Letter to the Editor

Epithelial barrier dysfunction in desmoglein-1 deficiency

To the Editor:

Mutations in the desmoplakin *(DSP)* and desmoglein-1 *(DSG1)* genes have been implicated in patients with the inherited inflammatory skin disease known as severe dermatitis, multiple allergies, and metabolic wasting (SAM) syndrome (MIM#603165, see Tables E1 and E2 in this article's Online Repository at www.jacionline.org).[1,2] The *DSP* and *DSG1* genes encode desmosome components that are critical for the structure of intercellular junctions and maintenance of epithelial barrier integrity. DSP and DSG1 are also key regulators of signaling pathways involved in differentiation, epidermal homeostasis, and carcinogenesis. DSG1 promotes keratinocyte differentiation by inhibiting epidermal growth factor receptor/extracellular signal-regulated kinase signaling through ERBB2-interacting protein (ERBIN), a scaffolding and signaling protein.[3] Through characterization of a new syndrome featuring severe allergic dermatitis and DSG1 deficiency, we highlighted the pivotal role of the functional DSG1/ERBIN interaction as an inhibitor of skin inflammation through the nuclear factor κB (NF-κB) signaling pathway.

Patients 1 and 2 (13 and 9 years old, respectively) are 2 unrelated boys born to healthy parents. They were referred for life-long desquamative erythroderma associated with sparse and wooly hair and dysplastic enamel. Both patients had painful palmoplantar keratoderma and dystrophic nails (Fig 1, *A* and *B*). Skin manifestations combined recurrent and painful erythrodermic skin flares triggered by infections and episodes of aseptic pustular psoriasiform dermatitis. Patient 1 (but not patient 2) displayed failure to thrive, eosinophilic esophagitis, colitis, and a variety of food allergies (total serum IgE level, 2968 kIU/mL [n < 114]). Cardiac examination of patient 1 revealed an asymptomatic, biventricular, dilated cardiomyopathy. At 9 years of age, patient 2 received a heart transplant because of a severe left-dominant arrhythmogenic cardiomyopathy.

For both patients, cutaneous histopathology showed epidermal acantholysis and dermal inflammatory lymphocytic infiltration (Fig 1, *C*, and see Fig E1 and the Methods section in this article's Online Repository at www.jacionline.org). Ultrastructural examination revealed large numbers of abnormal clusters of desmosomes in the epidermis (patient 1; Fig 1, *D*). Histopathology of the explanted heart showed the characteristic fibro-fatty myocardial infiltration of arrhythmogenic dysplasia (see Fig E1).

Two different heterozygous *de novo* missense mutations were identified by using whole-exome sequencing in exon 14 of the *DSP* gene: c.A1757C (p.H586P, patient 1) and c.T1828C (p.S610P, patient 2). Substitution of H586 or S610 by a proline is expected to induce a kink in the α-helix of DSP plakin domain that perturbs DSP's 3-dimensional structure (see Fig E1).

In both patients skin immunohistochemistry showed the following features: (1) low DSP and DSG1 expression in the epidermis (as in patient 1's primary keratinocytes), (2) irregular and less intense DSP and DSG1 staining at the keratinocyte plasma membrane, and (3) abnormal cytoplasmic accumulation of DSP and DSG1 proteins in keratinocytes (Fig 1, *E*). Esophageal immunohistochemistry revealed a low level of DSP staining, which was irregular and mottled at the cell border, and the absence of DSG1 expression (patient 1; Fig 1, *F*). Expression of DSP protein was also low in heart tissue (patient 2; see Fig E1; DSG1 is not expressed in the heart).

