given the low percent predicted FVC values at baseline in study patients (mean 39.9%), a relative increase of 15% is of particular importance. The American Thoracic Society defines a >15% relative change in FVC occurring over a one-year period as being clinically meaningful [26]. Similarly, the 54.5 m mean increase in 6MWT distance also is considered to be a clinically meaningful improvement, based on a study of adult men with chronic obstructive pulmonary disease [25]. The 6MWT is a sub-maximal exercise test that is a composite assessment of cardiac, respiratory, and musculoskeletal function. Because all three of these organ systems are involved in the MPS disorders, walking tests have been widely used as primary efficacy endpoints in clinical trials of enzyme replacement therapy for other MPS disorders, including MPS I [29,30] and MPS VI [31].

We observed no evidence for an effect of race on immunogenicity or safety. IgG antibodies were detected in 60% (6/10) of patients treated with idursulfase, which is similar to the 49.6% rate seen in the Phase 2/3 study that enrolled predominantly Caucasian and other non-Asian patients [20]. In addition, the adverse event profile was similar in all respects; infusion-related reactions occurred in 50% of patients in the current study compared to 69% of patients receiving weekly idursulfase in the Phase 2/3 study [20].

Limitations of this study include its open-label treatment, lack of control group, and small sample size. Other aspects of the study design, however, including the treatment dose and regimen, study duration, and efficacy and safety assessments were identical or very similar to those used in the Phase 2/3 study [20]. A placebo effect in this study cannot be excluded, especially for effort-dependent assessments such as the 6MWT and active joint range of motion. Nevertheless, the magnitude of change in the 6MWT distance was similar to those observed in previous studies of idursulfase [19,20]. Determination of FVC by spirometry is less susceptible to a placebo effect given the requirement for test–retest reproducibility at each assessment [21]. This study enrolled only 10 patients, which may not have had sufficient power to detect a statistically significant clinical response even if clinical improvements were present. On the other hand, the biomarkers of lysosomal GAG clearance, i.e. liver and spleen volumes and urinary GAG level, did have sufficiently large effect sizes (change/standard deviation of change) to show statistically significant differences. Finally, the study involved only adult males, all of whom had a substantial pre-existing disease burden. This study shows that many disease features of seriously ill patients, including diminished cardiorespiratory function, restricted joint range of motion, and hepatosplenomegaly can improve with idursulfase treatment. An even better response is expected in young children prior to final organ maturation and the development of chronic tissue damage. In this regard, a study in MPS II patients ⩽5 years of age is underway.

Conclusions

Idursulfase was generally well-tolerated and produced clinical improvements in adult Japanese patients with attenuated MPS II treated with the labeled dose, 0.5 mg/kg administered intravenously once weekly. Treatment with idursulfase also resulted in substantial reductions in hepatosplenomegaly and urinary GAG excretion, indicating efficient clearance of lysosomal GAG. The safety profile and immunogenicity of idursulfase appear to be similar between Japanese and previously studied Caucasian patients.

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皮膚症状から鑑別診断へ

新関寛徳*

はじめに

皮膚疾患の鑑別診断は2つのステップで行われる。ひとつめは目の前の患者がもつ皮疹がどのような発疹なのか，紅斑なのか紫斑なのか，あるいは色素沈着なのかなどである。ふたつめは鑑別した皮疹の性状からさらにどのような疾患を考えるかである。筆者がいただいた本稿のテーマは主に後者であると考えるが，発疹別に鑑別診断をまとめたので，その発疹の特徴についても簡単にふれることにする。

「目でみて」診断するのがわれわれ皮膚科診療の主体であるので，そのすべてを限られた紙数で表現することは困難である。小児医療従事者に関係の深い事柄を中心に，紙数の許すかぎり概説したい。

I 周囲の皮膚と同じ高さの発疹

皮膚の発疹はまず色調が最初の判断材料と考えておられた読者もいるかもしれない。まず高さ，次に色調で分類している。

1．斑（macule）

平坦な限局性色調変化である。

1）紅斑（erythema）

文字どおり紅色の「斑」。真皮乳頭層の血管拡張・充血による。赤い色調は血管内に充満する赤血球のヘモグロビンの色調を反映する。ガラス板で圧迫すると，血管内にある赤血球が圧排されるので褪色する。

以下は紅斑と関連しているが，区別（鑑別）すべき皮疹である。

●**毛細血管拡張**（telangiectasia）

紅斑は通常，**炎症性**反応の結果生じる**可逆的な**血管拡張・充血を指すのに対して，毛細血管拡張は**非炎症性**の**持続性**血管拡張を指す（例：クモ状血管腫）。

●**膨疹**（wheal）

皮膚の限局性浮腫性隆起で，痕跡を残さず短時間で消褪する。紅斑を伴うことが多いため，紅斑関連皮疹としたが，膨疹の定義自体には色調は関係しない（例：蕁麻疹）。

2）**紫斑**（purpura）

紫紅色の斑で，真皮（～皮下）の赤血球血管外漏出による。すなわち，単に紫色の斑というだけではなく，出血斑であることが定義である。ガラス板で圧迫しても赤血球の行き場がないため，色は消褪しない（例：アナフィラクトイド紫斑）。

紫斑の大きさにより，点状出血（径1～5 mm），斑状出血（溢血斑）（径1～5 cm）という用語もある。

3）**色素斑**（pigmented spot）（**表**）

皮膚の色素が増加したために生ずる限局性の色調の変化をいう。色素の量が増加するにつれて黒い色調を帯びてくる。色素の存在位置が深くなればなるほど，青紫色の色調が強くなる。

4）**白斑**（leukoderma）

色素脱出（depigmentation），脱色素斑（depigmented spot）。白色の斑で，多くはメラニン色素減少による（例：尋常性白斑）。完全色素脱出と不完全色素脱出がある。

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表　色素沈着の部位と色調

色　調	褐　色	黒～黒褐色	灰褐色	青　色
色素および色素細胞の分布	～～～～～～～～	～～～～～～～～	～～～～～～～～	～～～～～～～～
特徴的変化	表皮基底層にメラニン色素が増加	表皮と真皮上層に多量のメラニン色素および色素細胞が存在	真皮上層にメラニン色素を貪食した担色細胞が存在	真皮中・下層に真皮メラノサイトが存在
代表的疾患	扁平母斑，肝斑	母斑細胞母斑，悪性黒色腫	炎症性色素沈着	蒙古斑，青色母斑

（斉藤[1] 2005 より一部改変）

しかし，白色調になる程度であれば，血管収縮による赤血球減少（例：貧血母斑，Raynaud 現象）でもみられる。すなわち，ヘモグロビンも皮膚の色を規定する因子のひとつであることは紅斑と同じである。

II 周囲の皮膚より隆起している発疹（図 1）

1．丘疹（papule），結節（nodule），腫瘤（phyma；tumor）

隆起性病変を指す。径 0.5 cm 以下を丘疹，0.5～3 cm を結節，3 cm 以上を腫瘤とよぶ。

漿液性丘疹は，頂点に微小水疱を有する丘疹で，急性期の湿疹でみられる。それに対して充実性丘疹という語もある。

結節・腫瘤と丘疹との違いは大きさであり，丘疹より大きい限局性隆起を結節という。結節よりもさらに大きいのが腫瘤である。丘疹に対し，結節・腫瘤は，増殖傾向の強いという意味あいも含んで使用する傾向にある。

