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Increased β-endorphin and autotaxin in patients with prurigo

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Abstract

Objectives: Prurigo is a treatment-resistant inflammatory disease of unknown etiology. Persistent and severe itch is a major and important clinical symptom, but pathological mechanisms and/or actual mediators for itch in prurigo remain to be defined.

Methods: We investigated blood levels of β-endorphin, dynorphin A, and autotaxin in adult patients with prurigo (n = 18), including prurigo nodularis, prurigo chronica multiformis, and other forms. Patients with hemodialysis, hepatic dysfunction, and pregnancy were excluded. Immunofluorescence staining for β-endorphin and autotaxin in lesional skin was also performed. In addition, β-endorphin and autotaxin synthesis of cultured epidermal keratinocytes in response to several cytokine stimulation was assessed in vitro.

Results: Blood levels of β-endorphin were higher in patients with prurigo than in control subjects (n = 20), while dynorphin A levels were comparable in the two groups. Blood autotaxin levels also were increased in patients with prurigo compared to control subjects. On the one hand, epidermal expression of β-endorphin was increased in skin lesions of prurigo chronica multiformis, but not in prurigo nodularis. On the other hand, enhanced expression of autotaxin was observed in the epidermis in both prurigo nodularis and prurigo chronica multiformis. In vitro studies showed that β-endorphin-expressing epidermal keratinocytes increased following stimulation with TNF-α, interleukin-31, and/or IFN-γ. Autotaxin was also detected in the cytoplasm of cultured epidermal keratinocytes. The fraction of autotaxin-expressing cells increased when stimulated with TNF-α and/or interleukin-31.

Conclusions: Imbalance of opioid receptor signals and/or the autotaxin-lysophosphatidic acid axis may contribute, at least in part, to the pathogenesis and intractable pruritus in some of the patients with prurigo.

KEYWORDS

Autotaxin, lysophosphatidic acid, prurigo chronica multiformis, prurigo nodularis, β-endorphin

1 | INTRODUCTION

Prurigo is a reactive inflammatory skin disease characterized by itchy papulo-nodular lesions. Patients with prurigo seem to exhibit heterogeneity in morphology and pathoetiology. On the one hand, one form of prurigo, prurigo nodularis, is considered a secondary skin change resulting from severe scratching because of persistent pruritus associated with a variety of cutaneous and systemic diseases.[1,2] On the other hand, the pathological mechanisms of other forms of prurigo, including prurigo chronica multiformis and prurigo subacuta, have not been defined.

Irrespective of the type, the various forms of the prurigo share a common clinical symptom, a severe pruritus, which is generally difficult to treat with conventional therapies, such as H1 receptor antagonists, and the mediators for itch in those conditions are still to be fully elucidated. In addition to histamine, IL-31, substance P, nerve growth factors, and eosinophil granule proteins also may participate in chronic itch in prurigo nodularis.[3] In hemodialysis and chronic hepatic diseases, imbalances of opioid receptor signals in the central nervous systems (eg, predominant production of β-endorphin, an intrinsic agonist of μ-type opioid receptor providing pruritogenic signals compared with dynorphin A, and an agonist of κ-type opioid receptor antagonizing μ-type opioid receptor signals) have been implicated in intractable chronic pruritus.[4,5] Apparently, prurigo nodularis commonly affects those patients.

Autotaxin (ATX) is a 125-kD type II ectonucleotide pyrophosphatase/phosphodiesterase (ENPP2) that was originally identified among factors released from melanoma cells. ATX also has lysophospholipase D activity, generating lysophosphatidic acid (LPA) from lysophosphatidylcholine.[6] LPA is a water-soluble phospholipid that has a variety of biological functions, including roles in cell migration, cytokine production, and platelet activation.[7,8] Notably, high serum levels of ATX were detected in cholestatic patients,[9] and thus, LPA is considered a potential candidate cause of cholestatic pruritus.[7,10] In mouse models, LPA mediates itch through the LPA5 receptor.[11]

To see whether opioid signal imbalance and/or ATX-LPA signals are involved in intractable itch in prurigo, we analyzed blood β-endorphin, dynorphin A, and ATX levels in a series of patients with prurigo, focusing on patients not associated with hemodialysis and hepatic diseases. Expression of these pruritogens in lesional skin also was assessed.

