fact that both aforementioned skin conditions; atopic dermatitis and psoriasis involves substantial epidermal hyperplasia[5], in addition to PAR-2 associated sensory symptoms, most prominently itch, cutaneous inflammation and mild pain.

It is unclear if PAR-2 is involved in the pathogenesis of inflammatory skin conditions or whether aberrations in PAR-2-signaling is a consequence of preceding etiological processes, which could be distinct between different pathophysiological conditions. However, PAR-2 does appear to play a significant role in skin physiology, inflammation and the related cutaneous sensory symptoms such as itch and pain. This makes the receptor an interesting pharmaceutical target for inflammatory skin diseases.

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Betamethasone Butyrate Propionate Inhibits the Induction of Thymic Stromal Lymphopoietin in Cultured Normal Human Keratinocytes

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Dear Editor:

Thymic stromal lymphopoietin (TSLP), a key epithelial cell and keratinocyte-derived cytokine, has been shown to directly trigger allergic inflammation and the atopic march[1,2]. Therefore, suppression of TSLP expression should be a rational therapeutic strategy for allergic disorders such as atopic dermatitis (AD). In addition, topical treatments seem to be suitable for this strategy, since TSLP is produced in epithelia such as the epidermis. Topical glucocorticoid (GC) is the most popular treatment for AD and therefore, it is rational to examine the effects of GC on the expression of TSLP in keratinocytes. In fact, it has been shown that a GC, such as dexamethasone (Dex), but not calcineurin inhibitors, suppresses the expression of TSLP

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in cultured normal human keratinocytes (NHK)[3]. In addition, it has been reported that the other GCs such as prednisolone (PSL), betamethasone, fluticasone propionate and clobetasol propionate showed an inhibitory effect on TSLP induction in NHK in the similar degree to Dex[3]. In the present study, we examined the effects of betamethasone butyrate propionate (BBP) on the expression of TSLP in NHK compared with Dex.

Culture of NHKs was performed as previously reported[4]. When NHK reached 70%～90% confluence, expression of TSLP was induced by stimulation with poly I:C (10 μg/ml; GE Healthcare), which is a toll-like receptor 3 ligand and mimics viral double-stranded RNA, tumor necrosis factor (TNF)-α (20 ng/ml; R&D Systems) and interleukin (IL)-4 (100 ng/ml; R&D Systems) with or without BBP (a gift from Torii Pharmaceutical Co., Ltd.), Dex (Sigma), or PSL (Sigma) as previously reported[3,5]. During the stimulation, we used the medium without hydrocortisone but with an equivalent concentration of ethanol (0.0001%) that was used as vehicle for GCs in all experimental groups including the negative control (i.e., absence of GCs). The NHKs were harvested 6 h after stimulation for real-time polymerase chain reaction (PCR) analysis. The supernatants were harvested 24 h after stimulation for ELISA. Isolation of total RNA, reverse transcription, and real-time PCR for analysis of TSLP expression were performed as previously reported[6]. The primers used for real-time PCR were the following: upper primer for human TSLP is 5'-CAG GCT ATT CGG AAA CTC AGA T-3'; lower primer for human TSLP is 5'-GTA ATT GTG ACA CTT GTT CCA GAC-3'; upper primer for human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is 5'-TGA ACG GGA AGC TCA CTG G-3'; and lower primer for human GAPDH is 5'-TCC ACC ACC CTG TTG CTG TA-3'. Relative gene expression was calculated using the comparative Ct method or from a standard curve included in each run. Relative mRNA expression levels were normalized with a housekeeping gene, GAPDH. The quantity of TSLP in cell culture supernatants was determined by Quantikine ELISA for TSLP (R&D Systems) according to the manufacturer's instruction. All experiments were analyzed by two-tailed Student's t-test. All results are presented as means±SEMs of N experiments. A p-value of less than 0.05 was considered statistically significant.

BBP suppressed the TSLP expression induced by poly I:C, TNF-α, and IL-4 at both mRNA and protein levels in a dose-dependent manner (Fig. 1). BBP reduced the expression of TSLP more strongly than either Dex or PSL at the same dose which was lower than that in the previous report[3], suggesting that the suppressive effect of BBP was stronger than that of either Dex or PSL (Fig. 2).

