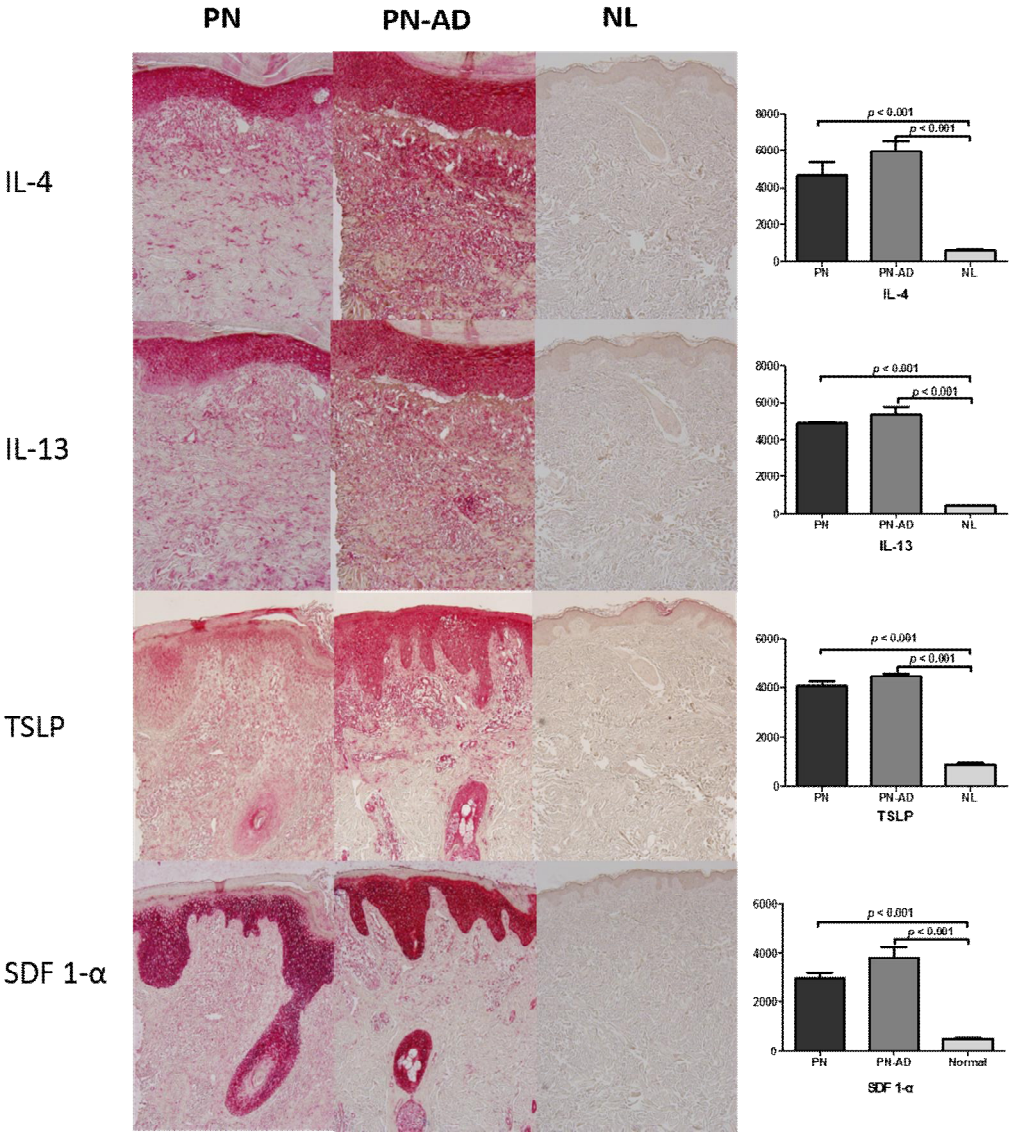


Figure 4. Immunohistochemical analyses of IL-4, IL-13, TSLP, and SDF 1-α. The stains were positive for both PN and PN-AD groups, and the staining intensities of were significantly higher than that of normal group. The intensities were higher in PN-AD group than PN group, but the differences between the two groups were not significant.

