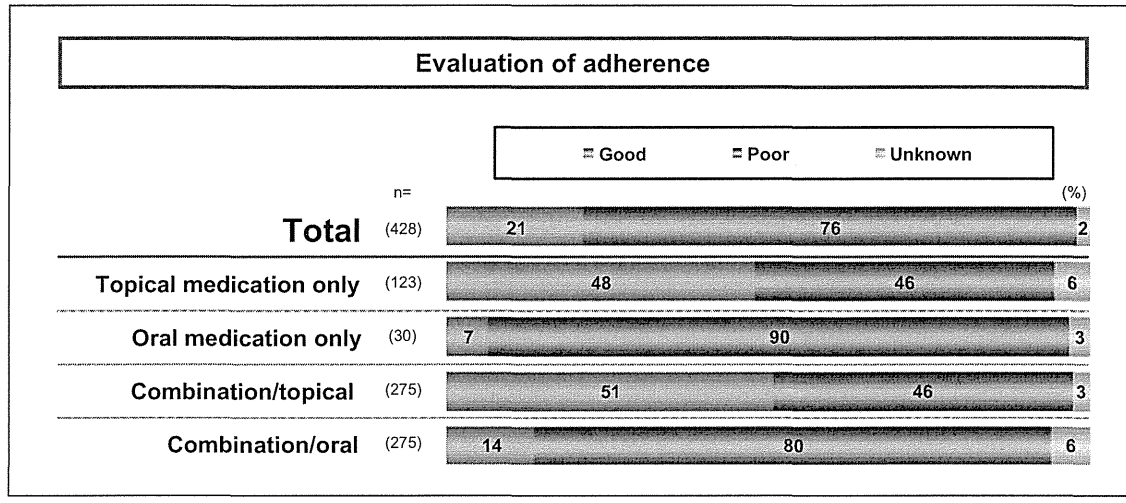


Fig. 2. Overview of adherence. Combination/topical = Use of a topical medication in any combination regimen; combination/oral = use of an oral medication in any combination regimen.

in those with mild acne, 48% in those with moderate acne, and 78% with severe acne). Additionally, patients who were treated with a combination of topical and oral medications were more likely to improve compared to those who were treated with either topical medication only or oral medication only (56 vs. 26 and 33%, respectively).

Change in Treatment at Study Visit

Treatment was changed in 17% of the cases, and the rate at which treatment was changed was similar regardless of acne severity. A total of 21% of subjects treated with oral and topical medications had a change in treatment, as did 10% of those treated with topical medications only and 3% of those treated with oral medications only. Usually, the change was to add or switch medication. The reasons given for changing therapy were insufficient efficacy (28%), an expectation of improvement in efficacy with change (24%), poor tolerability (10%), poor adherence (6%), and other (23%).

Dermatology Life Quality Index Scores

The acne sufferers in this study had a mean score on the DLQI of 3.5, indicating acne had a small impact on life. Mean scores were higher in females (3.7 vs. 3.1 in males) and those with more severe disease (4.3 for those with severe acne, 3.6 with moderate acne, and 2.4 with mild acne). Bothersome symptoms (rated as 'fairly' or 'very' bothersome) reported by subjects included itching/pain (11%) and embarrassment (23%).

Adherence Rates

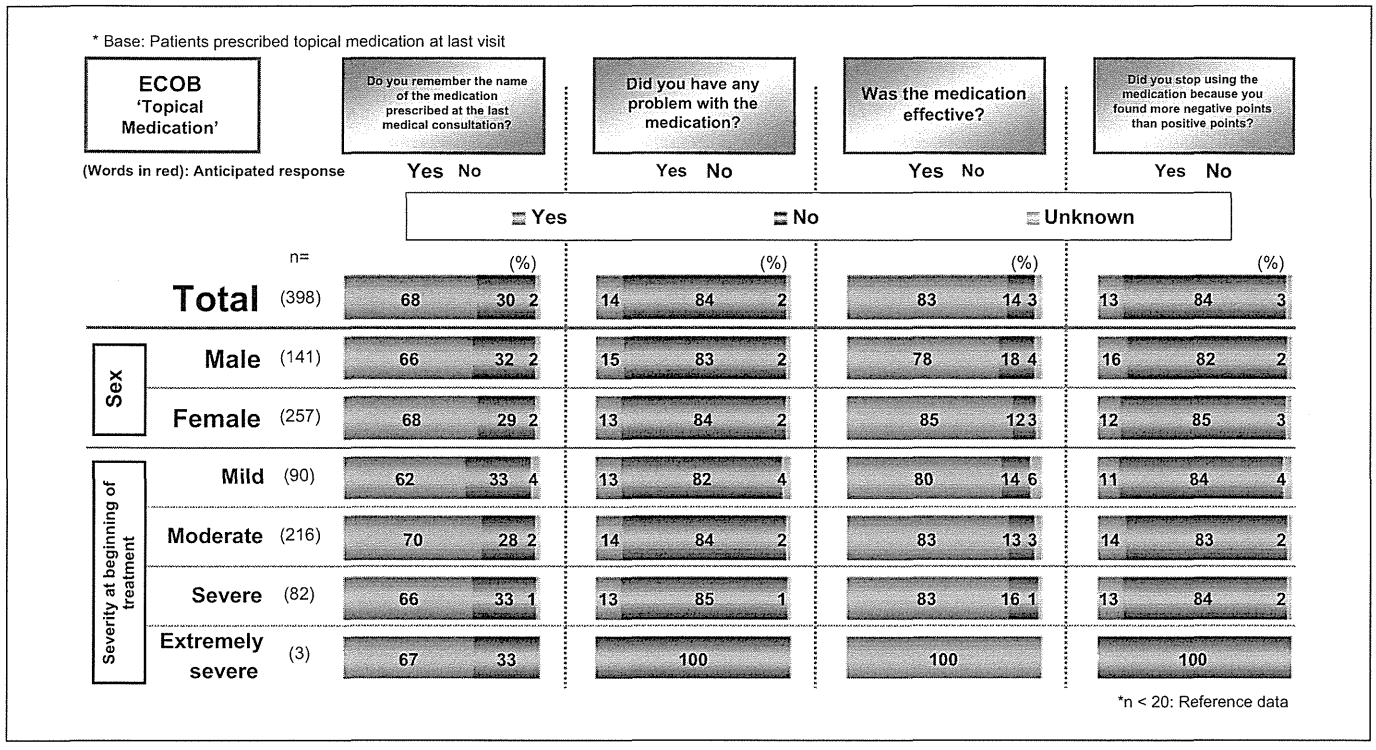
Figure 2 gives an overview of adherence in this study; the ECOB questionnaire is in two parts, one which assesses topical therapy and the other oral therapy. There was an overall rate of potentially poor adherence in 76% of subjects. Adherence to topical medication was judged likely to be good in 48% of those treated with a topical agent only (n = 123). Among those taking combination therapies (n = 275), adherence to the topical portion of therapy was good in 51% of subjects. The likelihood of good adherence to oral medication was lower, both when it was administered alone (n = 30, 7% good adherence) and when given as part of a combination regimen (n = 275, 14%). Figure 3 shows the responses to individual questions on the ECOB questionnaire.

Factors That Impacted Adherence

Factors associated with poor adherence included lack of satisfaction with treatment (OR 3.59) and to a lesser degree DLQI score of 6–10 (OR = 1.89), use of an over-the-counter topical antibacterial (OR = 1.75), and experience of a side effect (OR = 1.71).

Characteristics of Acne Consultation and Educational Efforts

The study investigators estimated their average length of consultation for acne as 9.6 min for a first visit and 4.7 min for a follow-up visit. Somewhat less than half (44%) of investigators indicated they distributed written materials about inflammatory acne and its treatment, 32% said



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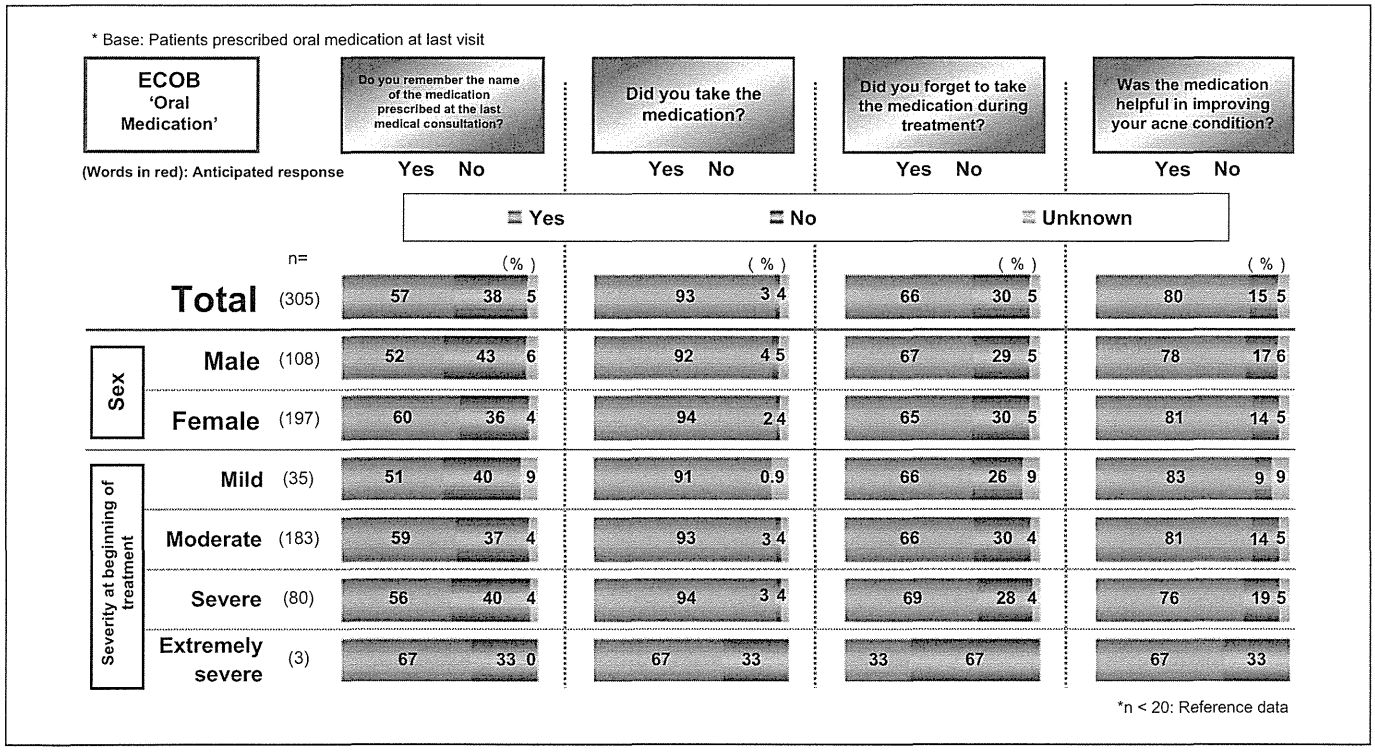