2 | MATERIALS AND METHODS

2.1 | Study participants

Eighteen patients (14 male and 4 female; mean age, 71.5 years) with prurigo lasting longer than 6 weeks were assessed in this study (Table 1). These cases included prurigo nodularis (PN) (n = 3), prurigo chronica multiformis (PCM) (n = 6), and other forms of prurigo (prurigo,

TABLE 1 Clinical profiles of patients

No.	Age	Sex (M/F)	Types of prurigo	Past histories[a]	VAS	β-endorphin (pg/mL)	Dynorphin A (pg/mL)	Autotaxin (×10²ng/mL)
1	67	M	Prurigo, others	HT, HU	8.5	3.25	1.19	3.72
2	76	M	Prurigo, others	DM	2.3	3.54	1.52	2.46
3	49	M	PN	None	6.3	2.65	1.31	1.83
4	60	M	PCM	None	8.1	7.69	1.7	3.85
5	81	M	Prurigo, others	None	8.9	3.53	1.2	2.88
6	90	M	PCM	None	5.2	9.49	1.3	2.53
7	57	F	Prurigo, others	HL	4.6	3.11	1.39	2.36
8	87	M	PN	DM	9.6	9.65	2.58	2.81
9	82	F	PN	None	1.1	6.87	1.46	7.07
10	26	F	Prurigo, others	None	8	1.78	0.85	3.66
11	90	M	Prurigo, others	HL	4.7	2.63	0.55	3.51
12	78	M	PCM	DM	6.6	8.1	0	2.13
13	77	M	PCM	HT, HU	1.5	5.86	0	3.11
14	74	M	Prurigo, others	None	9.1	4.33	0	2.39
15	90	M	Prurigo, others	HT	4.7	2.78	1.46	2.94
16	64	M	PCM	None	3	4.98	2.07	2.97
17	82	M	PCM	None	7.4	15.2	1.37	2.61
18	57	F	Prurigo, others	None	7.2	2.12	1.1	3.72

PN, prurigo nodularis; PCM, prurigo chronica multiformis; VAS, visual analogue scale; HT, hypertension; DM, diabetes mellitus; HU, hyperuricemia; HL, hyperlipidemia.

[a]Subjects with hemodialysis, hepatic dysfunction, and ultraviolet therapy were excluded.

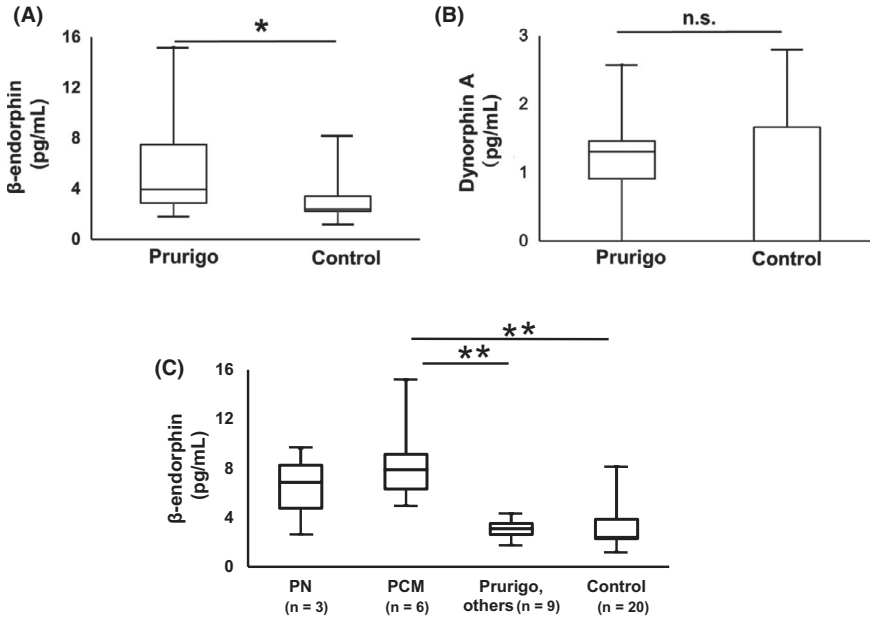

FIGURE 1 Plasma opioids in patients with prurigo. Plasma levels of (A) β-endorphin and (B) dynorphin A were measured by ELISA. *$P < 0.05$, as assessed by Mann-Whitney *U* test. ns, not statistically significant. The boxes indicate the range from 25th percentile to 75th percentile, the horizontal lines indicate the median values, and the whiskers extend from 10th percentile to 90th percentile. (C) Plasma β-endorphin levels in each type of prurigo. **$P < 0.01$, as assessed by Steel-Dwass's multiple comparison test

others), including prurigo simplex subacuta and unclassified forms (n = 9). Treatments were as follows: topical corticosteroids, vitamin D3 analogues, and/or oral antihistamines. Patients with systemic corticosteroids, immunosuppressants, and/or UV radiation therapies were excluded. The control group consisted of twenty subjects (seven male and 13 female; mean age, 68.5 years) who did not have itchy diseases. These cases included benign skin tumors (n = 8), malignant skin tumors (n = 9) (Bowen's disease, basal cell carcinoma, extramammary Paget's disease, and porocarcinoma), and three healthy volunteers. Subjects with hemodialysis, hepatic dysfunction, and age of less than 20 years were excluded. Prurigo gestationis also was excluded. Itch intensity was assessed by VAS (visual analogue scale, maximum: 10) scores. This study was conducted according to the Declaration of Helsinki Principles. Written informed consent was obtained from all subjects. This study was approved as Protocol No. 2445 by the ethical committee of the National Defense Medical College.