6. Immunohistochemical studies of galectin 10 and IL-22 stained positive weakly positive for PN and PN-AD groups

Additional immunhistochemical studies were done for galectin 10 and IL-22 (Fig. 5). In the tissues of PN and PN-AD groups, the stains were both slightly positive. When the intensities were assessed, the increase, however, were not significant when compared with normal. Also, no remarkable difference was observed between PN and PN-AD groups.

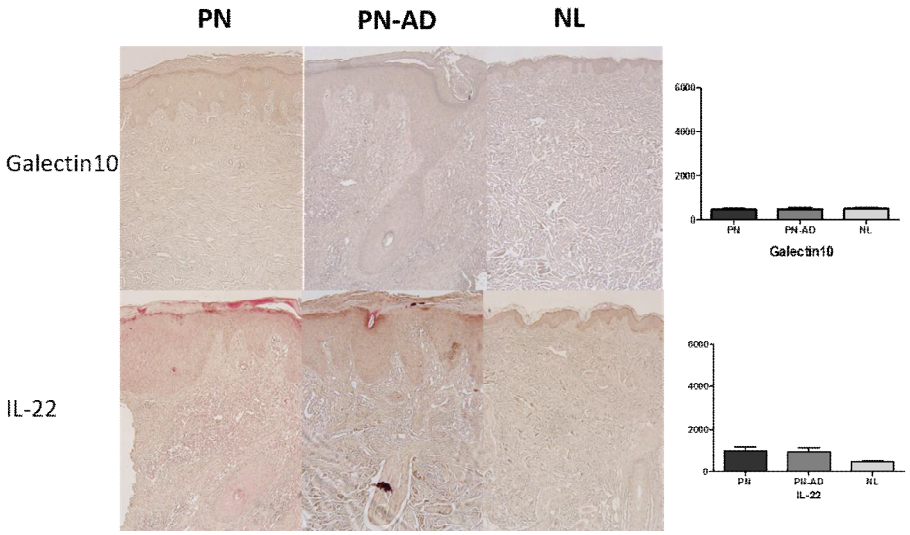


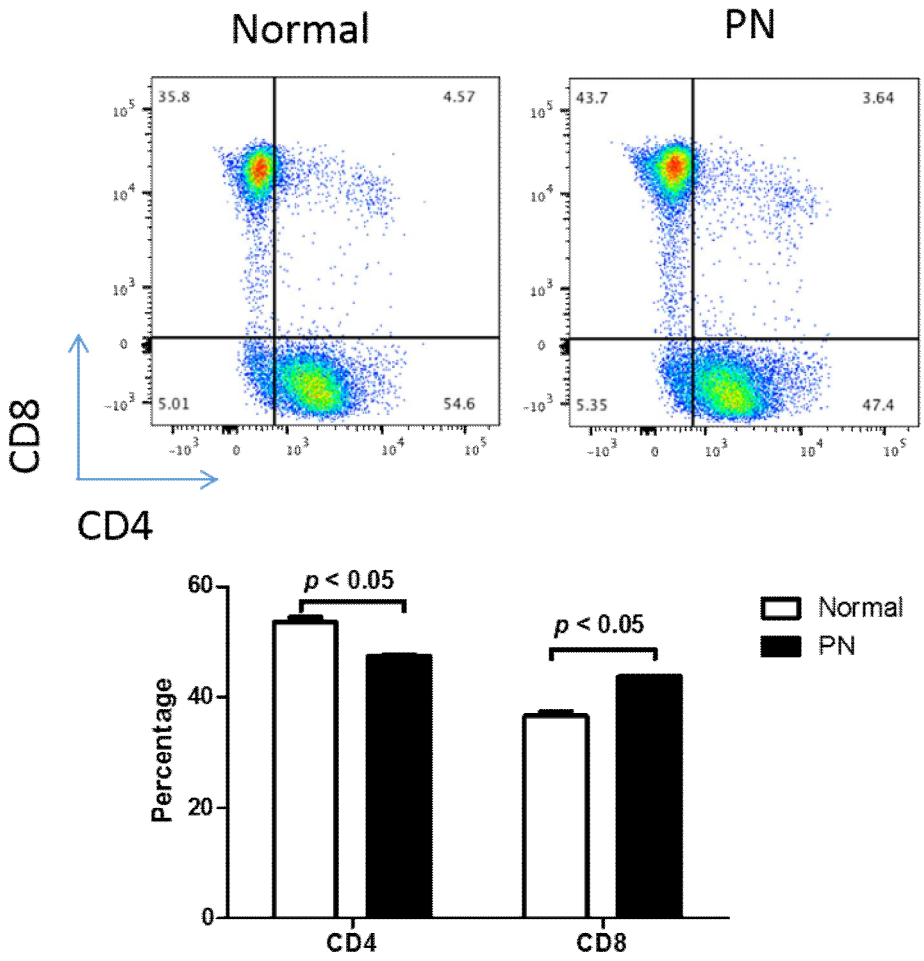
Figure 5. Immunohistochemical analyses of galectin 10 and IL-22. The stains were weakly positive for both PN and PN-AD groups, but the staining intensities were not significantly increased when compared with normal.

