HLA-DQB1*03:01 as a Biomarker for Genetic Susceptibility to Bullous Pemphigoid Induced by DPP-4 Inhibitors

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TO THE EDITOR

Dipeptidyl peptidase-4 inhibitor (DPP-4i) has been widely used to treat type 2 diabetes. DPP-4 inactivates incretins by catalyzing the cleavage of those proteins to inactive forms (Drucker, 2007). DPP-4i works by inhibiting the action of this enzyme and improves glycemic control (Aschner and Kipnes, 2006). DPP-4i has been known as a safe drug; however, an increased risk of bullous pemphigoid (BP) during DPP-4i exposure has been reported in diabetic patients administered DPP-4i (Béné et al., 2016).

BP is the most common autoimmune blistering disorder, and it is characterized by itchy edematous erythema and tense blisters on the whole body. It is mainly caused by autoantibodies to a major hemidesmosomal component at the dermal-epidermal junction of the skin, type XVII collagen (COL17 or BP180). The noncollagenous 16A (NC16A) domain of COL17 contains a major pathogenic epitope (Giudice et al., 1993). Although several factors have been reported as triggers of BP, the etiology of BP remains largely unknown.

The exact mechanism behind the association of DPP-4i exposure and BP has yet to be elucidated. Because several studies have reported an association between HLAs and drug-induced reactions (Chung et al., 2004; Wang et al., 2013), we examined HLA alleles in Japanese patients with BP who had been taking DPP-4i for type 2 diabetes for at least 3 months before BP onset (DPP-4i-BP). We recently reported that DPP-4i-BP tends to show a noninflammatory phenotype with few erythematous lesions, in sharp contrast to conventional BP unrelated to DPP-4i intake (Izumi et al., 2016). We encountered 30 patients with DPP-4i-BP in the last 3 years and found that most patients (21/30) showed the noninflammatory phenotype (Figure 1a). Based on the scores for erythema/urticaria in the bullous pemphigoid disease area index (BPDAI) (Murrell et al., 2012), DPP-4i-BP was clearly divided into two groups, inflammatory (BPDAI: erythema/urticaria $\geq$ 10) and noninflammatory (BPDAI: erythema/urticaria $<$ 10) (Figure 1b), and the clinical appearance of the patients with noninflammatory disease was distinct from that of those with conventional BP (Figure 1a). BPDAI scores for erosions/blisters showed no significant difference between DPP-4i-BP and conventional BP patients (Figure 1c). The antibody titers to full-length COL17 were similar between the two groups (Figure 1d), whereas those to the NC16A domain of COL17 were significantly lower in the noninflammatory DPP-4i-BP patients (Figure 1e). Histologically, eosinophil counts in the upper dermis of periblister lesions were significantly lower in noninflammatory DPP-4i-BP than in inflammatory DPP-4i-BP (Figure 1f). From these findings, we considered this unique noninflammatory subgroup to be distinct from inflammatory DPP-4i-BP and conventional BP, and this study focuses on this subgroup (Figure 1b red square [blue dots], and see Supplementary Tables S1 and S2 online). The collection of human samples was approved by the local ethics committee and the institutional review board of Hokkaido University and Keio University and by the research ethics committee of RIKEN. Written informed patient consent was obtained from the patients.

Surprisingly, 86% (18/21) of noninflammatory DPP-4i-BP patients in our sample carry HLA-DQB1*03:01 (Table 1). The frequencies of carriers of alleles HLA-DQB1*03:01, -DQA1*05:05, -DRB1*11:01, and -DRB1*12:01 were significantly higher, and those of carriers of alleles HLA-DQA1*01:03 and -DQB1*06:01 were significantly lower, in DPP-4i-BP than in Japanese general population control individuals (Table 1, and see Supplementary Tables S3–S9 online). We also compared the six HLA alleles in conventional BP patients with those in Japanese general population control individuals and found that none of those alleles was significantly different (Table 1). We next compared the six alleles in DPP-4i-BP with those in DPP-4i–tolerant patients with type 2 diabetes who were exposed to DPP-4i for at least 2 years (see Supplementary Table S10 online) and found that the frequencies of carriers of alleles HLA-DQB1*03:01 and -DRB1*12:01 were significantly higher in DPP-4i-BP (Table 1). These findings clearly show that the two alleles are significantly associated with DPP-4i-BP but not with conventional BP nor with type 2 diabetes. HLA-DQB1*03:01 was present in 19 (31%) of the 61 DPP-4i–tolerant control individuals, suggesting that this allele has 86% sensitivity and 69% specificity when we apply HLA-DQB1*03:01 as a risk predictor for noninflammatory DPP-4i-BP in the Japanese population. In addition to the allele frequencies, the two- or three-locus haplotype frequencies for

Abbreviations: BP, bullous pemphigoid; BPDAI, Bullous Pemphigoid Disease Area Index; COL17, type XVII collagen; DPP-4i, dipeptidyl peptidase-4 inhibitor

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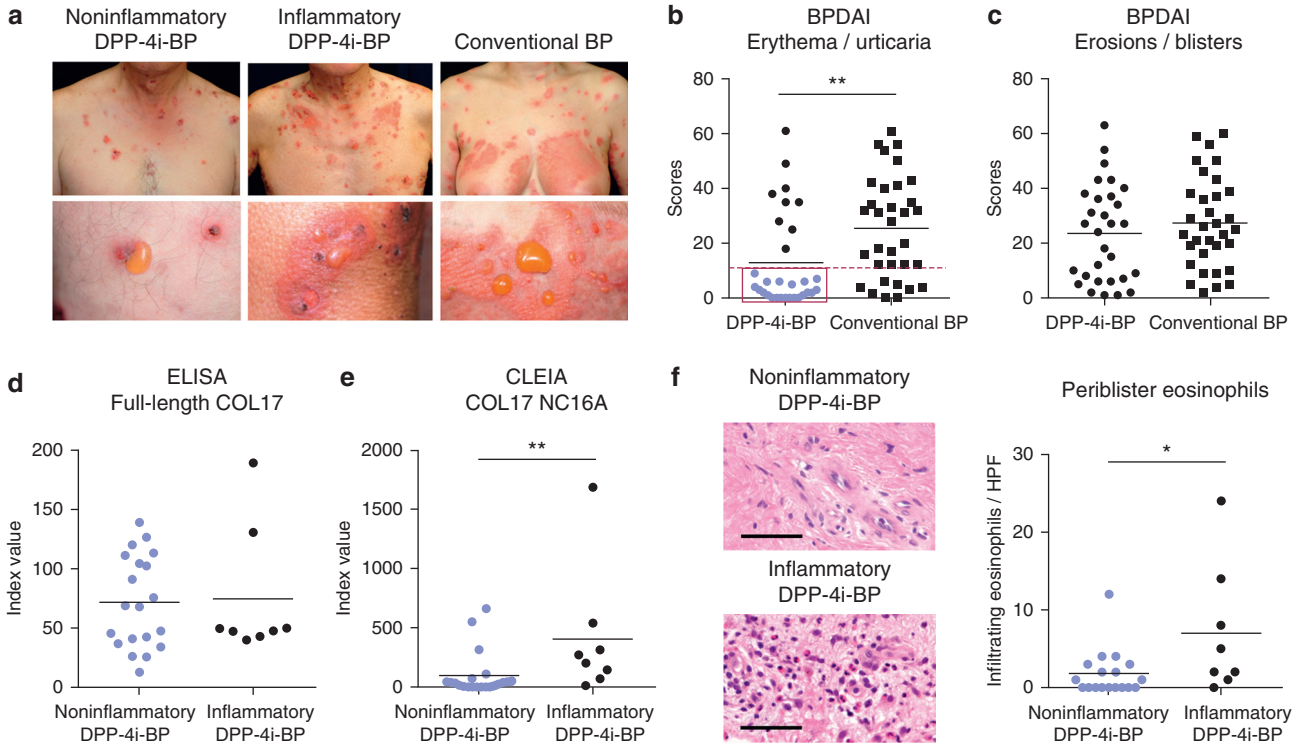

Figure 1. DPP-4i-BP shows a unique noninflammatory phenotype with few erythematous lesions. (**a**) The clinical appearance of noninflammatory DPP-4i-BP, inflammatory DPP-4i-BP, and conventional BP. (**b**) BPDAI scores for erythema/urticaria. The dashed line indicates a BPDAI (erythema/urticaria) score of 10. The red square indicates a group of patients with noninflammatory DPP-4i-BP. The blue dots represent scores of noninflammatory DPP-4i-BP. (**c**) BPDAI scores for erosions/blisters. (**d**) Full-length COL17 ELISA index. (**e**) COL17 NC16A CLEIA index. (**f**) Histopathological findings of representative noninflammatory DPP-4i-BP and inflammatory BP. Hematoxylin and eosin staining, scale bar = 100 μm. (**d**) Comparison of the number of infiltrating eosinophils between noninflammatory DPP-4i-BP and inflammatory BP. Bars represent mean values. $*P < 0.05$, $**P < 0.01$ using Mann-Whitney test. BP, bullous pemphigoid disease; BPDAI, Bullous Pemphigoid Disease Area Index; CLEIA, chemiluminescent enzyme immunoassay; COL17, type XVII collagen; DPP-4i, dipeptidyl peptidase-4 inhibitor; HPF, high-powered field.

HLA-DQA1, -DQB1 and -DRB1 were compared between DPP-4i-BP and control groups. HLA-DRB1*12:01-DQB1*03:01 showed the lowest *P*-value in 243 haplotypes ($P = 2.16 \times 10^{-8}$), which was greater than that of HLA-DQB1*03:01 alone ($P = 5.86 \times 10^{-11}$), indicating that HLA-DQB1*03:01 will be the more useful biomarker in predicting DPP-4i-BP before administration to Japanese patients (Table 1).

Six patients with conventional BP suffered from type 2 diabetes at the onset of BP. We found that BPDAI scores for erosions/blisters were similar in those with DPP-4i-BP and conventional BP with diabetes, whereas scores for erythema/urticaria were significantly higher in those with conventional BP with diabetes (see Supplementary Figure S1 online), suggesting that the noninflammatory phenotype in DPP-4i-BP correlates with the intake of DPP-4i rather than with the existence of type 2 diabetes. Furthermore, none of the patients with conventional BP with diabetes carried HLA-DQB1*03:01.

Eight patients with conventional BP had noninflammatory disease, and 37.5% (3/8) of those patients carried HLA-DQB1*03:01. This frequency is similar to that for patients with inflammatory conventional BP (16/64 [25%]) and inflammatory DPP-4i-BP (4/9 [44%]) and lower than that for those with noninflammatory DPP-4i-BP (18/21 [86%]), suggesting that HLA-DQB1*03:01 is associated with noninflammatory DPP-4i-BP rather than with noninflammatory conventional BP or inflammatory DPP-4i-BP.

To our knowledge, the association of HLA-DQB1*03:01 with noninflammatory DPP-4i-BP is the strongest association that has been described between a class II HLA and a drug-related autoimmune disease. HLA-DQB1*03:01, also reported to be associated with mucous membrane pemphigoid in Caucasian patients (Ahmed et al., 1991; Delgado et al., 1996), seems to be a risk factor for DPP-4i-BP in Japanese. To confirm this, the incidence of DPP-4i-BP among diabetic patients carrying HLA-DQB1*03:01 should be investigated. In addition, to determine whether the noninflammatory phenotype is a distinctive feature of DPP-4i-BP or just a mild form of BP, further investigations are required. The findings of this study give us important clues about the breakdown of self-tolerance that results from the interaction of genetic background and drug intake.

Table 1. Frequency of HLA alleles and haplotypes in cases and controls

HLA Allele	DPP-4i-BP (Noninflammatory), n (%) (n = 21)	Conventional BP, n (%) (n = 72)	Japanese General Population Control Individuals, n (%) (n = 873)	DPP-4i–Tolerant Diabetes Patients, n (%) (n = 61)	DPP-4i-BP (Noninflammatory) Patients Versus General Population Control Individuals		Conventional BP Patients Versus General Population Control Individuals		DPP-4i-BP (Noninflammatory) Versus Tolerant Control Individuals	
					OR (95% CI)	*P*-Value[1]	OR (95% CI)	*P*-Value	OR (95% CI)	*P*-Value[2]
DQB1*03:01	18 (86)	19 (26)	156 (18)	19 (31)	27.6 (8.0–94.8)	5.86×10^{-11}	1.6 (0.9–2.9)	8.24×10^{-2}	13.3 (3.5–50.5)	**2.13×10^{-5}**
DQA1*05:05	10 (48)	10 (14)	60 (7)	11 (18)	12.3 (5.0–30.2)	8.11×10^{-7}	2.2 (1.1–4.5)	5.58×10^{-2}	4.1 (1.4–12.1)	1.79×10^{-2}
DRB1*12:01	10 (48)	8 (11)	68 (8)	7 (11)	10.8 (4.4–26.2)	2.34×10^{-6}	1.5 (0.7–3.2)	3.63×10^{-1}	7.0 (2.2–22.4)	**1.08×10^{-3}**
DQA1*01:03	0 (0)	18 (25)	368 (42)	18 (30)	0.0 (0.0–0.5)	1.52×10^{-5}	0.5 (0.3–0.8)	4.07×10^{-3}	0.1 (0.0–0.9)	**4.31×10^{-3}**
DRB1*11:01	7 (33)	6 (8)	37 (4)	9 (15)	11.3 (4.3–29.7)	2.99×10^{-5}	2.1 (0.8–5.0)	1.32×10^{-1}	2.9 (0.9–9.1)	1.07×10^{-1}
DQB1*06:01	0 (0)	18 (25)	359 (41)	18 (30)	0.0 (0.0–0.6)	3.67×10^{-5}	0.4 (0.3–0.8)	3.45×10^{-2}	0.1 (0.0–0.9)	**4.31×10^{-3}**
DRB1*12:01-DQB1*03:01	10 (48)	6 (8)	39 (4)	1 (0)	19.4 (7.8–48.5)	2.16×10^{-8}	1.9 (0.8–4.8)	1.46×10^{-1}	54.5 (6.3–470.1)	**1.56×10^{-6}**
DQA1*05:05-DQB1*03:01	10 (48)	8 (11)	58 (7)	11 (18)	12.8 (5.2–31.3)	6.09×10^{-7}	1.8 (0.8–3.8)	1.51×10^{-1}	4.1 (1.4–12.1)	1.79×10^{-2}
DQA1*01:03-DQB1*06:01	0 (0)	16 (22)	357 (41)	18 (30)	0.0 (0.0–0.6)	3.65×10^{-5}	0.4 (0.2–0.7)	1.62×10^{-3}	0.0 (0.0–0.9)	**4.31×10^{-3}**

Abbreviations: BP, bullous pemphigoid; CI, confidence interval; DPP-4i, dipeptidyl peptidase-4 inhibitor; HLA, human leukocyte antigen; OR, odds ratio.
[1]All values in this column are significant after Bonferroni correction: $P < 1.27 \times 10^{-4}$ (0.05/152 HLA-A, -B, -C, -DRB1, -DPB1, -DQA, -DQB1 alleles; 76 DRB1-DQA1-DQB1 haplotypes; 52 DRB1-DQA1 haplotypes; 68 DRB1-DQB1 haplotypes; and 47 DQA1-DQB1 haplotypes).
[2]Boldface values in this column are significant after Bonferroni correction: $P < 5.56 \times 10^{-3}$ (0.05/6 HLA alleles, 1 DRB1-DQB1 haplotype, and 2 DQA1-DQB1 haplotypes).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2017.11.023.

