Elevated Expressions of BTN3A1 and RhoB in Psoriasis Vulgaris Lesions by an Immunohistochemical Study

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Abstract: Psoriasis is a chronic, immune-mediated inflammatory disease which pathogenesis is closely linked to γδ T cells. Recently, a critical role for butyrophilin 3A1 (BTN3A1) in mediating the activation of Vγ9Vδ2 T cells, which are reported to redistribute from blood to the perturbed skin lesions in psoriasis, has been proposed. Additional molecular partners, including RhoB and periplakin, have also been speculated to interact with BTN3A1 in modulating Vγ9Vδ2 T-cell activation. Immunohistochemical staining was performed to examine the expressions of BTN3A1, RhoB, and the plakin family members, including periplakin, epiplakin, and envoplakin in the psoriasis vulgaris lesions as compared with the normal control. The expressions of BTN3A1 and RhoB were found significantly upregulated in the psoriatic lesions. Besides, a downregulation of periplakin and an upregulation of epiplakin were noticed in the psoriasis vulgaris lesions. Our data suggest that BTN3A1 and RhoB might participate in the pathogenesis of psoriasis through Vγ9Vδ2 T-cell responses. In addition, a potential involvement of the plakin protein family, especially periplakin and epiplakin, in psoriasis pathology was proposed.

Key Words: psoriasis vulgaris, BTN3A1, RhoB, plakin protein family

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Psoriasis is a chronic inflammatory skin disease affecting ~0.09% to 5.1% of the general population.[1] The pathologic histology of psoriasis characteristically shows epidermal hyperplasia, parakeratosis, and dermal infiltration by mixed inflammatory cells, including dermal dendritic cells, macrophages, T cells, and neutrophils.[2] The interplay between interleukin (IL)-23 and IL-17 is now known to be at the core of psoriasis immune dysregulation.[3] γδ T cells are greatly increased in the psoriatic lesions and are thought to be the major IL-17 producers in the skin.[4]

γδ T cells are critical to the maintenance and regulation of the immune barrier and are essential in preventing cutaneous neoplasms at epithelial surfaces.[5,6] Vγ9Vδ2 T cells, the major γδ T-cell subset in human peripheral blood, are specifically activated by a set of pyrophosphate metabolites collectively named phosphoantigens (pAgs), including microbial (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate and endogenous isopentenyl pyrophosphate which is the intermediate of the mammalian mevalonate pathway.[7] These pAgs are sensed by butyrophilin 3A1 (BTN3A1) protein, and eventually leading to the activation of Vγ9Vδ2 T cells.[8] In addition to BTN3A1, RhoB and periplakin have also been reported as critical mediators for Vγ9Vδ2 T cells activation. Sebestyen et al[9] claimed that Vγ9Vδ2 T-cell activation was modulated by RhoB GTPase activity, which facilitated the spatial redistribution of BTN3A1 by promoting cytoskeletal trapping in the plasma membrane and subsequently induced the binding of pAgs to BTN3A1. Moreover, previous research suggested that the plakin protein family, including periplakin, epiplakin, and envoplakin, was essential for tissue homeostasis and the regulation of immune responses in the epithelium.[10] Among the plakin family, periplakin was found to significantly interact with BTN3A1, demonstrating a functional role in Vγ9Vδ2 T-cell activation.[11] A schematic diagram which depicts the possible role of BTN3A1, RhoB, and periplakin in the activation of γδ T cells and psoriasis pathogenesis is shown in Figure 1.

Taken into account the specific character of γδ T cell in the pathology of psoriasis, and the correlation between BTN3A1, RhoB, and periplakin with the activation of Vγ9Vδ2 T cells, we examine the expressions of BTN3A1, RhoB, and the plakin family members including periplakin, epiplakin, and envoplakin in psoriasis vulgaris lesions as compared with the normal skin by immunohistochemical staining.