7. FACS analysis of the PBMC of the patients revealed increased CD8+ T cells, IL-22, IL-17, and IFN-γ.

Three patients with pure PN (without AD) were compared with 3 normal subjects without any histories of allergic diseases or other comorbidities. The subsets of T cells were analyzed in PBMC of the subjects (Fig. 6A). The average percentage of CD8+ T cells in PN group were 43.6% and when compared with the results of the normal group (36.5%), the difference was statistically significant ($p < 0.05$). For CD4+ T cells, the average percentages of the PN group was significantly lower than that of normal group ($p < 0.05$).

IL-4, IL-22, IL-17, and IFN-γ were also analyzed using FACS, separately in CD4+ cells and CD8+ cells. For CD4+ cells, the average percentages of IL-17 and IFN-γ of PN group were significantly higher than that of normal group ($p < 0.01$). For IL-22 and IL-4, no significant differences were observed between the two groups (Fig. 6B). When the cytokines were assessed in CD8+ cells, the percentage of IFN-γ was also found to be higher in PN group than that of the normal group ($p < 0.01$). For IL-4, however, the percentage in PN group was found to be significantly lower in PN patients than the normal subjects. No significant differences were observed in the results of IL-17 and IL-22 between the two groups (Fig. 6C).

(A)



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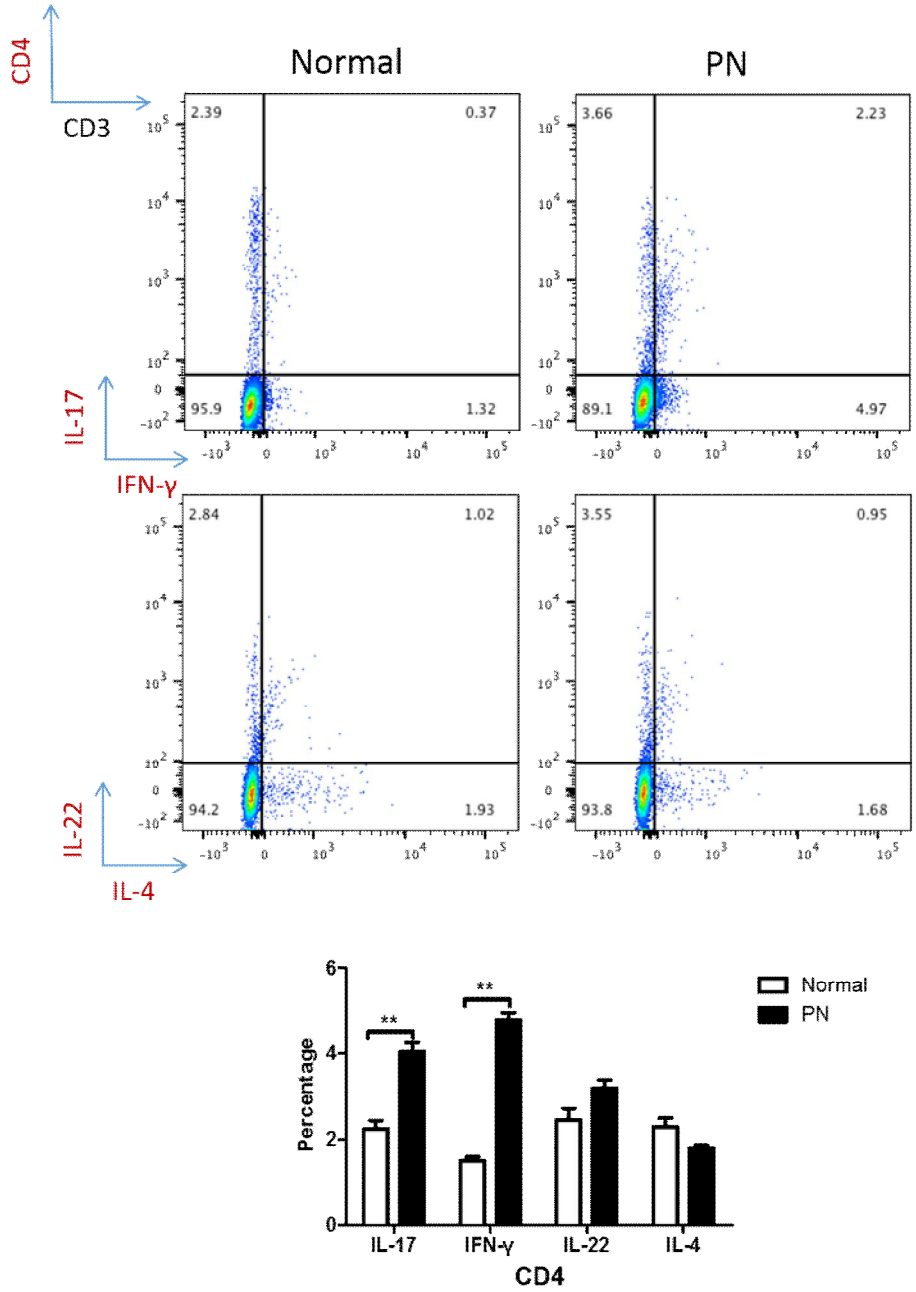