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LETTER TO THE EDITOR

Case of bullous pemphigoid associated with teneligliptin accompanied by severe mucous membrane involvement

Dear Editor,

Cases of bullous pemphigoid (BP) associated with dipeptidyl peptidase (DPP)-4 inhibitors have been reported.[1] Here, we describe a case of BP with severe mucous membrane involvement, which was difficult to be discriminated from mucous membrane pemphigoid (MMP) associated with a DPP-4 inhibitor.

A 73-year-old Japanese woman with type 2 diabetes mellitus (DM) developed blistering lesions both on the oral and pharyngeal mucosae and on the trunk (Fig. 1a,b). The patient had taken teneligliptin for 11 months for her DM. Histopathological examination of a blister on the shoulder showed it to be a subepidermal blister (Fig. 1c). Direct immunofluorescence (IF) revealed a linear deposition of immunoglobulin (Ig)G and C3 at

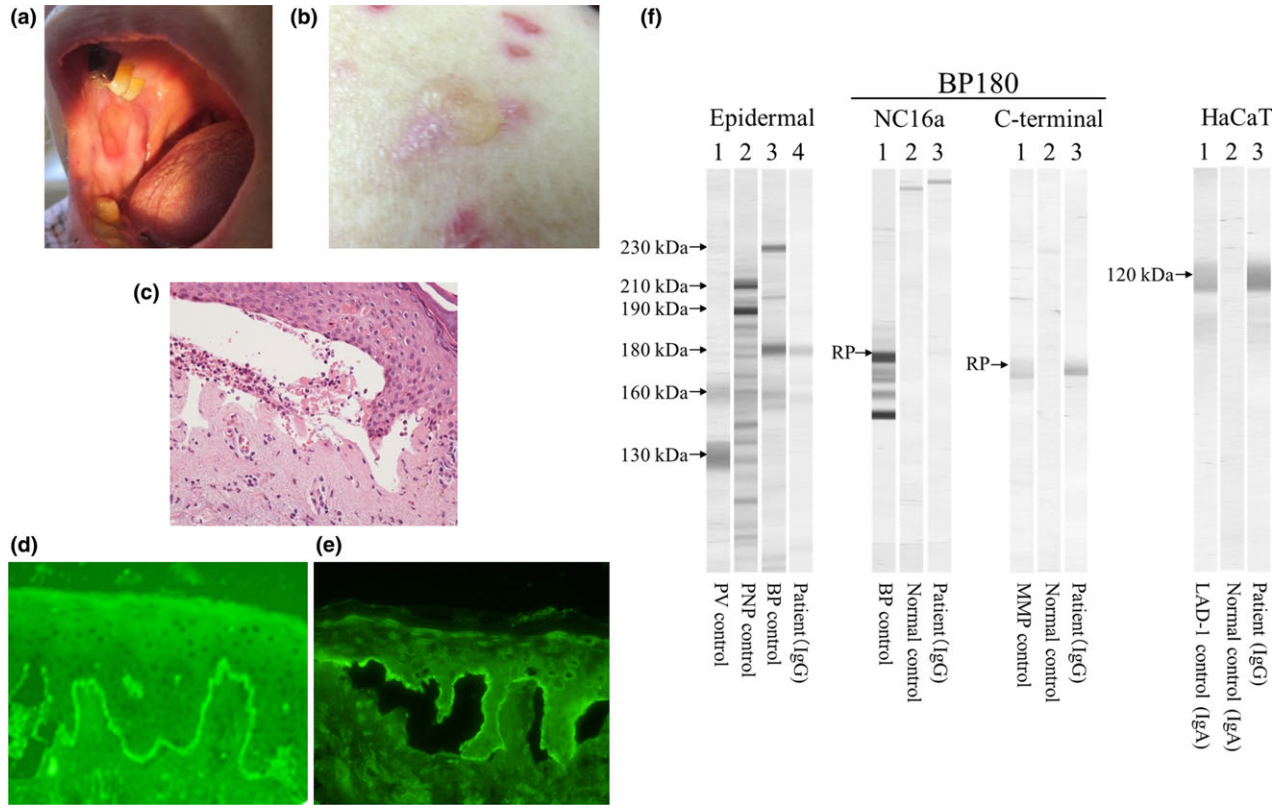

Figure 1. Clinical manifestation and histopathology of the case. (a) Oral erosion. (b) Blistering erythema on the patient's shoulder. (c) Histopathology of the skin lesion on the shoulder, showing a subepidermal blister (hematoxylin–eosin, original magnification ×200). (d) Direct immunofluorescence showing immunoglobulin (Ig)G deposition at the epidermal basement membrane zone (×200). (e) Indirect immunofluorescence of 1 mol/L sodium chloride-split skin section, showing IgG reactivity with epidermal side (×200). (f) Results of the immunoblot analyses using normal human epidermal extract (Epidermal), recombinant protein (RP) of BP180 NC16a domain (BP180 NC16a), RP of BP180 C-terminal domain (BP180 C-terminal) and concentrated culture supernatant of HaCaT cells (HaCaT). IgG antibodies of this patient reacted with the intact 180-kDa BP180 (lane 4 in the Epidermal panel), the BP180 C-terminal domain (lane 3 in the BP180 C-terminal panel), and the 120-kDa LAD-1 (lane 3 in the HaCaT panel), but not with the BP180 NC16a domain (lane 3 in the BP180 NC16a panel). BP, bullous pemphigoid; LAD-1, leukocyte adhesion deficiency-1; MMP, mucous membrane pemphigoid; PNP, paraneoplastic pemphigus; PV, pemphigus vulgaris.

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the basement membrane zone (Fig. 1d). Indirect IF using 1 mol/L sodium chloride-split skin sections revealed IgG antibodies reactive to the epidermal side of the split (Fig. 1e). Enzyme-linked immunosorbent assay (ELISA) for both IgG antibodies against the BP180 NC16a domain and BP230 yielded negative results, but an antibody against full-length BP180 was strongly positive (ELISA index, 163.3; cut-off, <4.64).[2] Immunoblot analysis showed positive IgG reactivity with intact BP180 in the normal human epidermal extract, with a recombinant protein of BP180 C-terminal domains, and with the 120-kDa leukocyte adhesion deficiency-1 (LAD-1), a C-terminal ectodomain of BP180 in the concentrated culture supernatant of HaCaT cells (Fig. 1f). Teneligliptin was discontinued, and 30 mg/day oral prednisolone (0.5 mg/kg per day) was started. Subsequently, the patient underwent three cycles of plasma exchange. The mucocutaneous lesions rapidly improved, along with a decline in full-length BP180 ELISA values (index, 6.27). The steroid dose was tapered without any recurrences.

Although teneligliptin is a DPP-4 inhibitor, no BP cases associated with this drug have been reported. We considered that this case was induced by teneligliptin for the following two reasons. First, the mucocutaneous lesions rapidly improved after teneligliptin withdrawal, concomitant with a reduction in the IgG index against full-length BP180. Second, IgG antibodies reacted with LAD-1, which we previously reported to be frequently observed in DPP-4 inhibitor-induced BP.[2] In addition, full-length BP180 without NC16a BP cases are strongly associated with DPP-4 inhibitors.[2]

This case was difficult to be discriminated between BP and MMP. The case had severe oral mucosal and pharyngeal lesions. Furthermore, positive IgG reactivity with BP180 C-terminal domains may indicate the diagnosis of MMP.[3] However, because mucosal lesions are observed in some BP patients, and because our patient had erythema and erosions on the skin with the BP disease area indices of 10, 8 and 27 for skin erosions/blisters, urticaria/erythema and mucosal erosions/blisters, respectively before treatment, we eventually diagnosed this case as BP rather than MMP. The low incidence of MMP may be the reason for the lack of reported DPP-4 inhibitor-related MMP cases, despite the high frequency of BP cases reported. The direct effect of DPP-4 inhibitors on development of autoantibodies to BP180 has not been elucidated. In view of the wide use of DPP-4 inhibitors, further studies are needed to determine the pathomechanisms.

CONFLICT OF INTEREST: None declared.

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症　例

高用量のステロイド内服加療を要したメシル酸ガレノキサシンによる多発性固定薬疹の1例

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要　旨

26歳，男性。咽頭炎に対しメシル酸ガレノキサシン（ジェニナック®）を含む複数の薬剤を内服した数時間後に発熱，口唇腫脹および四肢，体幹に多発する紅斑が出現した。固定薬疹が疑われ被疑薬を中止したが，症状が悪化したためPSL 60 mg/日（1 mg/kg/day）内服治療を行ったところ，症状は軽快した。また，被疑薬3剤の貼付試験および薬剤誘発リンパ球幼若化試験はすべて陰性であったため再投与試験を施行したところ，メシル酸ガレノキサシン40 mg（1回常用量の1/10量）内服後に高熱を伴う重篤な症状が誘発され，PSL 60 mg/日内服治療を要した。以上より，自験例をメシル酸ガレノキサシンによる多発性固定薬疹と診断した。同薬剤による薬疹では，自験例を含め多発性固定薬疹の報告が多く，再投与試験により原因薬と同定される例が多かった。自験例では，1回常用量の1/10量の再投与試験で重篤な症状が誘発された。今後，本剤の再投与試験を施行する際には投与量に関する注意が必要と考えられた。

(J Environ Dermatol Cutan Allergol, 11 (2) : 169-174, 2017)

キーワード：多発性固定薬疹，ニューキノロン系抗菌薬，メシル酸ガレノキサシン，再投与試験

はじめに

メシル酸ガレノキサシン（ジェニナック®）は，2007年10月にわが国で発売されたニューキノロン系抗菌薬である。同薬剤は，キノロン骨格の第6位にフッ素原子をもたないなど，既存のニューキノロン系抗菌薬とは異なる化学構造をもち，抗菌力が強く，抗菌スペクトルが広いことが特徴である。わが国における既存のキノロン系抗菌薬による薬疹の報告では光線過敏型薬疹が最も多かったのに対し，メシル酸ガレノキサシンでは光線過敏型薬疹の報告はなく，固定薬疹の報告が多い。今回，われわれは，治療に高用量のステロイド内服を要したメシル酸ガレノキサシンによる多発性固定薬疹の症例を経験したので，若干の文献的考察を加えて報告する。

症　例

患者：26歳，男性。

初診：2014年11月。

主訴：発熱，口唇腫脹。

家族歴：特記事項なし。

既往歴：慢性扁桃炎（扁桃腺摘出術後）。

現病歴：2013年にメシル酸ガレノキサシン（ジェニナック®）を含む複数の薬剤投与後に口唇腫脹が出現し，数日で症状が軽快した既往がある。2014年11月，39℃台の発熱があり，その3日後に

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近医を受診した。咽頭炎と診断され，セフトリアキソン（ロセフィン®）2g静注，メシル酸ガレノキサシン400mg，アセトアミノフェン（カロナール®）400mgを内服したところ，数時間後に発熱，口唇の腫脹と手足に瘙痒を伴う紅斑が出現し，翌日，当科紹介受診となった。

現症：初診時（第2病日），眼球結膜の軽度充血，口唇の腫脹，下口唇のびらん，右足第1第2趾間に境界明瞭な円形の紅斑が認められた。発熱はなかった。固定薬疹が疑われ，被疑薬と考えられたメシル酸ガレノキサシン，アセトアミノフェン，セフトリアキソンの投与を中止し，プレドニゾロン（PSL）30mg/日内服を開始した。初診の4日後（第6病日）に口唇の腫脹は増強し，口唇，口腔粘膜のびらんの新生，拡大がみられ，出血を伴っていた。右手母指と小指の腹側，左手掌，右大腿，陰茎に境界明瞭な円形の紅斑の新生がみられ，手掌の紅斑で水疱形成が認められた（図1）。右大腿の皮疹より皮膚生検を施行した。