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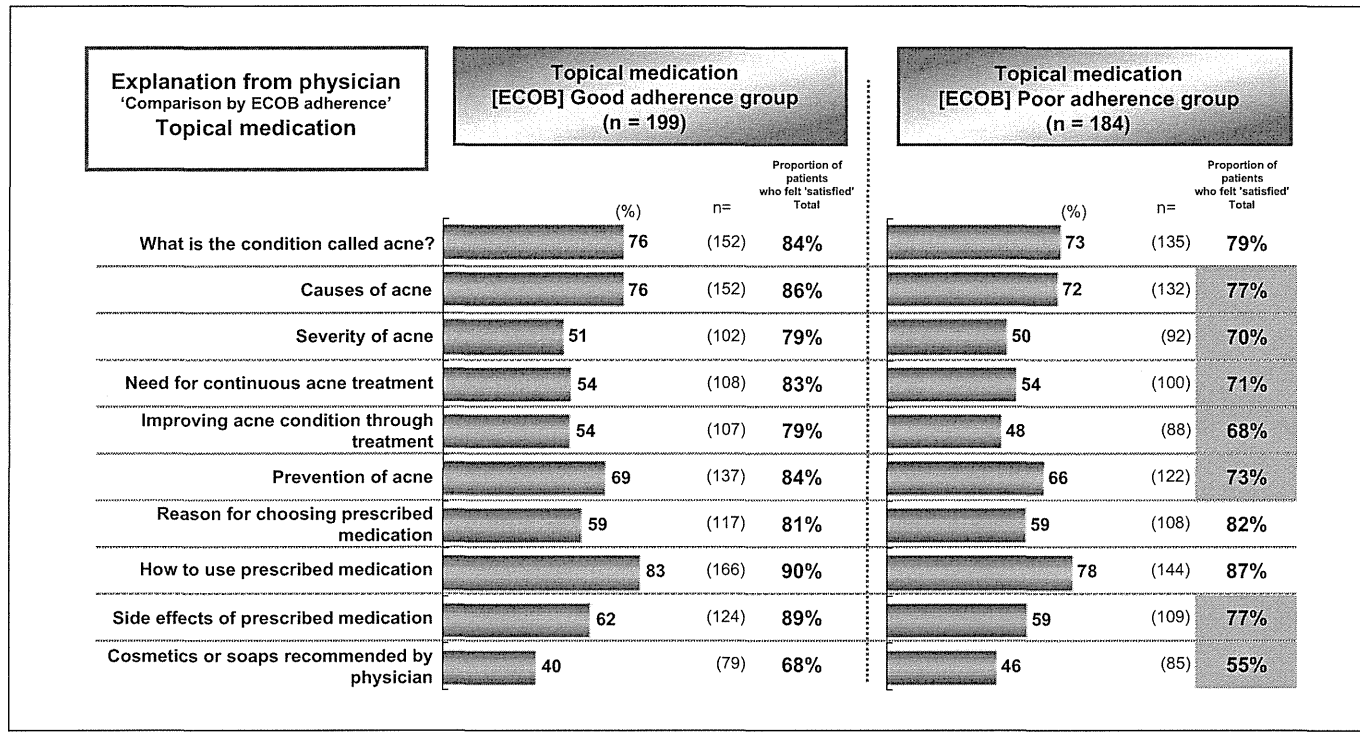
Fig. 3. Responses to individual questions on ECOB questionnaire. **a** Topical medication. **b** Oral medication.



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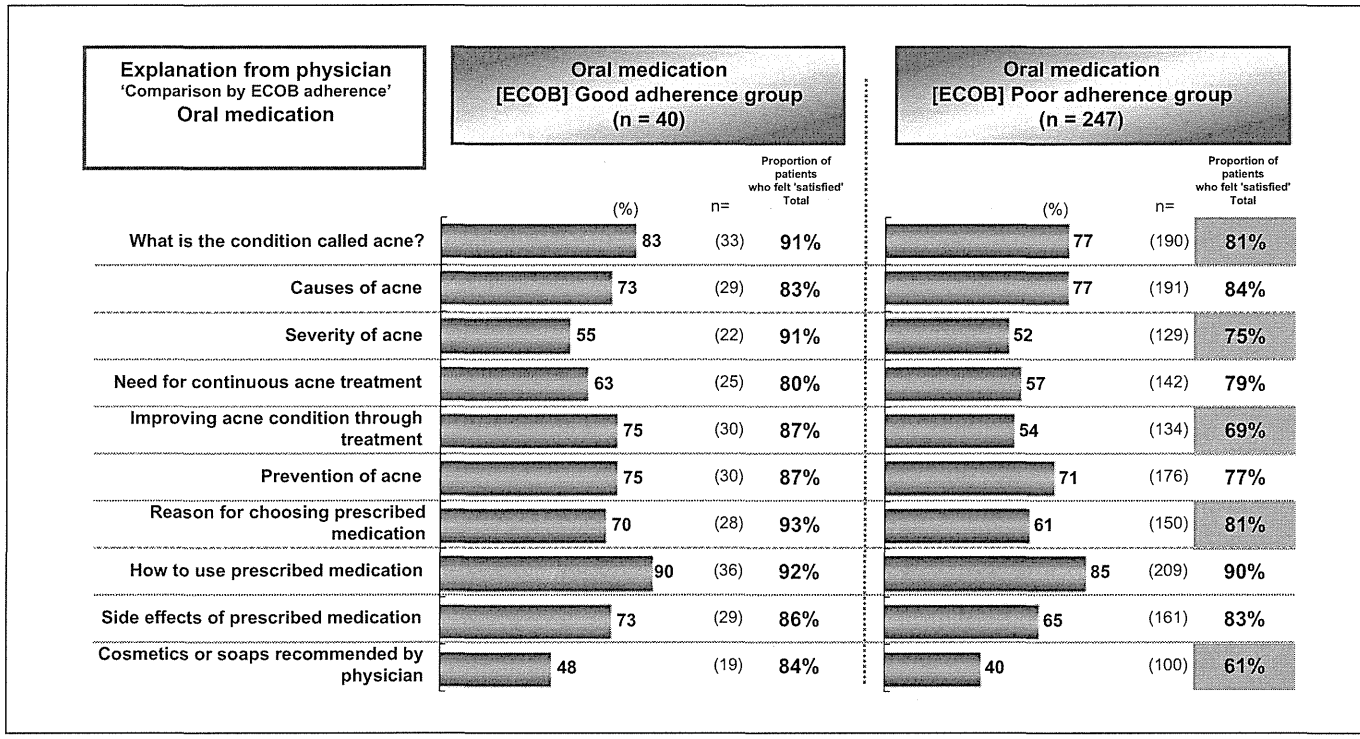

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Fig. 4. Relationship of good adherence to knowledge. **a** Topical medication. **b** Oral medication. Concerning the total proportion of patients who felt 'satisfied', highlighted numbers indicate proportions of the poor adherence group that were at least 10 points lower compared to those of the good adherence group.

they did not give materials, and 24% said they did some of the time. Investigators were asked what guidance they provided to acne patients; the most frequent response was lifestyle guidance (70%) followed by recommendations for hypoallergenic soap/cleansing products (14%), and recommendations or prescription for a moisturizing agent (10%).

When asked about their perception of treatment need, 83% of subjects said they were satisfied with their dermatologists' explanation of acne therapy and 73% indicated that the conversation with their physician had a positive impact on their motivation to use acne treatments. Further, subjects with good adherence were more likely to report that physician interactions had an impact on their behavior compared with those who had poor adherence (87 vs. 77%, respectively felt they had enough explanation; 77 vs. 70% felt the physician motivated them, 93 vs. 82% reported they were using medication according to instructions, and 92 vs. 90% indicated willingness to following treatment instructions). Patients who were dissatisfied with the dermatologists' explanations were most likely to report being unsure of why acne needed continuous treatment, what to expect in acne improvement during treatment, and how to prevent acne lesions.

Understanding of Acne and What to Expect from Treatment

Subjects were asked about knowledge of acne and its treatment; 50% felt they knew nothing or a little about acne and 62% knew little about its treatment. Women were more likely to report knowing 'something' or 'a lot' about acne compared with men (51 vs. 42%, respectively) as were those with severe acne compared with milder acne (54% severe, 50% moderate, and 46% mild acne). Women also felt more knowledgeable about acne treatment (42% knew something or a lot vs. 32% of men). Subjects who felt they had poor knowledge were more likely to be poorly adherent (fig. 4).

While many subjects reported being satisfied or very satisfied, 23% of subjects treated with topical medications and 24% of those treated with oral medications were only somewhat satisfied or not satisfied with therapy. Males were less likely than females to be satisfied with topical treatment (27 vs. 20% low satisfaction, respectively).

Discussion

This group had some characteristics that differed from other populations in adherence studies. First, our group had a relatively high mean age (24.4 years) and 21% were classified as having severe acne, which is interesting given the perception that Japanese have primarily mild acne; 42% had acne scarring. In an international study that included 1,191 Asian patients (from Hong Kong, India, the Philippines, and Singapore) with acne, the mean age was 23.1 ± 6.4 years and 3.9% of patients had severe acne, while 62% had scarring (pers. commun.) [4]. Kubota et al. [13] studied 1,443 Japanese adolescents, and reported that 59.5% had acne; of these, 52.9% said their acne had been present for at least 1 year but did not report the rate of scarring or severe acne. However, severe acne was present in 17% of subjects in a recent study of acne treatment in Japan by Kubota et al. [14]. Our study also included a relatively high proportion of adults (40%) who reported suffering from continuous acne since its onset.

Females consulted physician for acne at mean age of 19.9 vs. 18.3 years for males. It is interesting that females consulted physicians later than males, because in both our study and the epidemiological study of Kubota et al. [13], there were more females than males with acne. Most of our subjects had insurance with a co-pay (83%) or no out-of pocket cost (16%), suggesting that financial considerations may not have been the reason for a delay in seeking treatment and may or may not have impacted adherence. Patients had been treated on average for 8 weeks prior to study visit; in our study 17% had a change in therapy, usually addition of another acne treatment.

There was an overall rate of potentially poor adherence in 76% of subjects. Adherence to topical medication was judged likely to be good in 48% of those treated with a topical agent only (n = 123). Among those taking combination therapies (n = 275), adherence to the topical portion of therapy was good in 51% of subjects. The likelihood of good adherence to oral medication was lower, both when it was administered alone (n = 30, 7% good adherence) and when given as part of a combination regimen (n = 275, 14%). The high rate of non-adherence with oral medications is somewhat surprising. In an international study using the same tool to assess adherence (n = 3,339), Dreno et al. [4] reported an overall rate of poor adherence of 50% worldwide, but a rate of 48% in Asian patients (n = 1,191). Dreno et al. [4] also found a risk of poor adherence of 60% among patients taking a combination of both systemic and topical therapy, and a higher likelihood of poor adherence to the systemic arm of treatment compared to the topical arm (54 vs. 44%, respectively). Other studies utilizing questionnaires and/or single questions to assess adherence have found rates of poor adherence ranging from 76 to 0% [2, 3, 5, 15–17]. Studies using objective measures such as not keeping appoint-

ments, pill counts, weighing medication, number of medication refills, and electronic caps have reported adherence rates ranging from 28 to 88.3% [6–8, 18]. Clearly, more study is needed to fully understand adherence rates; however, it seems reasonable for clinicians to expect that approximately half of their patients may fail to follow prescribing instructions.

Topical retinoids were used by 47% of patients. Retinoids are new in Japan and strategies to optimize their use are evolving. In 2011, Kubota et al. [14] reported excellent success with initial treatment with clindamycin phosphate 1% gel twice daily plus adapalene 0.1% gel once daily, both in resolving acne lesions and in tolerability. Further, they found that maintenance therapy involving adapalene 0.1% gel once daily or twice weekly maintained improvements and allowed continued control of acne [14].

We found that satisfaction with treatment and side effects had an impact on adherence with acne therapy. Other studies have found that these factors impact treatment adherence as well, and also quality of life, older age, female gender, and employment [1]. Yentzer et al. [19] reported better adherence with once-daily combination products. We feel strongly that improving education will also enhance adherence.

Conclusions

There is a very high rate of non-adherence among acne patients. This probably contributes to poor outcomes in at least some cases. Selecting medications that are well tolerated and have a simple dosing regimen is likely to optimize adherence and, in turn, clinical outcomes.

Acknowledgments

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Narrowband ultraviolet B phototherapy decreased CCR4$^+$ CD8$^+$ T cells in a patient with palmoplantar pustulosis

Editor

Palmoplantar pustulosis (PPP) is characterized by symmetrical erythematous scaly plaques with sterile and numerous non-bacterial pinpoint pustules restricted to the palms and soles. As PPP exhibited inflammatory plaque and sterile accumulation of neutrophils, PPP is sometimes regarded as a localized pustular psoriasis. CC chemokine receptor (CCR) 4 has been used as a cell surface marker for type 2 helper and cytotoxic T (Th2/Tc2) cells. Recent studies showed that CCR4$^+$ CD8$^+$ T cells produced interleukin (IL)-4, interferon-γ and tumour necrosis factor-α effectively,[1] and that CCR4$^+$ CD8$^+$ T cells were increased in peripheral blood mononuclear cells (PBMC) in patients with psoriasis.[2] These reports indicate that CCR4$^+$ CD8$^+$ T cells may be involved in the pathogenesis of psoriasis, but no such increase has been reported in patients with PPP. Here, we report a case of PPP successfully treated with narrowband ultraviolet B (NB-UVB) phototherapy and subsequently exhibiting a decreased CCR4$^+$ CD8$^+$ T cells.