2．水疱，膿疱

1）水疱（bulla），小水疱（vesicle）

透明な水様の内容を有する病変。径 0.5 cm 以下の水疱は，小水疱という。

a）弛緩性水疱（flaccid bulla）

病理組織学的に，表皮内水疱は水疱蓋が薄いため，すぐに破れて弛緩性水疱となる（例：尋常性天疱瘡，伝染性膿痂疹）。

b）緊満性水疱（tense bulla）

一方で，表皮下水疱は水疱蓋が厚いため破れにくく，緊満性水疱になる（例：水疱性類天疱瘡）。

2）膿疱（pustule）

表皮内水疱の内容が白血球の遊走により膿性になっているもの。多くは細菌性であるが，無菌性のこともある（例：膿疱性乾癬）。

3．膿瘍（abscess）

真皮，または皮下組織に膿汁が貯留したもので，皮膚表面が発赤しゆるやかな丘のように隆起する。波動を触れる。

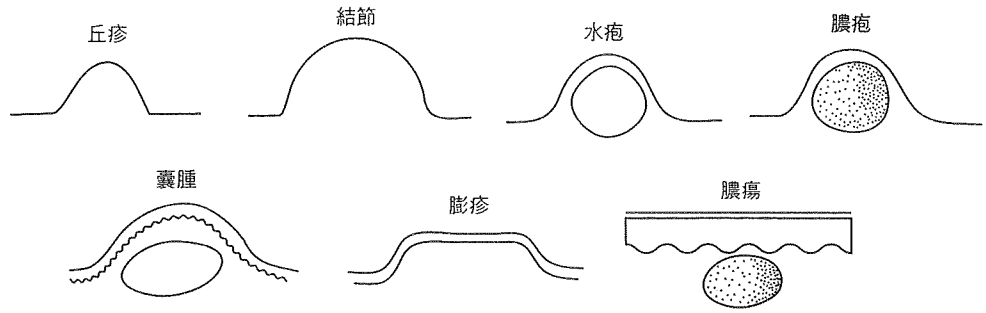

図 1　周囲の皮膚より隆起している発疹（佐々木[2] 2000 より一部改変）

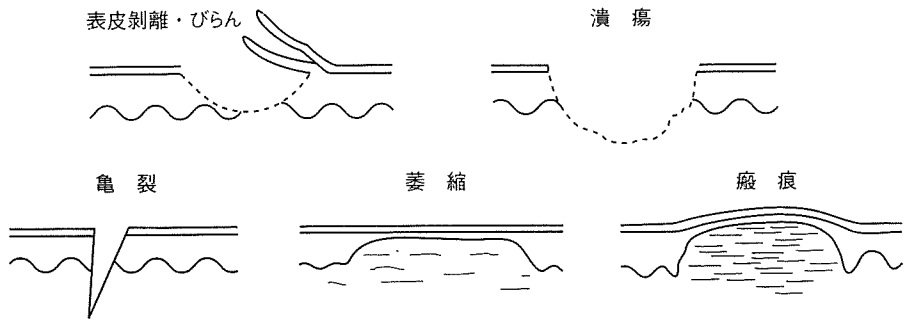

図 2　周囲の皮膚より陥凹している発疹（佐々木[2] 2000 より一部改変）

Ⅲ 周囲の皮膚より陥凹している発疹（図 2）

1．表皮剝離（excoriation）

掻破，外傷などにより表皮の部分的欠損をきたしたもの。深いと漿液や血液が漏出する。表皮剝離は軽い瘢痕や，数か月間続く色素沈着を残すことがある。

2．びらん（erosion）

小水疱，水疱，膿疱などが破れた後に生じる表皮剝離状態をさす。多くは有棘層まで，深くても基底層までの剝離欠損であり，数か月続く色素沈着を残すことはあっても，瘢痕は形成しない。

3．潰瘍（ulcer）

びらんよりも深く，真皮から皮下組織に及ぶ組織が欠損したもの。しばしば膿苔や痂皮で被われる。次第に肉芽組織を形成し，上部に表皮が再生して治癒する（例：褥瘡）。

4．亀裂（fissure）

表皮深層から真皮に達する細く深い線状の裂け目を指し，出血を伴う場合が多い。通常，瘢痕形成せずに治癒する。

5．萎縮（atrophy）

皮膚組織の退行変性により，皮膚組織全体が菲薄となったもの。表面平滑で光沢があり，やがて表面は細かい皺ができ，多少周囲の皮膚より陥凹した状態になる。

6．瘢痕（scar）

潰瘍，膿瘍，創傷治癒後に組織欠損を埋めた結合組織肉芽腫と，これを被う表皮により形成された局面を指す。瘢痕表面は，皮野がなく平滑で光沢がある。附属器は欠損し，色素脱失あるいは沈着をきたす。

Ⅳ その他（図 3）

1．鱗屑（scale）

角層が正常より厚くなり脱落しかかっている状態をいい，この現象を落屑（desquamation）という。鱗屑が細かい小さいものを粃糠疹（pityriasis）という。

2．痂皮（crust）

びらん，潰瘍から分泌された漿液，膿汁，壊死塊が乾いて固まったもの。俗称で「かさぶた」ともいう。血液が乾固したものを血痂とよぶ。

図 3　その他の発疹（佐々木[2] 2000 より一部改変）

3．胼胝（べんち，callus）

表皮角層の限局性の増殖肥厚を指す。圧迫などの外的刺激が頻繁にくり返して加わる部位に生じる。

V 皮疹から診断へ

1．紅斑をみたら（図4）

最初に，狭義の紅斑と血管拡張を鑑別する。分枝状の血管拡張であるクモ状血管腫などは，形態によりすぐに判別できる。斑状になっている血管拡張（非炎症性で非可逆的）の単純性血管腫などは，境界が明瞭，色調が均一，滲出傾向を欠くことなどから炎症性の結果生じる紅斑と鑑別する。

紅斑の形状から種々の疾患を導きだす。次いで，分布状態から原因を類推する。顔面など局所のみなのか，播種状（全身性）なのかなど。

標的様（虹彩様）紅斑は臨床的に特徴があり，図のような疾患を考える。

顔面の紅斑は日光との関連があるかどうかを検討することにより，膠原病を鑑別する。

環状を呈する疾患は限られており，かなり疾患を絞り込むことができる。

紅斑は日々変化していくため，種々の手がかりから診断にいたるときには典型疹が残っているとは限らない。図を参考になるべく皮疹の形態を記載し，（後日）成書の記載と照らし合わせるとよい。

2．紫斑をみたら（図5）

1）紫斑をきたす病態を考えながら，必要な検査を行って鑑別を進める。

2）血管炎により生じる紫斑は特徴がある。アナフィラクトイド紫斑では，真皮上層での細胞浸潤，浮腫も伴うため，他の病態の紫斑と異なり，palpable purpura（軽度隆起し，浸潤を触知する紫斑）がみられる。