病理検査所見：表皮真皮境界部に軽度の空胞変性が認められた。真皮浅層に多数のメラノファージがみられ，組織学的色素失調の所見を呈していた（図2）。

治療と経過：PSL 30mg/日内服で病勢が抑えられていないと判断し，同日，緊急入院とし，PSL 60mg/日（1mg/kg/日）内服へ増量した。以後，症状は改善傾向となり，PSLを漸減，第19病日に中止し退院となった。皮疹部は色素沈着を残して治癒した。第65病日に，被疑薬3剤の貼付試験（皮疹部および無疹部），薬剤誘発リンパ球幼若化試験（DLST）を実施したところ，いずれも陰性であった。第289病日に，被疑薬3剤について，入院させて再投与試験を施行したところ，メシル酸ガレノキサシン40mg（1回常用量の1/10量）内服の1時間30分後に，口唇および手掌に瘙痒を伴う紅斑，左右大腿や右足第1第2趾間に紅斑が生じた（図3）。さらに投与約6時間30分後には，手掌紅斑の拡大，口唇腫脹，眼球結膜充血や38℃台の発熱が出現し，症状の悪化が認められた。左大腿の紅斑以外は，いずれも初診時の皮疹出現部位と一致していた。PSL 60mg/日内服を開始したところ症状は改善し，PSLを漸減して退院となった。アセトアミノフェン，セフトリアキソンの再投与試験はいずれも陰性であった。以上より，自験例をメシル酸ガレノキサシンによる多発性固定薬疹と診断した。

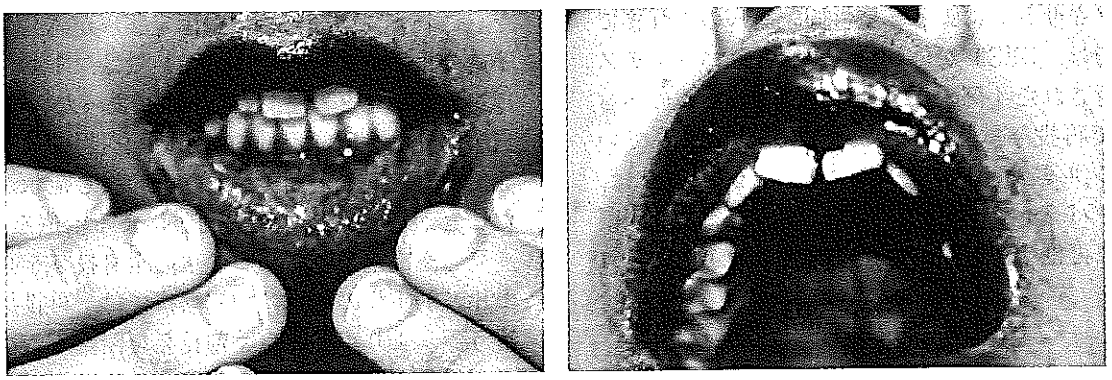
考　察

自験例は，ニューキノロン系抗菌薬であるメシル酸ガレノキサシンによる多発性固定薬疹であった。今回，PSL 30mg/日で病勢が抑えられず，口唇，口腔粘膜のびらんの新生・拡大，手掌に水疱形成を伴う紅斑の新生がみられたことから重症薬疹への進展が懸念され，PSL 60mg/日に増量して治癒させることができた。

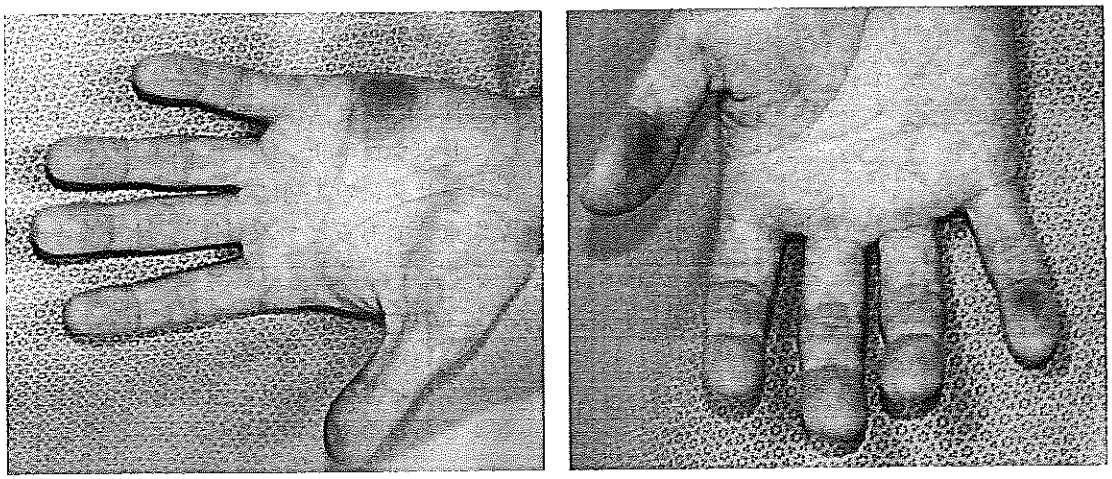
わが国におけるキノロン系抗菌薬による薬疹は，1980年から2015年までに373例の報告があった。このうち，光線過敏症が218例と最も多く，固定薬疹が66例と二番目に多かった。2007年にメシル酸ガレノキサシンがわが国で発売されてから本薬剤による光線過敏症の報告はなく，固定薬疹が13例のうち9例と最も多くを占め，多形紅斑，薬剤性過敏症症候群，紅皮症，アナフィラキシーが各1例ずつであった[1]。さらに，固定薬疹として報告された9例のうち8例が，自験例と同じ多発性固定薬疹であった。

メシル酸ガレノキサシンは，キノロン骨格の第6位にフッ素をもたないなど，既存のニューキノロン系抗菌薬と構造式が異なっていることが特徴である（図4）。これまでの報告では，ニューキノロン系抗菌薬の構造において，キノロン骨格の第6位と第8位のフッ素および第7位のピペラジン環が光線過敏症の発症に関与しているとされ[2]，特に第8位のフッ素は光毒性，光アレルギー性のいずれの発症機序においても重要であると考えられている[3]。さらに，UVA照射により第8位のフッ素が容易に変性することでニューキノロン系抗菌薬の光毒性が増強するといわれている[3]。ニューキノロン系抗菌薬のなかで強い光毒性を有する代表的な薬剤として知られているスパルフロキサシン，フレロキサシン，塩酸ロメフロキサシンは，いずれもキノロン骨格の第6位と第8位にフッ素が存在する。一方，光毒性が弱いとされるシプロフロキサシンやノルフロキサシンは，第8位にフッ素をもたない。メシル酸ガレノキサシンは，キノロン骨格の第6位と第8位のフッ素，第7位のピペラジン環のいずれの構造ももたないため，既存のニューキノロン系抗菌薬と比べて光毒性が低く光線過敏症を生じにくい薬剤ではないかと推察された。

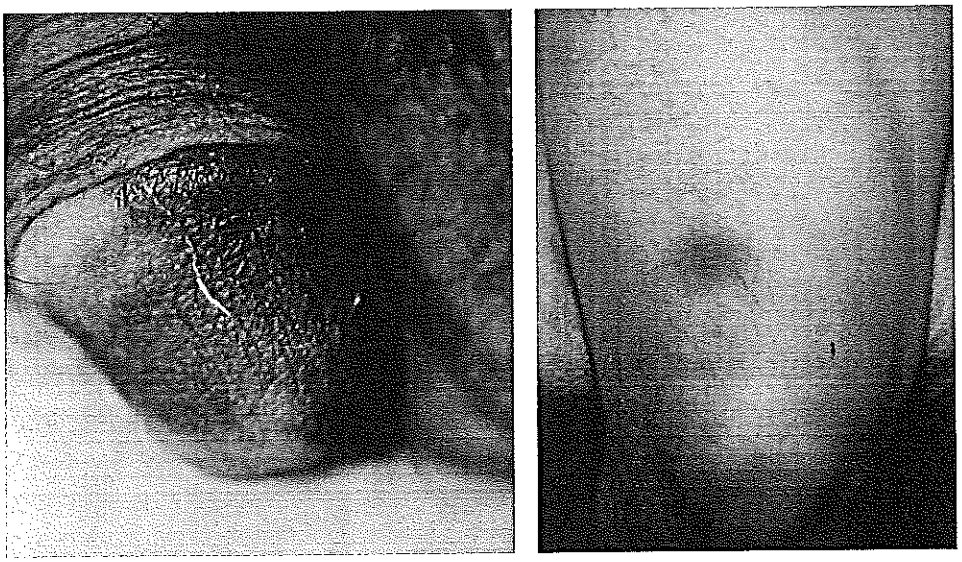
自験例では，DLSTの施行時期が適切でなかった可能性があるものの，DLSTやパッチテストは陰性で，再投与試験により原因薬と同定できた。メシル



(a)



(b)



(c)

図1：初診4日後（第6病日）の臨床像
（a）口唇，口腔粘膜のびらん，出血が認められた。
（b）右手母指と小指腹側，左手掌に境界明瞭な紅斑がみられた。
（c）陰茎亀頭部，右大腿にも境界明瞭な紅斑がみられた。

酸ガレノキサシンによる多発性固定薬疹のこれまでの報告では，DLST が施行された 3 例，パッチテストが施行された 4 例のすべてが陰性で，記載のあった全例で再投与試験により原因薬が同定されていた。メシル酸ガレノキサシンによる薬疹では，DLST やパッチテストが陽性となりにくく，原因薬の同定に再投与試験が必要となる可能性が推察された。これまでの報告では，固定薬疹が誘発された同薬剤の再投与試験における投与量は，1 回常用量の 1/20 量から 1/2 量までさまざまであった[4~11]（表 1）。自験例では 1 回常用量の 1/10 量を投与したところ，発熱と皮膚症状が誘発され，その後も症状が進行したことから，ステロイド内服による治療を要した。多発し，高用量のステロイドの全身投与を必要とした比較的重症の多発性固定薬疹では，再投与試験を行う際，より少量から薬剤投与を開始するなど，慎重な対応が必要と考えられた。

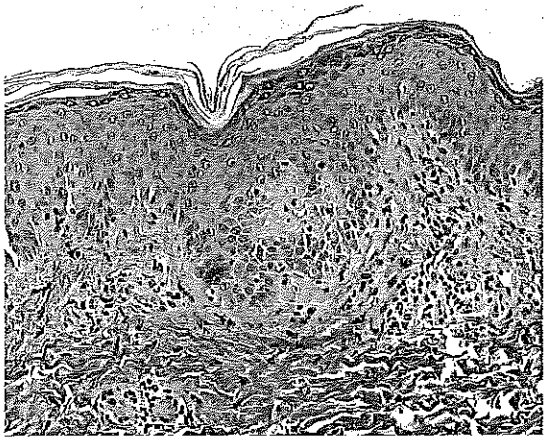


図 2：大腿部皮疹の病理組織像（HE 染色）
表皮真皮境界部に軽度の空胞変性と，真皮上層にメラノファージがみられた。

自験例では，今後，患者がほかのニューキノロン系抗菌薬を服用できるか否かが問題となる。これまで，ニューキノロン系抗菌薬の固定薬疹の経口負荷試験による交叉感作に関する検討で，オフロキサシン，レボフロキサシン，塩酸ロメフロキサシン，フレロキサシンの 4 剤に共通するメチルピペラジニル基が重要であるとする報告[12]，オフロキサシン，エノキサシン，ノルフロキサシン，シプロフロキサシンの 4 剤に共通するピペラジン環が重要であるとする報告[13]，シプロフロキサシンとオフロキサシンの 2 剤に共通するキノリン環およびピペラジニル基が重要であるとする報告[14]がある。メシル酸ガレノキ

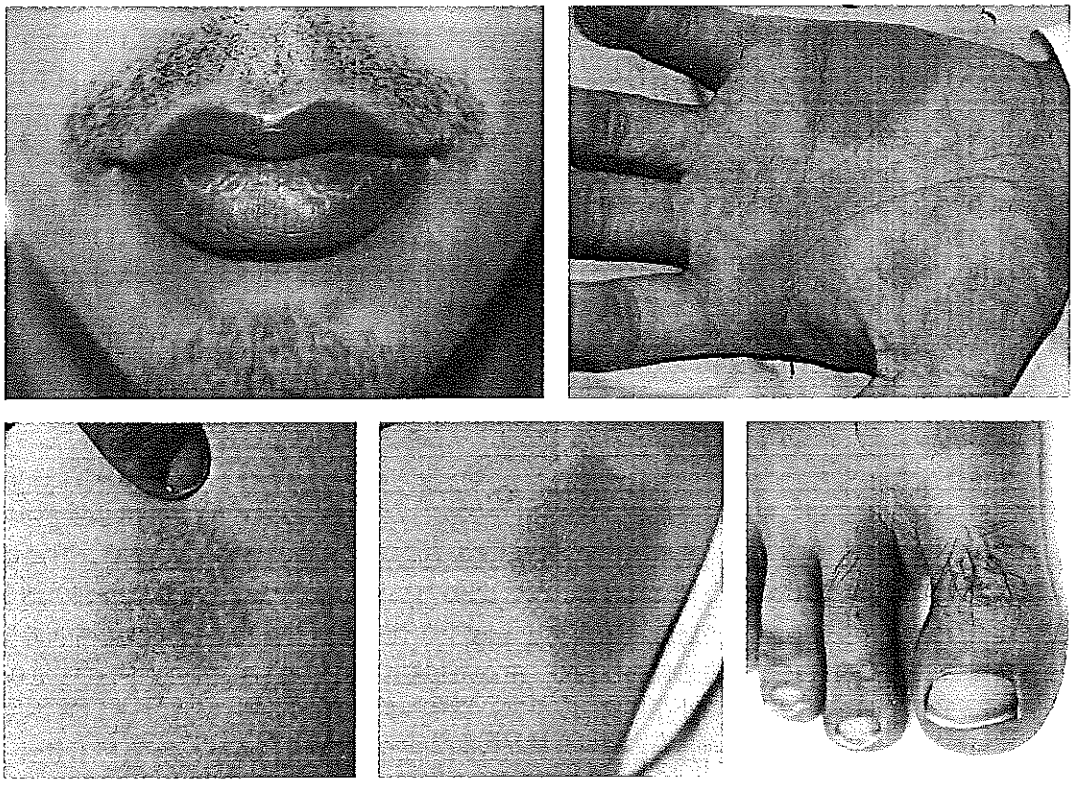
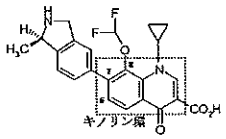
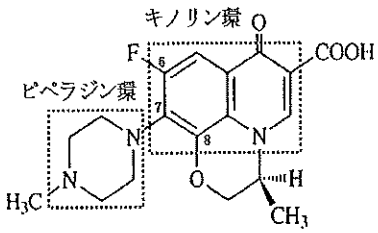