A 63-year-old woman presented with a 10-year history of erythema with pustules and prominent hyperkeratosis on her soles and palms. She had been treated with topical steroid cream prescribed at another clinic over 5 years. As this treatment was not providing sufficient relief of her symptoms, she was referred to our clinic.

Physical examination revealed prominent yellowish hyperkeratosis with pustules and vesicles on her soles and palms. Peripheral blood showed a normal leucocyte count. The patient was otherwise healthy. We diagnosed this patient as PPP and started NB-UVB treatment at an initial UVB dose of 0.3 J/cm^2 three times each week. The dose was increased by 0.1 J/cm^2 every 2 weeks to a maximum dose of 0.7 J/cm^2 UVB.

The patient's hyperkeratosis with pustules and vesicles improved remarkably within 2 months. In addition, we evaluated the frequency of CCR4$^+$ CD8$^+$ T cells by means of flow cytometry.

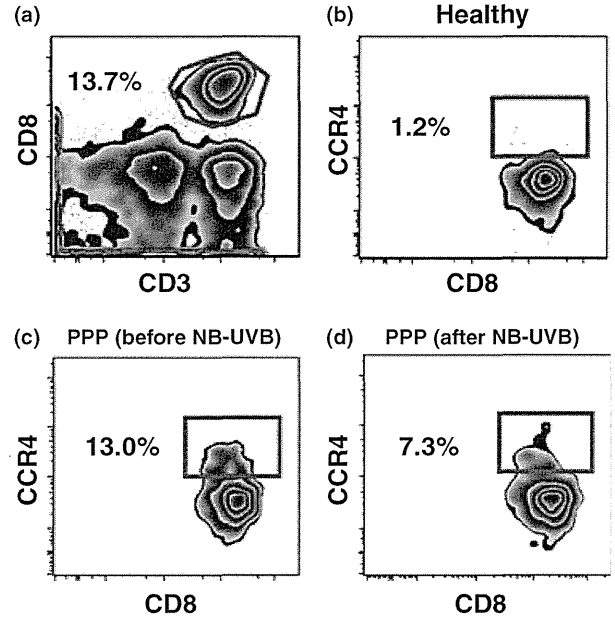

Figure 1 CCR4$^+$ CD8$^+$ T cells in PBMC. (a) CD8$^+$ T cells are recognized as CD3$^+$ and CD8$^+$ cells. (b) The proportion of CCR4$^+$ T cells in CD8$^+$ T cells from healthy control and from our PPP patient before (c) and after (d) NB-UVB phototherapy.

APC conjugated anti-CD3 antibody, PE-Cy7 conjugated anti-CD8 antibody and FITC conjugated anti-CCR4 antibody were purchased from eBioscience (San Diego, CA, USA). CD3$^+$ CD8$^+$ cells were regarded as CD8$^+$ T cells (Fig. 1a). The frequency of CCR4$^+$ cells in CD8$^+$ T cells of the PPP patient before phototherapy (13%) (Fig. 1b) was considerably higher than that of healthy donors (mean ± SD, 2.5 ± 1.4%) (Fig. 1c). After phototherapy, the proportion of CCR4$^+$ CD8$^+$ T cells had decreased from 13.0% to 7.3% (Fig. 1d). These results suggest that CCR4$^+$ CD8$^+$ T cells may be involved in the pathogenesis of not only psoriasis but also PPP. Previous reports showed that NB-UVB reduced CD8$^+$ memory-effector T cells in psoriatic lesions,[3] although we did not evaluate the infiltration of CCR4$^+$ CD8$^+$ T cells in the skin lesion of PPP. One possible explanation of systemic effect by localized NB-UVB phototherapy is that CCR4$^+$ CD8$^+$ T cells may be mainly derived from the skin. In fact, the skin has a potential to develop, maintain and expand memory T cells.[4,5] Although it is limited to a single case and we still have much to learn, our observation on CCR4$^+$ CD8$^+$ T cells in PPP may shed light on the mechanisms underlying PPP.

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Type 2 segmental Darier disease with twin spot phenomenon

Editor

Darier disease (DD) is an autosomal dominant skin disorder because of mutations in the gene ATP2A2. Two segmental forms of the disease have been distinguished. Type 2 manifestations are very infrequent. To date, the coexistence of band-like areas of excessive and absent involvement has been reported only once.[1]

A 50-year-old man presented with a chronic skin disorder involving the trunk, face and limbs, which had begun at the age of 12 years. The lesions had appeared on the ears and buttocks. Over the years, they had tent to generalize and became confluent. He complained of moderate itching and malodor. The disease worsened in summer. The diagnosis of DD had been established in another centre at the age of 28, and the patient had been taken oral retinoids since then with a poor response. He had no family history of DD.

Physical examination showed diffuse, brownish, keratotic papules affecting both sides of the trunk, arms and face (Fig. 1). Flat, skin-coloured papules were found on the hands. Numerous interruptions of rete ridges were noted on the palms. Mucosal whitish papules were seen on the palate. There were no nail alterations. Remarkably, in the posterior aspect of both legs, there was no diffuse involvement. Instead, several band-like zones of rather pronounced involvement with concomitant streaks of healthy skin, both following the pattern of the lines of Blaschko, were noted (Fig. 2). On the middle of the back, there was also a linear arrangement of the lesions alternating with bands of healthy skin.

Histopathological examination revealed suprabasilar acantholysis with prominent dyskeratosis. Dyskeratotic corps ronds and grains were also noted.

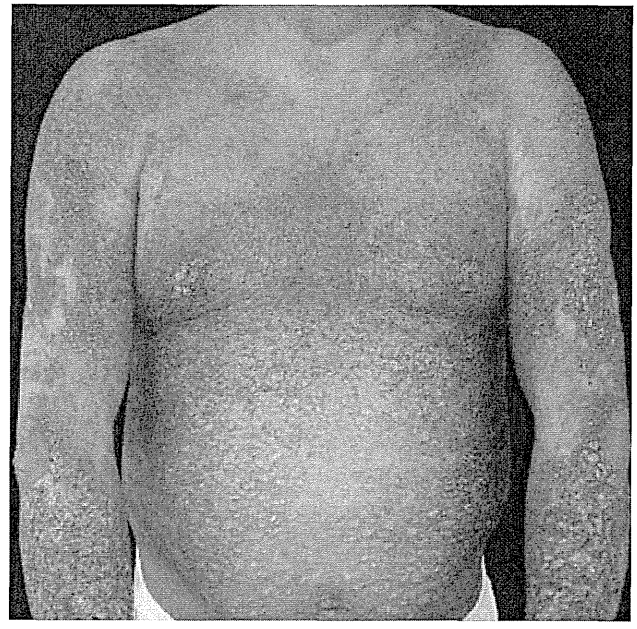

Figure 1 Diffuse and bilateral brownish, keratotic papules located on the trunk and arms.

Darier disease is an autosomal dominant skin disorder characterized by a defective adhesion between keratinocytes. This alteration gives rise to characteristic histopathological changes in the form of acantholysis and dyskeratosis. The causative mutations have been identified in the gene ATP2A2 on the chromosome 12q24.1, which encodes a sarco/endoplasmic reticulum calcium ATPase pump.

In various autosomal dominant skin disorders, including DD, segmental forms reflecting mosaicism have been reported. Nowadays, two types of segmental manifestation can be distinguished.[2] Type 1 reflects heterozygosity resulting from a postzygotic mutation very early in embryogenesis.[2] The existence of a true mosacism has been confirmed at the molecular level.[3] Clinically, these patients show segmental lesions whose severity corresponds to that observed in the non-mosaic state, and the skin outside the segmental manifestation is perfectly normal. Type 2 is characterized by areas of excessive involvement superimposed on the usual phenotype. In these cases, an individual with a heterozygous germline mutation would suffer a postzygotic mutation, such as mitotic recombination, nondisjunction or deletion that would lead to a population of cells either homozygous or hemizygous for the underlying mutation.[2] Happle and Köning hypothesized that if the loss of heterozygosity resulted from a postzygotic recombination, it would give rise to two different daughter cells homozygous for either the mutant or the normal allele.[4] This would clinically manifest as a twin spot phenomenon, with a severe segmental manifestation coexisting with a segmental area of perfect normal skin. In the reviewed literature, we have found six cases of type 2 segmental

Prostaglandin E_2–prostoglandin E receptor subtype 4 (EP4) signaling mediates UV irradiation-induced systemic immunosuppression

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UV radiation induces systemic immunosuppression. Because nonsteroidal anti-inflammatory drugs suppress UV-induced immunosuppression, prostanoids have been suspected as a crucial mediator of this UV effect. However, the identity of the prostanoid involved and its mechanism of action remain unclear. Here, we addressed this issue by subjecting mice deficient in each prostanoid receptor individually or mice treated with a subtype-specific antagonist to UV irradiation. Mice treated with an antagonist for prostaglandin E receptor subtype 4 (EP4), but not those deficient in other prostanoid receptors, show impaired UV-induced immunosuppression, whereas administration of an EP4 agonist rescues the impairment of the UV-induced immunosuppression in indomethacin-treated mice. The EP4 antagonist treatment suppresses an increase in the number of $CD4^+$/forkhead box P3-positive ($Foxp3^+$) regulatory T cells (Treg cells) in the peripheral lymph nodes (LNs) and dendritic cells expressing DEC205 in the LNs and the skin after UV irradiation. Furthermore, the EP4 antagonist treatment down-regulates UV-induced expression of receptor activator of NF-κB ligand (RANKL) in skin keratinocytes. Finally, administration of anti-RANKL antibody abolishes the restoration of UV-induced immunosuppression by EP4 agonism in indomethacin-treated mice. Thus, prostaglandin E_2 (PGE_2)–EP4 signaling mediates UV-induced immunosuppression by elevating the number of Treg cells through regulation of RANKL expression in the epidermis.

It has been known for more than 3 decades that UV radiation in the UVB (280–320 nm) range induces immunosuppression in animals. Fisher and Kripke (1) first noticed that UV-irradiated mice were more susceptible to transplanted cancer than nonirradiated mice and that lymphoid cells from irradiated mice failed to eliminate cancer cells. Based on these findings, they suggested that UV radiation induces systemic immunosuppression in animals and that this immunosuppression contributes indirectly to causing skin cancer. Systemic immunosuppression induced by UV was confirmed later in several animal models including contact hypersensitivity (CHS) (2), delayed-type hypersensitivity (3), allergic asthma (4), and experimental autoimmune encephalomyelitis (EAE) (5). This immunosuppressive effect also is exploited clinically, and UV radiation is used to treat a variety of diseases, such as psoriasis, vitiligo, and atopic dermatitis (6).

Several immune modulatory factors and immune cells are implicated in UV-induced systemic immunosuppression, including TNF-α, IL-4, IL-10 (7), platelet-activating factor (8), histamine (9), *cis*-urocanic acid (10), and natural-killer T cells (NKT cells) (11). In the early 1980s, T lymphocytes were found to play an important role in UV-induced systemic immunosuppression (12). Quite recently, regulatory T cells (Treg cells) were implicated in UV-induced systemic immunosuppression (13, 14). Loser et al. (13) reported that epidermal receptor activator for NFκB ligand (RANKL) is associated with UV-induced Treg cells and immunosuppression. They suggested that UV exposure upregulates RANKL expression in keratinocytes, leading to the induction of Treg cells through activating epidermal dendritic cells (DCs) expressing DEC205, which recently were confirmed to be specialized to induce forkhead box P3-positive ($Foxp3^+$) Treg cells (15). However, definite proof of the involvement of RANKL and Treg cells in UV-induced systemic immunosuppression has yet to be obtained.

Among the factors involved in UV-induced immunosuppression are prostanoids. Prostanoids, comprising prostaglandin (PG) E_2, PGD_2, $PGF_{2\alpha}$, PGI_2, and thromboxane (TX) A_2, are oxygenated metabolites of arachidonic acid produced by sequential catalysis of cyclooxygenase (COX) and respective synthases. They are produced in large amounts in inflammatory sites in response to various stimuli, including UV, and exert a variety of physiological and pathophysiological actions by acting on G protein-coupled receptors that includes four subtypes of PGE receptor (EP_1, EP_2, EP_3, and EP_4), PGD receptors (DP1 and DP2), PGF receptor (FP), PGI receptor (IP) and TXA receptor (TP) (16). Implication of prostanoids in UV irradiation-induced immunosuppression has been indicated by many studies showing that nonsteroidal anti-inflammatory drugs (NSAIDs), including indomethacin, that exert their effect through COX inhibition can reverse the immunosuppressive effect of UV radiation (8, 9, 17). Combined with the fact that prostanoids such as PGE_2 are produced abundantly by keratinocytes upon UV exposure (18, 19), this reversal strongly suggests that prostanoids are involved in UV-induced immunosuppression. However, the identity of the prostanoid involved and how it is related to other proposed mechanisms of UV-induced immunosuppression remain unknown. We combined genetic and pharmacological approaches and addressed this long-standing question on the role of prostanoids in UV-induced systemic immunosuppression.