Abnormally high levels of mRNAs encoding proinflammatory cytokines (*IL6*, *IL8*, and *IL1B*), 3 NF-κB target genes, and *TSLP* were found in patient 1's keratinocytes (Fig 2, *A*). Overexpression of IL-6 was confirmed by means of ELISA (see Fig E2 in this article's Online Repository at www.jacionline.org). In contrast, mRNA levels of *TNFA* and other pro-T_H2 cytokines (*IL13*, *CCL5*, and *IL4*; data not shown) were not increased. Inhibition of the NF-κB signaling pathway by ML120B, which selectively targets the catalytic subunit of inhibitor of NF-κB kinase (IKK) β, restored normal expression of *IL8* mRNA by patient 1's keratinocytes (see Fig E2). In view of the primary DSG1 deficiency reported in patients with SAM syndrome and the very low levels of DSG1 protein expression in our patients, we hypothesized that DSG1 could play a role in the inflammatory phenotype. We demonstrated that DSG1 (but not DSP) inhibits NF-κB reporter activity in a dose-dependent manner after stimulation by IL-1β or TNF-α in HEK293T cells (Fig 2, *B*, and see Fig E2). Concomitant downregulation of *IL6* and *IL8* expression was observed on transfection with the DSG1-encoding plasmid (see Fig E2). Silencing of *DSG1* by short hairpin RNA (with a mean decrease of *DSG1* mRNA of 32%) enhanced transcription of *IL6*, *IL8*, *IL1B*, *TNFA*, and *TSLP* genes in control keratinocytes (regardless of stimulation with IL-1β; Fig 2, *C*, and see Fig E2). Retroviral transduction of wild-type *DSG1* into patient 1's keratinocytes rescued the inflammatory cellular phenotype by restoring *IL8* production (Fig 2, *D*).

Given that DSG1 might regulate ERBIN's cellular localization and thus modulate the latter's ability to block extracellular signal-regulated kinase signaling, we hypothesized that ERBIN can play a role in DSG1-mediated NF-κB inhibition.[3] We observed little ERBIN/DSG1 colocalization at the cell membrane and an accumulation of ERBIN in the cytoplasm in a skin biopsy specimen from patient 1 relative to healthy control values (mean proportion of ERBIN/DSG1 colocalization, 40.7% vs 71.2%; $P < .001$; see Fig E3 in this article's Online Repository at www.jacionline.org).

Experiments in control keratinocytes highlighted recruitment of ERBIN to the cell membrane and an increase in ERBIN/DSG1 colocalization after stimulation by IL-1β. Interestingly, this recruitment did not occur in patient 1's keratinocytes (see Fig E3). Contrary to patient 1's keratinocytes, the level of ERBIN protein increased after stimulation of control keratinocytes by IL-1β (see Fig E3). These results demonstrated that localization of ERBIN at the cell membrane and membrane colocalization of ERBIN/DSG1 in keratinocytes were regulated by inflammatory stimuli (eg, IL-1β).

In a reporter transactivation assay, we found that ERBIN transfection in both nonstimulated and IL-1β–stimulated HEK293T cells led to NF-κB activation in a dose-dependent manner (Fig 2, *E*). *Erbin*$^{-/-}$ keratinocytes, derived from *Erbin*$^{-/-}$ mice, showed abnormally low mRNA expression of the proinflammatory cytokines *IL6* and *IL1B* (Fig 2, *F*). In addition, abnormally low expression of *IL1B* mRNA were found in the *Erbin*$^{-/-}$ mouse