2.2 | Preparation of blood samples

Peripheral blood was obtained from each subject, and serum and EDTA-anticoagulated plasma were collected following centrifugation. The resulting samples then were stored at −20°C until use. Plasma opioids, such as β-endorphin and dynorphin A, were measured with ELISA kits (Phoenix Pharmaceuticals, Inc, Burlingame, California, USA) (detection limits: 0.5 pg/mL) at Toray Research Center, Inc (Tokyo, Japan). Serum ATX was assayed with a sandwich ELISA system using Quantikine Human ENPP-2/Autotaxin ELISA Kit (R&D systems, Minneapolis, MN, USA) (detection limit: 15.6 ng/mL).

2.3 | Immunohistochemistry

Cryosections of biopsied specimens were reacted with either rabbit anti-β-endorphin antibody (Ab) (1:100; Chemicon, Temecula, CA, USA) or rabbit anti-ENPP2/autotaxin (ATX) Ab (1:50; Abgent, San Diego, California, USA) followed by incubation with fluorescein-conjugated goat anti-rabbit IgG secondary Ab (1:200, Chemicon). Normal rabbit IgG (Abcam, Cambridge, England) was used as a negative control. Stained tissue sections were observed under a fluorescence microscope (BZ-X710; Keyence, Osaka, Japan). Immunofluorescence intensities were evaluated with the BZ-H3M measurement module (Keyence).

2.4 | Culturing and stimulation of normal human keratinocytes

Normal human keratinocytes (Epidermal Keratinocyte Progenitors (single donor); Axol Bioscience, Ltd., Cambridgeshire, UK) were subjected to serial culturing in HuMedia-KG2 (Kurabo Industries, Ltd., Osaka, Japan) in 24-well plates at 37℃ and 5% CO_2. Before the third passage achieved 70% confluency, the cells were stimulated with either IL-4, (Sigma-Aldrich, Inc, Saint Louis, Missouri, USA), TNF-α (Sigma-Aldrich), IL-17A (R&D Systems, Inc, Minneapolis, Minnesota, USA), IFN-γ (R&D Systems), or IL-31 (Sigma-Aldrich) for 16 hours

Stimulated cells were fixed with BD Fixation Permeabilization solution (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA) and stained with a rabbit anti-β-endorphin Ab or a rabbit anti-ENPP2/ATX Ab followed by the incubation with fluorescein-conjugated goat anti-rabbit IgG secondary Ab. Stained cells were assessed under a fluorescence microscope (Keyence). Image analysis was performed using the BZ-X710 Hybrid Cell count image analysis program (Keyence). For a given field, approximately 100 cells stained with DAPI were counted and assessed for the β-endorphin and ATX expressions. Fifteen fields per sample were evaluated, and data were expressed as percentages of positive cells.

2.5 | Statistical analyses

Statistical analyses were performed with either two-tailed Student's *t* test or the two-tailed Mann-Whitney *U* test for comparisons of

two groups. Steel's test or Steel-Dwass's test was used for multiple comparison analyses. Correlation coefficients were determined by Spearman's rank correlation test. *P* values of < 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Levels of plasma opioids in patients with prurigo

Plasma β-endorphin levels in patients with prurigo were significantly higher than those in control subjects (Figure 1A). On the other hand, plasma dynorphin A levels in patients with prurigo were comparable to those in control subjects (Figure 1B). The ratios of β-endorphin to dynorphin A were not calculated, as dynorphin A was undetectable in some of the patients. Figure 1C shows plasma β-endorphin levels in each prurigo subtype. Patients with PCM tended to have higher plasma β-endorphin levels than patients with other forms, although the comparison with PN was not ideal because of a small sample size (n = 3).

3.2 | Levels of ATX in patients with prurigo

LPA production is strongly correlated with ATX levels.[6] Since LPA is an unstable substance, we measured serum ATX levels, which reflect LPA levels. Patients with prurigo had higher serum levels of ATX than control subjects (Figure 2). Unlike plasma β-endorphin, serum ATX levels were not apparently influenced by the type of prurigo (data not shown).

3.3 | Expression of β-endorphin and ATX in lesional skin

We next attempted to determine epidermal expression of β-endorphin and ATX. β-endorphin was expressed in epidermal keratinocytes in normal skin, consistent with a prior report.[12] Compared to normal skin, PCM (n = 5) skin exhibited a higher level of β-endorphin expression, whereas PN epidermis (n = 5) showed a comparable β-endorphin expression to normal skin (Figure 3). ATX expression also was observed in normal epidermis. Epidermis from both PN (n = 5) and PCM (n = 5) expressed higher levels of ATX than did normal skin (Figure 4).