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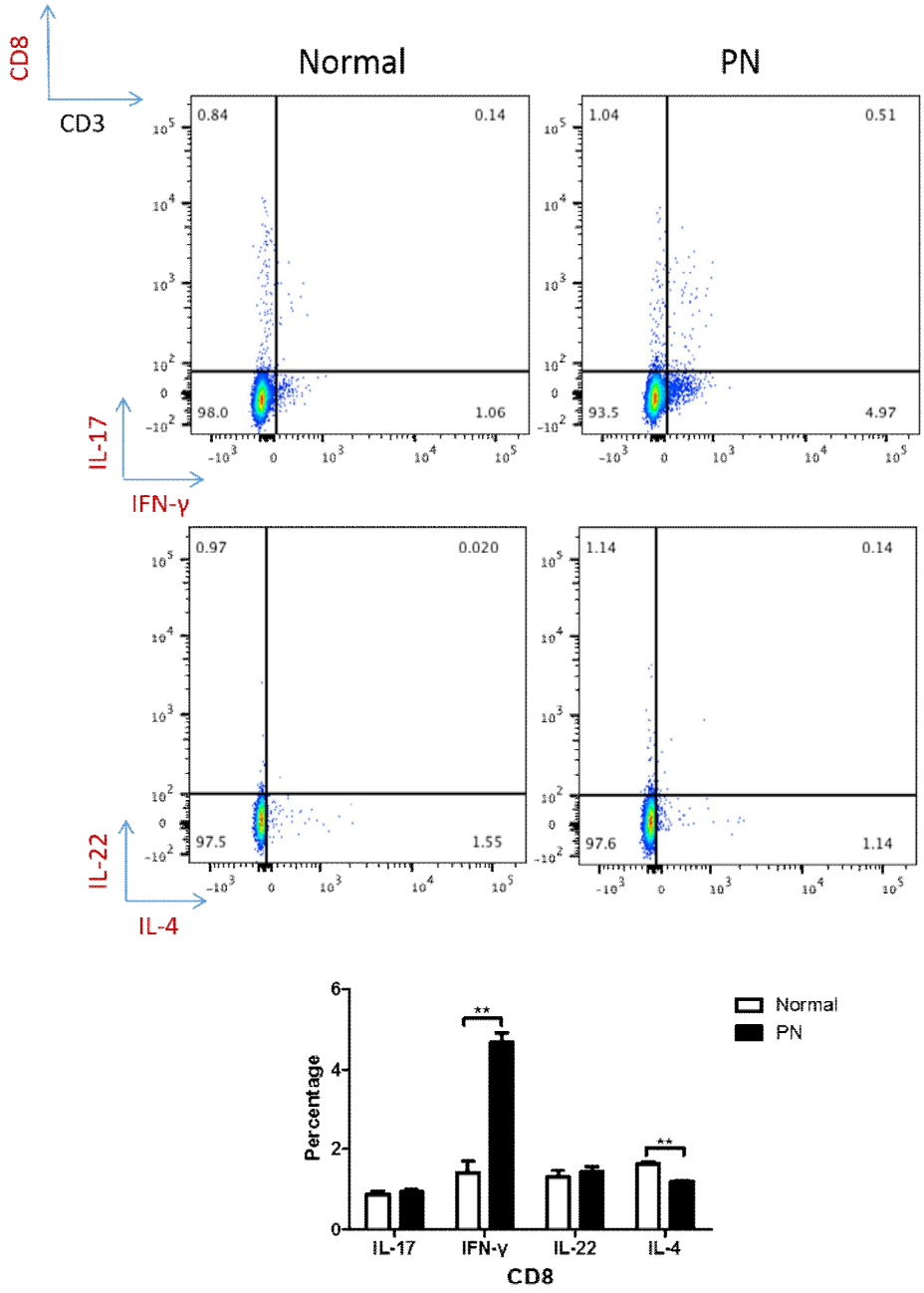

Figure 6. FACS analyses of 3 PN patients compared with 3 normal subjects. (A) The subsets of T cells were analyzed, in which the percentage of CD8+ T cells were significantly higher in PN group (40.70%) than the normal group (34.67%), while the CD4+ T cells were significantly lower in PN group ($p < 0.05$). (B) The percentages of the cytokines IL-4, IL-22, IL-17, and IFN- γ were assessed on CD4+ cells, in which the results of IL-17 and IFN-γ of PN group were significantly higher than that of normal group ($p < 0.01$). (C) The percentages of the cytokines IL-4, IL-22, IL-17, and IFN- γ were also assessed on CD8+ cells, in which the results of IFN-γ and IL-4 of PN group were significantly higher than that of normal group ($p < 0.01$).

IV. DISCUSSION

PN is a skin disorder with multiple pruritic hyperkeratotic papules on nodules on whole body and the treatment has been considered to be difficult[16]. Studies have tried to elucidate its pathogenesis, and cutaneous neuropathy and the involvement of Th2 cytokines have been found to be associated[7,12,13]. Also, PN is a disease known to be associated with various underlying diseases that may induce pruritus, with AD regarded as one of the most common diseases. The mechanisms of PN, however, remain largely unknown and only few studies have investigated on the associations between PN with AD versus PN without AD.