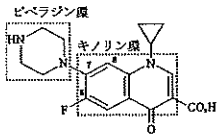
図 3：メシル酸ガレノキサシンの再投与試験時の臨床像
メシル酸ガレノキサシン 40 mg（1 回常用量の 1/10 量）内服 1 時間 30 分後に口唇の著明な発赤，腫脹と手掌，右大腿，左大腿，右足第 1 第 2 趾間に境界明瞭な紅斑が出現した。



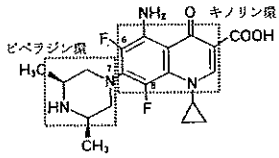
メシル酸ガレノキサシン（ジェニナック®）



レボフロキサシン（クラビット®）



シプロフロキサシン（シプロキサン®）



スパルフロキサシン（スパラ®）

図4：ニューキノロン系抗菌薬の構造式

表1：メシル酸ガレノキサシンによる固定薬疹の本邦報告例

症例	単発/多発	パッチテスト	DLST	経口負荷テスト	文献
40歳　男性	多発	記載なし	記載なし	記載なし	2011　久保田
21歳　男性	多発	記載なし	記載なし	記載なし	2011　久保田
33歳　女性	多発	陰性	記載なし	陽性：1/2量（200 mg）	2013　Miyake
43歳　女性	多発	施行せず	記載なし	陽性：1/2量（200 mg）	2013　佐藤
40歳　女性	単発	記載なし	記載なし	記載なし	2013　井上
34歳　女性	多発	記載なし	記載なし	陽性：1/20量（20 mg）	2014　田中
54歳　男性	多発	陰性	陰性	陽性：（内服量不明）	2014　平岩
39歳　女性	多発	陰性	記載なし	陽性：1/2量（200 mg）	2015　関根
42歳　女性	多発	記載なし	陰性	陽性：1/2量（200 mg）	2015　竹林
26歳　男性	多発	陰性	陰性	陽性：1/10量（40 mg）	自験例

サシンは，これらの交叉感作に重要と推定されたピペラジン環をもたず，既存のニューキノロン系抗菌薬と構造が異なるため，自験例の患者が他のニューキノロン系抗菌薬を服用しても薬疹を生じる可能性が低いのではないかと推察された。現時点でメシル酸ガレノキサシンと既存のニューキノロン系抗菌薬との交叉感作に関する報告はないが，今後，症例の集積と詳細な検討が待たれる。

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A Case of Multiple Fixed Drug Eruption Induced by Mesilate Garenoxacin (Geninax®) Successfully Treated with High Dose Systemic Corticosteroids

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A 26-year-old man who had taken some medicines, including mesilate garenoxacin (Geninax®), for pharyngitis, developed lip swelling and multiple erythematous lesions on the limbs and the body with high fever. He was successfully treated with discontinuance of suspected medicines and high dose oral prednisolone (1 mg/kg ; 60 mg daily). Oral provocation test with mesilate garenoxacin was positive, whereas both patch test and DLST (drug-induced lymphocyte stimulation test) were negative. He was diagnosed with multiple fixed drug eruption caused by mesilate garenoxacin. Multiple fixed drug eruption has been most commonly reported among drug eruptions induced by mesilate garenoxacin. Severe cutaneous symptoms with high fever were induced after oral provocation test with mesilate garenoxacin at a dose of 40 mg (one-tenth of a single dose) in our case. Therefore, more attention should be paid to dosage of mesilate garenoxacin for oral provocation tests in order to not induce severe symptoms.

(J Environ Dermatol Cutan Allergol, 11 (2)：169-174, 2017)

Key words：multiple fixed drug eruption, new quinolone antibacterial agents, mesilate garenoxacin, oral provocation test

Discussion | A 30-minute daily or alternate-day facial exercise program sustained over 20 weeks may modestly improve the facial appearance of selected middle-aged women. Blinded ratings of validated photoscales showed significant improvement in upper and lower cheek fullness. Rater estimates of mean participant age showed a significant monotonic decrease from 50.8 years at baseline to 49.6 years at 8 weeks and 48.1 years at 20 weeks. Participants were highly satisfied, noting significant improvement in 18 of 20 facial features.

This study had limitations that may reduce its external validity. The sample was small, exclusively of middle-aged women, there were numerous dropouts, and there was no control group in the study. Another limitation is that participants were self-selected and may have been particularly willing to continue with an exercise regimen.

In conclusion, a regimen of at-home facial exercises maintained for 20 weeks seemed to improve mid-face and lower face fullness. The mechanism may be exercise-actuated hypertrophy of cheek and other muscles. Further research is warranted to isolate the causes and effects of exercise-related changes and to assess the generalizability of these findings.

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Study concept and design: Alam, Walter, Geisler, Sikorski, Tung.
Acquisition, analysis, or interpretation of data: Walter, Geisler, Roongpisuthipong, Poon.
Drafting of the manuscript: Alam, Geisler, Sikorski, Tung, Poon.
Critical revision of the manuscript for important intellectual content: Walter, Roongpisuthipong, Tung, Poon.
Statistical analysis: Roongpisuthipong, Poon.
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OBSERVATION

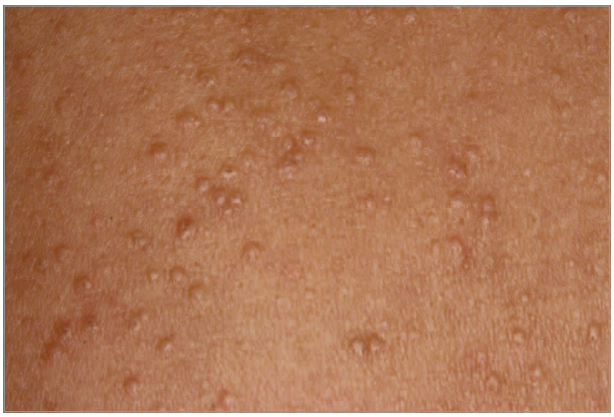
Generalized Lichen Nitidus Following Anti–PD-1 Antibody Treatment

Lichen nitidus (LN) is an uncommon skin disease characterized by minute flesh-colored papules on the abdomen, limbs, and genitalia.[1] Generalized LN is a rare form of LN that is more often seen in children and young adults.[1] Anti–programmed cell death 1 (PD-1) antibodies, such as nivolumab, are immune checkpoint inhibitors that prevent the binding of PD-1 to its ligands, thereby facilitating the activation of T lymphocytes in patients with cancers such as melanoma and non–small-cell lung carcinoma.[2] Here, we report a case of generalized LN following nivolumab treatment that was highly responsive to topical steroid therapy.

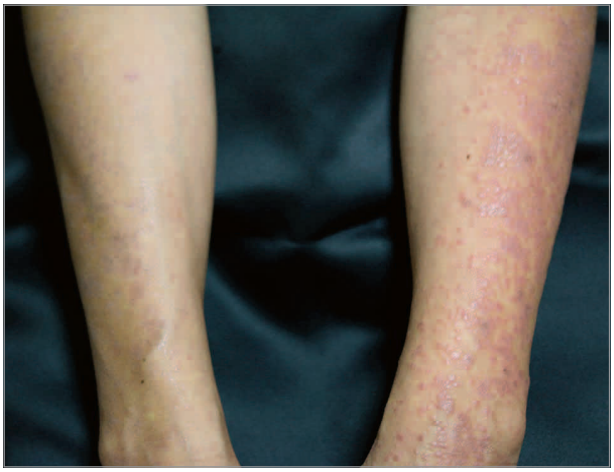
Report of a Case | A man in his 40s presented after developing multiple skin lesions. He had been diagnosed with metastatic lung adenocarcinoma the previous year and received 2 courses of radiotherapy to his head and leg and 4 cycles of carboplatin, pemetrexed, and bevacizumab followed by nivolumab (3 mg/kg) administered every 2 weeks. After 8 cycles of nivolumab over 5 months, he developed 1- to 2-mm shiny papules scattered on the upper limbs. Nivolumab therapy was continued, and similar lesions spread to the rest of his body over the subsequent 3 months (**Figure 1**A). Skin biopsy of the papular lesions showed typical histological features of LN: focal lymphohistiocytic infiltrates beneath a thinned epidermis circumscribed by elongated rete ridges, vacuoles in the dermoepidermal junction, and melanin incontinence (**Figure 2**). We therefore diagnosed the patient with generalized LN.

Figure 1. Clinical Presentation Before and After Treatment With Topical Steroids

A Before steroid treatment



B After steroid treatment of 1 arm

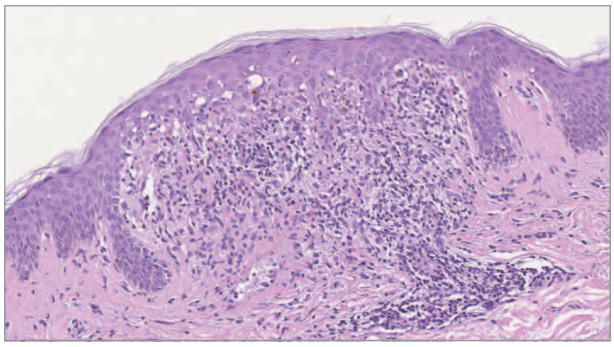


A, Numerous 1- to 2-millimeter skin-colored shiny papules developed on the upper back 5 months after starting nivolumab therapy. B, Improvement of the skin lesions following the use of steroids was remarkable on the treated right forearm compared with the untreated left forearm. The lesions on the untreated arm appeared coalesced and showed a plaque-like appearance similar to that of lichen planus.

He subsequently developed pruritus, and treatment with a topical steroid (betamethasone butyrate propionate ointment) was started on his right arm while nivolumab therapy was continued. One month later, the skin lesions on his right arm had completely resolved, while those on his untreated arm and body remained (Figure 1B). The lesions on his untreated arm also coalesced into plaques, displaying clinical appearance similar to that of lichen planus (LP) (Figure 1B). At last follow-up, nivolumab therapy was ongoing, with full resolution of LN by topical steroids.

Discussion | To our knowledge, there are 2 preceding reports of iatrogenic generalized LN, one of which was associated with tremelimumab, a monoclonal antibody against cytotoxic T-lymphocyte-associated antigen 4, an immune checkpoint inhibitor.[3,4] There have been no reports, however, of generalized LN developing after anti–PD-1 therapy. A prior study indicated that cutaneous adverse reactions to anti–PD-1 therapies exhibit histological features of lichenoid dermatitis despite variable clinical features, suggesting that these skin changes may be directly related to the PD-1 pathway.[5]

Figure 2. Histological Examination



Skin biopsy specimen obtained from the upper limbs. Note discrete foci of lymphocytes and histiocytes immediately beneath the epidermis, surrounded by elongated rete ridges (hematoxylin-eosin, original magnification ×10).

Two features were characteristic in the present case of generalized LN associated with anti–PD-1 therapy. First, the clinical appearances of the skin lesions partially shifted into LP-like lesions. Although LP and LN are thought to involve different immunological mechanisms, there has been a previous report of LP occurring subsequent to generalized LN.[6] An association may therefore exist in their pathogeneses, or this disease progression may be particular to skin lesions occurring with anti–PD-1 therapy. Second, the skin lesions in this patient responded well to topical steroids despite various earlier reports describing treatment resistance.[1,3,4] Similarly, lichenoid dermatitis occurring in association with anti–PD-1 therapy also appears to be responsive to steroid treatment.[5]

In conclusion, we report a case of generalized LN that developed during anti–PD-1 therapy and its atypical transformation into LP-like lesions and responsiveness to topical steroids. Such clinical progression in addition to the histopathological features distinguish the lesions seen in this case from other immune-related adverse effects and further contributes to ongoing studies on the cutaneous reactions to nivolumab, suggesting a disease progression of LN that may occur in relation to immune checkpoint inhibitors.