3.4 | β-endorphin and ATX expression in epidermal keratinocytes in vitro

We next assessed in vitro expression of β-endorphin in cultured normal human keratinocytes exposed to each of several stimulants, such as IL-4, TNF-α, IL-31, IL-17A, and IFN-γ. Consistent with a prior report,[13] the prevalence of β-endorphin-expressing cells increased in response to IL-31. In addition, TNF-α and IFN-γ also stimulated β-endorphin expression in keratinocytes in vitro (Figure 5).

We are not aware of any prior studies regarding ATX levels in the epidermis. Since ATX was detected in lesional epidermis, we also attempted to assess in vitro expression of ATX in cultured keratinocytes. Cultured epidermal keratinocytes expressed ATX in their cytoplasm with peripheral accentuation (Figure 6A). The prevalence of ATX-expressing cells increased in response to TNF-α or IL-31 (Figure 6B,C).

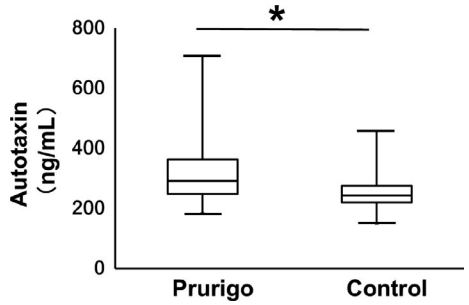

FIGURE 2 Serum autotaxin (ATX) in patients with prurigo. Serum levels of ATX were measured by ELISA. *P < 0.05, as assessed by Mann-Whitney U test. The boxes indicate the range from 25th percentile to 75th percentile, the horizontal lines indicate the median values, and the whiskers extend from 10th percentile to 90th percentile

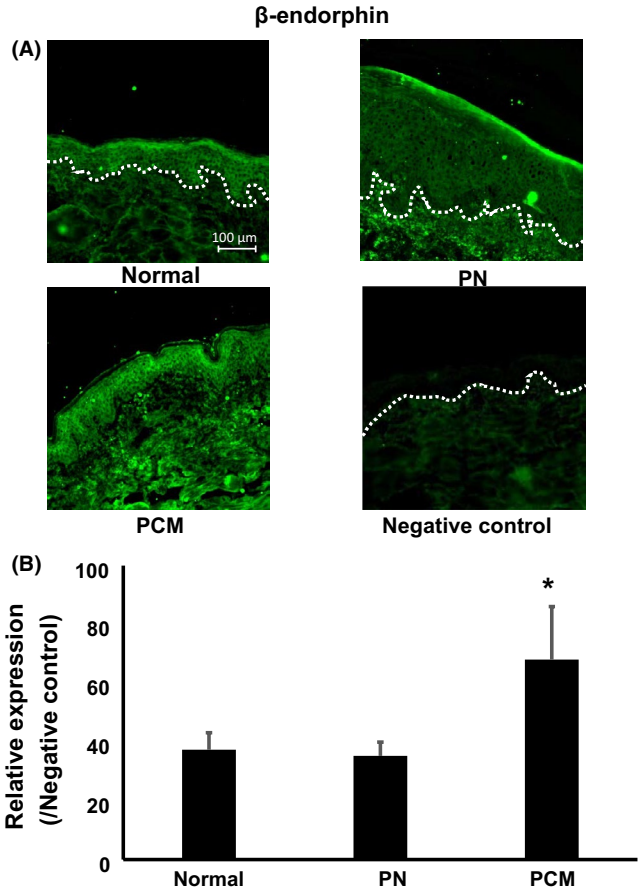

FIGURE 3 β-endorphin expression in lesional skin. A, Immunofluorescence staining features. Dotted lines indicate dermal-epidermal junction. B, Fluorescence intensities of epidermis were analyzed as described in the Materials and Methods and expressed as relative expression.*P < 0.01, compared with normal skin as assessed by Steel-Dwass's multiple comparison test. normal: normal skin, PN: prurigo nodularis, PCM; prurigo chronica multiformis