MATERIALS AND METHODS

Research Subjects

The pathologic tissue samples used in this study were obtained during biopsy on 10 patients with psoriasis

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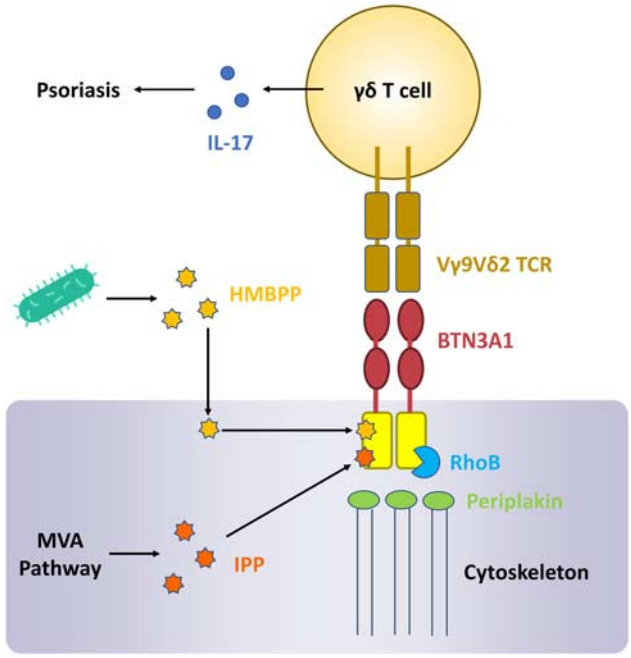

FIGURE 1. Proposed role of butyrophilin 3A1 (BTN3A1), RhoB, and periplakin in the pathogenesis of psoriasis through γδ T-cell activation. Vγ9Vδ2 T cells, the major γδ T-cell subset in human peripheral blood, can be activated by phosphoantigens (pAgs). Intracellular accumulation of phosphoantigens could originate from endogenous mevalonate pathway (MVA pathway) which produces isopentenyl pyrophosphate (IPP) or from microbials which release (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP). These pAgs are sensed by BTN3A1 and eventually leading to the activation of γδ T cells. Several partner molecules, such as RhoB and periplakin, which regulate cytoskeleton modifications might also be involved in the activation process. The proinflammatory cytokines produced by activated γδ T cells, such as interleukin (IL)-17, play an important role in pathogenesis of psoriasis.

vulgaris. All patients were treated at Shanghai Skin Diseases Hospital between 2013 and 2015. Diagnosis of psoriasis vulgaris was based on correlation of clinical and pathology findings. Clinical characteristics of the 10 psoriasis patients are shown in Table 1. The control group included 10 healthy subjects who came to the hospital seeking for surgical excision of melanocytic nevi on trunk or extremities. Normal skin peripheral to the melanocytic nevi was collected during surgical resection and used as a control in this study. The study was approved by the Ethics Committee of Shanghai Skin Diseases Hospital. Informed consent was obtained from all participants.

TABLE 1. Clinical Characteristics of Psoriasis Patients

Patients No.	Age	Sex	Biopsy Site
1	45	Female	Right lower limb
2	61	Female	Buttock
3	65	Female	Scalp
4	63	Female	Left lower limb
5	61	Male	Back
6	24	Male	Left lower limb
7	63	Male	Chest
8	63	Male	Glans penis
9	35	Male	Scalp
10	57	Male	Right lower limb

Immunohistochemical Staining

The expressions of BTN3A1, RhoB, periplakin, epiplakin, and envoplakin were evaluated by immunohistochemical analysis. Five-micron-thick paraffin slides were dewaxed with xylene for 30 minutes and then rehydrated using graded ethanol concentrations. Endogenous peroxidase was blocked with 3% hydrogen peroxide (Maixin, Fuzhou, China) for 10 minutes at room temperature. For the antigen retrieval procedure, we heated the slides at 98 to 99°C for 15 minutes in a pressure cooker. All slides were then incubated with goat serum (Maixin, Fuzhou, China) for 20 minutes to reduce nonspecific staining. Then, the slides were incubated at 4°C overnight with the following primary antibody, anti-RhoB antibody at 1:10 dilution (rabbit polyclonal antibody, ab-170611; Abcam, Cambridge, MA), antiperiplakin antibody at 1:50 dilution (rabbit polyclonal antibody, ab-131269; Abcam), antienvoplakin antibody at 1:500 dilution (rabbit polyclonal antibody, ab-185217; Abcam), antiepiplakin antibody at 1:500 dilution (rabbit polyclonal antibody; ThermoFisher), mouse anti-BNT3A1 antibody at 1:100 dilution (mouse polyclonal antibody, eBioscience, San Diego, CA). Biotinylated antimouse antibody (for BNT3A1) and antirabbit antibody (for RhoB, periplakin, envoplakin, and epiplakin) were deposited in a humidity chamber for 15 minutes at room temperature. Finally, a DAB Kit (Maixin, Fuzhou, China) was used for the final chromogen analysis. Phosphate buffered solution was used as the negative control.