Results

UV Irradiation Induces Systemic Immunosuppression and Increases the Number of Treg Cells Responsible for UV-Induced Systemic Immunosuppression. UV-induced systemic immunosuppression usually is examined in mice by CHS response. We adopted a protocol of previous studies (7, 9) with some modifications (Fig. 1*A*). We shaved the back skin of C57BL/6 mice on day 0, irradiated them on the back with UV on day 1, sensitized them on the abdomen with 2,4-dinitrofluorobenzene (DNFB) on day 5,

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challenged them on the ear on day 10, and evaluated ear swelling on day 11. We first determined an optimal UV dose producing effective systemic immunosuppression by applying various doses of UV. UV doses more than 5 kJ/m^2 showed effective immunosuppression as evidenced by significantly lower CHS responses in these groups of mice than in nonirradiated control mice (Fig. 1*B*). Although the skin was severely injured in mice irradiated with UV at more than 7 kJ/m^2, irradiation at 5 kJ/m^2 caused only slight erythema on the skin. We therefore used 5 kJ/m^2 of UV in our subsequent study. The immunosuppression induced by this dose of UV lasted for at least 2 wk (Fig. S1). To confirm the immunosuppression at the cellular level, we collected regional lymph nodes (LNs) on day 10 and cultured LN cells in the presence of 2,4-dinitrobenzene sulfonic acid (DNBS), a water-soluble form of DNFB. Consistent with the CHS response being driven mainly by IFN-γ–producing T cells, the cells from the UV-irradiated mice showed significantly lower cell proliferation and significantly less IFN-γ production than cells from nonirradiated mice (Fig. 1 *C* and *D*). Because it has been suggested that Treg cells play a prominent role in the immunosuppressive effect of UV (14), we monitored the number of Treg cells in the LNs after UV irradiation. The number of CD4$^+$Foxp3$^+$ Treg cells (20) increased in a time-dependent manner and reached a significant increase 3 d after UV irradiation (Fig. 1*E*), a result that is in agreement with our finding in CHS (Fig. S1) and with a previous report that systemic immunosuppression in mice starts not earlier than 3 d after the irradiation (21). We then assessed the significance of Treg cells in UV-induced systemic immunosuppression by using Foxp3$^{hCD2/hCD52}$ mice. This line of mice expresses human CD2 and human CD52 chimeric protein in Foxp3$^+$ Treg cells (22). As reported, Treg cells in these mice, but not in wild-type mice, were markedly depleted using Mabcampath, an anti-human CD52 antibody (Fig. S2). Treg-depleted Foxp3$^{hCD2/hCD52}$ mice irradiated with UV showed significantly higher CHS response than UV-irradiated wild-type mice (Fig. 1*F*). This result thus shows that Treg cells are required for UV-induced systemic immunosuppression.

PGE$_2$–EP4 Receptor Signaling Mediates UV-Induced Systemic Immunosuppression. Using the system defined above, we next examined involvement of COX and PGs in UV-induced immunosuppression in our system. COX-2, but not COX-1, was strongly induced in skin keratinocytes of mice by UV irradiation (Fig. S3 *A* and *B*). Quantitative analysis of arachidonate metabolites revealed that, in comparison with other PGs, PGE$_2$ was produced substantially in the skin 24 h after UV irradiation, and its production was suppressed significantly by indomethacin treatment (Fig. S3*C*). We then treated mice with 4 mg kg^{-1} d^{-1} indomethacin added in drinking water for 3 d beginning 24 h before UV irradiation. Treatment with indomethacin alone did not affect the CHS response. However, in agreement with previous studies (8, 9, 17), the indomethacin treatment reversed the UV-induced immunosuppression, as shown by significantly higher CHS response in the treated mice than in the control UV-irradiated mice (Fig. 2*A*). Similarly, treatment with a selective COX-2 inhibitor, SC-236, but not with a selective COX-1 inhibitor, SC-560, reversed the UV-induced immunosuppression (Fig. S4). Because these results suggest the involvement of PG in our experiment, we subjected mice deficient in each PG receptor individually (23) to our UV-induced systemic immunosuppression model. We excluded EP4-deficient mice from the experiment because they have a mixed genetic background of 129/Ola and C57BL/6 and show impaired CHS response (24). Instead we used an EP4 antagonist, ONO-AE-3–208 (24), and administered it to wild-type C57BL/6 mice to block the EP4 receptor pharmacologically. We also administered a TP/DP2 antagonist, ramatroban (25), to TP-deficient mice to examine involvement of DP2. We noted that UV irradiation induced immunosuppression in mice lacking DP1, EP1, EP2, EP3, FP, IP, or TP and in TP-deficient mice treated with ramatroban to a level similar to that found in controls (Fig. 2*B*), suggesting that these PG receptors do not play a crucial role in the induction of the systemic immunosuppression by UV exposure. To examine the involvement of EP4 receptor signaling, various doses of the EP4 antagonist were applied to wild-type mice in drinking water for the same period as described for indomethacin treatment. Notably, administration of the EP4 antagonist at 50 mg kg^{-1} d^{-1} and 100 mg kg^{-1} d^{-1} significantly prevented the UV-induced immunosuppression (Fig. 2*C*). The EP4 antagonist treatment restored the CHS response to a level found in mice treated with indomethacin or SC-236 (Fig. S4). Histological examination of the ear 24 h after the challenge showed that UV irradiation considerably decreased cell infiltration and edema in the dermis and that these

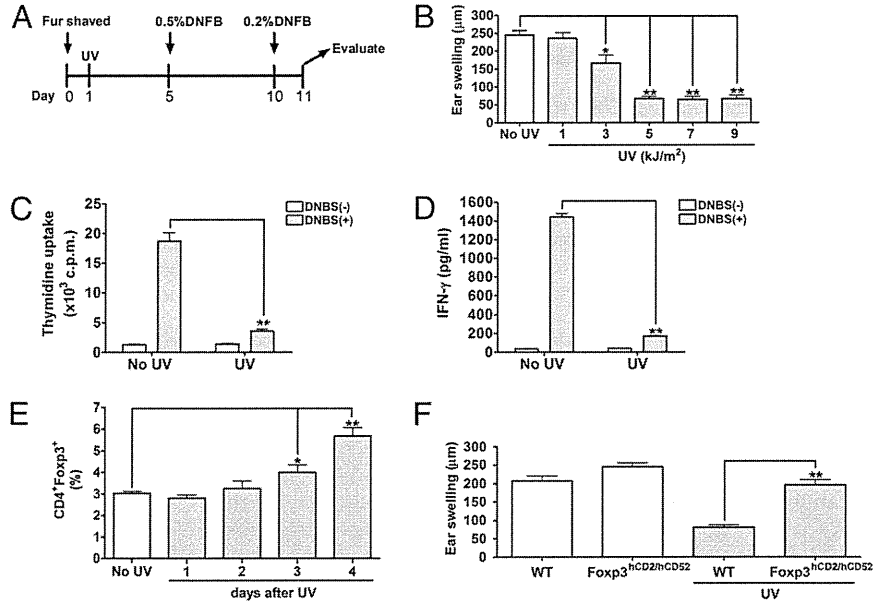

Fig. 1. UV irradiation suppresses CHS responses in mice and induces Treg cells in draining LNs. (*A*) The experimental protocol. Mouse back skin is shaved on day 0, subjected to UV or sham irradiation (No UV) on the back on day 1, sensitized with 0.5% DNFB applied to the shaved abdomen on day 5, and challenged by applying 0.2% DNFB on the ear. Ear swelling is evaluated 24 h after the challenge. (*B*) Dose-dependent suppressive effect of UV on CHS responses. Ear swelling was measured 24 h after challenge in sham-irradiated mice (No UV) and mice irradiated with indicated doses of UV (n = 4 mice per group). (*C*) Reduced DNBS-induced proliferation of LN cells from UV-irradiated mice. Cells were prepared from draining LNs of sham-irradiated mice (No UV) or mice irratiated with 5 kJ/m^2 UV (UV) 5 d after sensitization and were cultured in the presence or absence of DNBS for 72 h. Cell proliferation was measured by [^{3}H]thymidine incorporation (n = 3 mice per group). (*D*) Reduced DNBS-induced IFN-γ production in LNs cells from UV-irradiated mice. LN cells were prepared as above and were cultured with or without DNBS for 48 h. The amount of IFN-γ in the culture medium was measured by ELISA (n = 3 mice per group). (*E*) Increase in Treg cells in LNs after UV irradiation. LNs were collected from mice at indicated times after UV irradiation or from control mice without irradiation (No UV), and CD4$^+$Foxp3$^+$ Treg cells in LNs were quantified by flow cytometry (n = 3 mice per group). (*F*) Suppressed UV-induced immunosuppression in Treg-depleted Foxp3$^{hCD2/hCD52}$ mice. Foxp3$^{hCD2/hCD52}$ and wild-type mice were injected i.v. with 0.5 mg of Mabcampath 1 d before irradiation. Ear swelling was measured 24 h after challenge (n = 4 mice per group). Data are representative of at least three independent experiments with similar results and are shown as mean ± SEM. *P < 0.05; **P < 0.01.

changes were reversed by treatment with the EP4 antagonist (Fig. S5). Consistent with such changes, LN cells taken from EP4 antagonist-treated, UV-irradiated mice exhibited significantly increased cell proliferation and IFN-γ production in response to DNBS compared with LN cells from control irradiated mice (Fig. 2 *D* and *E*). These results suggest the importance of PGE_2–EP4 signaling in UV-induced immunosuppression. To verify this hypothesis, we examined whether administration of an EP4 agonist (ONO-AE-1-329) (26) can restore immunosuppression in irradiated mice treated with indomethacin. Mice treated with 4 mg kg^{-1} d^{-1} indomethacin added to drinking water from day 0 to day 3 were subjected to UV irradiation on day 1 and were injected s.c. with different doses of the EP4 agonist immediately and 12 h after the UV irradiation (Fig. 3*A*). We confirmed that this injection of ONO-AE-1–329 can induce systemic EP4-blocking effects in mice injected with LPS (Fig. S6). Administration of ONO-AE-1–329 alone did not affect CHS responses (Fig. 3*B*). However, this compound dose-dependently restored the immunosuppression in the indomethacin-treated, UV-irradiated mice (Fig. 3*B*). This effect was mimicked by CAY10580, another EP4 agonist with a structure different from ONO-AE-1–329, but not by agonists for DP, EP2, or IP, which, like EP4, activate Gs protein (16) (Fig. S7). These findings affirm the action of PGE_2–EP4 signaling in UV-induced systemic immunosuppression.

PGE_2–EP4 Signaling Facilitates Increase of Treg Cells in Regional LNs After UV Irradiation. To explore the mechanism of immunosuppressive action of PGE_2–EP4 signaling, we collected axillary and inguinal LNs from UV-irradiated mice 4 d after irradiation and examined the effect of the EP4 antagonist on the number and composition of cells in these LNs. The UV irradiation significantly increased the total number of the LN cells, and the treatment of the EP4 antagonist did not affect this increase (Fig. 4*A*). The numbers of cells in the CD4 ($CD4^+CD8^-$), CD8 ($CD8^+CD4^-$), B ($B220^+$), NK ($DX5^+Thy1.2^-$), and NKT ($DX5^+Thy1.2^+$) cell population also increased in the peripheral LNs after UV irradiation, but, again, there was no significant difference in the cell number of each of these populations between the mice treated with the EP4 antagonist and mice treated with the control vehicle (Fig. S8). However, the increase in Treg cells (defined as $CD4^+Foxp3^+$ cells) in the regional LNs after the irradiation was significantly lower both in number and percent in mice treated with either the EP4 antagonist or indomethacin than in control mice treated with vehicle (Fig. 4 *B* and *C* and Fig. S9). These results indicate the involvement of PGE_2–EP4 signaling in increase in Treg cells after UV irradiation.