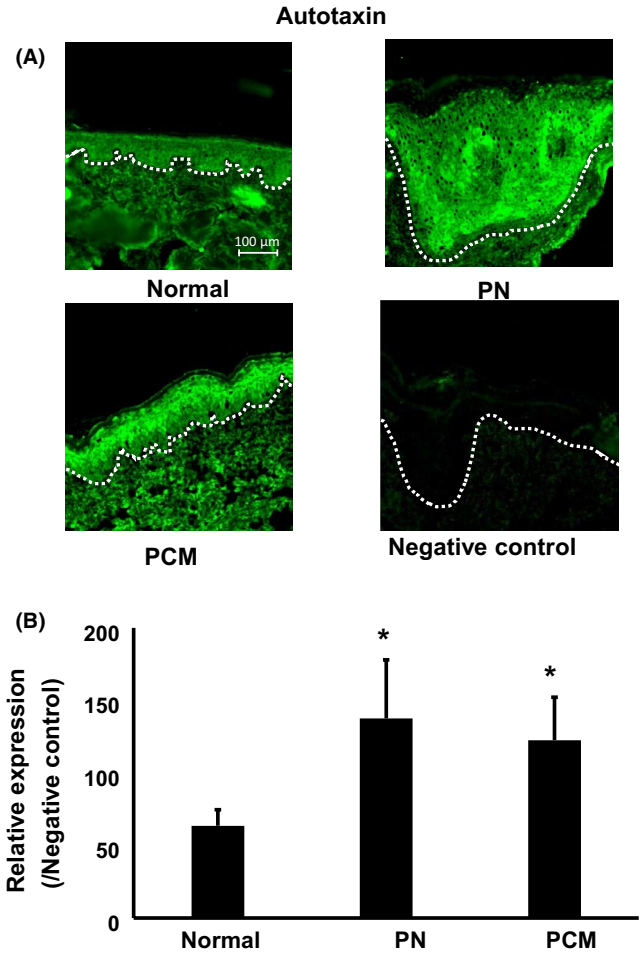

FIGURE 4 Autotaxin expression in lesional skin. A, Immunofluorescence staining features. Dotted lines indicate dermal-epidermal junction. B, Fluorescence intensities of epidermis were analyzed as described in the Materials and Methods and expressed as relative expression. *$P < 0.01$, compared with normal skin as assessed by Steel-Dwass's multiple comparison test. normal: normal skin, PN: prurigo nodularis, PCM; prurigo chronica multiformis

4 | DISCUSSION

The actual factors that precipitate severe itch in patients with prurigo remain unclear. In this study, we demonstrated increased production of plasma β-endorphin in patients with prurigo. There was no significant difference in plasma dynorphin A level between patients with prurigo and normal subjects, suggesting that μ-opioid receptors are activated to higher levels than are κ-opioid receptors in patients with prurigo. Regulatory agents for opioid receptors, such as nalfurafine hydrochloride, a κ-receptor agonist, may exert therapeutic effects on pruritus in some patients with prurigo, irrespective of renal and/or hepatic dysfunction. We also observed increased production of ATX in the serum of patients with prurigo. Since blood ATX levels correlate with blood LPA levels,[6] our result implies that patients with prurigo have high blood levels of LPA. In a prior report, high levels of ATX also were detected in the blood of patients with atopic dermatitis.[14] In other work (data not shown), we have found that plasma β-endorphin levels did not correlate with ATX levels. This observation indicated that β-endorphin is regulated independently from ATX and that different mediators and/or pruritogens participate in the prurigo reactions for individual patients.

β-endorphin has been shown to be expressed in the epidermis of patients with atopic dermatitis and psoriasis.[12,15] In the present study, we observed that β-endorphin is expressed in epidermal keratinocytes in the lesional skin of prurigos. However, β-endorphin expression of PN epidermis was not necessarily enhanced compared with normal skin. This result was in contrast to prior findings that *IL-31* mRNA levels are increased in skin lesions of PN and that human keratinocytes generate β-endorphin in response to IL-31 in vitro.[13,16] Induction of β-endorphin in IL-31-stimulated keratinocytes was also confirmed in our in vitro studies (Figure 4). The basis of this discrepancy remains unclear, but these differences may reflect the use by patients with PN of topical therapies, such as corticosteroids and/or activated vitamin D3 analogues. Even if those topical therapies were administered before the study, such treatments may have exerted long-term inhibitory effects on epidermal β-endorphin expression in these patients. Alternatively, IL-31's contribution to the pathogenesis of PN may vary depending on the stages of skin lesions and the nature of PN, given that PN has multiple etiologies and can arise from a variety of skin conditions.[2] On the other hand, epidermis from patients with PCM showed higher expression of β-endorphin than did epidermis from normal patients. This result may be consistent with the observation that some patients with PCM had high blood levels of β-endorphin (1C).

In this study, we also found that keratinocytes expressed β-endorphin in response to TNF-α and IFN-γ. These results imply that immune reactions biased toward Th1 may induce epidermal β-endorphin expression. Further studies are needed to assess epidermal β-endorphin expression and its biological significance in other dermatological diseases.