Quantitative Evaluation of the Immunohistochemical Staining

The immunohistochemical staining was quantitatively evaluated using Image Pro Plus 6.3 analysis system (Media Cybernetics, Silver Spring, MD) introduced by Xavier. The images were acquired with a DP70 digital camera equipped with an Olympus microscope (image resolution: 1360×1024; Olympus Co. Ltd, Japan). For each section, the integrated optical density of the immunohistochemical staining was counted in 3 random fields of each sample and average optical density (average optical density = integrated optical density/area) was used for further statistical analysis.

Statistical Analysis

All data analysis was performed with R 3.5.3 using the package tidyverse 1.2.1. Descriptive statistics were expressed as mean (SD). Student t test was used for comparison between 2 groups. $P < 0.05$ (2 tailed) was considered as statistically significant.

RESULTS

Expression of BTN3A1 in the Normal Skin and the Psoriatic Lesions

BTN3A1 in normal skin was scarcely found in the epidermal keratinocytes but was shown in some

perivascular inflammatory cells scattered in the dermis with a membranous pattern. BTN3A1 positive reactivity was observed in the psoriatic lesions of all samples. While BTN3A1 positive immunostaining was predominant in the keratinocytes of the basal layer and the lower spinous layer of the epidermis of the psoriatic lesions, the expression of BTN3A1 was significantly increased in the infiltrating inflammatory cells in the dermis (Fig. 2).

Statistically enhanced expression of BTN3A1 was notably found between the psoriatic lesions and the control group in both the epidermis [46.9(30.8) vs. 2.6(1.8); $P < 0.05$] and the dermis [66.2(39.1) vs. 3.4(2.8); $P < 0.05$] (Fig. 5).

Expression of RhoB in the Normal Skin and the Psoriatic Lesions

In normal skin, a mild to moderate immunohistochemical reactivity of RhoB was found in all the epidermal keratinocytes, depicting a nuclear and cytoplasmic pattern. In the dermis, RhoB staining was observed in hair follicles and some inflammatory cells. In comparison to the normal skin, intense immunohistochemical reactivity of RhoB was detected in the epidermal keratinocytes, as well as the dense infiltrating inflammatory cells, including lymphocytes and neutrophils, of the psoriatic lesions (Fig. 3).

Quantitative analysis revealed a statistically enhanced expression of RhoB in the psoriatic lesions as compared with the normal skin in the dermis [57.9(28.2) vs. 33.5(21.3); $P < 0.05$]. A numerically elevated expression of RhoB was simultaneously detected in the epidermis [76.5(36.9) vs. 42.5(37.3); $P > 0.05$], yet without statistical significance (Fig. 5).

Expressions of Periplakin, Epiplakin, and Envoplakin in the Normal Skin and the Psoriatic Lesions

Immunohistochemical staining of skin specimens with antiperiplakin antibody revealed a membranous pattern, outlining the cell membranes of the keratinocytes. Stained cells were scarcely detected in the dermis (Figs. 4A, B). Meanwhile, a noticeably increased immunochemical reactivity of epiplakin was demonstrated in the epidermis of the psoriatic lesions (Figs. 4C, D). No evident difference was found for the expression of envoplakin between the psoriatic lesions and the normal control (Figs. 4E, F).

Statistical analysis of the expression of epiplakin revealed a remarkable increase in the epidermal immunostaining intensity in the psoriatic lesions when compared with the normal skin [103.1(37.7) vs. 43.1(25.1); $P < 0.05$]. However, no significant difference was found in the expressions of periplakin [40.7(26.2) vs. 34.5(17.5); $P > 0.05$] and envoplakin [50.5(20.0) vs. 30.6(23.5); $P > 0.05$] in the epidermis between psoriatic lesions and normal control. A significant downregulation of periplakin was found in the dermis of psoriasis lesion compared with normal skin [3.8 (2.9) vs. 19.4(11.9); $P < 0.01$]. A statistically enhanced expression of epiplakin was found in the psoriatic lesions as compared with the normal skin in the dermis [38.5(24.2) vs. 12.5(11.0); $P < 0.01$] (Fig. 5).