Clinical investigations of PN with and without AD reveal that the mean age of the patients with AD are lower, although the difference was insignificant. This can be supported by the previous study which has shown that the development of PN in those with AD is earlier than those without AD[6]. The mean total IgE level and mean eosinophil counts were both significantly higher in those with AD, which is also consistent with the findings of previous studies that suggested PN with AD to possibly be another disease entity[5,6]. PN has been considered as disease associated with various comorbidities, including diabetes mellitus, hypertension, and chronic kidney disease[3], in which these diseases have also been found to be accompanied in patients of PN without AD in this study. Through these findings, in PN associated with AD, sensitization through allergens may be associated with the pathogenesis, unlike PN without AD.

The histologic features of PN are hyperkeratosis, acanthosis, dermal cellular infiltration and vertically-oriented collagen bundles[2]. In this study, the thicknesses of the epidermis and dermis and the number of infiltrated inflammatory cells were compared between those PN with and without AD and normal. The epidermal thickness was found to be significantly increased in the tissues of PN, both with and without AD, when compared to the normal group. The number of inflammatory cells infiltrated in the dermis were also found to be increased, whereas no remarkable differences were found in the dermal thicknesses. In addition, the number of dermal vasculatures were found to be significantly increased in PN with and without AD when compared with normal. No remarkable differences of epidermal and dermal thicknesses and number of inflammatory cells were found between PN with and without AD. Through these findings, epidermal acanthosis and dermal inflammatory cellular infiltration can be considered as key histologic changes in PN, regardless of the presence of AD.

Through immunohistochemical studies, the associations of various cytokines have been analyzed. When compared with normal, stains of SDF 1- α, TSLP, IFN- γ, IL-4, IL-13, IL-18, IL-31, and IL-33 were found to be increased in both tissues of PN with and without AD when compared with normal. galectin 10 and IL-22 stained weakly positive, in which the measured intensities were not significantly higher than that of normal. Based on the staining intensities, TSLP, IL-4, IL-13, and SDF 1- α were most strongly stained, thus Th2 cytokines can

be suggested to be most associated with the pathogenesis. Previous studies have found increased expressions of IL-4, IL-17, IL-22, and IL-31 in lesional skins of PN[13], as well as high expressions of STAT6 transcription factor in the lesional epidermis[12], which are consistent with the findings of this study.

The immunohistochemical results were then compared between the tissues of PN with and without AD. For PN with AD, the staining intensities of SDF 1- α, TSLP, IL-4, and IL-13 were higher than those of PN without AD, although the differences were not statistically significant. For PN without AD, IFN- γ, IL-18, and IL-33 were found to have higher staining intensities than those with AD, of which the intensities were statistically higher. Through these findings, in PN with AD, although similar features may be shared with pure PN, the pathogenesis may be more associated to AD, as suggested by the higher increase in Th2 cytokines. In addition, in PN without AD, IFN- γ and IL-18 are increased, in which Th1 cytokines may also be associated. Also, because the staining intensities of IL-18, IL-33, and IFN-γ of PN-AD group were significantly lower than that of the PN group, the effects of IL-18, IL-33, and IFN-γ are likely to be more related.

Through FACS analysis of PBMC of the patients with PN, the percentage of CD8+ T cells were found to be statistically higher than that of the normal control, while the percentage CD4+ T cells between PN and normal groups showed no significant difference. This finding may suggest that CD 8+ T cells may have important roles in the pathogenesis of PN, as a previous study has

found increased CD8+ T cells in PN patients through immunohistochemistry[14]. Studies have found the production of IFN-γ, IL-13, IL-17, and IL-22 in AD and psoriasis by CD8+ T cells[17], thus CD8+ T cells may be involved by producing such cytokines.

Several cytokines have been additionally assessed by FACS analysis, in which IL-17, IL-22, and IFN-γ were found to be increased in patients with PN. However, for IL-4, no significant difference was observed between the PN and normal groups. Comparing with the results of immunohistochemistry, the data of IFN-γ was consistent. For IL-22 and IL-4, however, the findings between immunohistochemistry and FACS analyses were different. IL-17 and IL-22 belong to Th17 cytokines, in which IL-17 has been found to be associated with skin remodeling in AD and contact dermatitis[18], while IL-22 is also known to cause skin remodeling as well as epidermal hyperplasia[19]. According to the results of FACS analysis, these cytokines may be associated with the increased epidermal thickness, but since IL-22 was not significantly increased in immunohistochemistry, further studies may be needed to confirm the inconsistent results.