In contrast to the results with β-endorphin expression, we observed enhanced epidermal ATX expression in both PN and PCM. Intriguingly, in vitro experiments showed that keratinocytes expressed ATX in their cytoplasm and that the fraction of ATX-expressing cells increased in response to TNF-α or IL-31 stimulation. Increases in blood levels of ATX in patients with prurigo may reflect, at least in part, local production of ATX from lesional skin. It also can be assumed that locally generated ATX in keratinocytes catalyzes the conversion of lysophosphatidylcholine to LPA, provoking itch via LPA receptors on local peripheral nerve endings.

Unfortunately, neither blood levels of β-endorphin nor ATX correlated with itch intensity as assessed by VAS scores (data not shown). Thus, we were unable to directly prove that β-endorphin and/or ATX contribute to itch in prurigo patients. However, this observation could reflect the fact that there seems to be a heterogeneity in the pathoetiologies of prurigo; multiple (rather than single) pruritogens might have contributed to itch in individual patients. Moreover, VAS scores seemed to depend not only on the presence and areas of generalized pruritus, but also on

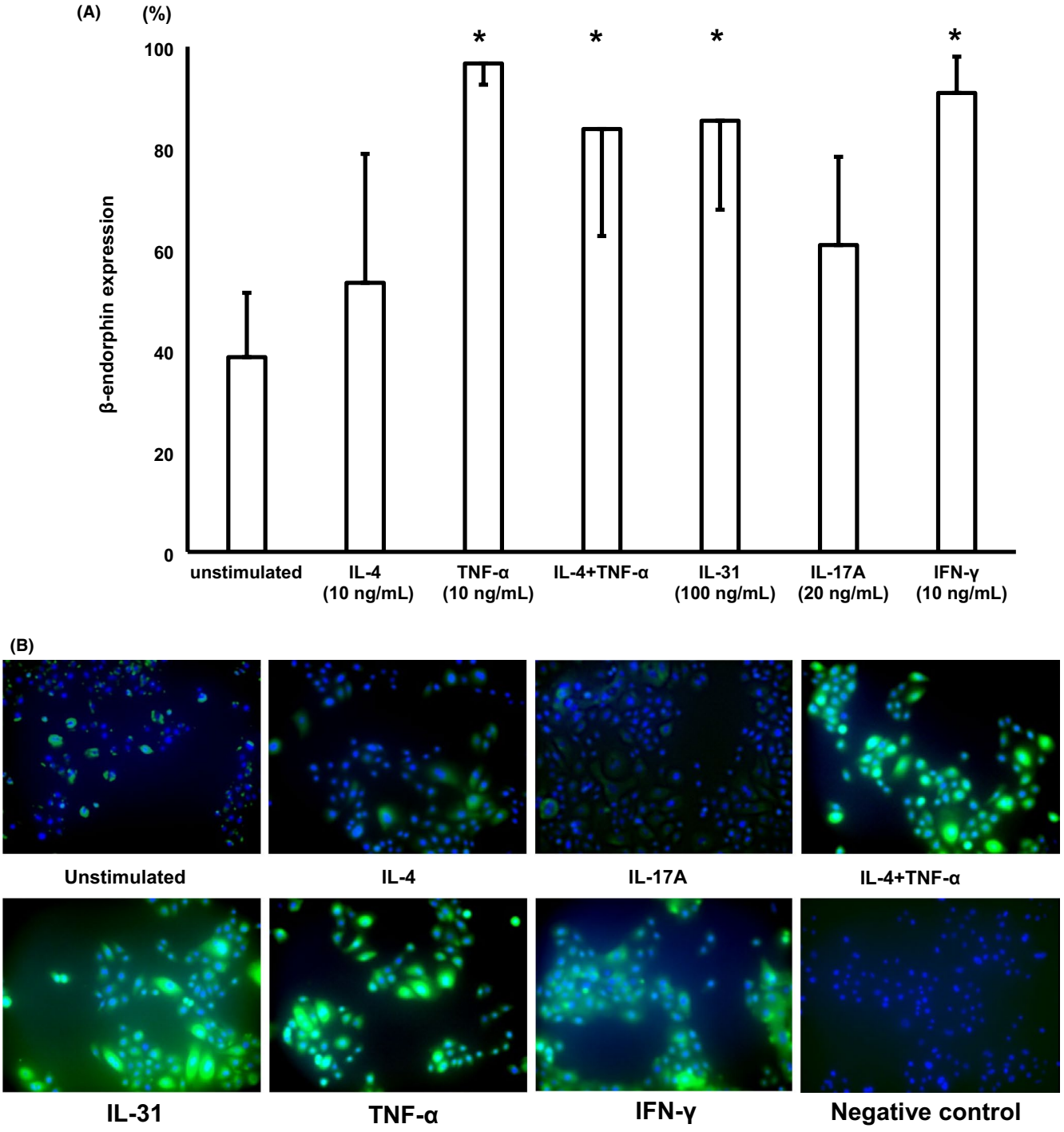

FIGURE 5 β-endorphin expression in normal human keratinocytes. A, Human keratinocytes were stimulated with IL-4, TNF-α, IL-31, IL-17A, or IFN-γ. Percentages of β-endorphin-expressing cells were analyzed as described in the Materials and Methods. *$P < 0.01$ compared with unstimulated cells (Steel's multiple comparison test). Vertical bars indicate standard deviation. Experiments were repeated three times. B, Representative features of β-endorphin-expressing cells in response to cytokine stimulation. Nuclei were stained with DAPI