DISCUSSION

This is the first study of the immunohistochemical expression pattern of BTN3A1 and RhoB in psoriasis lesions. Previous study has demonstrated dermal γδ T cells as critical IL-17-producing cells in the pathogenesis of psoriasis.[4] Prior research observed a striking reduction in circulating Vγ9Vδ2 T cells and an increased presence of Vγ9Vδ2 T cells in psoriatic lesions as compared with the healthy control, which indicated a redistribution of Vγ9Vδ2 T cells from blood to the skin compartment in psoriasis.[12] Vγ9Vδ2 T cells, the major γδ T-cell subset in human peripheral blood, is known to be specifically activated by exposure to pAgs, isopentenyl pyrophosphate and (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate.[13] The activation of human Vγ9Vδ2 T cells was found linked to

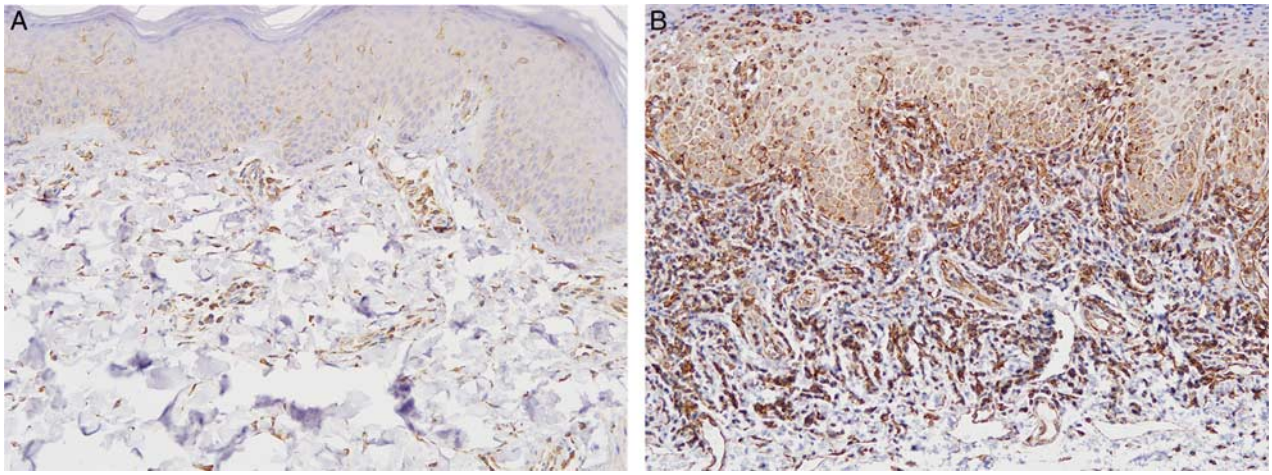

FIGURE 2. Expression of butyrophilin 3A1 (BTN3A1) in normal skin and psoriatic lesion. A, (BTN3A1 ×200) In normal skin, weak positive BTN3A1 immunoreactivity was scarcely found in keratinocytes in the epidermis. B, (BTN3A1 ×200) In psoriatic lesion, strong and disseminated BTN3A1 immunoreactivity with a membranous pattern was found in keratinocytes in the basal and spinous layers of the epidermis, together with the dense perivascular inflammatory cells in the dermis. full color online