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Erythema Nodosum–like Eruption in the Setting of Sorafenib Therapy

Treatment with sorafenib, a small-molecule multikinase inhibitor (MKI) of the serine-threonine kinases CRAF and BRAF; vascular endothelial growth factor receptors 1, 2, and 3; and platelet-derived growth factor receptor β, may result in numerous cutaneous toxic effects, including hyperkeratotic hand-foot skin reaction, maculopapular or papulopustular eruptions, erythema multiforme, generalized keratosis pilaris–like eruption, and alopecia.[1] Here we report the first case to our knowledge of erythema nodosum (EN)-like eruption concurrent with sorafenib therapy.

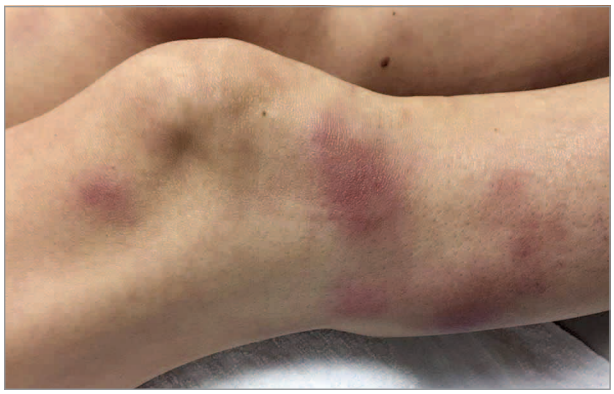
Report of a Case | A woman in her 60s with familial adenomatous polyposis complicated by desmoid tumors of the pancreatic head and duodenum presented with tender lesions on the lower extremities of 3 weeks' duration. Treatment with sorafenib, 400 mg/d, had been initiated 8 weeks earlier for enlarging desmoid tumors. There were no other medication changes or recent illnesses. Her other medication regimens were long-standing and included insulin lispro (Humalog; Lilly), lipase-protease-amylase, and diazepam. A review of systems revealed fevers, fatigue and arthralgias, which all preceded the development of the skin lesions.

Physical examination was notable for approximately 12 red, tender subcutaneous plaques and nodules on the bilateral shins (**Figure 1**). Scaly hyperkeratotic plaques on the palms and soles were consistent with grade 1 hand-foot skin reaction.

A 4-mm punch biopsy demonstrated septal and lobular panniculitis with a mixed inflammatory infiltrate composed of lymphocytes, histiocytes, and rare neutrophils with focal fat degeneration and occasional multinucleated giant cells, suggesting a panniculitis with clinical features of EN (**Figure 2**). Fibrinoid necrosis and vasculitis were not observed. Additional laboratory workup findings were negative for antistreptolysin O titer and Quanitferon-TB Gold assay.

Sorafenib treatment was discontinued, and the patient was treated with clobetasol, 0.05%, ointment twice daily. There was rapid improvement over the course of 2 weeks. Sorafenib therapy was resumed at a lower dose of 200 mg/d without recurrence of the lesions. Repeat imaging revealed a radiographic response with interval reduction in the size of the desmoid tumor and adjacent lymphadenopathy.

Figure 1. Clinical Appearance of Erythema Nodosum–Like Eruption Concurrent With Sorafenib Treatment



The patient developed red, tender subcutaneous plaques and nodules on the bilateral shins.

Discussion | Given the onset of lesions in this patient 8 weeks after initiation of sorafenib therapy, and clinical improvement after treatment interruption, we postulate that sorafenib was the likely etiologic agent. To our knowledge, this is the first report of EN-like eruption in the context of sorafenib treatment.

The occurrence of panniculitis in patients taking RAF inhibitors may correspond to a class effect. EN-like lesions have occurred in patients with *BRAF* V600-mutant melanoma treated with the selective BRAF inhibitors vemurafenib and dabrafenib, with variable degrees of lobular or septolobular panniculitis and leukocytoclastic vasculitis.[2,3] An additional case has been reported in a patient treated with regorafenib, a second-generation MKI that also targets BRAF.[4] In general, EN-like lesions present as tender, erythematous nodules typically on the lower extremities, as seen in the present case. A prior report of sorafenib-induced panniculitis with necrotizing vasculitis described unilateral manifestation, on 1 leg only,[5] unlike the bilateral presentation of EN-like lesions in the present case.

Treatment for EN-like eruption depends on the severity of the case. Asymptomatic or mildly tender lesions without accompanying fevers or arthralgias may be managed with high-potency topical steroids and nonsteroidal anti-inflammatory drugs. For severe cases, dose interruption of the causative drug should be considered, and treatment with systemic steroids may be warranted. EN-like lesions may resolve, persist, or recur after continuation of targeted therapy.[2-5]

The pathomechanism of EN-like eruption has not been fully elucidated. The panniculitis may be triggered by the medication, underlying malignancy, or BRAF inhibition on the mitogen-activated protein kinase pathways, which partially regulate neutrophilic migration.[2,6]

Recognizing common dermatologic toxic effects associated with sorafenib as well as rarely reported events such as EN-like eruption is crucial for their prompt diagnosis and management. While severe reactions may lead to dose interrup-

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LETTER TO THE EDITOR

Case of diffuse panbronchiolitis developed in a patient with epidermodysplasia verruciformis

Dear Editor,

Epidermodysplasia verruciformis (EV) is a rare genodermatosis characterized by susceptibility to infection by human papillomavirus (HPV) strains and subsequent risk of malignant transformation.[1] These HPV types are commonly referred to as EV-associated HPV types and include HPV-5, -8, -9, -12, -14, -15, -17, -19–25, -36–38 and -49.[2] Diffuse panbronchiolitis (DPB) is an idiopathic inflammatory disease, principally affecting the respiratory bronchioles.[3] Both disorders are related to immunodeficiency conditions; however, there has been no report on the association between these two diseases. Here, we report a case of EV accompanying DPB.

A 36-year-old Japanese man was referred to our department for evaluation of his skin lesions that had begun in his adolescence. He had had DPB as an underlying disease since his early childhood. He had no family history of consanguineous marriage or other disease. Physical examination showed multiple round lesions such as red plaques with slight scales or vitiligo of 1–2 cm in diameter distributed on the trunk (Fig. 1a). He also presented verruca-like papillomatous lesions on his hands and feet. Laboratory tests showed no abnormal findings, including the CD4/8 T-cell ratio and T-cell proliferation ability. A skin biopsy from his right shoulder revealed epidermal acanthosis and parakeratosis with a characteristic steel blue cytoplasm within keratinocytes (Fig 1b). Polymerase chain reaction analysis using two different primers, CP65/70 and MY09/MY11, was performed to determine HPV type. The results indicated that HPV-22 was present in the lesion. Based on these findings, we diagnosed the patient as classic EV.

The exact pathogenesis of EV is not fully known, although two inactivating mutations of the EVER1/TMC6 and EVER2/TMC8 genes have been identified in familial forms.[1] Data from a recently published gene atlas of the human protein-encoding transcriptome indicate that both EVER1 and EVER2 are highly expressed on T cells, B cells and natural killer (NK) cells.[4] Specific abnormalities in NK cell cytotoxicity and T-cell activation have been reported in EV patients.[5] Although the etiology of DBP remains unknown, it has been reported that human leukocyte antigen is associated with the disease, and that DBP is association with human T-cell leukemia virus type 1 infection.[4] These results suggest that immunodeficiency, such as T-cell dysfunction, is related to the pathogenesis of DBP.[4]

Therefore, both disorders may share some underlying mechanisms in common, such as immunodeficiency due to T-cell dysfunction, but these two disorders are so rare that no cases of EV accompanied with DBP have been reported. Although the clinical manifestation suggested that T-cell functions may be attenuated, T-cell proliferation capacity *in vitro* and CD4/8 differentiation among peripheral blood mononuclear cells were intact in our case. Although it is limited to a single case and we still do not know the underlying common pathogenesis, our case may improve our understanding of the pathogenesis of EV and DBP.

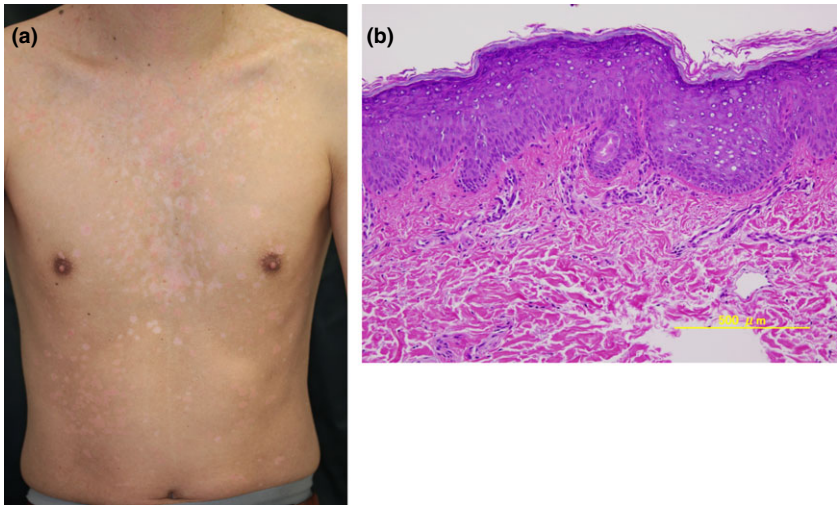

Figure 1. Clinical manifestations and histology of the patient. (a) Multiple round lesions such as red plaques with slight scales or vitiligo of 1–2 cm in diameter distributed on the trunk. (b) Skin biopsy from the white patch on his right shoulder revealed epidermal acanthosis and parakeratosis with a characteristic steel blue cytoplasm within keratinocytes (hematoxylin–eosin, original magnification ×20).

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CONFLICT OF INTEREST: None declared.

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LETTER TO THE EDITOR

Drastic effect on giant lung metastatic melanoma by sequential administration of nivolumab with ipilimumab/radiation combination therapy

Dear Editor,

Ipilimumab with local radiotherapy is reported to have a synergistic effect on malignant melanoma.[1–3] Despite the effectiveness of the ipilimumab/radiation combination therapy, a considerable number of cases are reported to result in unsatisfactory outcomes.[1–3] We experienced a case of metastatic melanoma of the lung that drastically shrunk after ipilimumab/radiotherapy, followed by nivolumab (an anti-programmed death-1 antibody). Our case suggests that administrating nivolumab after ipilimumab/radiation therapy may have efficacy in the treatment of metastatic melanoma.

An 84-year-old man developed malignant melanoma on the left side of his back. Computed tomography (CT) identified a metastasis in the left axillary lymph node (LN). The patient underwent extensive surgery of the primary back lesion with an entire axillary LN dissection. Nineteen months after the surgery, a recurrence occurred on his left back (Fig. 1a). We surgically removed the lesions and started nivolumab (2 mg/kg) treatment every 3 weeks. After six cycles of nivolumab therapy, CT showed occupying lesions at his right axillary LN, hilar LN and right lung (Fig. 1b). Because laboratory data showed an elevated lactate dehydrogenase level and unchanged white blood cell count and C-reactive protein, we diagnosed the lesion as metastatic melanoma. We switched nivolumab to ipilimumab. After three cycles of ipilimumab therapy, the patient complained of neurogenic pain at his back. Fractionated radiation to a total dose of 39 Gy was applied to his left back for pain mitigation, which ameliorated his symptom. CT at 1 month after four cycles of ipilimumab therapy showed a progression of the metastatic lesions (Fig. 1c). Because he did not have *BRAF* mutation, nivolumab (3 mg/kg) every 2 weeks was restarted. After three cycles of the third-line nivolumab therapy, CT depicted a drastically shrunk lung lesion (Fig. 1d). Other metastatic lesions were augmented on CT. A continuous decrease of the lung metastases was seen after 3 months of nivolumab administration (Fig. 1e). The patient had developed uveitis (grade III, Common Terminology Criteria for Adverse Events version 4.0) after the third-line nivolumab was

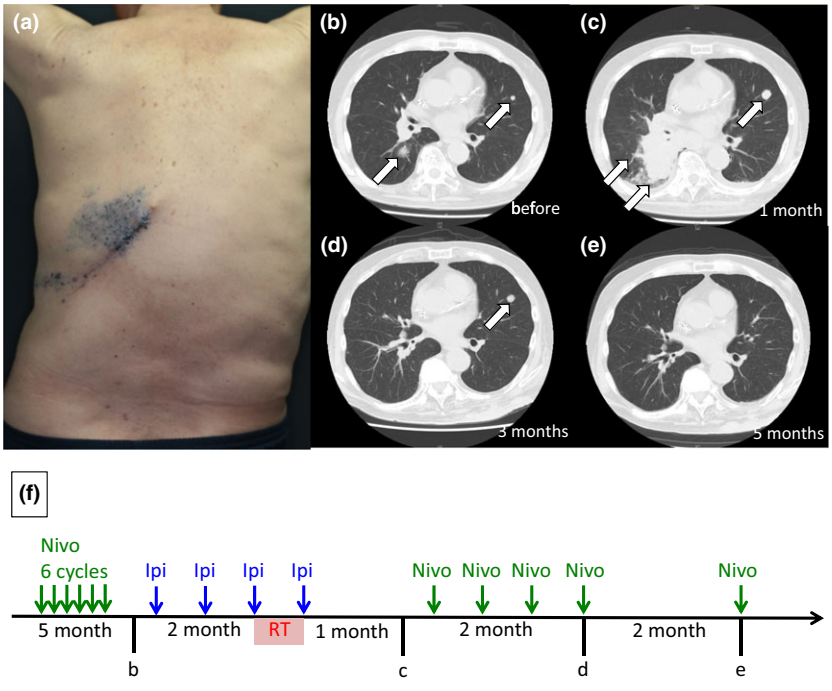

Figure 1. A clinical image and computed tomography (CT) of melanoma. (a) Melanoma lesions on the left back. (b–e) CT of lung metastatic lesions before and after ipilimumab/radiation therapy at the indicated time points. White arrows indicate metastases. (f) A schematic clinical course of the case. Ipi, ipilimumab; Nivo, nivolumab; RT, radiation therapy.