itch severity of individual prurigo lesions, as patients with limited numbers of localized prurigo lesions occasionally presented with high VAS scores. In this regard, circulating β-endorphin/ATX in the blood may have paralleled generalized pruritus in some patients, while epidermal expression of β-endorphin/ATX may have reflected local pruritus of prurigo lesions, in addition to many other unidentified pruritogens. We should also be aware that β-endorphin/ATX in blood and skin levels may be differentially regulated, and thus, blood levels are not solely determined by β-endorphin/ATX generated from the skin.

Although a larger study with each type of prurigo, in particular PN, is needed for the actual assessment of the involvement of

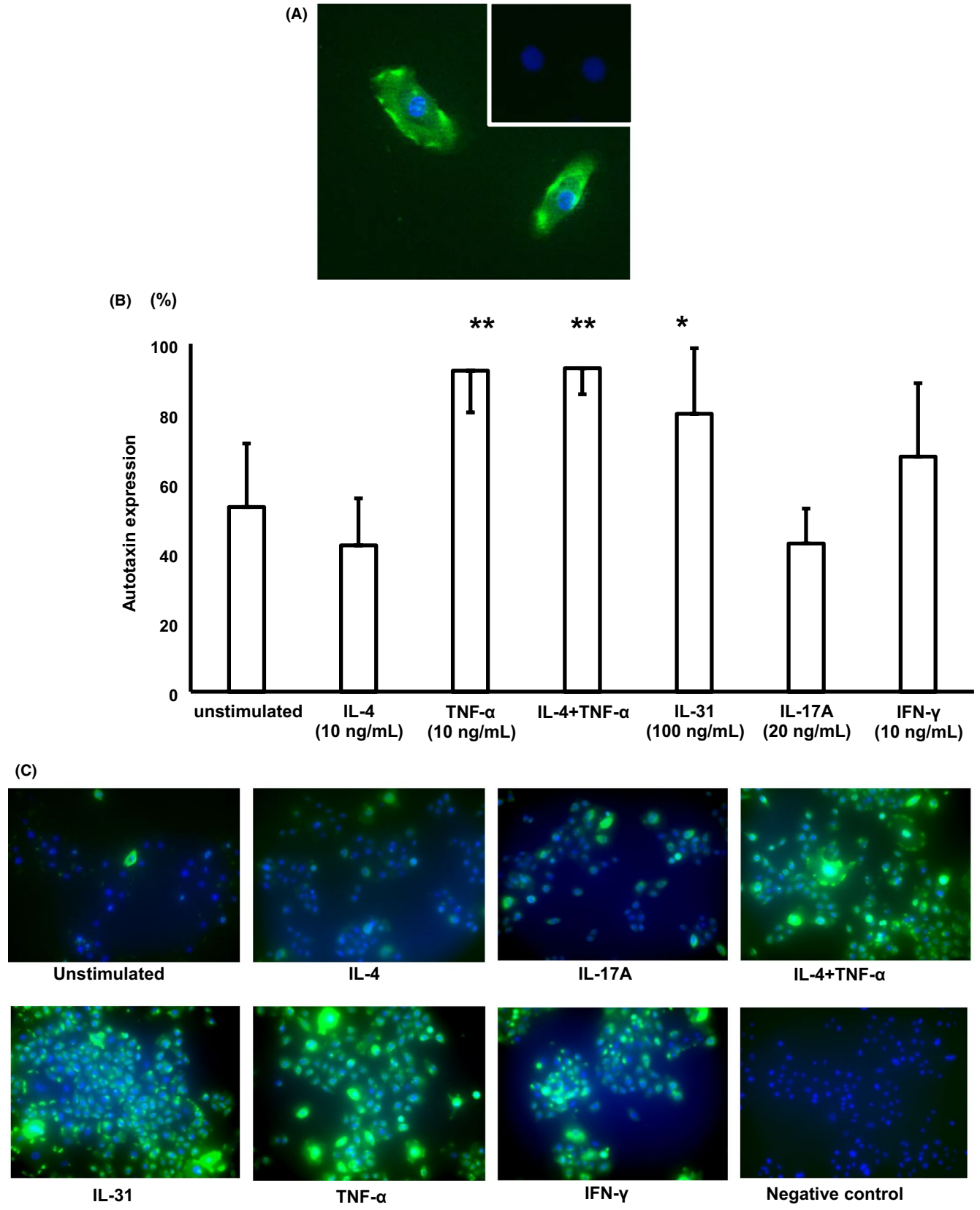

FIGURE 6 Autotaxin (ATX) expression in normal human keratinocytes. A, ATX was immunohistochemically detected in the cytoplasm and cell membrane. Nuclei were stained with DAPI. Right upper panel: negative control staining. B, Human keratinocytes were stimulated with IL-4, TNF-α, IL-31, IL-17A, or IFN-γ. Percentages of ATX-expressing cells were analyzed as described in the Materials and Methods. *$P < 0.05$, **$P < 0.01$, compared with unstimulated cells (Steel's multiple comparison test). Vertical bars indicate standard deviation. Experiments were repeated three times. C, Representative features of ATX-expressing cells in response to cytokine stimulation. Nuclei were stained with DAPI

β-endorphin and ATX in pruritus, data presented herein suggested that μ-type opioid receptor signals and/or ATX-LPA signals are involved, at least in part, in the pathomechanisms of prurigo.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Ständer S, Weisshaar E, Mettang T, Szepietowski JC, Carstens E, Ikoma A, et al. Clinical classification of itch: a position paper of the International Forum for the Study of Itch. Acta Derm Venereol. 2007;87:291-4.
2. Iking A, Grundmann S, Chatzigeorgakidis E, Phan NQ, Klein D, Ständer S. Prurigo as a symptom of atopic and non-atopic diseases: aetiological survey in a consecutive cohort of 108 patients. J Eur Acad Dermatol Venereol: JEADV. 2013;27:550-7.
3. Raap U, Gunther C. [Pathogenesis of prurigo nodularis]. Hautarzt. 2014;65:691-6.