Through the findings of the study, PN accompanied by AD may have stronger associations with Th2 cytokines than PN without AD. TSLP, IL-4, and IL-13 had relatively stronger staining intensity in PN-AD group, in which atopic diathesis and increased sensitization to allergens lead to persistent scratching, increasing TSLP and subsequently activating innate allergic responses and Th2

cytokines associated with AD[20-22]. Although the exact mechanisms leading to the development of PN in AD need to be discovered, the findings in this study suggest that the inflammation in AD underlies the pathogenesis of PN, unlike pure PN (without AD).

Recently, IL-18 has also been suggested as a cytokine to be associated in the itch-scratch response[23]. IL-18 is a member of IL-1 family of cytokines, which is produced by various cells including keratinocytes as inactive forms. Stimuli such as pathogen-associated molecular patterns or danger-associated molecular patterns, that may be increased due to epidermal injuries, activate IL-18 into its active form. IL-18 then induces IFN-γ, subsequently causing inflammation[24,25]. IL-33 is also a member of IL-1 family of cytokines, which may be released by epithelial and endothelial cells after tissue injury. IL-33 primarily induces Th2 immune response by enhancing Th2 cytokine secretions, such as IL-5 and IL-13. IL-33 also has the potential to promote Th1 cytokines such as IFN- γ[26]. Thus, IL-18 and IL-33, which are members of IL-1, can be suggested to have crucial roles in the pathogenesis of pure PN, subsequently inducing IFN- γ, and possibly leading to the epidermal proliferation as a result.

IFN- γ is a cytokine known to be increased in chronic inflammatory skin diseases, especially in AD, which is known to induce chronic inflammation [27]. Recent studies have found that IFN- γ induces IL-33 in keratinocytes and IL-33 induces further release of IFN- γ by T cells, suggesting that these cytokines are closely associated to epidermal inflammation in AD and also contributes to

driving skin inflammation towards chronic responses[28]. Through the findings of the previous studies, PN, especially when unaccompanied by AD, can be suggested to be associated to IL-18, IL-33, and IFN- γ, in which continuous scratching of the lesions causing epidermal injury leads to the production of IL-18 and IL-33, which then subsequently induce IFN- γ, leading to cutaneous inflammation.

V. CONCLUSION

In conclusion, PN is a disease that is related not only to Th2 immune responses, but also Th1 responses, in which complex mechanisms may be associated. The pathogenesis of PN with and without AD may be different, in which inflammation associated with allergen sensitization and Th2 cytokines may be more associated in those with AD, whereas scratching that causes epidermal injuries and the increase of cytokines IL-18, IL-33, and IFN- γ may be the primary cause of PN (Fig. 8).