3．色素斑をみたら（図6）

1）メラニン以外にもヘモジデリンや異物沈着も念頭におく。

2）色調により色素沈着の深さが類推できる。

4．白斑をみたら（図7）

1）局所性貧血を除外する。局所性貧血は，血行障害や血管の攣縮で生じる。

2）いつ出現したか。先天性，後天性の疾患がある。

3）分布はどうか。限局性と全身性があり，代謝性疾患では全身性にみられることが多い。

4）白斑以外に皮膚症状，皮膚外症状がみられるか。母斑症では白斑以外の皮膚症状や，皮膚以

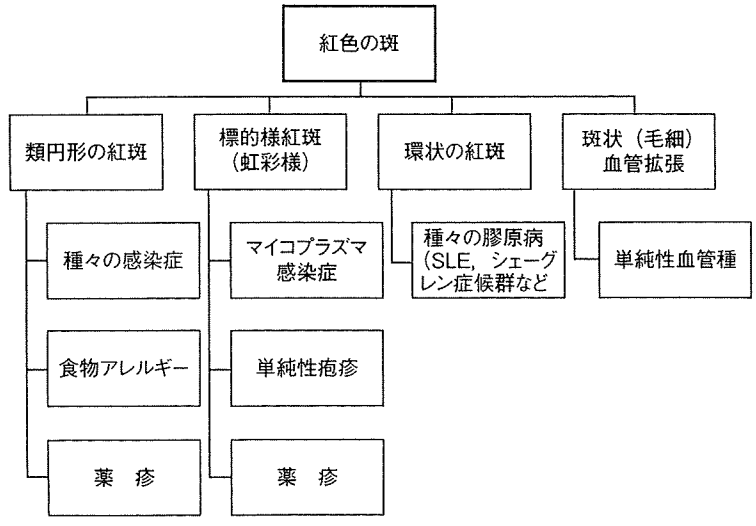

図4　紅斑をみたら（斉藤[1] 2005より一部改変）

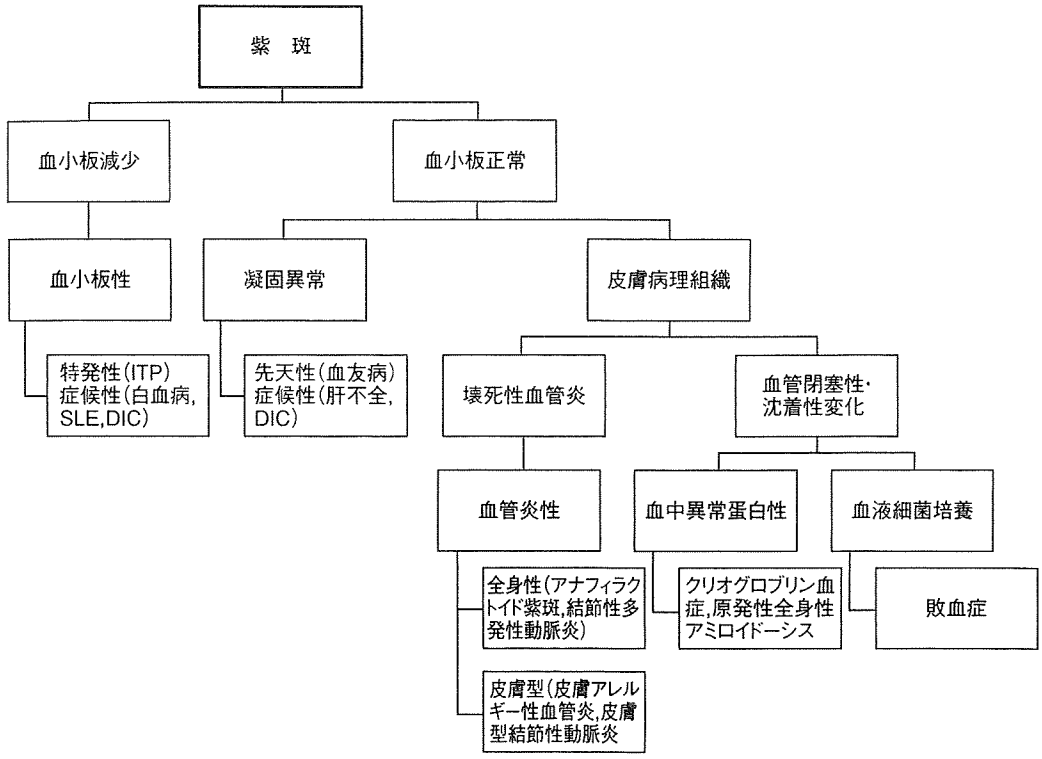

図 5　紫斑をみたら（土田[3] 2006 より一部改変）

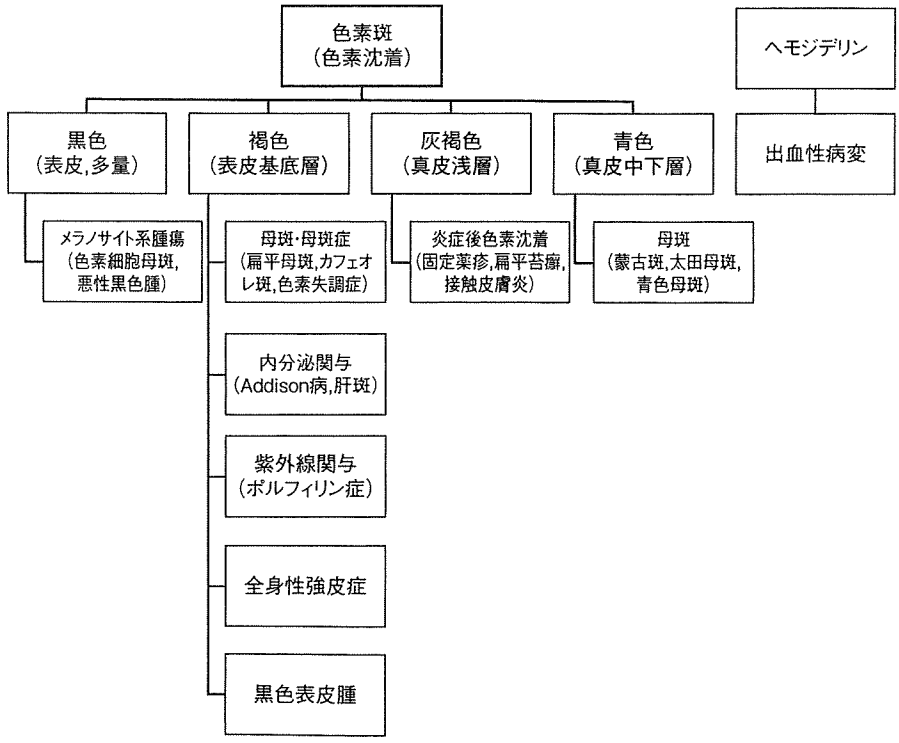

図 6　色素斑をみたら（土田[3] 2006 より一部改変）

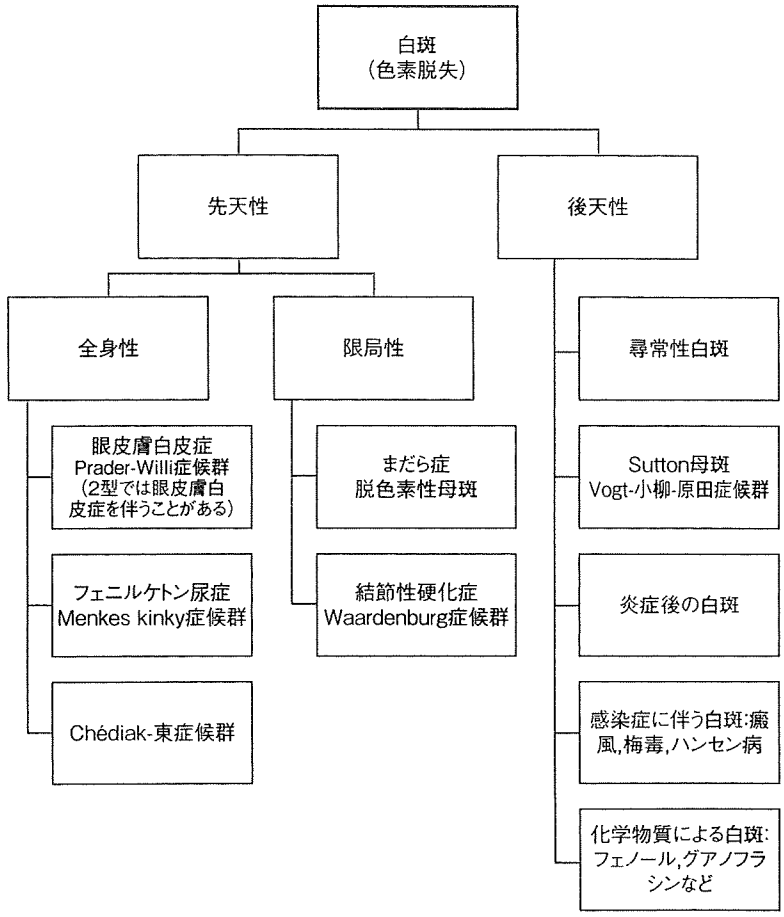

図 7　白斑をみたら（斉藤[1] 2005 より一部改変）

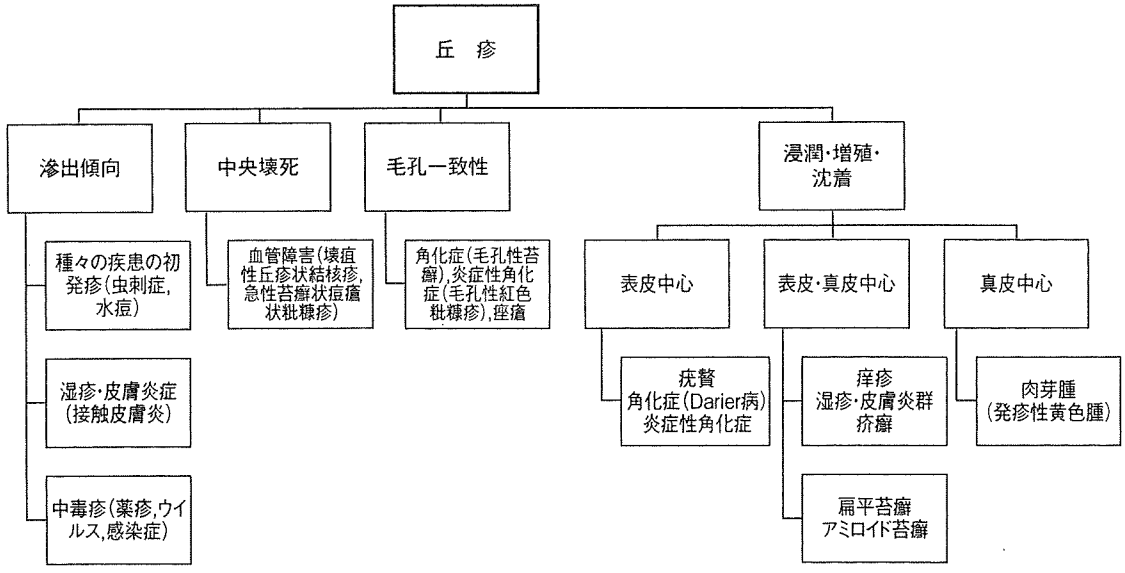

図 8　丘疹をみたら（土田[3] 2006 より一部改変）

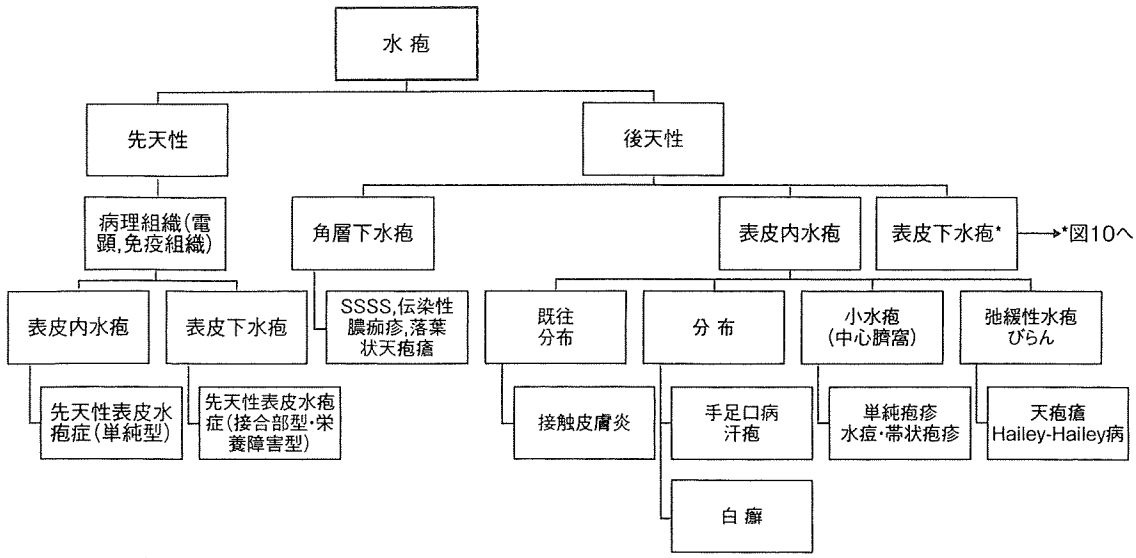

図 9　水疱をみたら（土田[3] 2006 より一部改変）

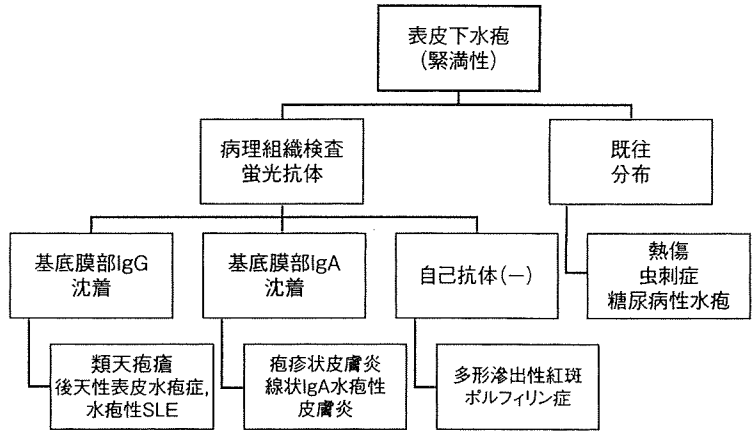

図 10　表皮下水疱の鑑別（土田[3] 2006 より一部改変）

外（内臓病変）にも診断に有用な症状がみられることがあるのでよく検討する（例：結節性硬化症）。

5．丘疹をみたら（図 8）

紅斑同様，組織学的変化を考えながら鑑別を進める。

6．水疱をみたら（図 9，10）

日常診療でみかけるさまざまな疾患で水疱が生じることを念頭においたうえで，まれではあるが自己免疫性水疱症を見逃さないようにする。

おわりに

以上，駆け足で皮膚発疹学，診断学のさわりをご紹介させていただいた。引用した文献はいずれもすぐれたものであるので，是非ご覧いただくことを願うと同時に著者の先生には，引用させていただいたことにこの場を借りてお礼申し上げます。