4. Kumagai H, Ebata T, Takamori K, et al. Efficacy and safety of a novel k-agonist for managing intractable pruritus in dialysis patients. Am J Nephrol. 2012;36:175-83.
5. Kumada H, Miyakawa H, Muramatsu T, Ando N, Oh T, Takamori K, et al. Efficacy of nalfurafine hydrochloride in patients with chronic liver disease with refractory pruritus: A randomized, double-blind trial. Hepatol Res. 2017;47:972-82.
6. Sun Y, Zhang W, Evans JF, Floreani A, Zou Z, Nishio Y, et al. Autotaxin, Pruritus and Primary Biliary Cholangitis (PBC). Autoimmun Rev. 2016;15:795-800.
7. Kremer AE, Martens JJ, Kulik W, Ruëff F, Kuiper E, van Buuren HR, et al. Lysophosphatidic acid is a potential mediator of cholestatic pruritus. Gastroenterology. 2010;;139(3):1008-1018.e1.
8. van Meeteren LA, Moolenaar WH. Regulation and biological activities of the autotaxin-LPA axis. Prog Lipid Res. 2007;46:145-60.
9. Kremer AE, van Dijk R, Leckie P, Schaap FG, Kuiper E, Mettang T, et al. Serum autotaxin is increased in pruritus of cholestasis, but not of other origin, and responds to therapeutic interventions. Hepatology. 2012;56:1391-400.
10. Beuers U, Kremer AE, Bolier R, Elferink R. Pruritus in cholestasis: facts and fiction. Hepatology. 2014;60:399-407.
11. Kittaka H, Uchida K, Fukuta N, et al. Lysophosphatidic acid-induced itch is mediated by signalling of LPA5 receptor, phospholipase D and TRPA1/TRPV1. J Physiol. 2017;595:2681-98.
12. Tominaga M, Ogawa H, Takamori K. Possible roles of epidermal opioid systems in pruritus of atopic dermatitis. J Invets Dermatol. 2007;127:2228-35.
13. Lee CH, Hong CH, Yu WT, et al. Mechanistic correlations between two itch biomarkers, cytokine interleukin-31 and neuropeptide beta-endorphin, via STAT3/calcium axis in atopic dermatitis. Br J Dermatol. 2012;167:794-803.
14. Nakao M, Sugaya M, Suga H, Kawaguchi M, Morimura S, Kai H, et al. Serum autotaxin levels correlate with pruritus in patients with atopic dermatitis. J Invest Dermatol. 2014;134:1745-7.
15. Taneda K, Tominaga M, Negi O, Tengara S, Kamo A, Ogawa H, et al. Evaluation of epidermal nerve density and opioid receptor levels in psoriatic itch. Br J Dermatol. 2011;165:277-84.
16. Sonkoly E, Muller A, Lauerma AI, Pivarcsi A, Soto H, Kemeny L, et al. IL-31: a new link between T cells and pruritus in atopic skin inflammation. J Allergy Clin Immunol. 2006;117:411-7.

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