PGE_2–EP4 Signaling Mediates Epidermal RANKL Induction and Induces Epidermal DCs Expressing DEC205 in UV-Irradiated Mice. Loser et al. (13) previously reported that UV irradiation up-regulated RANKL expression in the epidermis and induced DCs expressing

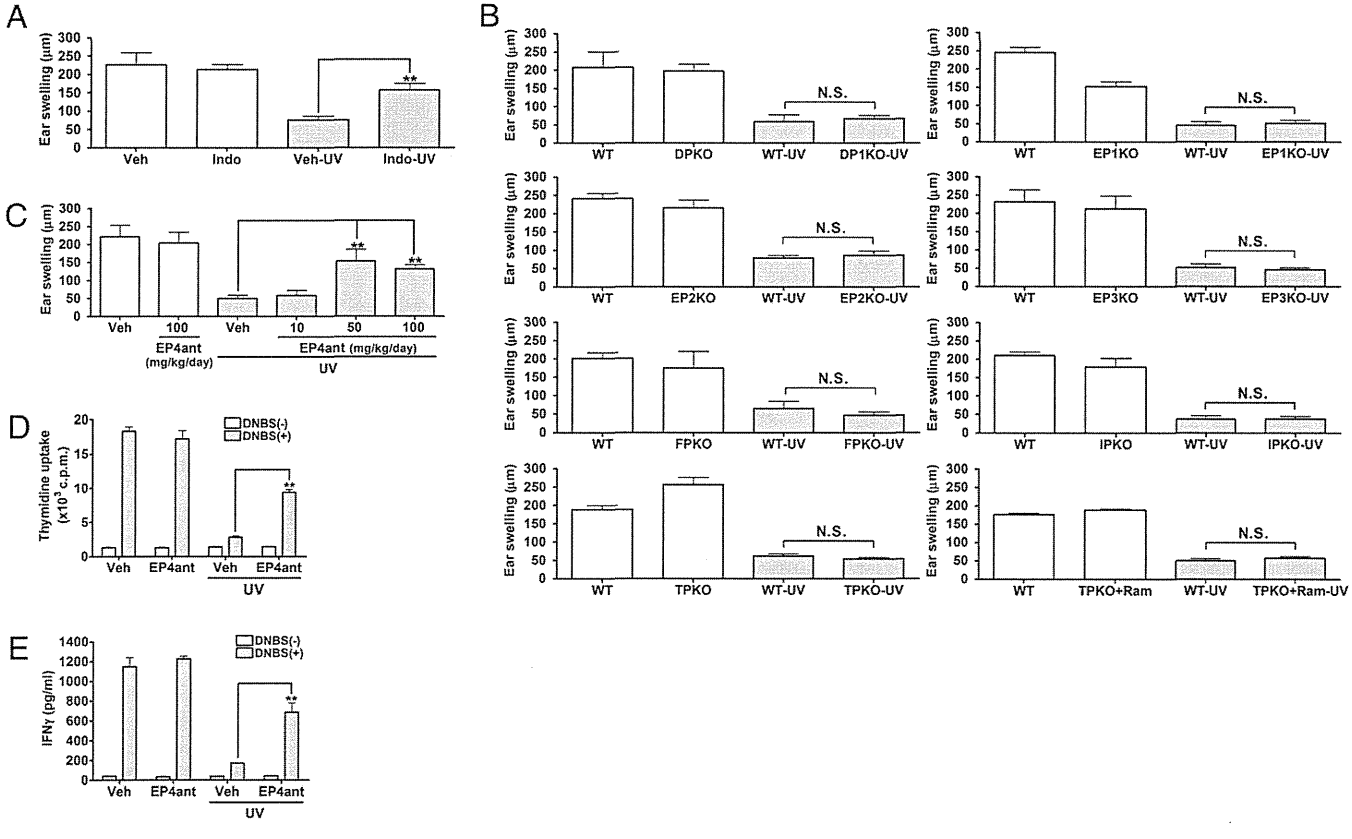

Fig. 2. Reversal of UV-induced immunosuppression by COX inhibition or EP4 receptor antagonism. (*A*) Effects of indomethacin. Mice were administered 4 mg kg^{-1} d^{-1} of indomethacin (Indo) or vehicle (Veh) in drinking water from day 0 to day 3, and the CHS response was measured 24 h after challenge. (*B*) Effects of PG receptor deficiency. Mice deficient in DP1, EP1, EP2, EP3, FP, IP, or TP (DP1KO, EP1KO, EP2KO, EP3KO FPKO, IPKO, and TPKO, respectively) and TP-deficient mice treated with 10 mg kg^{-1} d^{-1} of ramatroban (TPKO+Ram) were used with their wild-type counterparts as control. Ear swelling was measured 24 h after challenge (n = 4 mice per group). (*C*) Effects of ONO-AE3-208. Mice were administered the indicated doses of an EP4 antagonist, ONO-AE3-208 (EP4ant) or vehicle (Veh) dissolved in drinking water from day 0 to day 3. Ear swelling was measured 24 h after challenge (n = 4 mice per group). (*D* and *E*) Effects of the EP4 antagonist on LN cell proliferation and IFN-γ production. Mice administered 50 mg kg^{-1} d^{-1} ONO-AE3-208 or vehicle from day 0 to day 3 were subjected to UV or sham irradiation on day 1 and then were sensitized with 0.5% DNFB on day 5. LNs were excised on day 10, and LN cells were subjected to DNBS-induced cell proliferation (*D*) and IFN-γ production (*E*) (n = 3 mice per group). Data are representative of three experiments with similar results and are shown as mean ± SEM. $^{**}P < 0.01$; N.S., not significant.

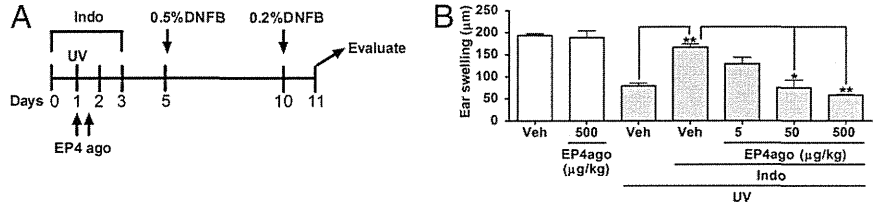

Fig. 3. Restoration of UV-induced immunosuppression in indomethacin-treated mice by administration of an EP4 agonist. (*A*) The experimental protocol. Mice treated with indomethacin (Indo) or vehicle (Veh) as in Fig. 2*A* were irradiated with 5 kJ/m^2 UV on day 1 and were injected s.c. with an EP4 agonist, ONO-AE1-329 (EP4ago), or vehicle (Veh) immediately and 12 h after the irradiation. The mice then were subjected to CHS induction as in Fig. 1*A*. (*B*) Effects of the EP4 agonist on indomethacin-induced impairment of UV-induced immunosuppression. Mice were treated with vehicle or the indicated doses of ONO-AE1-329, and the CHS response was measured (n = 4 mice per group). Data are representative of three experiments with similar results and are shown as mean ± SEM. $*P < 0.05$; $**P < 0.01$.

DEC205 there, both of which they suggested might be responsible for induction of Treg cells in the peripheral lymphoid organs. We therefore analyzed the effect of the EP4 antagonist on the expression of RANKL in the skin. Real-time RT-PCR analysis of the skin exhibited a marked increase in *RANKL* mRNA after UV irradiation, and this increase was significantly suppressed by treatment with the EP4 antagonist (Fig. 5*A*). We also immunostained for RANKL in the skin of control mice and mice treated with the EP4 antagonist. The UV irradiation markedly enhanced immunofluorescent signals for RANKL in keratinocytes that were costained with an antibody to cytokeratin, a marker for keratinocytes. Additionally, keratinocytes formed multiple layers after the irradiation, suggesting that they underwent activation. Treatment with the EP4 antagonist substantially suppressed RANKL expression in the epidermis to the level seen in nonirradiated control mice and prevented multilayer formation (Fig. 5*B*). We also found that treatment with indomethacin elicited the same result as treatment with the EP4 antagonist, and the addition of the EP4 agonist could restore keratinocyte activation and RANKL production after UV irradiation in mice treated with indomethacin (Fig. 5*B*). These results indicate that PGE_2–EP4 signaling mediates RANKL expression induced by UV irradiation in keratinocytes. We next examined the number of DCs expressing DEC205 ($CD11c^+DEC205^+$) specialized to induce $Foxp3^+$ Treg cells (15) in the LNs. We detected a markedly increased number of $CD11c^+DEC205^+$ cells in the peripheral LNs 2 d after UV irradiation, and this increase of $CD11c^+DEC205^+$ cells was suppressed significantly by treatment with the EP4 antagonist (Fig. 5*C*). Although these results indicate that fewer $DEC205^+$ DCs are present in the LNs to induce Treg with the EP4 antagonism, we also noted that the total number of $CD11c^+$ DCs in the LNs decreased with the EP4 antagonist treatment 2 d after UV irradiation (Fig. 5*C*). These results might reflect the interference of DC migration by the EP4 antagonist, as we previously reported (24), and raised a question whether the EP4 antagonism suppressed induction of $CD11c^+DEC205^+$ cells by UV irradiation in situ in the skin. We therefore isolated epidermal sheet 2 d after UV irradiation and examined the number of $CD11c^+DEC205^+$ cells in the epidermis. UV irradiation significantly increased the population of $CD11c^+DEC205^+$ cells in the epidermal cells, and the treatment with the EP4 antagonist suppressed this increase of $CD11c^+DEC205^+$ cells (Fig. 5*D*). These results clearly show that the lack of PGE_2–EP4 signaling leads to reduced induction of $CD11c^+DEC205^+$ cells in the skin. To verify further that PGE_2–EP4 signaling mediates UV-induced systemic immunosuppression by regulating epidermal RANKL, we treated mice with either anti-RANKL or the isotype control antibody 2 d before UV irradiation. Anti-RANKL treatment could diminish immunosuppression by UV irradiation to a degree similar to indomethacin treatment (Fig. 6). There was no additive effect of treatments with indomethacin and anti-RANKL antibody. Further, treatment with the EP4 agonist did not restore immunosuppression in indomethacin-treated mice cotreated with anti-RANKL. These results suggest that RANKL is indispensable for the PGE_2–EP4 signaling to mediate UV-induced systemic immunosuppression. It also is noted that administration of either anti-RANKL antibody or indomethacin did not restore the immune response fully to the level observed in control non–UV-irradiated mice.

Discussion

UV radiation is not only carcinogenic but also suppresses immunity. Here, we answered a long-standing question regarding the role of prostanoids in UV-induced systemic immunosuppression by showing that PGE_2–EP4 signaling mediates UV-induced systemic immunosuppression. We showed impairment of the immunosuppressive effect of UV by EP4 antagonism and reversal of the indomethacin-induced impairment of immunosuppression by EP4 agonism. Notably, treatment with the EP4 agonist alone, without UV irradiation, did not result in immunosuppression (Fig. 3*B*). Because several immune-modulatory mediators have been reported to play roles in the UV-induced systemic immunosuppression (7–10), it is likely that PGE_2 acts in

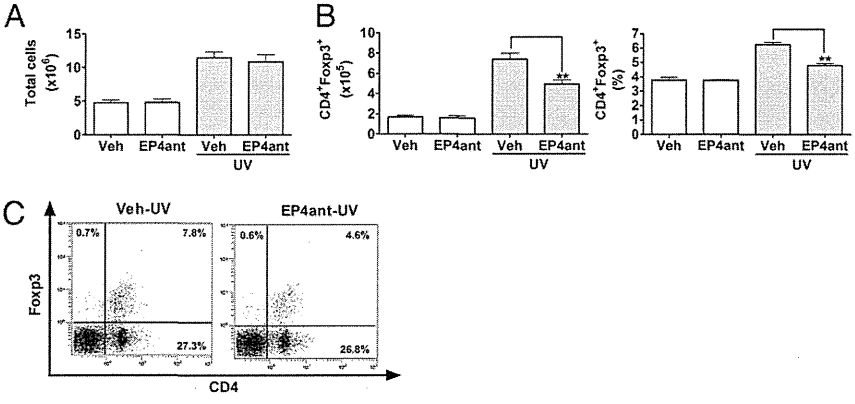

Fig. 4. Selective suppression of the UV irradiation-induced increase in Treg cells in LNs by EP4 antagonism. Mice were administered 50 mg kg^{-1} d^{-1} of ONO-AE3-208 (EP4ant) or vehicle (Veh) from day 0 to day 3 and were subjected to UV irradiation and sensitization. Draining LNs were excised on day 5, and the number and composition of Treg cells were analyzed (n = 4 mice per group). (*A*) Total cell number of LNs. (*B*) The number and percent of $CD4^+Foxp3^+$ Treg cells in LNs. $**P < 0.01$. (*C*) Representative dot plot analysis of $CD4^+Foxp3^+$ cells in LNs from the vehicle-treated UV-irradiated mice and EP4 antagonist-treated UV-irradiated mice. Data are representative of three experiments with similar results and are shown as mean ± SEM.