Key Points

❶鑑別疾患を考えるまえに，まず皮疹の性状を記載する：紅斑なのか紫斑なのか，あるいは色素沈着なのかなど。

❷紅斑では，狭義の紅斑と血管拡張を鑑別する：クモ状血管腫，単純性血管腫など。

❸色素斑では，色調により色素沈着の深さが類推できる。

❹白斑では，白斑以外に皮膚症状，皮膚外症状がみられるかを検討する：結節性硬化症など。

❺水疱では，まれではあるが自己免疫性水疱症を見逃さないようにする。

文　献

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Prostaglandin E_2–EP_3 signaling suppresses skin inflammation in murine contact hypersensitivity

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Background: Prostaglandin (PG) E_2 exerts a variety of actions through 4 G protein–coupled receptors designated as EP_1, EP_2, EP_3, and EP_4. We have reported that PGE_2 acts on EP_3 in airway epithelial cells and exerts anti-inflammatory actions in ovalbumin-induced murine allergic asthma. Although EP_3 is also expressed in skin and PGE_2 is produced abundantly during skin allergic inflammation, the role of PGE_2-EP_3 signaling in skin allergic inflammation remains unknown.

Objective: We sought to investigate whether PGE_2-EP_3 signaling exerts anti-inflammatory actions in skin allergic inflammation.

Methods: We used a murine contact hypersensitivity (CHS) model and examined the role of EP_3 by using an EP_3-selective agonist, ONO-AE-248 (AE248), and EP_3-deficient mice. The inflammation was evaluated by the thickness and histology of the hapten-challenged ear. Inflammation-associated changes in gene expression and effects of AE248 were examined by means of microarray analysis of the skin. Localization of EP_3 was examined by staining for β-galactosidase knocked in at the EP_3 locus in EP_3-deficient mice. EP_3 action was also examined in cultured keratinocytes.

Results: Administration of AE248 during the elicitation phase significantly suppressed CHS compared with that seen in vehicle-treated mice. Microarray analysis revealed that administration of AE248 inhibited the gene expression of neutrophil-recruiting chemokines, including CXCL1, at the elicitation site. X-gal staining in EP_3-deficient mice revealed EP_3 expression in keratinocytes, which was further confirmed by anti-EP_3 antibody in wild-type mice. In cultured keratinocytes AE248 suppressed CXCL1 production induced by TNF-α.

Conclusion: PGE_2-EP_3 signaling inhibits keratinocytes activation and exerts anti-inflammatory actions in murine CHS. (J Allergy Clin Immunol 2009;124:809-18.)

Key words: *Prostaglandin E_2, EP_3 receptor, contact hypersensitivity*

Murine contact hypersensitivity (CHS) is widely used as a model for contact dermatitis, a common allergic skin disorder of human subjects. The CHS model is composed of 2 phases: the sensitization phase, in which skin dendritic cells take up antigens, migrate to regional lymph nodes, and stimulate T-cell activation and differentiation, and the elicitation phase, in which effector T cells evoke immune inflammation on exposure to antigens.[1] Although the elicitation reaction is known to be mediated by IFN-γ–producing T_H1 cells and T cytotoxic type 1 cells, it is suggested that initial neutrophil infiltration is required for subsequent recruitment of T cells and development of inflammation.[2,3] On exposure to antigens in the elicitation phase, keratinocytes produce neutrophil-recruiting chemokines, such as CXCL1 and CXCL2, as well as T cell–recruiting chemokines, such as CCL17 or CCL27, which contribute to neutrophil recruitment within 12 hours after elicitation and after T-cell infiltration, respectively.[3-5] At an inflammatory site, other than chemokines or cytokines, lipid mediators, such as prostanoids, are produced abundantly, which might regulate CHS responses.[6,7]

Prostanoids, including prostaglandin (PG) D_2, PGE_2, $PGF_{2\alpha}$, PGI_2 (prostacyclin), and thromboxane A_2, are oxygenated metabolites of arachidonic acid produced by sequential catalysis of COX and respective synthases. They are produced in large amounts during inflammation in response to various stimuli and exert a variety of actions, including inflammatory swelling, pain sensation, and fever generation. Prostanoids exert these actions by acting on a family of G protein–coupled receptors, which include PGD receptor, 4 subtypes of PGE receptor (EP_1, EP_2, EP_3, and EP_4), PGF receptor, PGI receptor, and thromboxane A receptor.[8] In addition, another receptor belonging to the chemokine receptor family, CRTH2, also responds to PGD_2. PGE_2 and PGD_2 are abundantly produced in the skin during the elicitation phase of CHS.[6,9] It has been shown that PGD_2 promotes neutrophil infiltration through CRTH2 and contributes to progression of inflammation during elicitation.[9] However, the role of PGE_2 in the elicitation phase has not been fully investigated. Furthermore, if the above action of the PGD_2-CRTH2 signaling is the only PG-mediated action involved in elicitation of a CHS response, nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit COX and suppress PG production would suppress or lessen allergic inflammation in the skin. However, NSAIDs are usually without significant effects on the inflammation of CHS, suggesting the presence of other PG receptor–mediated processes that suppress inflammation.

On the basis of this hypothesis, we have examined the action of PGE_2 in allergic skin inflammation. Among EPs, we focused on

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Abbreviations used
AE248: ONO-AE-248
CHS: Contact hypersensitivity
DNFB: 2,4-Dinitrofluorobenzene
HE: Hematoxylin and eosin
LT: Leukotriene
NSAID: Nonsteroidal anti-inflammatory drug
PG: Prostaglandin
PMN: Polymorphonuclear leukocyte
WT: Wild-type

EP_3 because EP_3 is expressed abundantly in the skin[10,11] and mediates suppression of allergic inflammation in the murine allergic asthma model.[12] Although EP_3 has been reported to have both proinflammatory and anti-inflammatory roles in patients with acute skin inflammation,[13,14] the role of EP_3 signaling in allergic skin inflammation has not been investigated. Here we used an EP_3-selective agonist and EP_3-deficient *(Ptger3*$^{-/-}$*)* mice and examined whether PGE_2-EP_3 signaling has anti-inflammatory action during the elicitation phase of CHS.

METHODS

Materials

Female 8- to 12-week-old C57BL/6 mice (Japan SLC, Shizuoka, Japan) and mice lacking EP_3 that were backcrossed to a C57BL/6 background for more than 10 generations[15] were used. Mice were bred at the Institute of Laboratory Animals of Kyoto University on a 12-hour light/dark cycle under specific pathogen-free conditions. All experimental procedures were approved by the Committee on Animal Research of Kyoto University Faculty of Medicine. The EP agonists ONO-DI-004 (EP_1 agonist), ONO-AE1-259 (EP_2 agonist), ONO-AE-248 (AE248; EP_3 agonist), and ONO-AE1-329 (EP_4 agonist) were kindly provided by Ono Pharmaceutical Co (Osaka, Japan). The structures, ligand-binding affinities and selectivities, and pharmacokinetic properties of each EP agonist were described.[8] 2,4-Dinitrofluorobenzene (DNFB) was purchased from Nacalai Tesque (Kyoto, Japan). Indomethacin was purchased from Sigma (St Louis, Mo).

CHS experiment

CHS was induced as previously described.[16] Briefly, mice were shaved and painted on the abdomen with 25 μL of 0.5% DNFB in acetone/olive oil (4:1). Five days later, the mice were challenged by painting with 10 μL of 0.3% DNFB on both sides of the ear. Ear thickness was measured with a thickness gage (Teclok, Nagano, Japan) before and 24 hours after the challenge, and the difference was used as a parameter of ear swelling. AE248 was diluted with saline or acetone and administered either subcutaneously in the dorsal skin or topically applied to the ear 3 times a day (30 minutes before and 3 and 8 hours after the DNFB challenge, respectively) at indicated doses. For repeated DNFB application, mice were sensitized first by means of topical application of 20 μL of 0.15% DNFB to both ears and challenged with 20 μL of 0.15% DNFB on both ears once a week for 4 weeks. Vehicle (acetone) or indomethacin (0.2 mg/mL in acetone, 20 μL per ear) was applied 30 minutes before each challenge.

Bone marrow transplantation

Bone marrow cells were taken from femurs from wild-type (WT) or *Ptger3*$^{-/-}$ donor mice and transplanted to recipient WT green fluorescent protein transgenic mice (2×10^6 cells for each mouse from the tail vein) irradiated with 8 Gy. Four weeks after transplantation, more than 97% of whole blood cells were reconstituted with donor-derived cells, which was confirmed by analyzing the expression of green fluorescent protein–positive cells among blood cells with flow cytometry, and we used those mice for experiments.