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conducted, which was cured with ocular instillation of betamethasone valerate after 2 months of cessation of nivolumab (Fig. 1f).

Local radiotherapy combined with ipilimumab is reported to have a synergistic effect on antitumor activity.[1–3] In our case, a drastic antitumor effect was obtained after ipilimumab/radiation therapy. Because the lung metastases obviously progressed during the ipilimumab/radiation therapy, we considered that ipilimumab/radiation therapy itself was not effective in our case.

Although the first-line nivolumab and subsequent ipilimumab/radiation therapy could not suppress the tumor activity, the lung metastases shrunk drastically following the third-line nivolumab treatment. We hypothesize that sequential administration of nivolumab followed by ipilimumab therapy has an effect on antitumor effect, especially when radiotherapy is combined.

Although it is limited to a single case, we experienced that giant metastatic lung melanoma disappeared after ipilimumab/radiation therapy followed by nivolumab. Even when first-line nivolumab treatment was not effective for metastatic melanoma, the subsequent nivolumab course may exert a booster effect on the immune responses to the tumor, especially after ipilimumab/radiation therapy.

CONFLICT OF INTEREST: None declared.

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LETTER TO THE EDITOR

Total cell necrosis of metastatic malignant melanoma at the regional lymph node in a patient treatment with nivolumab

Dear Editor,

Nivolumab is a monoclonal antibody to the immune checkpoint molecule programmed death 1 (PD-1).[1] Nivolumab facilitates T-cell activation and exerts antitumor effects in various cancers including melanoma.[2] Nivolumab-induced antitumor responses are thought to result from the direct injury of tumor cells by T cells.[3] Herein, we report a unique histological case of total cell necrosis of the lymph node (LN) after nivolumab treatment.

A 69-year-old man developed malignant melanoma (MM) on his left heel. A sentinel LN biopsy from the popliteal LN showed metastasis of MM. The patient underwent extensive surgery of the left heel with complete LN dissection. One year after surgery, both clinical progress at site and computed tomography (CT) indicated a relapse of MM on the left heel, and in the popliteal and inguinal LN. Nivolumab (2 mg/kg) treatment was commenced every 3 weeks from June 2016 because the relapsed tumor lesions were spread around multiple sites and were unresectable. The subcutaneous papules on the left heel rapidly diminished. The CT scans taken every 2 months showed a decrease in the size of all the LN except one in the inguinal LN. The inguinal LN gradually grew over the 4 months following the start of nivolumab treatment (Fig. 1a,b). We therefore surgically removed this LN 5 months after nivolumab treatment.

Histological analysis showed total cell necrosis of the LN cells with surrounding hyalinization (Fig. 1c). Thrombotic large

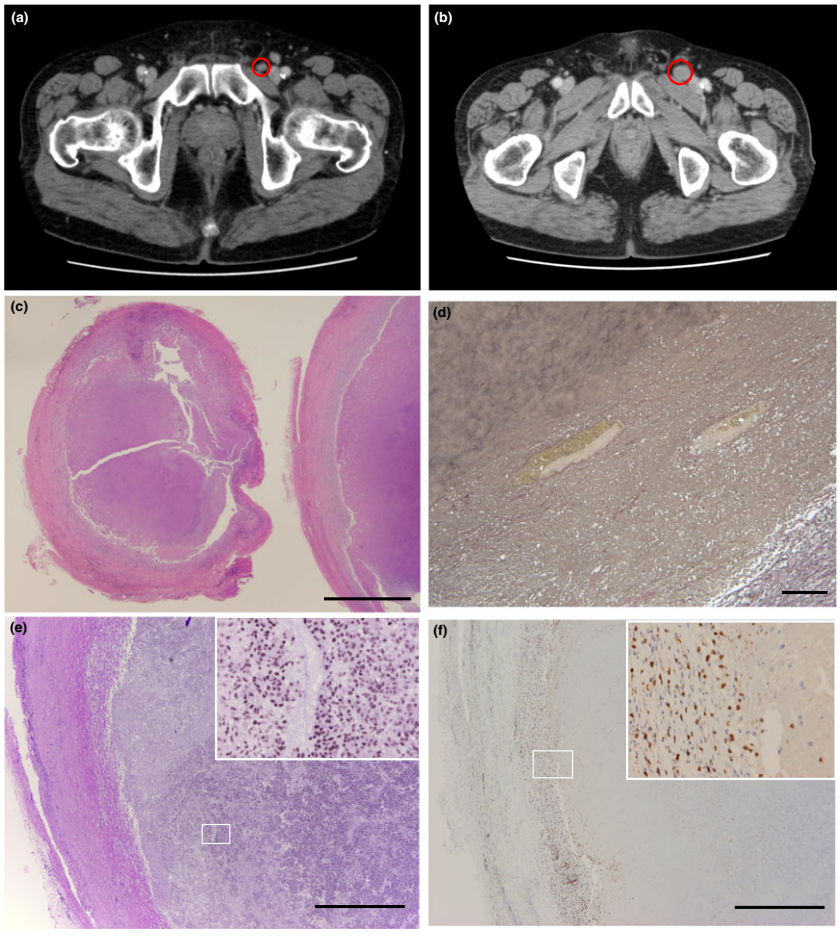

Figure 1. Computed tomography and histological findings of the inguinal lymph node (LN) after nivolumab treatment. Computed tomography of the inguinal LN (a) before surgery and (b) after nivolumab treatment. The red circles indicate growing inguinal lymph nodes. (c) Hematoxylin–eosin staining of the inguinal LN (original magnification ×1.25, scale bar 2 mm). (d) Elastica van Gieson staining of the inguinal LN. Large vessels were seen in hyalinized areas of the LN (×10, scale bar 200 μm) in multiple places. (e,f) Immunohistochemistry with anti-Sox-10 or anti-CD3 antibody (×4, scale bar 1 mm). The squares on the upper right show high-magnification images. The square indicates the region of high magnification.

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vessels were involved at the hyalinized area of the LN (Fig. 1d). Immunohistochemistry was positive for Sox-10 and Melan-A at the necrotic areas (Fig 1e and data not shown). CD3-positive cells were marginally observed in a limited part of the necrotic areas, but their aggregation was observed in the peripheral area of the LN (Fig. 1f).

Nivolumab facilitates T-cell activity to attack tumor cells directly by infiltration into the tumor parenchyma.[4] In our case, although all MM cells in the LN were necrotizing, there were a few infiltrations of CD3-positive T cells at the center of the tumor cluster (Fig. 1f).[3] This observation may indicate that direct T-cell attack was not the main inducer of tumor necrosis at the center of LN.

Total cell necrosis of the LN is developed by vascular thrombosis induced by inflammation.[5] We observed hyalinized tumor cells and large vessels around the tumor mass suggesting that strong inflammation involving large vessels occurred in the past (Fig. 1d). This strong inflammation may be caused by T cells that were activated with nivolumab treatment, triggered thrombosis of LN and induced total cell necrosis of the LN. Alternatively, circumferential T-cell-mediated tumor injury around hyalinized areas in the past may induce total cell necrosis of the LN as seen in our case.

In conclusion, we experienced a case with a unique histology of total cell necrosis of the LN after nivolumab treatment. This case suggests that nivolumab induces strong immune responses that may trigger total cell necrosis of the LN through infarction of large vessels surrounding the LN.

CONFLICT OF INTEREST: None declared.

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inflammation, and IgE production, whereas development of these is attenuated in mice deficient in ADAM 10.[9]

Intriguingly, ADAM 10 is also the cellular receptor for *Staphylococcus aureus* α-hemolysin toxin,[2] suggesting that ADAM 10–dependent responses to allergens and infections, both viral and bacterial, may represent a signaling nexus in chronic severe disease exacerbations, which merits further examination in the clinic.

Additional information is available (see this article's Methods, Results, and References section in the Online Repository at www.jacionline.org).

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Local inflammation exacerbates cutaneous manifestations in a murine autoimmune pemphigus model

To the Editor:

Autoantibodies directed against antigens in extravascular tissues must pass through the blood vessel wall to exert their pathogenicity. How their extravasation is regulated *in vivo* remains largely unknown. Pemphigus is one of such autoantibody-mediated disorders, which is caused by pathogenic autoantibodies against epidermal desmosomal cadherins, desmoglein (Dsg) 1 and Dsg3. Because these autoantibodies can directly disrupt epidermal cell-cell adhesion,[1] their concentration in the epidermis would be linked to the disease severity. Such anti-Dsg antibody deposition in the epidermis has been evaluated by direct immunofluorescence (DIF) assay in the skin biopsy specimens. Besides, the elicitation of blistering after external stimuli such as mechanical trauma or sun exposure has been reported in patients with pemphigus.[2,3] These observations imply that the local inflammation, which permits extravasation of macromolecules,[4] might also promote extravasation and subsequent deposition of autoantibodies in the skin. Herein, we tested this hypothesis by using a murine pemphigus model.

We first evaluated the dynamics of epidermal antibody deposition in inflamed and noninflamed skin in mice after intravenously injecting fluorescein isothiocyanate–conjugated AK18, a nonpathogenic antimouse Dsg3 antibody that belongs to the IgG_1 subtype.[5] We used this nonpathogenic antibody to avoid keratinocyte dissociation and local inflammation that may hinder the subsequent analysis. Intravital observation by 2-photon microscopy demonstrated that epidermal AK18 deposition occurred gradually over 6 hours in the inflamed ears treated with topical phorbol myristate acetate (PMA), whereas no deposition was observed in the untreated ears (Fig 1, *A*), suggesting that local inflammation enhances AK18 deposition.

For more precise evaluation, we used a flow cytometric analysis. Twenty-four hours after intravenous injection of AK18 simultaneously with PMA application to the ear skin, the concentration of AK18 in the ear epidermis was quantified by measuring the mean fluorescent intensity (MFI) of IgG_1 in $CD45^-$ E-$cadherin^+$ keratinocytes (hereafter referred to as IgG_1-MFI) (Fig 1, *B*; see Fig E1, *A* and *B*, in this article's Online Repository at www.jacionline.org). IgG_1-MFI was significantly increased in the PMA-painted ears compared with the untreated ears (Fig 1, *C*). Neither the expressions of Fcγ receptors nor IgG_1-MFI following control-IgG_1 injection was elevated on keratinocytes after PMA application, suggesting that this assay specifically detects AK18 deposition in the epidermis (Fig E1, *C* and *D*). In both the untreated and PMA-painted ears, IgG_1-MFI positively correlated with the dose of AK18 injected (1-1000 μg) (Fig 1, *D*).

In the PMA-painted ears, 10 μg of intravenous AK18 injection to mice seemed to be sufficient to induce the equivalent IgG_1-MFI in the untreated ears of mice that received 100 μg of AK18 (Fig 1, *D*). Consistently, DIF assay revealed epidermal antibody deposition in the PMA-painted ears that received as little as 5 μg of AK18, whereas antibody deposition was undetectable in the untreated ears of mice that received as much as 50 μg of AK18 (Fig 1, *E*). Similar enhancement in antibody deposition in the PMA-painted ears was observed irrespective of IgG subclasses (IgG_1 or IgG_4) when injecting serums from 2 patients with pemphigus (Fig 1, *F*; see Fig E2 in this article's Online Repository at www.jacionline.org). At least 100 μL of each serum was required to obtain apparently positive DIF results in the untreated ears, whereas positive DIF results were observed in the PMA-painted ears when injecting 20 μL of each serum. Taken together, these findings allowed us to consider that local skin inflammation can enhance the autoantibody deposition in the epidermis *in vivo*.