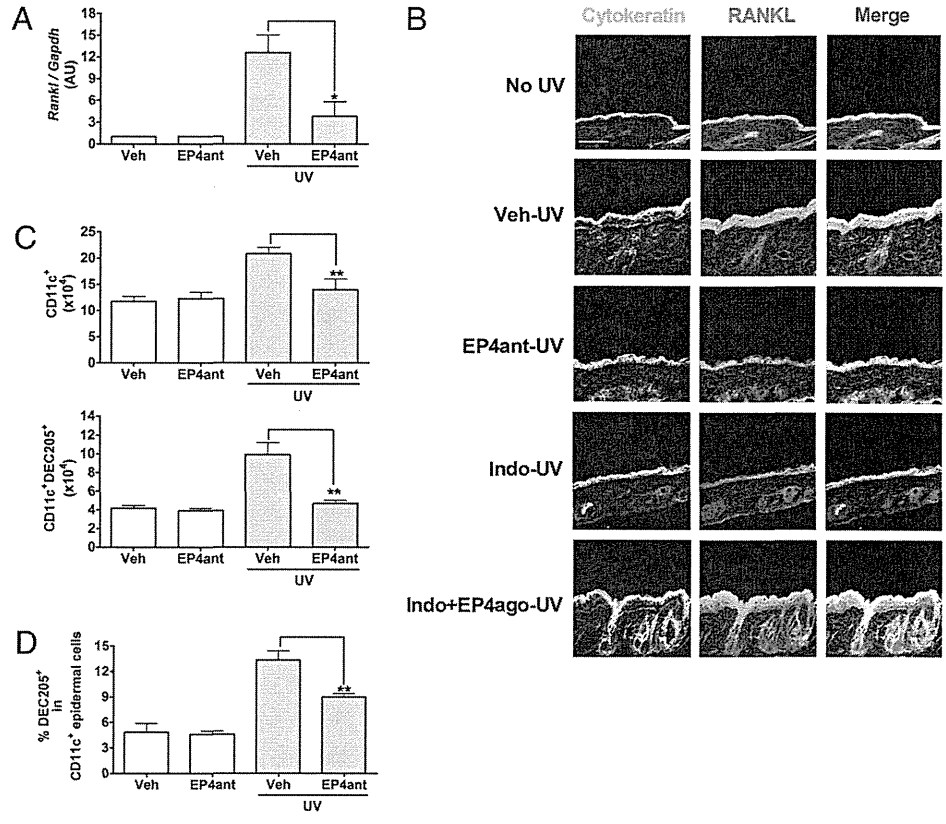

Fig. 5. PGE_2–EP4 signaling mediates the expression of RANKL in keratinocytes and induces DCs expressing DEC205 in UV irradiation. Mice were treated with 50 mg kg^{-1} d^{-1} ONO-AE3-208 (EP4ant) or vehicle (Veh) from day 0 and were subjected to UV or sham irradiation on the back on day 1. (*A*) Effects of EP4 antagonism on UV-induced expression of *RANKL* mRNA in the skin. The back skin was excised 1 d after irradiation, and *Rankl* mRNA expression in the skin was evaluated by quantitative real-time PCR analysis and is shown in arbitrary expression units (AU). *Rankl* expression is normalized to that of *Gapdh* ($n = 3$ mice per group). (*B*) Effect of EP4 antagonism and EP4 agonism on UV-induced increase in RANKL protein in keratinocytes. For EP4 agonist treatment, mice were treated with 4 mg kg^{-1} d^{-1} indomethacin (Indo) from day 0 on, were subjected to UV irradiation, and were injected s.c. with 500 μg/kg ONO-AE1-329 (EP4ago), or vehicle immediately and 12 h after the irradiation. The back skin was excised 2 d after irradiation and was stained for RANKL (red) and cytokeratin (green). Cell nuclei were stained with Hoechst-33342. (Scale bar, 50 μm.) (*C*) Effects of EP4 antagonism on the increase of $DEC205^+$ DCs in LNs after UV irradiation. LNs were collected 2 d after UV irradiation, and the number of $CD11c^+$ and $CD11c^+DEC205^+$ cells in LNs was analyzed by flow cytometry ($n = 4$ mice per group). (*D*) Effects of EP4 antagonism on the UV-induced increase of $DEC205^+$ DCs in the skin. Epidermal cells were prepared from back skins excised 2 d after irradiation, and $CD11c^+$ cells were isolated by magnetic-activated cell sorting (MACS). $DEC205^+$ cells in isolated $CD11c^+$ cells were analyzed by flow cytometry ($n = 4$ mice per group). Data are representative of three experiments with similar results and are shown as mean ± SEM. $*P < 0.05$; $**P < 0.01$.

collaboration with other mediators to induce the immunosuppression after UV irradiation. Indeed, we previously showed that PGs such as PGE_2 and PGI_2 collaborate with IL-1β and enhance induction of various cytokines and remodeling factors, including RANKL, in a model of collagen-induced arthritis (27). Which mediator collaborates with PGE_2 to induce immunosuppression remains to be determined. By examining the cell populations of the peripheral LNs after UV irradiation, we found that only an increase in the Treg cell population after UV irradiation was significantly suppressed by treatment with the EP4 antagonist, whereas the EP4 antagonist did not affect increases in numbers of other LN cell populations, including NKT cells, which previously were reported to mediate systemic immunosuppression after UV irradiation (11). Using $Foxp3^{hCD2/hCD52}$ mice, we further showed that Treg cells play an indispensable role in UV-induced systemic immunosuppression. We also used anti-RANKL antibody and showed that RANKL makes a major contribution to this process. Our results, however, do not exclude an involvement of signaling molecules other than PGE_2 in immunosuppression in a process independent of PGE_2–EP4–RANKL signaling, because the EP4 antagonism did not completely suppress the increment of Treg cells after UV irradiation, and anti-RANKL antibody did not restore ear swelling to the level found in control mice.

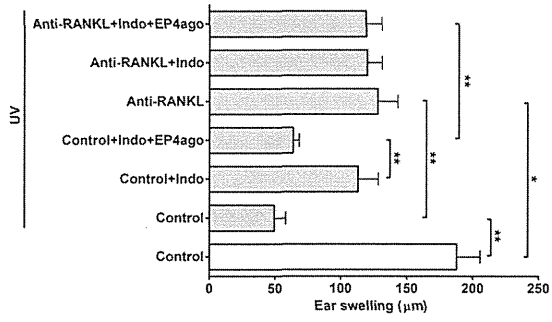

Fig. 6. RANKL is indispensable for EP4-mediated, UV-induced systemic immunosuppression. Mice were injected s.c. with 100 μg of either anti-RANKL or isotype control antibody (Control) 2 d before UV irradiation. Indomethacin (Indo), 4 mg kg^{-1} d^{-1}, and ONO-AE1-329 (EP4ago), 500 μg/kg, were administered as in Fig. 3*A*. The CHS response was measured ($n = 4$ mice per group). Data are representative of two experiments with similar results and are shown as mean ± SEM. $*P < 0.05$; $**P < 0.01$.

Here we identified PGE_2–EP4 signaling as an initiating factor for RANKL expression in keratinocytes. Classically, PGE_2 was known as an osteolytic factor that functions in osteoblasts downstream of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α in induction of osteoclasts from bone marrow precursor cells. We previously identified the receptor mediating this action as EP4 (28). The molecule induced by PGE_2 treatment and responsible for osteoclastogenesis was identified as RANKL (29). Thus, our present study has revealed that the identical PGE_2–EP4–RANKL signaling operates in different types of cells with different consequences, one for osteolysis and the other for immunomodulation. It also is noted that PGE_2–EP4 signaling can exhibit apparently opposite immunomodulatory actions in different situations. We recently used the EAE mouse model and showed that PGE_2–EP4 signaling promotes immune inflammation through Th1 cell differentiation and Th17 cell expansion (30), whereas our current study showed that the identical signaling promotes immunosuppression. How such context-dependent differences arise should be defined clearly so that the EP4 antagonist can be used properly and safely in therapeutics in the future. In addition, UV-induced immunosuppression has been implicated in nonmelanoma skin cancers caused by UV radiation (1, 12, 31), and

the incidence of nonmelanoma skin cancers was lower in subjects receiving a selective COX-2 inhibitor, celecoxib, than in subjects receiving placebo (32). We hope that the action of PGE_2–EP4 signaling we have described here is exploited in various clinical settings, including this malignancy.

Materials and Methods

Animals. Mice lacking each type or subtype of PG receptor individually were generated and backcrossed more than 10 times onto C57BL/6 background as described previously (23). $Foxp3^{hCD2/hCD52}$ mice with C57BL/6 background were generated as described (22). Female mice of each genotype were used at age 8–10 wk. Wild-type C57BL/6CrSlc mice (Japan SLC) were used as controls. All mice were maintained on a 12-h/12-h light/dark cycle under specific pathogen-free conditions. All experimental procedures were in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* and were approved by the Committee on Animal Research of Kyoto University Faculty of Medicine.

UVB Irradiation and CHS. A bank of sunlamps emitting 280–360 nm with a peak emission at 313 nm (FL 20SE; Toshiba) arranged in parallel was used as a source of UVB. The irradiance, measured by an UVR-305/365D radiometer (Tokyo Kogaku), was 5 $J/m^2\ s^{-1}$ at a distance of 40 cm. On day 0, back fur was shaved with electric clippers. On day 1, mice were exposed to 5 kJ/m^2 of UVB on the shaved back with their ears and eyes protected. They were sensitized by applying 25 μL of 0.5% (wt/vol) DNFB (Sigma) in acetone/olive oil (4/1, vol/vol) on the shaved abdomen on day 5. Then, on day 10, the mice were challenged by application of 20 μL of 0.2% DNFB to the dorsal and ventral surfaces of both ears. The ear thickness of each mouse was measured before and 24 h after elicitation at a predetermined site with a micrometer, and the difference was expressed as ear swelling.

For drug treatment, 4 mg $kg^{-1}\ d^{-1}$ of indomethacin (Nacalai) or 10–100 mg $kg^{-1}\ d^{-1}$ of ONO-AE-3–208 in drinking water was given ad libitum from day 0 to day 3; 5 mg kg^{-1} of Ramatroban (Tocris) was injected s.c. every 12 h to TP-deficient mice from day 0 to day 3; and 5–500 μg/kg of ONO-AE-1–329 in 0.9% saline was injected s.c. immediately and 12 h after UV irradiation.

For anti-RANKL treatment, mice were injected s.c. with 100 μg of anti-mouse RANKL (Oriental Yeast) 2 d before UV irradiation. The same amount of rat IgG2a antibody (eBioscience) was used as a control.

Lymphocyte Culture. Single-cell suspensions were prepared from inguinal and axillary LNs of mice 5 d after sensitization with 0.5% DNFB. The cells were cultured at 5×10^5 cells per well in a 96-well plate in 200 μL of RPMI-1640 containing 10% FBS with or without 100 μg/mL DNBS (Sigma) for 3 d and were pulsed with 0.5 μCi [^{3}H]thymidine for the last 24 h of culture. The acid-insoluble radioactivity was determined by liquid scintillation counting. For measurement of cytokine production, the cells were cultured for 48 h, and the culture supernatants were collected. IFN-γ in the supernatants was measured using an IFN-γ ELISA kit (Endogen).