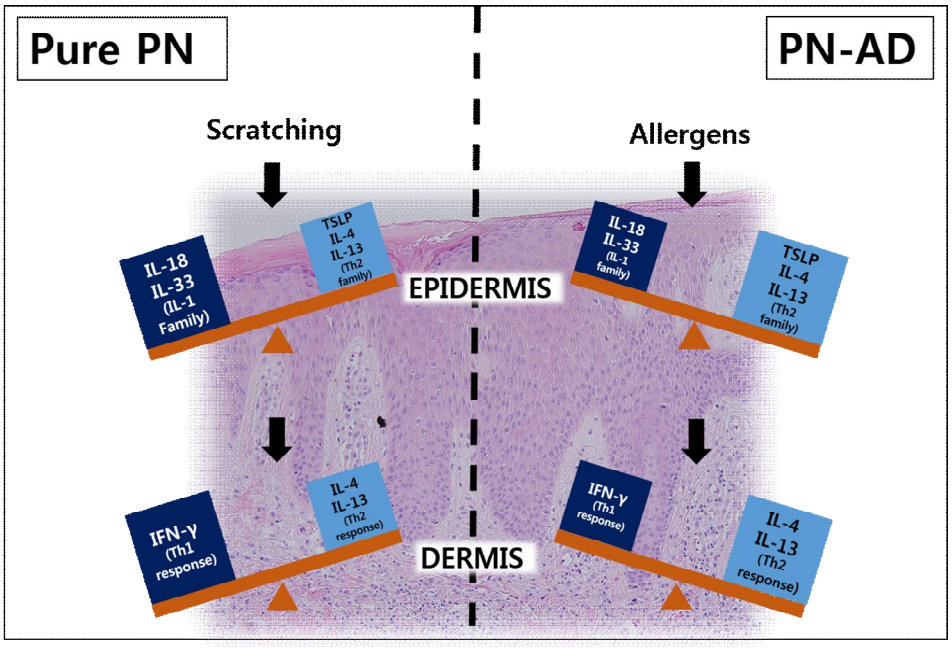

Figure 7. A schematic diagram of the summary of the pathogenesis of (pure) PN and PN-AD, in which scratching causes epidermal injury that leads to the increase of IL-1 family cytokines IL-18 and IL-33, which induces IFN-γ in PN, whereas in PN-AD, allergens possibly contribute to increase of Th2 cytokines, including IL-4, IL-13, and TSLP, contributing to the development of PN.

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ABSTRACT (IN KOREAN)

임상적, 조직학적, 면역화학적 분석을 통한 결절성 소양진에서의 표피 두께 증가의 원인에 대한 고찰

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곡 원 호

결절성 소양진은 만성적인 가려움증을 동반하는 피부 질환으로 다수의 과각화된 결절을 특징으로 한다. 여러 가설들이 제시되어 왔으나 아직 발병기전에 대해서는 명확히 밝혀져 있지는 않다. 또한 아토피피부염이 연관이 되어 있는 것으로 알려져 있으나 아직 이와 연관성은 잘 알려져 있지 않다.

이 연구에서는 결절성 소양진 환자 (PN), 아토피피부염이 동반된 결절성 소양진 환자 (PN-AD), 그리고 정상 대조군과 임상적, 조직학적, 그리고 면역화학적으로 비교를 하였다.

PN과 PN-AD 환자들을 비교한 결과, PN-AD군에서 혈청 전체 IgE 수치와 eosinophil count가 통계적으로 유의미하게 높았다. 조직학적으로 분석을 하였을 때, PN과 PN-AD 군에서 표피의 두께 및 진피에 침윤된 염증 세포의 수가 정상 대조군에 비해서

유의미하게 증가하였다. 면역화학적 분석에서는 SDF 1- α, TSLP, IFN- γ, IL-4, IL-13, IL-18, IL-31 및 IL-33에 대한 염색이 PN과 PN-AD군에서 증가되었다. 염색된 강도를 분석하였을 때 PN-AD군에서 SDF 1- α, TSLP, IL-4 및 IL-13의 염색 강도가 PN군에 비해서 더 높게 나타났다. 반면에 PN군에서는 IL-18, IFN- γ 그리고 IL-33의 염색 강도가 PN-AD군에 비해서 유의미하게 강하게 나타났다.

이러한 결과들을 통해서 아토피피부염이 동반되지 않은 결절성 소양진의 발병 기전은 아토피피부염이 동반된 경우와 다를 것으로 생각되며, IL-18, IFN- γ 그리고 IL-33 이 연관이 있을 것으로 사료된다. 따라서 아토피피부염이 동반되지 않은 결절성 소양진의 경우 긁음 등 경미한 기계적 자극에 의해 손상된 표피에서 증가된 IL-18 과 IL-33 이 초기 염증 작용을 하여 궁극적으로 표피의 만성화에 관여하는 IFN- γ을 유도함으로써 결절성 소양진의 표피의 증가, 즉 극세포증을 일으키고 침윤된 염증 세포가 이 과정에 관여하리라 생각된다.

핵심되는 말: 결절성 소양진, 아토피피부염, 면역화학 분석, 사이토카인