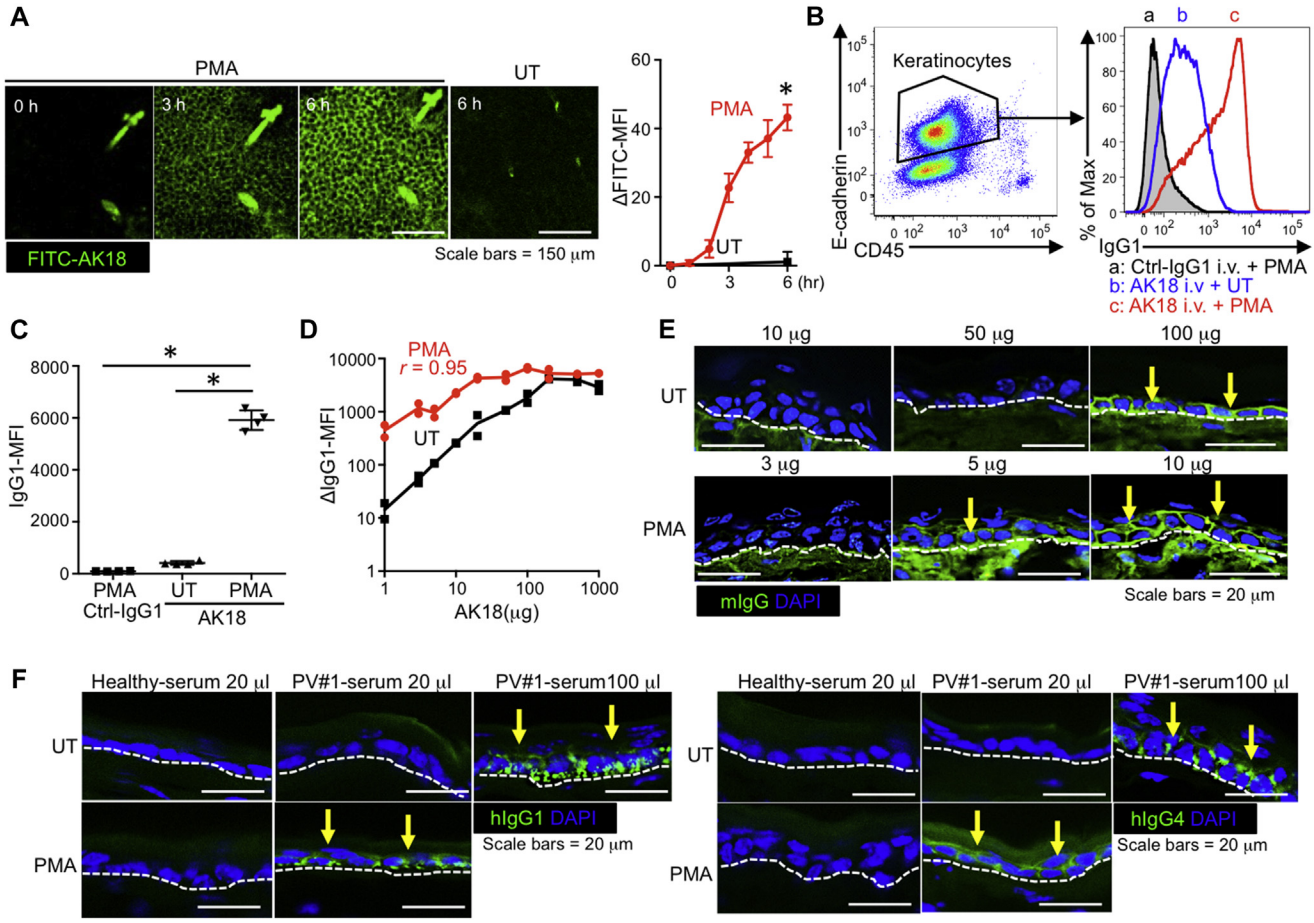

FIG 1. Anti-Dsg3 antibody deposition in the untreated and PMA-painted skin. **A,** Time-lapse images and ΔFITC-MFI of epidermis in the PMA-painted (PMA) or untreated (UT) ear after FITC-labeled AK18 injection. **B,** Identification of IgG_1 among keratinocytes. **C,** IgG1-MFI after 20 μg of AK18 or control IgG_1 injection. **D,** ΔIgG_1-MFI against AK18 injection doses. **E** and **F,** DIF after injection of AK18 (Fig 1, *E*), or serum from a healthy donor or a pemphigus patient **(F)**. *FITC*, Fluorescein isothiocyanate. **P* < .05.

To evaluate epidermal antibody deposition under more physiological skin inflammations, we used other inflammatory stimuli, such as a single painting of hapten (Fig 2, *A*) and tape-stripping (Fig 2, *B*). These treatments were sufficient to increase IgG_1-MFI. In addition, we irradiated mouse ears with ultraviolet B and demonstrated a positive correlation of IgG_1-MFI with the ultraviolet B irradiation dose ($r = 1.00$) (Fig 2, *C*) and with the intensity of skin inflammation as evaluated by ear swelling ($r = 0.90$) (Fig 2, *D*). These results indicate that deposition of autoantibody in tissue is correlated with the severity of local inflammation in mice.

Last, to confirm that local inflammation predisposes to the development of the pemphigus phenotype, we used a pathogenic antimouse Dsg3 antibody, AK23. As previously reported,[5] administration of a sufficient dose of AK23 resulted in hair loss in mice (>80-100 μg; see Fig E3 in this article's Online Repository at www.jacionline.org). In contrast, hair loss and epidermal antibody deposition was limited to the PMA-painted area when mice received a subpathogenic dose (50 μg) of AK23 (Fig 2, *E* and *F*). These observations demonstrate that pemphigus lesions tend to occur at the local inflammatory site in mice.

We have previously demonstrated that the histamine-induced vascular hyperpermeability evokes the extravasation of macromolecules *in situ*.[4] In this context, we hypothesized that cutaneous histamine prick[6] might be useful for enhancing the diagnostic sensitivity of DIF assay. We evaluated antibody deposition in the mouse ear after histamine prick with intravenous AK18 injection. As expected, IgG_1-MFI was increased by histamine prick, but not by vehicle-prick (Fig 2, *G*). This result suggests that preceding histamine prick is useful for enhancing antibody deposition in the skin.

In summary, our results in mice suggest that local inflammation might also enhance anti-Dsg3 antibody deposition in the skin in patients with pemphigus. Thus, taking skin biopsies from perilesional erythematous (presumably inflammable) areas in autoimmune blistering diseases might be beneficial to increase the DIF sensitivity. It might also explain the induction of blisters after external stimuli such as sun exposure, the pathogenesis of which remains unclear.[2,3] Likewise, the predisposition of pemphigus skin lesions on the back as well as intertriginous and seborrheic areas[7] might reflect the existence of multiple external stimulants, including frictions and mechanical pressure. This increase in autoantibody deposition (see Fig E4 in this article's Online Repository at www.jacionline.org) might be common for antibody-related autoimmune conditions and related to their symptoms, such as the sun exposure–induced skin

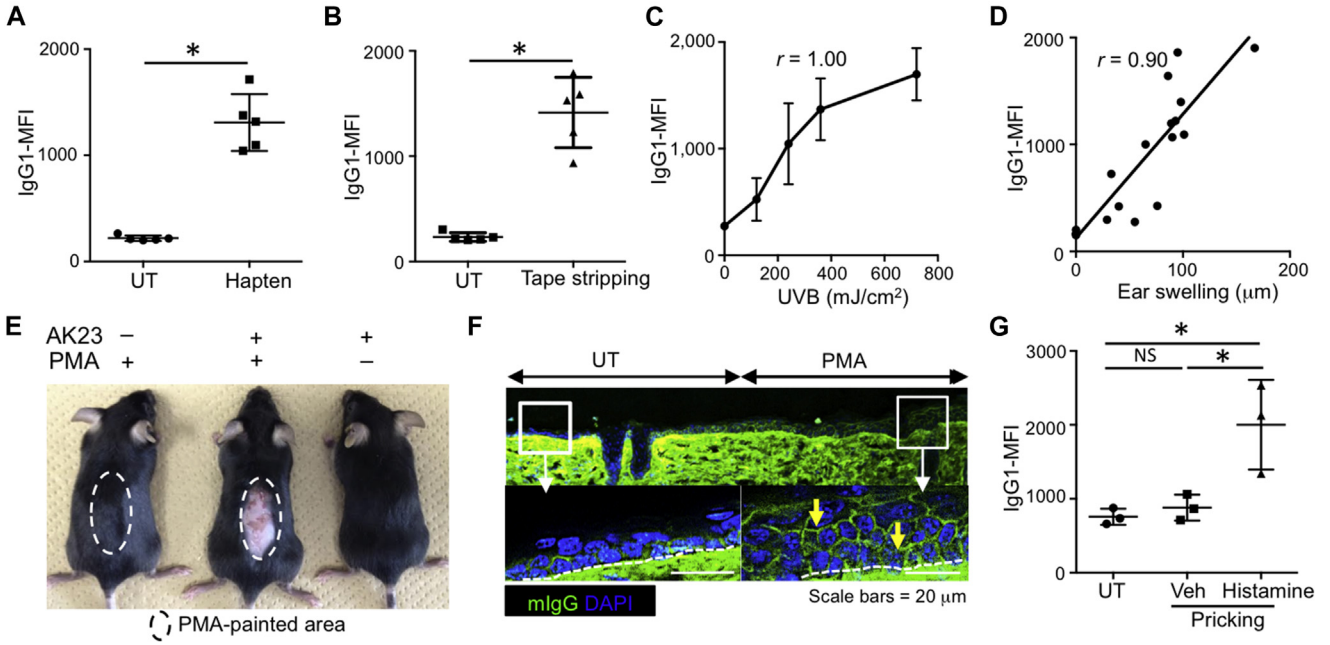

FIG 2. Anti-Dsg3 antibody deposition under various skin inflammatory conditions. **A-C,** IgG_1-MFI of hapten-painted (Fig 2, *A*), tape-stripped (Fig 2, *B*), and UVB-irradiated ears (Fig 2, *C*) compared with that of untreated ears. **D,** A plot of ear swellings against IgG_1-MFI in Fig 2, *C*. **E** and **F**, Hair loss (Fig 2, *E*) and AK23 deposition (Fig 2, *F*) limited to the PMA-painted area. **G**, IgG_1-MFI of the ear after a histamine- or vehicle (Veh)-prick. Precise information is located in this article's Methods section in the Online Repository at www.jacionline.org. *UT*, Untreated; *UVB*, ultraviolet B. *$P < .05$.

eruptions in lupus erythematosus.[8] Furthermore, our preliminary study demonstrated that a histamine pricking, which is commonly used in clinic as a positive control for type I hypersensitivity,[6,9] may improve the diagnostic sensitivity of DIF assay when administered before skin biopsy (data not shown). A cutaneous histamine prick might also assist in diagnosis of patients especially whose pemphigus lesions are confined to the oral mucosa, where taking a biopsy specimen is often hard to conduct.

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METHODS

Preparation of epidermal cell suspension for a flow cytometric analysis and calculation of IgG_1-MFI

AK18 or control mouse $IgG_{1\kappa}$ (Biolegend, San Diego, Calif) diluted in 500 μL PBS were injected via tail vein. In every examination otherwise indicated, the ear skins were collected 24 hours after intravenous injection of AK18, and dorsal halves were floated for 30 minutes at 37°C on 5 mg/mL dispase II (Wako Pure Chemical industries Ltd, Osaka, Japan) dissolved in RPMI 1640 (Invitrogen, Carlsbad, Calif) containing 10% heat-inactivated FCS. The epidermis was then manually separated from the dermis and floated on 0.25% trypsin-EDTA for 8 minutes at 37°C and filtered through 70-μm cell-strainer mesh (BD Bioscience, San Diego, Calif). The epidermal suspensions were stained with fluorochrome-conjugated antibodies to E-cadherin, CD45, and mouse IgG_1 (BD Bioscience). Cells were analyzed with FACS LSR Fortessa flow cytometric system (BD Bioscience) and FlowJo software (Tree Star, Ashland, Ore), and IgG_1-MFI was calculated in E-cadherin$^+$ CD45$^-$ epidermal keratinocytes. ΔIgG_1-MFI indicates the changes in MFI from vehicle control.

Time-lapse image acquisition by 2-photon microscope

Five milligram per milliliter of fluorescein isothiocyanate (FITC) in dimethyl sulfoxide, 1 mol sodium bicarbonate buffer at pH 9.1, and 1 to 2 mg/mL of AK18 were reacted for 2 hours at room temperature. FITC-conjugated AK18 were obtained after several dialyses. PMA was applied to the mouse ear to evoke inflammation 24 hours before intravenous injection of 100 μg of FITC-labeled AK18. Mice were positioned on the heating-plate on the stage of a 2-photon microscope, IX-81 (Olympus, Tokyo, Japan), and their ear lobes fixed beneath cover slips with a single drop of immersion oil. The tail vein was cannulated using polyethylene tubing (PE-10: Becton, Dickenson and Co, Franklin Lakes, NJ) with a 30-gauge disposable needle (Dentronics, Tokyo, Japan) connected to a syringe filled with FITC-conjugated AK18. The time course of *in vivo* AK18 deposition to the epidermal keratinocytes was evaluated after injection. To calculate ΔFITC-MFI, the changes in MFI from 0 hour were evaluated in 3 epidermal areas.

DIF analysis

As for DIF staining, the ears of mice were collected 24 hours after intravenous injection (otherwise indicated) of AK18, or serums from patients with pemphigus vulgaris (PV) or a healthy donor, fixed in 4% paraformaldehyde in PBS, and embedded in O.C.T. compound (Sakura Finetek, Torrance, Calif) for frozen sectioning. Five-micrometer slices were prepared and were incubated for 30 minutes at room temperature with goat antimouse IgG Alexa fluora 546 (Invitrogen), after treatment with Image-iT FX Enhancer (Invitrogen). Slices were mounted with antifade mounting reagent with 4′-6-diamidino-2-phenylindole, dihydrochloride (Invitrogen). In each picture, yellow arrows represent intercellular autoantibody deposition. Blue represents nuclei stained by 4′-6-diamidino-2-phenylindole, dihydrochloride. White dotted lines represent the border between the epidermis and the dermis.

DIF analysis using serums from patients with PV

Serums from 2 patients with PV (PV1 and PV2), who were positive for anti-Dsg3 antibody but not anti-Dsg1 antibody, were collected for the analysis. Among the IgG_1-IgG_4 subclasses, PV1 was positive for the IgG_1 and IgG_4 subclasses. PV2 was positive for the IgG_4 subclass. With intravenous injection of each serum of PV1 and PV2 to adult mice via tail vein, we determined that at least 100 μL of each patient's serum was required to obtain apparently positive DIF results in the untreated ears. In contrast, positive DIF results were observed in the PMA-painted ears of adult mice with injection of even under 20 μL of each patient's serum. Because of the limitation of the serum amount, the DIF experiments using patients' serums were performed twice with similar results for each serum.