Immunofluorescence. Immunostaining was performed on 10-μm cryostat sections of mouse back skin embedded in optimal cutting temperature (OCT) compound. Tissue sections were fixed in precooled acetone for 10 min. Anti-RANKL and anti-cytokeratin antibodies were used as primary antibodies. Biotinylated anti-rat IgG (Vector) and Alexa Fluor 488 anti-mouse IgG (Invitrogen) were used as secondary antibodies. The RANKL signal was amplified using the tyramide signal amplification (TSA) Plus Cyanine-3 System (Perkin-Elmer) according to the manufacturer's protocol. Cell nuclei were stained using Hoechst-33342 (Invitrogen) according to the manufacturer's instruction.

Statistical Analysis. Data were analyzed by Student's *t* test, and differences were considered significant at $P < 0.05$. All bar graphs represent the mean ± SEM.

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Multiple Follicular Pustules as an Atypical Cutaneous Manifestation of Drug-induced Hypersensitivity Syndrome

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Drug-induced hypersensitivity syndrome (DIHS), also known as drug rash with eosinophilia and systemic symptoms (DRESS), is a severe drug reaction presenting with generalized skin eruption, organ failure and haematological abnormality (1, 2). Typical cutaneous manifestations include maculopapular, lichenoid, purpuriform, target-like and possibly also other types of lesions. Erythroderma with or without exfoliation or desquamation may sometimes be observed. We report here a case of DIHS/DRESS presenting with multiple diffuse follicular pustules on the trunk and extremities.

CASE REPORT

A 15-year-old Japanese male student was admitted to our hospital with generalized pruritic rash and high fever after treatment with carbamazepine for epilepsy over 5 weeks. The patient exhibited marked oedema on the face (Fig. 1A), cervical lymphadenopathy, and small follicular pustules diffusely distributed on his trunk and extremities (Fig. 1B). Bacterial cultures of both blood and pustules were negative. A skin biopsy from the back revealed a spongiotic pustule in the follicular infundibulum with moderate upper-dermal perivascular infiltrations of lymphocytes, neutrophils and a few eosinophils (Fig. 1C). A blood test indicated leukocytosis (white blood cell count, 12,000/ml) containing up to 5% atypical lymphocytes, marked eosinophilia (2,280/ml), elevated levels of liver enzymes (aspartate aminotransferase (AST) 367 IU/l and alanine aminotranferase (ALT) 1,637 IU/l), and positivity for human herpes virus (HHV)-6 DNA. Treatment with 30 mg oral prednisone resulted in improvement in the patient's general condition and skin eruptions.

DISCUSSION

This case fulfils the criteria for both DIHS (7/7 of the Japanese consensus group criteria) and DRESS (8/9 of the Kardaun et al. criteria (7)) (Table I). The mechanism of DIHS/DRESS has not been fully elucidated. Immune responses by drug-reactive T cells, plasmacytoid dendritic cells, and activation of herpes viruses have been proposed (2, 3).

Before the concept of DIHS/DRESS was established, anticonvulsant hypersensitivity syndrome was recognized as a severe adverse drug reaction induced by anticonvulsants such as carbamazepine, phenytoin,

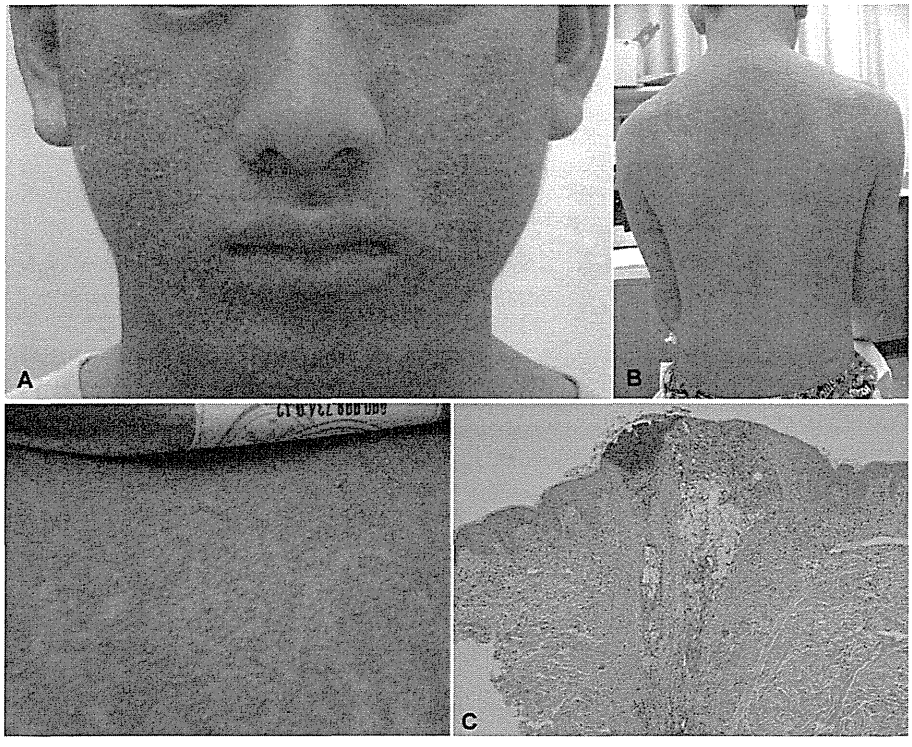

Fig. 1. Clinical and histological findings in a patient with drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms. (A and B) Clinical manifestations. The patient had marked oedema of the face and small, diffusely distributed, follicular pustules. (C) Histological examination revealed a spongiotic pustule in the follicular infundibulum with moderate upper-dermal perivascular infiltrations.

Table I. *Criteria for drug-induced hypersensitivity syndrome (DIHS; top) and drug rash with eosinophilia and systemic symptoms (DRESS; bottom). The patient met the criteria for DIHS (7/7) and DRESS (8/9)*

Criteria for typical DIHS (presence of all 7 criteria) (ref. 6)

1. HHV-6 reactivation
2. Prolonged clinical symptoms 2 weeks after discontinuation of causative drug
3. Maculopapular rash developing >3 weeks after starting with limited number of drugs
4. Fever >38°C
5. Lymphadenopathy
6. Liver abnormalities (alanine aminotransferase >100 U/l) or other organ involvement, e.g. renal involvement
7. Leukocyte abnormalities (at least one present)
   - Leukocytosis ($>11\times10^9$/l)
   - Atypical lymphocytosis (>5%)
   - Eosinophilia ($>1.5\times10^9$/l)

	Score −1	Score 0	Score 1	Score 2
*Scoring system for classifying DRESS cases as definite, probable, possible, or no case[a] (applicable items in **bold**) (ref. 7)*				
Fever ≥38.5°C	No/U	**Yes**		
Enlarged lymph node		No/U	**Yes**	
Eosinophilia		No/U		
Eosinophils			$0.7–1.499\times10^9l^{-1}$	$\mathbf{\geq1.5\times10^9l^{-1}}$
Eosinophils, if leucocytes $<4.0\times10^9l^{-1}$			10–19.9%	≥20%
Atypical lymphocytes		No/U	**Yes**	
Skin involvement				
Skin rash extent (% body surface area)		No/U	**≥50%**	
Skin rash suggesting DRESS	No	U	**Yes**	
Biopsy suggesting DRESS	No	Yes/U		
Organ involvement				
Liver, kidney, lung, muscle/heart, pancreas, other organ			**One organ**	Two or more organ
Resolution ≥15 days	No/U	**Yes**		
Evaluation of other potential causes				
Antinuclear antibody				
Blood culture				
Serology for HAV/HBV/HCV				
Chlamydia/mycoplasma				
If none positive and ≥3 of above negative			**Yes**	

[a]Total score <2: no case; 2–3; possible case; 4–5: probable case; >5: definite case.
U: unknown/unclassifiable; HAV: hepatitis A virus; HBV: hepatitis B virus; HCV: hepatitis C virus; HHV-6: human herpes virus 6.

and phenobarbital sodium (4). Several cases of acute generalized exanthematous pustulosis (AGEP) induced by anticonvulsants have been reported (5), but isolated pustules in the follicular infundibulum, as seen in our case, are a clear contrast to AGEP, which usually manifests histopathologically as confluent, flaccid pustules with non-follicular, subcorneal or upper-epidermal pustules.

We diagnosed the patient as typical DIHS, since we observed the reactivation of HHV-6 in addition to the clinical manifestations seen in DRESS. The case was diagnosed as DIHS/DRESS with reactivation of HHV-6. We describe here an atypical case of DIHS presenting with diffuse follicular pustules on the trunk and extremities, which was reasonably well controlled by conventional therapy with an oral steroid.

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Eosinophilic Pustular Folliculitis

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Introduction

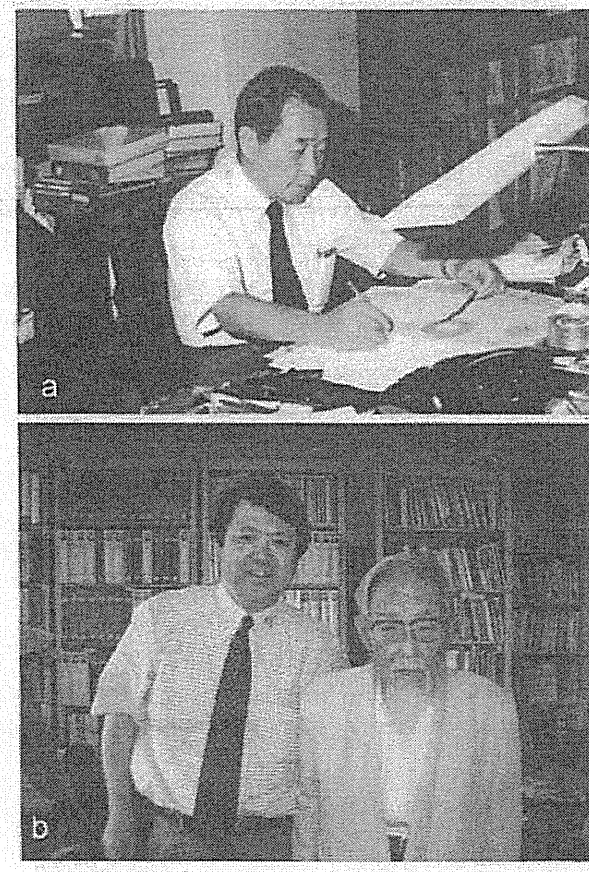
Eosinophilic pustular folliculitis (EPF) is a noninfectious inflammatory skin disease characterized by eosinophilic infiltration mainly of hair follicles. This clinical entity is very memorable for Asian dermatologists because it was first described by Prof. Shigeo Ofuji of Kyoto University, Japan (Fig. 1). Although classic EPF has been reported mainly from Japan as well as other Asian countries, global dermatology has paid a considerable attention to EPF because immunosuppression-associated EPF was recognized as an HIV-related skin disease. In this Chapter, EPF will be reviewed from the standpoint of its etiology and pathophysiology, emphasizing the great contribution of Asian dermatologists to this Asia-originated, globally-acknowledged skin disease.

Definition and Epidemiology

EPF was first reported in 1965 as a follicular variant of subcorneal pustular dermatosis[1], which was later named EPF in 1970 as a novel clinical entity by Ofuji[2]. This is why EPF is called Ofuji's disease, however, it should be noted that papuloerythroderma is sometimes referred as another Ofuji's disease[3] since it was also first described by Ofuji in 1984[4].

EPF manifests as chronic and recurrent itchy follicular papules and sterile pustules which enlarges peripherally with central clearing tendency. Histopathologically, it is characterized by the eosinophilic infiltrates in pilosebaceous unit.

EPF is now classified into 3 variants: Classic EPF, immunosuppression-associated EPF and infancy-associated EPF[5]. Classic EPF has been reported mainly from Asian countries. Epidemiological studies revealed that classic EPF occurs in young adults of 20-30 years old and the



Professor Ofuji, Kyoto University, Japan. (a) Before retirement in 1979. (b) Recent photograph in 2008 (Right), Left: Author (YM).

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male/female ratio is 4.8:1 in Japanese literature[6]. The most commonly affected lesion of classic EPF is the face (85%), however, the eruptions can be seen on the back, arms and the chest. Of great interest is that the eruption can be found on palms and soles in 22% of the patients[7]. One may infer that the term "folliculitis" seems to be inappropriate since 6% of the patients start only with palmoplantar pustular lesions, though 66% of them first appear on the face[8]. Although some papers proposed to use the term of sterile eosinophilic pustulosis[9] or eosinophilic pustular dermatosis[10], Prof. Ofuji insisted that the name of the disease should represent the characteristic features of the typical eruption, and thus the name of EPF should be kept irrespective of the palmoplantar lesions (personal communication). In his own review article, Prof. Ofuji mentioned that he personally had an impression that the classic EPF seemed to be more commonly observed in patients with a past history of acne[11].