Histology

Ears were isolated 24 hours after elicitation, fixed in 10% formalin, and embedded in paraffin. Sections of 7 μm in thickness were prepared and stained with hematoxylin and eosin (HE). The number of neutrophils per a ×40 field was determined in 4 randomly chosen fields, and the average counts were determined. For EP_3 localization, X-gal staining was performed as previously described.[12] The sections were then counterstained with HE or anti-keratin 5 antibody (R&D Systems, Minneapolis, Minn). For staining of EP_3, the rabbit polyclonal antibody reactive with murine EP_3 (Cayman, Ann Arbor, Mich) was used as previously described.[17]

Real-time RT-PCR

Total RNA was obtained from keratinocytes of murine ear skin by using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Complementary DNA was synthesized with Superscript III (Invitrogen, Carlsbad, Calif). The amount of mRNA for CXCL1 and glyceraldehyde-3-phosphate dehydrogenase was quantified by means of real-time RT-PCR with the LightCycler 2.0 (Roche Diagnostic, Foster City, Calif). The primer sequences of glyceraldehyde-3-phosphate dehydrogenase were previously described.[18] Primers used for CXCL1 were 5′-GCC TAT CGC CAA TGA GC-3′ (forward) and 5′-TGG ACA ATT TTC TGA ACC AAG-3′ (reverse). Data were analyzed by using LightCycler Software Version 4.0.

Keratinocyte culture and ELISA

Normal human epidermal keratinocytes were obtained from Kurabo (Okayama, Japan) and cultured in Humedia KG2 medium (Kurabo). Cells in the third passage were seeded in triplicate at 5×10^4 cells/well onto 24-well plates in 0.5 mL of Humedia KB2 and cultured for 24 hours. The cells were washed, incubated with 10 μmol/L AE248 for 15 minutes, and then incubated with 10 ng/mL TNF-α in the continued presence of AE248 in Humedia KB2 containing 1 μmol/L indomethacin for 6 hours. The supernatant was collected, and the amount of CXCL1 was determined by means of ELISA (R&D Systems).

DNA microarray analysis

Total RNA was prepared from DNFB-challenged ears by using TRIzol reagent (Invitrogen) and purified by using the RNeasy Mini Kit (Qiagen), and 3.5 μg of purified RNA was used for microarray analysis with a Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, Calif), according to the manufacturer's protocol. Data were analyzed by using Statistical Algorithm with the Affymetrix GeneChip Expression Analysis software (Microarray Suite 5.0). All microarray data are deposited in Gene Expression Omnibus (GEO).

Statistics

Data were expressed as means ± SEMs, and statistical analyses were performed by means of ANOVA or the Student *t* test, as appropriate. A P value of less than .05 was considered statistically significant.

RESULTS

Effect of EP_3 agonist on the elicitation phase of CHS

We first examined whether stimulation of EP_3 had an anti-inflammatory effect on CHS. To investigate this, we administered an EP_3 agonist CAE248, 100 μg/kg subcutaneously 3 times a day during the elicitation phase. This dose of AE248 exerts a significant effect *in vivo*.[12] The DNFB challenge caused ear swelling in both vehicle-treated and AE248-treated mice. However, the mice treated with AE248 showed significant reductions in swelling

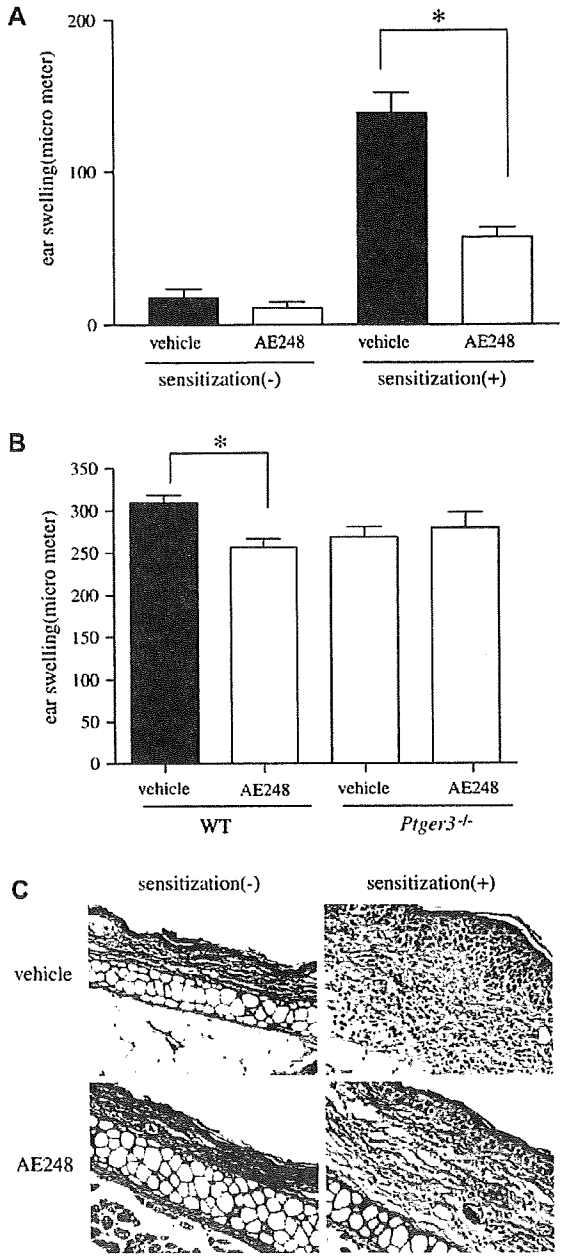

FIG 1. Suppressive effects of EP_3 agonist on the ear-swelling response in mice with CHS. A and B, Results are expressed as means ± SEMs (n = 5 in both groups). Data are representative of 3 experiments. C, HE staining of control- and DNFB-challenged ears treated with either vehicle or EP_3 agonist. Representative samples of each group are shown.

compared with that seen in the vehicle-treated mice 24 hours after elicitation (Fig 1, *A*). This suppressive effect of AE248 was completely absent in *Ptger3*$^{-/-}$ mice (Fig 1, *B*), suggesting that the effect was elicited through the EP_3 receptor. Histology of the ear from sensitized mice showed edema and marked inflammatory cell infiltration in the dermis 24 hours after elicitation (Fig 1, *C*). Consistently, the extent of the edema and inflammatory cell infiltration was markedly reduced in the AE248-treated mice compared with that seen in the vehicle-treated mice. These findings together demonstrate that EP_3 stimulation in the elicitation phase elicits suppressive effect on CHS.

We next examined the localization of EP_3 in the normal murine ear. X-gal staining was performed in *Ptger3*$^{-/-}$ mice, in which the β-galactosidase gene was knocked in at the EP_3 gene locus. Positive signals were detected mostly in the basal layer of epidermis in the skin of control mice (Fig 2, *A*). Similar signals were also observed in the ears of mice after elicitation, whereas little signals were detected in the cells infiltrating the dermis (data not shown). These findings suggest that the main cell species expressing EP_3 in the skin is keratinocytes and that they express it constitutively. To examine the EP_3 expression in keratinocytes of the basal layer, we costained for keratin 5, a specific marker of basal keratinocytes, and found that signals for keratin 5 colocalized with those of the X-gal staining (Fig 2, *A*). EP_3 expression in keratinocytes was confirmed by means of immunohistochemical analysis with anti-EP_3 antibody in WT mice (Fig 2, *B*). These results, together with our finding that little X-gal staining was detected in lymph nodes (data not shown), suggest a possibility that AE248 acts on keratinocytes and not on immune cells to exert its anti-inflammatory actions. Therefore we next examined the effects of topical application of AE248 to the ear in CHS. AE248 was dissolved in acetone and topically applied to the ear 3 times in the elicitation phase. This topical application of AE248 showed significant dose-dependent suppression of ear swelling in CHS 24 hours after elicitation (Fig 2, *C*), whereas that of agonists specific to other EP subtypes was without effect (Fig 2, *D*). Administration of AE248 showed a suppressive effect 24, 48, and 72 hours after elicitation, suggesting that the effect of AE248 did not induce just the delay in the development of inflammation (Fig 2, *E*). To confirm that the effect of AE248 was not caused by immune cells, we made bone marrow chimera in which stromal cells, such as keratinocytes, express EP_3, whereas bone marrow–derived cells do not express EP_3, as described in the Methods section. A suppressive effect of AE248 on ear swelling in CHS of the bone marrow chimera was detected (Fig 2, *F*), which supports our hypothesis that AE248 acts on EP_3 in keratinocytes to exert an anti-inflammatory effect.