The present study revealed that BBP not only has potent anti-inflammatory effects[7] but also has a potent inhibitory effect on induction of TSLP in keratinocytes. Therefore, topical treatment with BBP might also prevent the advancement of allergic states, namely the atopic march, via direct suppression of TSLP induction in keratinocytes, although such a preventive effect of topical treatment with GCs has been confirmed in neither animal models nor atopic patients. In addition, the present study showed not only that a different GC could have a different inhibitory potential on the induction of TSLP in NHK but also that

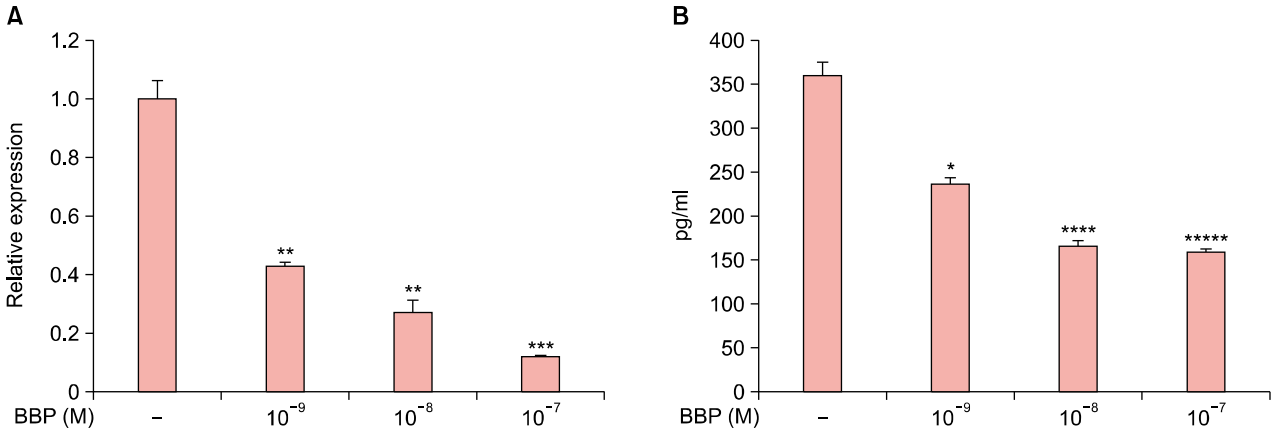

Fig. 1. Effects of betamethasone butyrate propionate on the expression of thymic stromal lymphopoietin (TSLP) in cultured normal human keratinocytes (NHKs). Expressions of TSLP in cultured NHKs were induced by stimulation with poly I:C (10 μg/ml), tumor necrosis factor-α (20 ng/ml) and interleukin-4 (100 ng/ml) with or without the indicated dose (M) of betamethasone butyrate propionate (BBP). NHKs for real-time polymerase chain reaction (A) and cultured supernatants for ELISA (B) were harvested at 6 h and 24 h after stimulation, respectively. n=5 in (A) and (B). Error bars represent means±SEMs. p-values, vs. experimental group without BBP: *$p<0.001$, **$p<0.0005$, ***$p<0.0001$, ****$p<0.00005$, *****$p<0.00001$.