Pathophysiology

At present, nobody can answer the questions why eosinophils are attracted in hair follicles, why peripheral number of eosinophils is increased, why the skin lesions flare up periodically, why pustules are observed in palmoplantar area or why EPF is frequently found in patients with HIV infection. However, the possible pathomechanism of EPF has gradually been elucidated in recent days.

As for the mechanism of eosinophilic accumulation to hair follicles, it has been reported that eosinophilic chemotactic factor was found in skin surface lipid products[12], which seems to be a reasonable explanation because most of the EPF lesions are distributed in seborrheic region of the patients with the past history of acne.

Since the clinical features of EPF resemble dermatophyte and/or *Malassezia* folliculitis, it is speculated that some unknown reactions may occur to these infectious microorganisms, though the pustules in EPF are definitely sterile. A favorable response to antifungal therapy in some cases may support this concept[13].

Elicitation of follicular inflammation by eosinophils can be attributable to eosinophilic cationic protein[14] and nitric oxide[15], which may provide a novel treatment tool for EPF by regulating these inflammatory factors.

Peripheral eosinophilia can partly be explained by micro environmental change of cytokines such as IL-5, because peripheral eosinophilia was observed in EPF patients with myelodysplastic syndrome[16] and drug-induced EPF[17]. The periodical flare up of EPF lesions may be partly due to the fluctuation of cytokine levels in view of interferon studies[18,19].

Most of the immunosuppression-associated EPF is seen in patients with AIDS, which shows both common and different features of classic EPF. It is reported that eotaxin-1 and Th2 cytokines play a crucial role in eosinophil recruitment and inflammation leading to the tissue injury of hair follicles[20]. Since immunosuppression-associated EPF can be found in patients with other hematologic malignancy such as lymphoma and leukemia, some common immunological impairment induced by whatever stimuli may be involved in the pathogenesis of EPF. In immunosuppression-associated EPF, superficial fungal infections are suspected to participate[21,22] because EPF displays similarities with dermatophyte folliculitis, however, no direct evidence supports this concept.

Infancy-associated EPF which is usually found in the scalp of children and shows good clinical response to corticosteroids[23-25], may be somewhat different from adult EPF but a variety of skin diseases such as scabies, insect bites and linear IgA-dermatosis though histopathology bears a close resemblance[26].

Clinical Manifestations and Laboratory Findings

Classic EPF occurs predominantly in middle-aged men, which affects face, trunk and arms. Characteristic features of classic EPF are pruritic follicular papules and sterile pustules which enlarge gradually making a well demarcated area (Fig. 2a). There is a central healing tendency leaving slightly scaly pigmentation (Fig. 2b). On face, slightly elevated maculoerythematous indurations with scattered papules and pustules are occasionally seen, which subside spontaneously in due course but flare up periodically re-

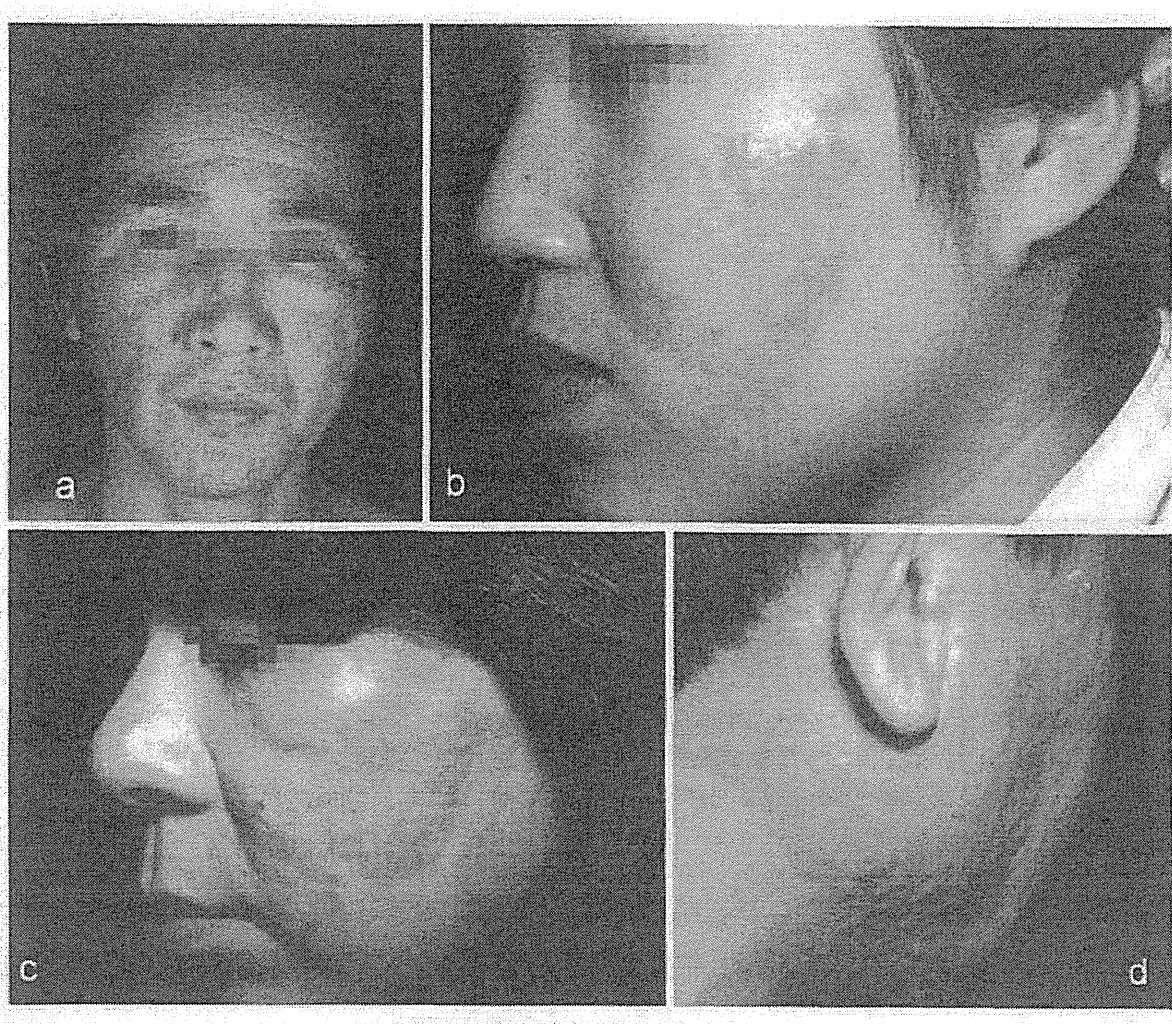

Fig. 2. Clinical features of eosinophilic pustular folliculitis. (a) Characteristic features of classic EPF are pruritic follicular papules and sterile pustules which enlarge gradually making a well demarcated area. (b) There is a central healing tendency leaving slightly scaly pigmentation. (c, d) On face, slightly elevated maculoerythematous indurations with scattered papules and pustules are occasionally seen, which subside spontaneously in due course but flare up periodically resulting in a chronic course.

sulting in a chronic course (Fig. 2c, d). When palmoplantar regions are involved, symmetrical erythema and pustules resembling palmoplantar pustulosis are observed with hypertrophic scaling. Over half patients complain of itching. Usually there is no prodrome or systemic symptoms noticed. Differential diagnosis of classic EPF includes inflammatory acne, rosacea, tinea corporis, pustular psoriasis, subcorneal pustular dermatosis and seborrheic dermatitis.

Mild to moderate eosinophilia is occasionally observed with elevated IgE level. Since EPF is occasionally accompanied with HIV infections and other immunosuppressive conditions such as hematologic malignancies, these complications should be carefully checked.

Histopathology

Immunosuppression-associated EPF and infancy-associated EPF are indistinguishable histologically from classic EPF regardless of their different clinical features. The histopathology of typical EPF is characterized by a dense inflammatory infiltrate of mononuclear cells and eosinophils around hair follicles and sebaceous glands (Fig. 3a). In early phase papular eruption, spongiosis of the outer root sheath of the follicles presumably due to the destruction mediated by eosinophils is observed (Fig. 3b). In advanced stage, eosinophilic infiltration extends to the whole hair follicles leading to the pustular formation.

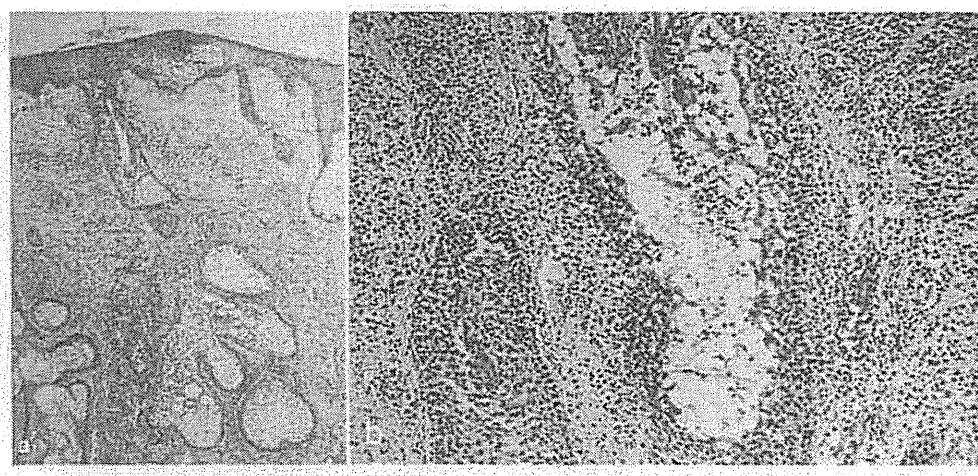

Fig. 3. (a) The histopathology of typical EPF is characterized by a dense inflammatory infiltrate of mononuclear cells and eosinophils around hair follicles and sebaceous glands. (b) In early phase papular eruption, spongiosis of the outer root sheath of the follicles presumably due to the destruction mediated by eosinophils is observed.

Mucinous degeneration of the sebaceous gland and outer root sheath may be seen. Moderate increases of tryptase-positive and chymase-negative mucous type mast cells are observed which might play some role in the pathogenesis[27]. Electron microscopic observation revealed that T cells may be involved in the pathomechanism of EPF[28]. As for the infancy-associated EPF, it is speculated that this type of EPF is not a distinctive inflammatory disease of the skin but a variety of different diseases, and the term "eosinophilic folliculitis" is better defined as a histopathologic pattern for infancy-associated EPF[26].

Treatment

Various treatments have been proposed for EPF, however, the endpoint of the treatment should be to control the disease with mild side effects, since EPF shows a chronic course with wax and wane. Topical corticosteroids might be the first choice of the drug, but the clinical effect is sometimes limited. Among other treatment options such as phototherapy[29], systemic steroids, dapsone[30], metronidazole[31], minocycline and retinoids, oral indomethacin seems to be the most promising choice of the treatment[32,33]. Topical indomethacin is also effective in some cases[34]. The mechanism of action by which indomethacin works on EPF still remains unclear, however, indomethacin either suppresses the production of cyclooxygenase-dependent eosinophilic chemotactic factor or alters the cytokine balance leading to the inhibition of prostanoids synthesis[18].

Recently, in addition to indomethacin, other treatments have been applied for EPF[35]. Oral cyclosporine[36] and topical tacrolimus[37] may be beneficial choices when patients have been resistant to previous conventional treatments. Tacrolimus may act against EPF presumably through the inhibition of several proinflammatory cytokines by T cells as well as the prevention of the cytokine release from mast cells[38].

Prognosis of EPF is relatively poor resulting in chronic course with the remission and relapsing for years in many patients.

Conclusion

EPF was first described by an Asian dermatologist as a noninfectious inflammatory skin disease characterized by eosinophilic infiltration mainly of hair follicles, which revealed later as a globally important skin disease because immunosuppression-associated EPF was recognized as an HIV-related skin disease. EPF may be induced by whatever antigenic stimuli and some unknown immunological reaction patterns with eosinophil participation mainly in

the hair follicles may be involved. Development of a new treatment may reveal not only the pathomechanism of EPF but also some hidden multifactorial immune responses in skin diseases mediated by cytokines and chemokines for eosinophils.

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