Reduced expression of genes related to inflammatory cell infiltration caused by topical treatment with AE248

Various inflammation-related genes, including those for chemokines, are upregulated during the elicitation phase of CHS.[5] We therefore compared gene expression between vehicle-treated control mice and mice treated with AE248 to examine the role of EP_3 in this process. We first examined the time course of gene expression during the elicitation phase in our model. Ears challenged with DNFB were isolated at 1, 3, 6, 12, and 24 hours after elicitation for microarray analysis by using an Affymetrix Mouse Genome 430 2.0 GeneChip that contains 45,101 genes. We screened for gene expression, which exhibited a more than 2-fold increase at any given time during the elicitation phase over basal expression at 0 hours (Table I). Among the genes with increased expression, we focused on chemokine genes. At 1 hour after challenge, 130 genes were detected as genes showing a more than 2-fold increase in expression, and none of chemokine genes was among those genes. At 3 hours, 263 genes were upregulated, and 4 kinds of chemokines were included in this group. The analysis similarly picked up 408 genes with 5 kinds of chemokine genes at 6 hours, 655 genes with 8 kinds of chemokine genes at 12 hours, and 902 genes with 14 kinds of chemokine

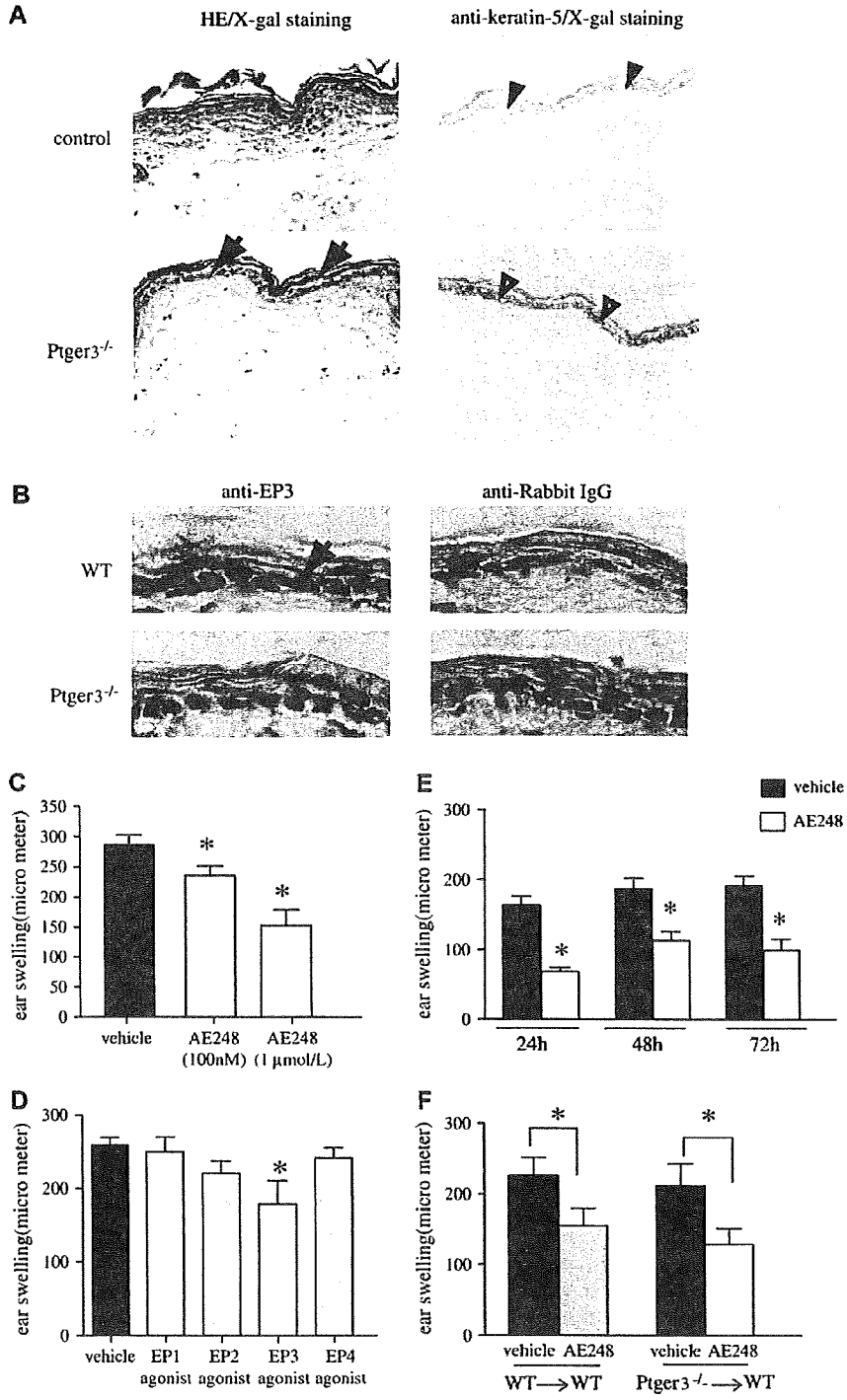

FIG 2. Localization of EP_3 receptors and effect of topical application of EP_3 agonist on mice with CHS. A, Histochemical staining for EP_3 (X-gal) counterstained with HE or anti-keratin 5 antibody. *Arrows*, Positive signaling (blue); *black arrowheads*, positive staining of keratin 5; *white arrowheads*, colocalization of positive signals in X-gal and anti-keratin 5 staining. B, Immunohistologic analysis for EP_3. The *arrow* indicates positive signals. C-E, Suppressive effects of topical administration of EP_3 agonist and effects of various EP agonists (1 μmol/L) on murine CHS (n = 5 per group [Fig 2, *C*] and n = 4 per group [Fig 2, *D* and *E*]). Data are representative of 2 experiments. F, Effect of AE248 on murine CHS of bone marrow chimera (n = 13-15 per group). Results are a combination of 3 independent experiments.

TABLE I. Time course of chemokine-related genes with expressions upregulated more than 2-fold in the elicitation phase

Time (h)	No. of genes	Gene title	Gene symbol	Probe set ID	Change ratio
1	130				
3	263	Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1419209_at	2.2
		Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1441855_x_at	1
		Chemokine (C-X-C motif) ligand 2	*Cxcl2*	1449984_at	1.3
		Chemokine (C-C motif) ligand 7	*Ccl7*	1421228_at	1.3
		Chemokine (C-C motif) ligand 20	*Ccl20*	1422029_at	1.2
6	408	Chemokine (C-C motif) ligand 17	*Ccl17*	1419413_at	2.6
		Chemokine (C-C motif) ligand 20	*Ccl20*	1422029_at	2.6
		Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1419209_at	2.5
		Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1457644_s_at	1
		Chemokine (C-X-C motif) ligand 16	*Cxcl16*	1449195_s_at	1.2
		Chemokine (C-C motif) ligand 9	*Ccl9*	1417936_at	1
12	655	Chemokine (C-X-C motif) ligand 9	*Cxcl9*	1456907_at	4.5
		Chemokine (C-X-C motif) ligand 9	*Cxcl9*	1418652_at	2.9
		Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1419209_at	3.8
		Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1441855_x_at	2.2
		Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1457644_s_at	2.1
		Chemokine (C-X-C motif) ligand 16	*Cxcl16*	1449195_s_at	1.4
		Chemokine (C-C motif) ligand 9	*Ccl9*	1417936_at	1.2
		Chemokine (C-X-C motif) ligand 10	*Cxcl10*	1418930_at	2.8
		Chemokine (C-C motif) ligand 20	*Ccl20*	1422029_at	2.4
		Chemokine (C-X-C motif) ligand 2	*Cxcl2*	1449984_at	1.7
		Chemokine (C-C motif) ligand 2	*Ccl2*	1420380_at	1.2
24	902	Chemokine (C-X-C motif) ligand 9	*Cxcl9*	1418652_at	5.2
		Chemokine (C-X-C motif) ligand 9	*Cxcl9*	1456907_at	5.1
		Chemokine (C-X-C motif) ligand 10	*Cxcl10*	1418930_at	4
		Chemokine (C-C motif) ligand 17	*Ccl17*	1419413_at	2.5
		Chemokine (C-C motif) receptor 1	*Ccr1*	1419609_at	2.4
		Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1419209_at	2.2
		Chemokine (C-C motif) ligand 12	*Ccl12*	1419282_at	2.1
		Chemokine (C-C motif) ligand 2	*Ccl2*	1420380_at	2
		Chemokine (C-X-C motif) ligand 2	*Cxcl2*	1449984_at	1.8
		Chemokine (C-C motif) ligand 9	*Ccl9*	1448898_at	1.4
		Chemokine (C-C motif) ligand 9	*Ccl9*	1417936_at	1.2
		Chemokine (C-C motif) receptor 2	*Ccr2*	1421186_at	1.3
		Chemokine (C-C motif) ligand 7	*Ccl7*	1421228_at	1.3
		Chemokine (C-C motif) ligand 19	*Ccl19*	1449277_at	1.3
		Chemokine (C-X-C motif) ligand 16	*Cxcl16*	1449195_s_at	1.2
		Chemokine (C-C motif) ligand 8	*Ccl8*	1419684_at	1

genes at 24 hours. Because these results show the most prominent change in gene expression at 24 hours after elicitation, we chose this time and compared gene expression in the ears of vehicle-treated mice with that in ears of AE248-treated mice (Fig 3). Among the 902 genes, the signal intensity of 178 genes was significantly decreased in the AE248-treated group compared with that seen in the vehicle-treated group (cluster A), and signal intensity of 183 genes was significantly increased in the AE248-treated group (cluster B). The signal intensity of the other 541 genes was not significantly different between the groups. As for chemokine genes of significant signal intensity, cluster A includes genes for 3 chemokines: CXCL1, CXCL9, and CXCL16. Among them, expression of CXCL1 was most strongly suppressed by the AE248 treatment; the signal intensity decreased to 52% compared with the control intensity (Table II), and the average intensity of the other 2 chemokine genes, CXCL9 and CXCL16, decreased to 62% and 69%, respectively, compared with the control intensity. On the other hand, 4 chemokine genes, CCL19, CCL9, CCL8, and CCL12, were detected in cluster B. The average signal intensity of CCL19, CCL9, CCL8, and CCL12 in the AE248-treated group was 129%, 268%, 368%, and 404%, respectively, of that in the vehicle-treated group. Thus the treatment with AE248 did not decrease all of the chemokines upregulated 24 hours after the challenge. However, it nonetheless suppressed the inflammatory response in CHS, indicating that suppression of the expression of the chemokine genes in cluster A (ie, CXCL1, CXCL9, and CXCL16) has an important role in the CHS response. CXCL1 binds to CXCR2 and is one of the strong neutrophil-attracting chemokines. CXCL9 recruits T_H1 cells by binding cell-surface CXCR3 and contributes to the development of CHS.[5] CXCL16 is known to bind and activate the chemokine receptor CXCR6, which is expressed on T cells and natural killer T cells.[19] Given the critical role of CXCL1 and neutrophils in the development of CHS,[2,3] we further examined the roles of CXCL1 and neutrophils in CHS. We performed real-time RT-PCR analysis and confirmed that the expression of CXCL1 mRNA was