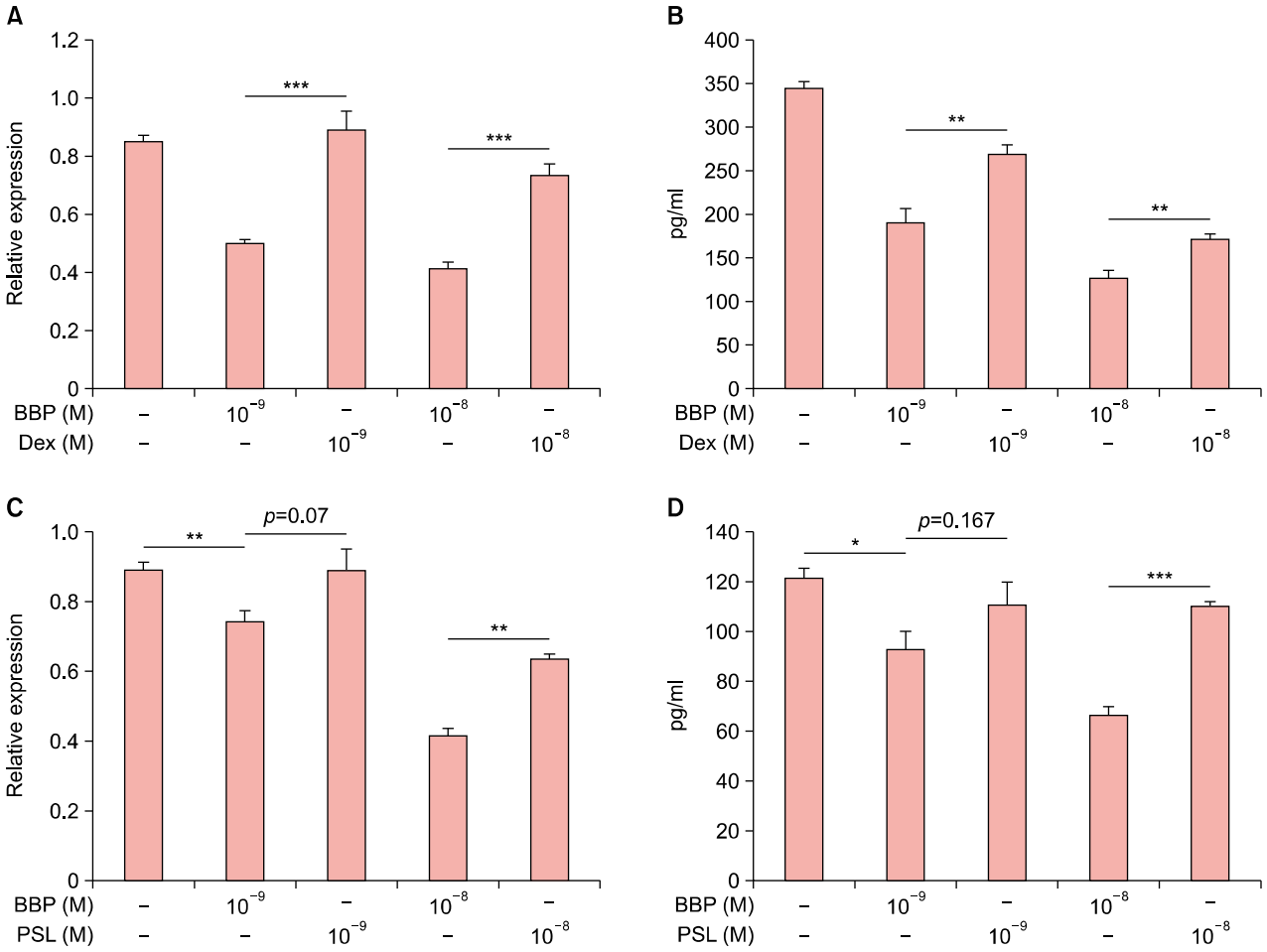

Fig. 2. Comparison of the effects on thymic stromal lymphopoietin (TSLP) expression in cultured normal human keratinocytes (NHKs) between betamethasone butyrate propionate (BBP) and dexamethasone (Dex) or prednisolone (PSL). Expressions of TSLP in cultured NHKs were induced by stimulation with poly I:C (10 μg/ml), tumor necrosis factor-α (20 ng/ml) and interleukin-4 (100 ng/ml) with or without indicated dose (M) of BBP, Dex (A, B), or PSL (C, D). NHKs for real-time polymerase chain reaction (A, C) and cultured supernatants for ELISA (B, D) were harvested at 6 h and 24 h after stimulation, respectively. n=5. Error bars represent means±SEMs. *$p<0.005$, **$p<0.001$, ***$p<0.0005$.

BBP might be more beneficial than other GCs, although the mechanism for this remains unclear.

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A Case of Premature Hair Graying Treated with Ferrous Sulfate

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Dear Editor:

Premature graying of hair is defined as the occurrence of the hair graying before the age of 20 in whites, 25 in Asians, and 30 in Africans[1]. Premature graying of hair, known as premature canities, has been uncleared about the exact etiology. A lot of pathologic conditions have been discussed about the association with a premature canities such as hypothyroidism, vitamin B12 deficiency, vitiligo, progeroid syndromes and medications[2]. However, there are few studies about the correlation of serum mineral content and the premature graying of hair. We report a case of a premature graying of hair that responded well to ferrous sulfate medication.

An 11-year-old male presented with a 1-year history of a slowly graying of hair on the vertex of his scalp. The physical examination revealed a lot of gray colored hair admixed with normal colored hair on the scalp (Fig. 1). He didn't have any family history of premature hair graying and autoimmune diseases including alopecia areata, vitiligo, autoimmune thyroid diseases, pernicious anemia and some related rare premature syndrome manifesting premature graying of hair. The initial laboratory findings were notable for decreased serum ferritin (2.6 ng ml^{-1}; normal, 20～80) and decreased Hb level (8.4 g/dl; normal, 10～15.5) consistent with iron deficiency anemia (IDA). Two years ago, he received an operation for pyloric stenosis and this may be the main cause of provocation of IDA. Other thyroid function test, serum calcium, serum iron, vi-

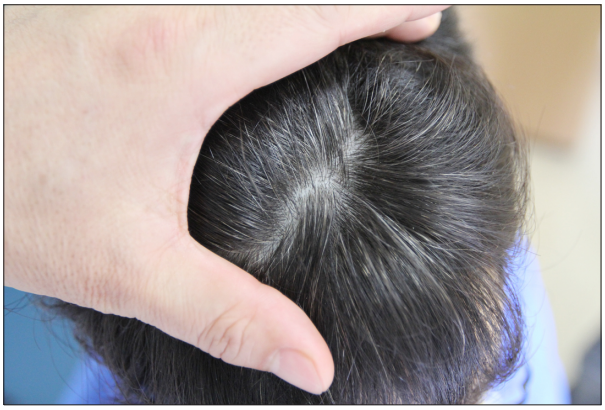

Fig. 1. A lot of gray hairs admixed with normal colored hairs on the scalp.

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