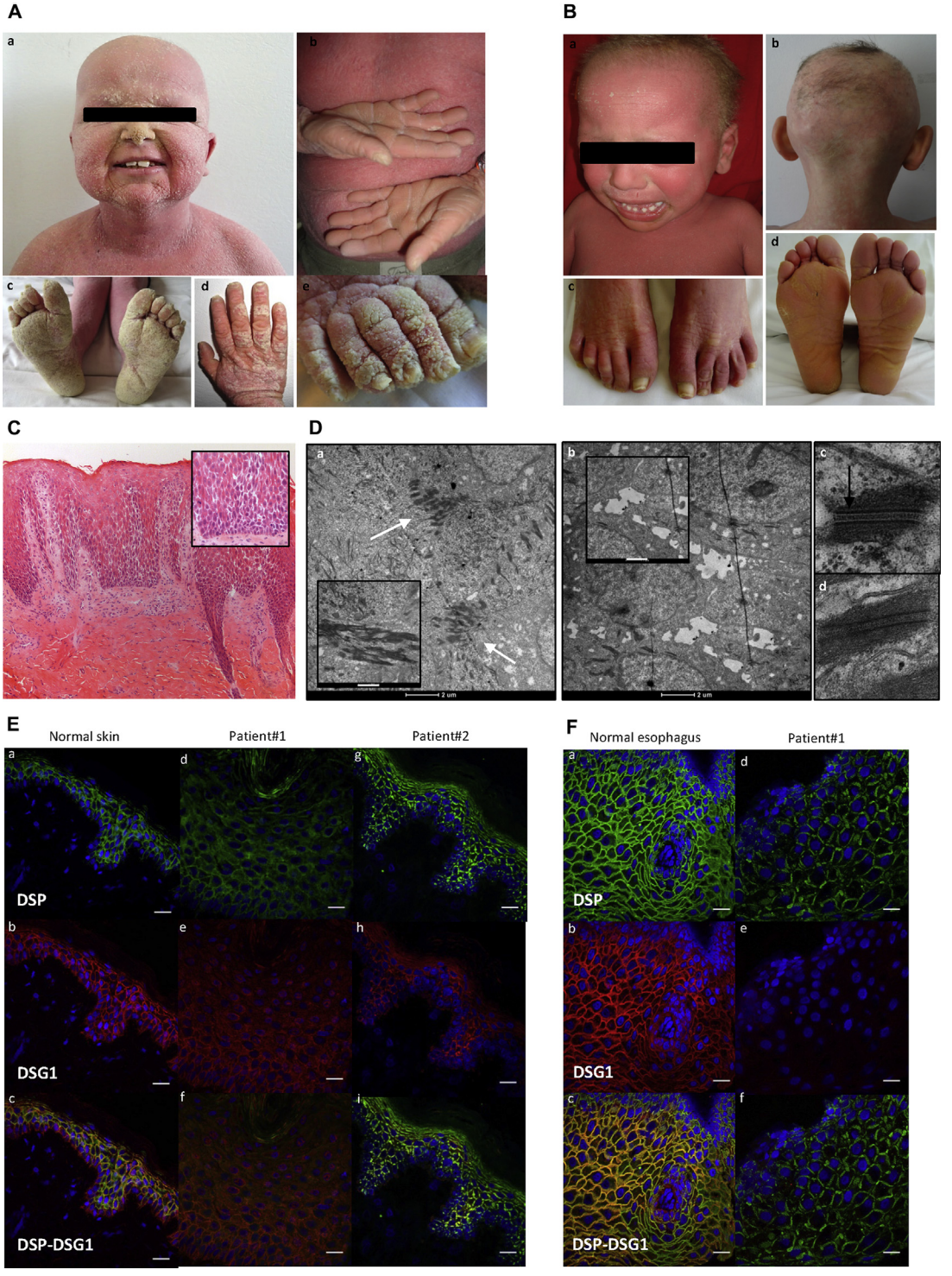

FIG 1. Clinical and histopathologic features of patients 1 and 2. **A,** Clinical phenotype of patient 1: desquamative erythroderma, thickening of the skin *(a-e)*, sparse hair *(a)*, diffuse palmoplantar keratoderma (*PPK*; *b* and *c*), and thickening of the nail plate (*d* and *e*). **B,** Clinical phenotype of patient 2: desquamative erythroderma *(a)*, sparse hair (*a* and *b*), onycholysis *(c)*, and plantar keratoderma *(d)*. **C,** Skin histology (patient 1) showing epidermal acanthosis, hyperorthokeratosis, extensive acantholysis *(inset)*, and inflammation in the superficial dermis (lymphocytes; ×100 magnification for Fig 1, *C*, and ×200 magnification for the *inset* of Fig 1, *C*). **D,** Ultrastructural features of a skin biopsy specimen from patient 1 (*a*, *b*, and d) and a healthy control subject *(c)*. *a*, Many desmosomes clustered in the upper epidermis *(arrows)*. Keratin filaments strongly aggregated to the desmosome *(inset)*. *b*, Complete absence of desmosomes and widened spaces between keratinocytes (cell membrane features filipodium-like processes; *inset*) in the lower epidermis. *d*, Patient 1's desmosome lacks the inner plaque compared with a control subject (*arrow*, *c*). Scale bar = 2 μm for Fig 1, *D*, *a* and *b*. Scale bar = 500 nm for the *inset* of Fig 1, *D*, *a*, and 1 μm for the *inset* of Fig 1, *D*, *b*. **E,** Immunofluorescence on skin sections from a healthy control subject *(a-c)*, patient 1 *(d-f)*, and patient 2 *(g-i)* showed drastic reduction and mislocalization in staining of DSP and DSG1 in both patients. Scale bar = 20 μM. **F,** Immunofluorescence on esophageal sections from a healthy control subject *(a-c)* and patient 1 *(d-f)*, showing low levels of DSP staining and absence of DSG1 staining in patient 1. Scale bar = 20 μM.