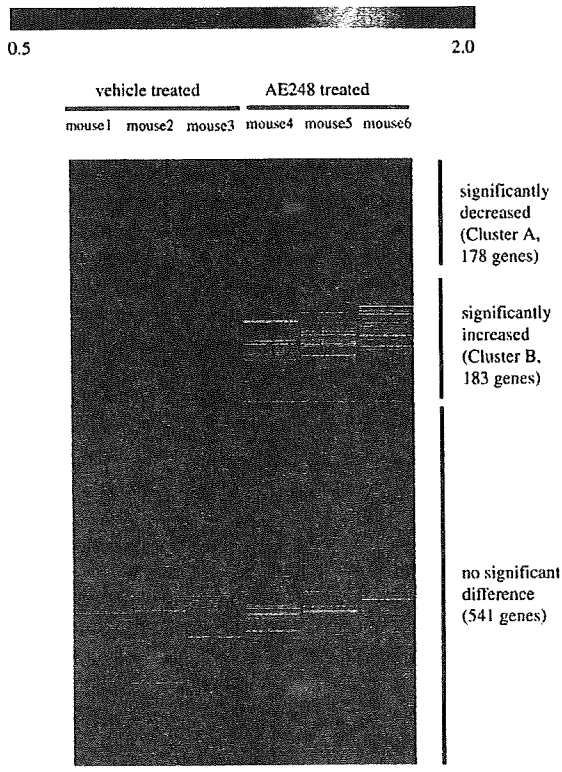

FIG 3. Microarray analysis for the effect of AE248 on gene expressions. Genes with a signal intensity that increased more than 2-fold in 24 hours at elicitation from baseline are selected (total of 902 genes). The signal intensity of each gene in the AE248-treated group (n = 3) was compared with the average signal intensity of each gene in the vehicle-treated group (n = 3), and the change ratio was indicated by means of color gradation.

significantly decreased in the AE248-treated group compared with that seen in the vehicle-treated group (data not shown). CXCL1 is produced mainly by keratinocytes in the elicitation phase of CHS. To confirm whether differences in gene expression result from differences in cell composition rather than from changes in gene expression in keratinocytes, we next examined the effect of AE248 on the mRNA expression of CXCL1 in keratinocytes from murine ear skin. We purified keratinocytes as shown in the Methods section of this article's Online Repository at www.jacionline.org. We then examined CXCL1 mRNA expression in purified keratinocytes and found that the AE248-treated group had significantly lower CXCL1 mRNA expression compared with that seen in the vehicle-treated group (Fig 4, *A*). We further examined the effect of AE248 on the production of CXCL1 by cultured keratinocytes *in vitro*. We found that keratinocytes activated with TNF-α produced a significant amount of CXCL1, and the administration of AE248 significantly suppressed the CXCL1 production (Fig 4, *B*). We next compared neutrophil (polymorphonuclear leukocytes [PMNs]) infiltration in the ears of the vehicle-treated and AE248-treated mice, as shown in the Methods section. We detected a number of PMNs in the dermis of the ear 24 hours after elicitation, whereas only a few cells were detected in the dermis of control animals. We also detected PMNs in the ears of the AE248-treated mice. However, the number of PMNs was significantly reduced in the AE248-treated mice compared with that seen in the vehicle-treated group (Fig 4, *C*).

Because the average signal intensity of CXCL2 in microarray analysis, which is another important chemokine for neutrophil recruitment, was 44% of the vehicle-treated group in the AE248-treated group, we examined the CXCL2 mRNA expression in purified keratinocytes. We found that the AE248-treated group had significantly lower CXCL2 mRNA expression compared with that seen in the vehicle-treated group (see Fig E1 in this article's Online Repository at www.jacionline.org). These results combined together suggest a possibility that EP_3 exerts its anti-inflammatory effect by acting directly on keratinocytes and inhibiting neutrophil infiltration through downregulation of neutrophil-recruiting chemokines.

Involvement of endogenous PGE_2-EP_3 signaling in the development of CHS

We next examined the involvement of endogenous PG signaling in the development of CHS by applying indomethacin topically to the elicitation site. Because the effects of indomethacin were hard to detect in the usual CHS protocol (data not shown), we adopted the repetitive challenge model of CHS (repeated-challenge CHS) and examined the effect of indomethacin on the model as in the Methods section. Mice treated with indomethacin showed significantly increased ear swelling compared with the vehicle-treated mice (Fig 5, *A*), suggesting that endogenous PG produced locally in the challenged skin plays a suppressive role in inflammation of repeated-challenge CHS. We next examined the involvement of PGE_2-EP_3 signaling in this process by subjecting *Ptger3*$^{-/-}$ mice to repeated-challenge CHS. Similarly to the indomethacin-treated mice, *Ptger3*$^{-/-}$ mice exhibited significantly increased ear swelling compared with that of WT mice, and this enhancement continued 72 hours after elicitation (Fig 5, *B*). HE staining showed increased inflammatory cell infiltration in *Ptger3*$^{-/-}$ mice compared with that seen in WT mice (Fig 5, *C*). These results suggest that PGE_2-EP_3 signaling functions endogenously to negatively modulate the development of repeated-challenge CHS.

DISCUSSION

In the present study we have made the following findings. First, systemic administration of AE248, an EP_3 agonist, during the elicitation phase, can suppress inflammation in mice with CHS. Data of X-gal staining and immunohistochemical analysis demonstrated predominant EP_3 expression in keratinocytes, and topical application of AE248 to the ear skin resulted in suppression of CHS. Microarray analyses revealed that administration of AE248 modulates CHS-induced gene expression in the lesional skin either way; a decrease and an increase were demonstrated in clusters A and B, respectively. Among the chemokine genes regulated by AE248, CXCL1 was the most strongly suppressed. Consistently, AE248 suppressed CXCL1 production by TNF-α–activated keratinocytes *in vitro*. Finally, local treatment with indomethacin or the loss of EP_3 exacerbated inflammatory response to the repeated-challenge CHS. These results suggest that endogenous PGE_2 acts on EP_3 in keratinocytes *in situ* in the skin to modulate the extent of inflammation of CHS and that stimulation of PGE_2-EP_3 signaling with exogenously added agonist can control allergic inflammation in the skin.

The importance of keratinocytes in inflammatory skin diseases, such as contact dermatitis,[2,4] atopic dermatitis,[20] and psoriasis,[21,22]

TABLE II. Top 20 genes with signal intensities that were significantly decreased in AE248-treated mice compared with those in vehicle-treated mice

Gene title	Gene symbol	Probe set ID	Signal intensity		Percentage of AE248/vehicle
			Vehicle	AE248	
Solute carrier family 26, member 4	*Slc26a4*	1419725_at	2431	664.6	27.3
Matrix metallopeptidase 10	*Mmp10*	1420450_at	3371	1152	34.2
PG-endoperoxide Synthase 2	*Ptgs2*	1417262_at	3377	1345	39.8
SH2 domain containing 5	*Sh2d5*	1436100_at	2729	1118	41
Small proline-rich protein 2I	*Sprr2i*	1422963_at	13751	6136	44.6
Serine (or cysteine) peptidase inhibitor, clade E, member 1	*Serpine1*	1419149_at	801.6	359	44.8
Interferon-activated gene 202B	*Ifi202b*	1457666_s_at	15766	7122	45.1
Solute carrier family 29 (nucleoside transporters), member 2	*Slc29a2*	1447748_x_at	359.4	166.6	46.4
Fos-like antigen 1	*Fosl1*	1417487_at	3827	1785	46.6
IL-6	*Il6*	1450297_at	2010	955.7	47.6
Heparin-binding EGF-like growth factor	*Hbegf*	1418349_at	3090	1490	48.2
Cardiotrophin-like cytokine factor 1	*Clcf1*	1437270_a_at	1047	530.9	50.7
Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1419209_at	4085	2131	52.2
Nucleolar complex–associated 2 homolog (*S cerevisiae*)	*Noc2l*	1424323_at	1638	867.1	52.9
RIKEN cDNA 2310002A05 gene /// hypothetical protein LOC630971	*2310002A05Rik /// LOC630971*	1456248_at	25273	13462	53.3
Similar to late cornified envelope protein	*LOC545548*	1456001_at	19223	10243	53.3
Sulfiredoxin 1 homolog (*S cerevisiae*)	*Srxn1*	1426875_s_at	4664	2525	54.1
Defensin β 3	*Defb3*	1421806_at	17218	9328	54.2
Serine (or cysteine) peptidase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 9	*Serpina9*	1429285_at	1362	750.5	55.1
RIKEN cDNA 2310007F04 gene	*2310007F04Rik*	1429641_x_at	9952	5505	55.3

The average signal intensity of each gene was compared between vehicle-treated mice and AE248-treated mice.
EGF, Epidermal growth factor.