Induction of skin inflammation to the mouse ear

The ears of mice were irritated with either 0.1 mg/mL PMA in acetone, tape-stripping, 0.5% 1-Fluoro-2, 4-dinitrobenzen-fluorodinitrobenze (Nakarai Tesque, Kyoto, Japan) in acetone/olive oil as a hapten, or ultraviolet B (UVB) irradiation. A hapten is applied in nonsensitized mice. In every experiment otherwise indicated, mice were intravenously injected with 10 μg of AK18, and the ears were collected 24 hours later. As for PMA, a hapten, and a tape-stripping, they were applied to the ears of mice simultaneously with intravenous AK18 injection. As for UVB, intravenous AK18 injection was given just after the last irradiation to mice. In particular, irradiation was conducted as 120 mJ/cm^2 for 1 day (day 0, total 120 mJ/cm^2), 2 days (day −1 and day 0, total 240 mJ/cm^2), 3 days (day −2, day −1, and day 0, total 360 mJ/cm^2), or 240 mJ/cm^2 for 3 days (day −2, day −1, and day 0, total 720 mJ/cm^2) before intravenous AK18 injection at day 0. The ear swelling levels were measured 24 hours after the final UVB irradiation.

Induction of hair loss and DIF analysis in mice with intravenous AK23 injection

Fifty microgram of AK23 was intravenously injected to mice simultaneously with PMA application to the dorsal skin. The boundary lesion to cover both untreated and PMA-painted areas of the dorsal skin was evaluated for DIF analysis 2 days after PMA and AK23 administration.

Histamine prick to the mouse ears

Histamine dihydrochloride (10 mg/mL; Sigma-Aldrich) in PBS was administered with pricking of the dorsal ear with lancet needles. PBS was also administered as a control. Histamine prick was administered simultaneously with 20 μg of intravenous injection of AK18, and epidermis was collected 24 hours later for a flow cytometric analysis.

Statistical analysis

Unless otherwise indicated, data are presented as means ± SDs and each data point is representative of 3 independent experiments. Statistical analyses were performed using Graphpad prism (GraphPad Software, Inc, La Jolla, Calif), using either a parametric Student *t* test or 1-way ANOVA test. A *P* value of less than .05 was considered to indicate statistical significance, and indicated as asterisk. NS indicates not significant.

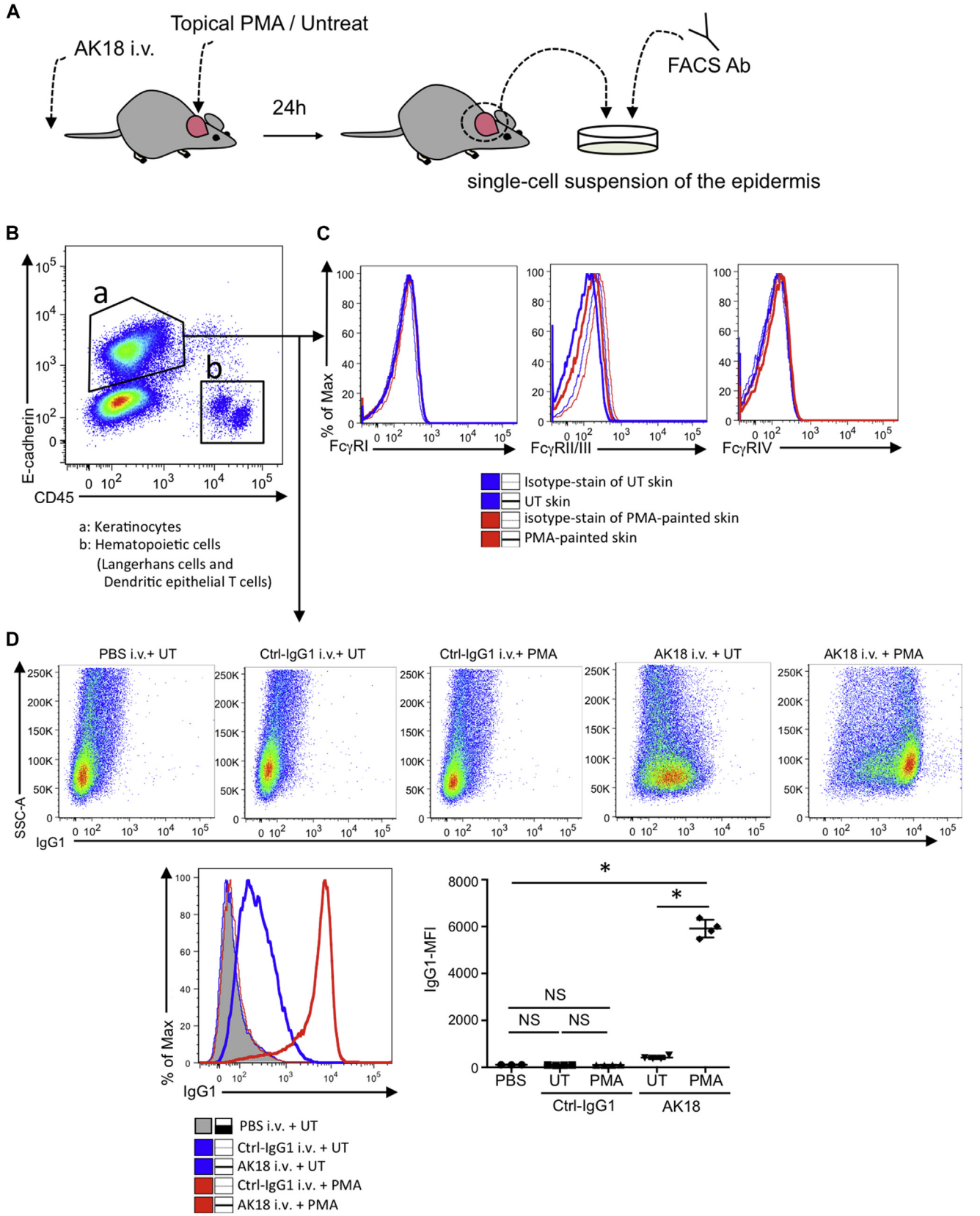

FIG E1. A flow cytometry–based analysis for detecting AK18 deposition in the murine epidermis. **A,** A schema of preparation of epidermal cell suspension. **B,** Identification of keratinocytes (a) and hematopoietic cells (b). **C,** Expression of Fcγ receptors among keratinocytes, compared with isotype-stained control. **D,** Identification of IgG_1 and IgG_1-MFI among keratinocytes after injection of PBS, 20 μg of AK18, or 20 μg of control IgG_1, in PMA-painted or untreated ears. *$P < .05$. *NS*, Not significant; *UT*, untreated.

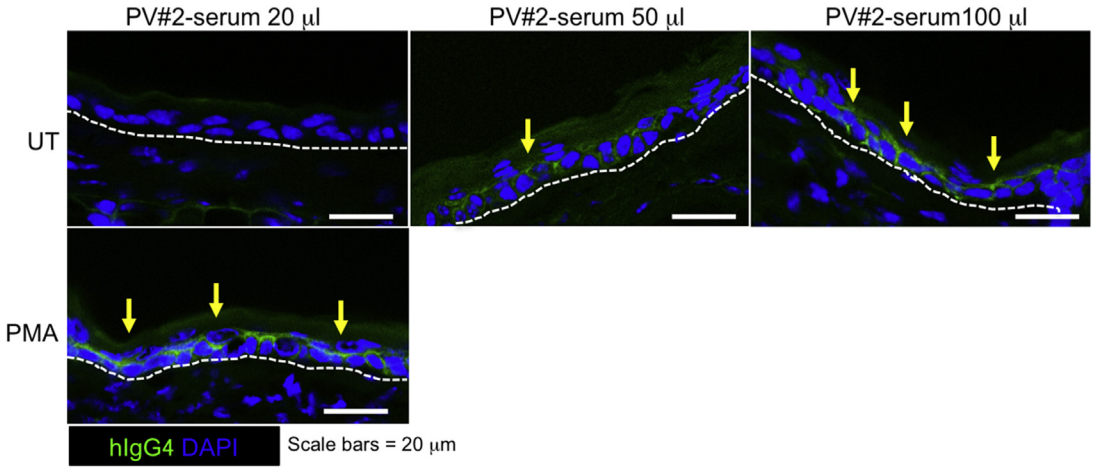

FIG E2. Human anti-Dsg3 antibody deposition in the untreated and PMA-painted mouse skin. Immunohistochemical evaluation for antibody deposition after intravenous injection of various doses of serum from a patient with PV (PV2) whose anti-Dsg3 antibodies were of IgG_4 subclass. *UT*, Untreated.

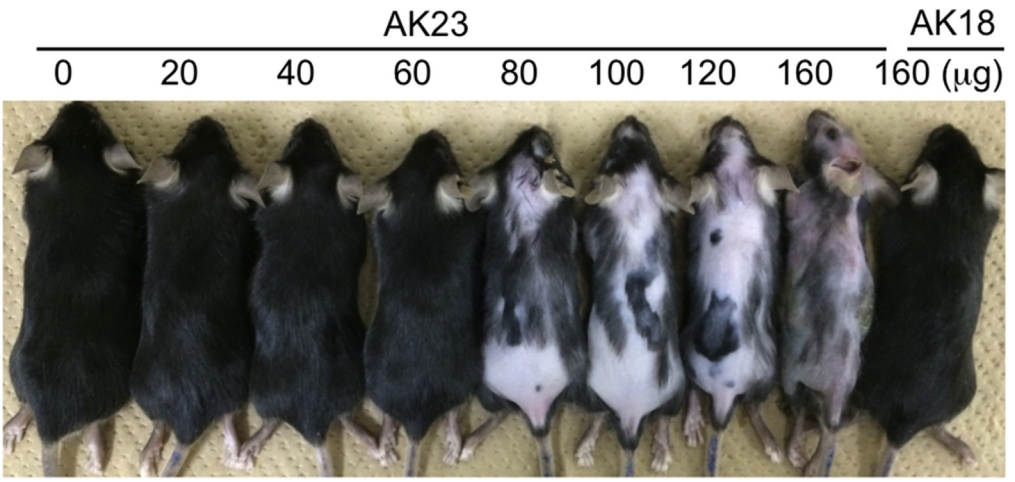

FIG E3. Phenotype of a murine pemphigus model. Manifestation of hair loss after intravenous injection of AK23 or AK18. The injection dose is indicated above each mouse.

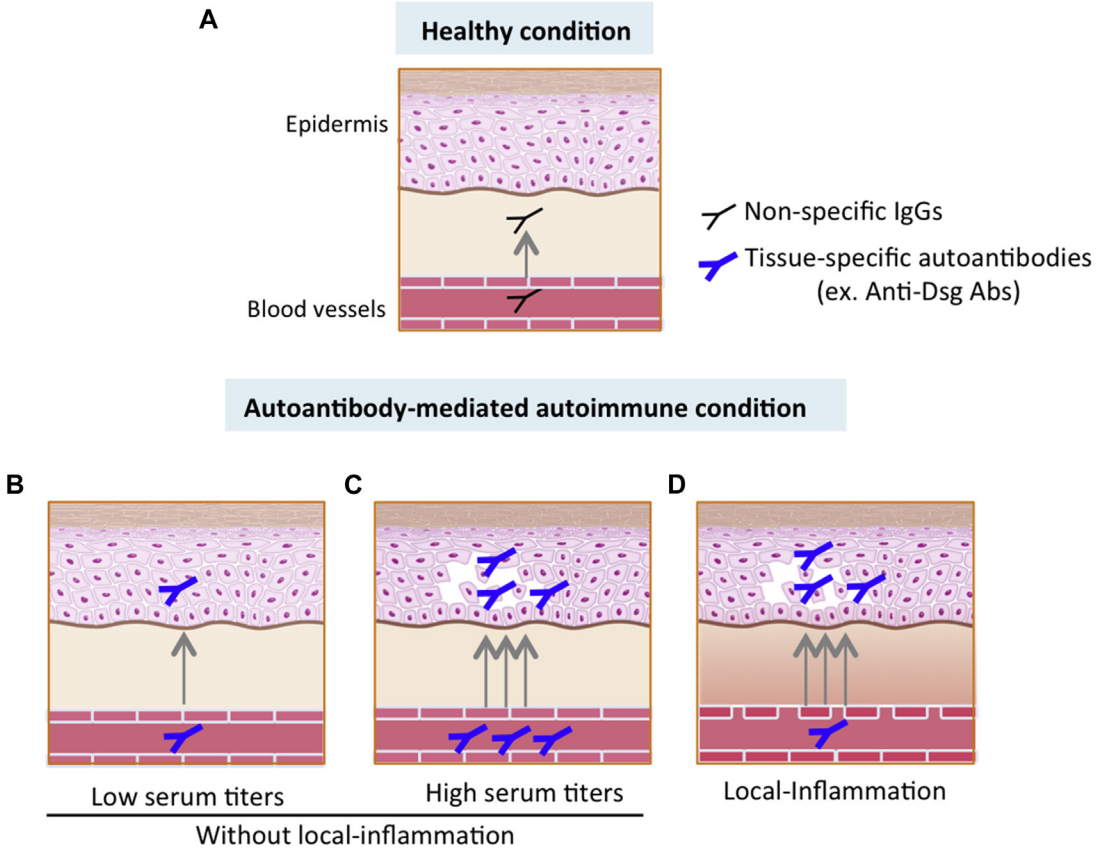

FIG E4. A conceptual drawing of kinetics of IgGs including anti-Dsg antibodies in the skin. **A,** No nonspecific IgGs deposition in the epidermis. **B,** Low epidermal anti-Dsg antibody deposition and unapparent skin manifestations with low serum antibody titers. **C,** Accumulation of epidermal anti-Dsg antibody deposition and induction of intraepidermal blistering with high serum antibody titers. **D,** Enhancement of epidermal anti-Dsg antibody deposition by local inflammation and induction of intraepidermal blistering with low serum antibody titers. *Abs*, Antibodies.