has now received much attention. In CHS keratinocytes produce various chemokines and regulate inflammatory cell infiltration. For example, it was previously shown that blockade of CCL27, which is produced from keratinocytes and attracts memory T cells to the skin, reduced T-cell infiltration and led to the suppression of CHS,[4] and inhibition of CCL8 produced from keratinocytes suppressed CHS.[22] In this study we found that EP_3 is expressed in keratinocytes and that administration of EP_3 agonist suppresses CXCL1 mRNA expression and its production in keratinocytes. CXCL1 is a strong attractant of neutrophils and is produced mainly by keratinocytes in the elicitation phase of CHS.[2,3] Recently, it has been reported that infiltration of neutrophils is required for the development of inflammation in CHS.[2] Depletion of neutrophils in hapten-challenged mice decreased the number of IFN-γ–producing T cells at elicitation sites, which resulted in the inhibition of CHS response, and injection of neutrophils into ears restored the CHS response.[2,3] Administration of anti-CXCL1 serum in the elicitation phase significantly inhibited the neutrophil infiltration in the challenged ear and suppressed CHS.[3] Furthermore, it has been reported that corticosteroids exert their anti-inflammatory effects in contact dermatitis mainly by targeting neutrophils and macrophages.[23] These findings are consistent with our results described above and suggest that one of the anti-inflammatory effects of AE248 is through EP_3-mediated downregulation of CXCL1

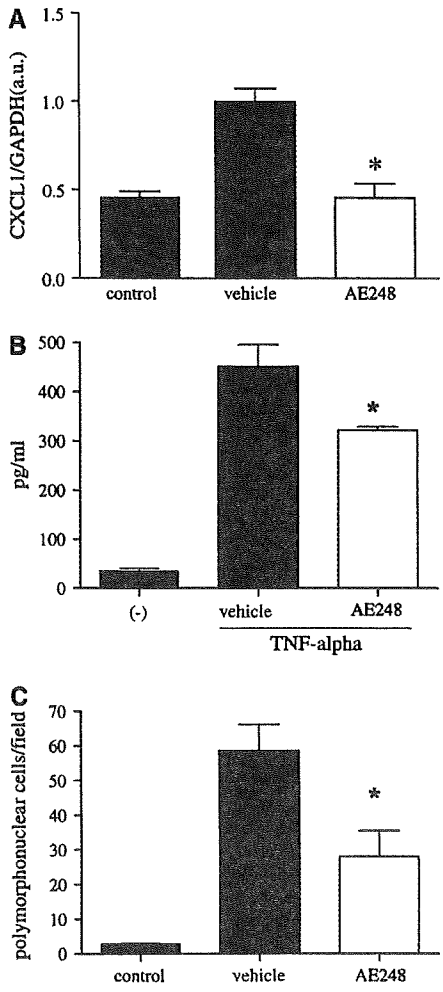

FIG 4. Effect of AE248 on CXCL1 production and neutrophil accumulation at the elicitation site. A, Real-time RT-PCR analysis on mRNA expression of CXCL1 in the keratinocytes in hapten-challenged ears of vehicle- or AE248-treated mice (n = 3 per group). B, ELISA analysis of CXCL1 production from cultured keratinocytes (n = 3 per group). Results are expressed as means ± SEMs and are representative of 3 independent experiments. C, Number of polymorphonuclear cells in the skin (n = 4 per group). *$P < .05$ versus the vehicle-treated group.

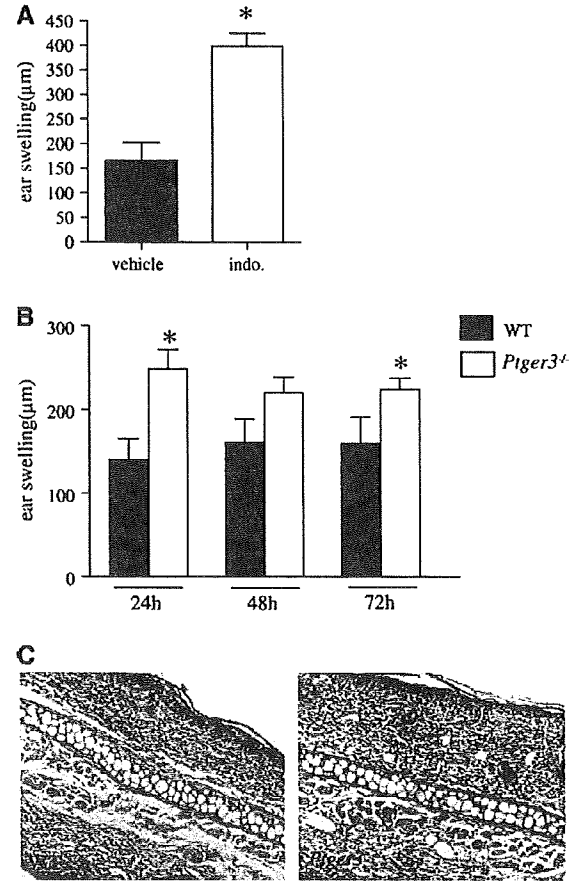

FIG 5. Enhanced inflammation of indomethacin *(indo)*-treated mice or *Ptger3* $^{-/-}$ mice in repeated-challenge CHS. A, Effect of topical administration of indomethacin on murine CHS (n = 4 per group). B, Increased ear swelling of *Ptger3* $^{-/-}$ mice in the CHS group (n = 4 per group). *$P < .05$ versus WT mice. Data are representative of 3 independent experiments. C, Representative HE staining of ear skin in WT and *Ptger3* $^{-/-}$ mice with repeated-challenge CHS at 24 hours after the fourth challenge.

production in keratinocytes and inhibition of neutrophil infiltration in the skin. On the other hand, our study showed that the EP_3 stimulation might have an effect on other chemokines opposite to that expected from the previous studies. For example, the expression of CCL8 was increased more than 3-fold in the AE248-treated group. In addition, we did not detect significant expression of CCL27 in our model. Given the finding that inhibition of CCL8 leads to suppression of inflammation, as described above, it is intriguing that EP_3 stimulation can reduce the inflammatory reaction in spite of such enhanced CCL8 gene expression.Unraveling this apparent discrepancy might help to reveal intricate relations among chemokines in inducing skin inflammation and define more correctly how EP_3 modulates their interaction. Taken together, chemokines from keratinocytes contribute much to the inflammation of CHS, and regulation of their production can be a useful strategy for the treatment of allergic dermatitis.

It should mentioned here that EP_3 is also expressed in mast cells in skin[24] and that mast cells can regulate the elicitation of CHS.[25,26] Indeed, expression of CXCL2 mRNA, which mast cells produce and was reported to promote neutrophil infiltration in elicitation sites, decreased to 44% of the control expression with EP_3 stimulation, although the difference was not statistically significant. However, we found that CXCL2 mRNA expression was significantly decreased in AE248-treated ears on purified keratinocytes. In addition, data of bone marrow chimera experiments indicate that most of the effect of AE248 was through stromal cells, suggesting that the anti-inflammatory effect of EP_3 derived from its action on keratinocytes.

EP_3 has 3 splice variants in the mouse, and 8 splice variants in human subjects,[27] among which at least 3 EP_3 variants are expressed in human keratinocytes.[10] They can couple Gs, Gi, and Gq and use cyclic AMP or Ca^{2+} as second messengers. EP_3 signaling can use ceramide as a second messenger in keratinocytes.[10] Our study does not clarify which signaling mechanism of EP_3 is responsible for its anti-inflammatory effect. The fact that a small dose of AE248 can induce anti-inflammatory effects is consistent with the Gi pathway because the cyclic AMP decrease mediated by Gi occurs at lower agonist

concentrations than other signaling. Indeed, the CB1 cannabinoid receptor that couples to Gi was recently reported to act on keratinocytes and suppress CHS inflammation.[28] However, this article reports that stimulation of CB1 inhibits CCL8 production from keratinocytes, which, as described, is opposite to our findings. These results indicate that multiple signaling pathways might function downstream of EP_3. It was shown recently that the Ca^{2+} signaling from EP_3 can inhibit nuclear factor κB activation in keratinocytes,[29] which is consistent with regulation of expression of CXCL1 mRNA by nuclear factor κB activity.[30] The precise molecular mechanisms underlying the anti-inflammatory effect of EP_3 remain to be elucidated.

Our results might also explain the anti-inflammatory effect of PGE_2 in skin inflammation and the adverse effect of NSAIDs in inflammatory skin diseases, such as psoriasis. Psoriasis is one of the inflammatory skin diseases, and constitutive activation of keratinocytes has been suggested as one of its causes.[21,22] Histologically, neutrophil infiltration in the epidermis is one of its characteristic features. Although PGE_2 is generally considered an inflammatory mediator by increasing vasodilation and edema formation, the anti-inflammatory effect of PGE_2 has been suggested in patients with psoriasis[31] or in animal models of neutrophil infiltration in skin.[32] On the other hand, administration of NSAIDs sometimes causes the exacerbation of psoriasis.[33,34] Thus far, the increase in levels of leukotrienes (LTs), such as LTB_4, a strong chemoattractant for neutrophils or T cells, has been suggested as one of its causative mechanisms.[33,34] Because arachidonic acid is used by both COX and lipoxygenase and NSAIDs block only COX activity, the use of NSAIDs might divert arachidonate metabolism to the lipoxygenase pathway, which leads to the increase of LTB_4. Such an argument was also made in aspirin-induced asthma. However, our study appears against such diversion mechanism. Alternatively, the PGE_2 pathway somehow modulates LT production. Our results suggest that one of the therapeutic effects of PGE_2 and an adverse effect of NSAIDs in skin inflammation is through modulation of PGE_2-EP_3 signaling.

In conclusion, stimulation of EP_3 signaling suppresses skin inflammation in CHS. Regulation of EP_3 signaling and keratinocyte function might be a novel approach for the treatment of skin inflammation, including allergy.

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Clinical implications: EP_3 in keratinocytes can be a target for the treatment of allergic skin inflammation.

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