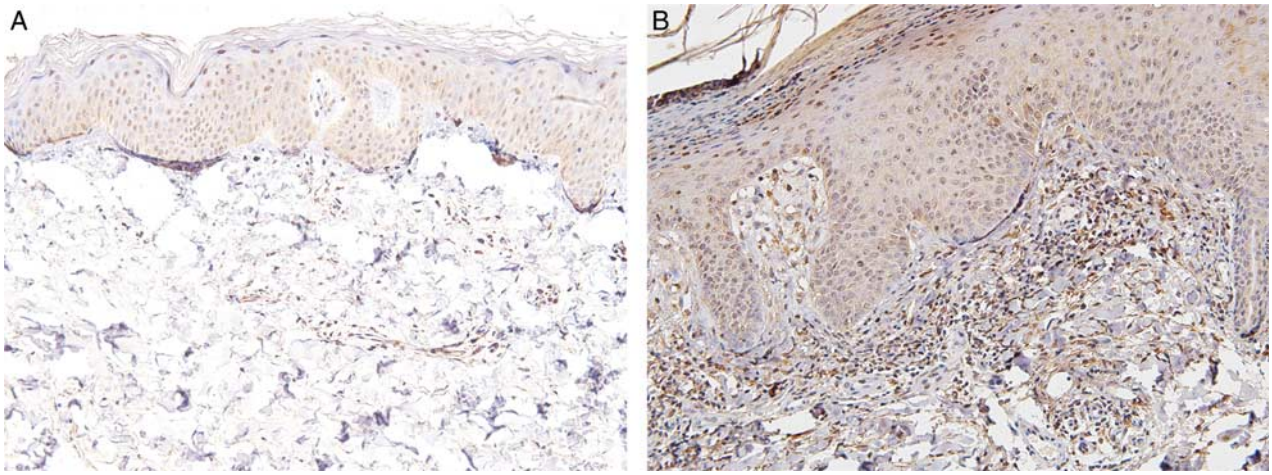

FIGURE 3. Expression of RhoB in normal skin and psoriatic lesion. A, (RhoB ×200) In normal skin, mild RhoB immunoreactivity was found in keratinocytes in the epidermis and some infiltrating inflammatory cells in the dermis. B, (RhoB ×200) In psoriatic lesion, intense RhoB immunoreactivity was detected in keratinocytes in the epidermis and the dense infiltrating inflammatory cells in the dermis. full color online

BTN3A1, a type I glycoprotein that belongs to the B7 family.[14] Two underlying mechanisms have been hypothesized: Harly et al[8] supposed that intracellular interaction between pAgs and the B30.2 domain of BTN3A1-induced conformational changes of BTN3A1, which were sensed by Vγ9Vδ2 T cells and subsequently caused their activation. In comparison, Vavassori et al[15] anticipated that intracellular pAgs were exported and afterwards bound to the extracellular domain of BTN3A1, serving as an antigen-presenting molecule for Vγ9Vδ2 T cells. Though the precise mechanism remains to be clarified, both conception demonstrated an indispensable role for BTN3A1 in the activation process. Considering the statistically upregulated expression of BTN3A1 in both the epidermis and the dermis of the psoriatic lesions, our data support that BTN3A1 might participate in the pathogenesis of psoriasis through Vγ9Vδ2 T-cell activation.

In addition to BTN3A1, RhoB was also reported as a critical mediator which linked pAgs accumulation to the BTN3A1-mediated triggering of Vγ9Vδ2 T-cell responses.[9] RhoB, a member of the Rho family of isoprenylated small GTPases, is well-known for controlling actin cytoskeleton and thus regulating cytoskeletal reorganization in cells.[16] As decreased mobility of the transmembrane protein BTN3A1 on tumor cells has been identified as a critical determinant of Vγ9Vδ2 T-cell activation, Sebestyen et al[9] suggested that RhoB contributed to this target recognition by orchestrating BTN3A1 in the plasma membrane through cytoskeletal rearrangements. In our study, a statistically enhanced expression of RhoB was shown in the dermis of the psoriatic lesions. Recognizing the interaction of RhoB and BTN3A1, this may indicate a possibility of RhoB participating in the pathology of psoriasis through modulating BTN3A1-mediated activation of Vγ9Vδ2 T cells.

The plakin protein family is involved in the cross-linking and organization of the cytoskeleton and adhesion complexes, modulating fundamental biological processes like cell adhesion, migration, polarization, or signaling pathways.[10] Among the plakin family, periplakin was identified as a BTN3A1 interacting molecule by Rhodes and colleagues through yeast 2-hybrid assays, implying a role for this cytoskeletal adaptor in the regulation of Vγ9Vδ2 T-cell responses. Rhodes et al[11] suggested that periplakin was recruited to the di-leucine motif of BTN3A1, which served to anchor or stabilize the BTN3A signaling complex in the cell membrane. Rhodes et al[11] further proposed a regulatory rather than mandatory role for periplakin in transmitting activation signals to γδ T cells, as knockdown of periplakin using siRNAs did not consistently block but caused dysregulated Vγ9Vδ2 T-cell responses to pAg exposure. In our study, while the expression of periplakin did not show a statistical difference between the psoriatic lesions and the normal skin in the epidermis, a significant downregulation of periplakin was found in the dermis (Fig. 5). Taking our results into account, periplakin may be dispensable for the BTN3A1-mediated activation of Vγ9Vδ2 T cells. Other factors may compensate for its absence in interaction with BTN3A1. An in-depth study investigating the correlation between BTN3A1 and periplakin in psoriasis vulgaris is further required.

Apart from periplakin, 2 additional plakin family members, epiplakin and envoplakin, were examined in our study. Whereas no evident difference was found between the normal skin and the psoriatic lesions for envoplakin, an enhanced expression of epiplakin was exhibited at both the epidermis and the dermis of the psoriatic lesions. Epiplakin belongs to the plakin family of cytolinker proteins and is one of the intermediate filament-related components. A previous study using an epiplakin-null mouse line showed that lacking epiplakin accelerates the migration of epidermal keratinocytes in mice, which may relate to changes in interactions between epiplakin and intermediate

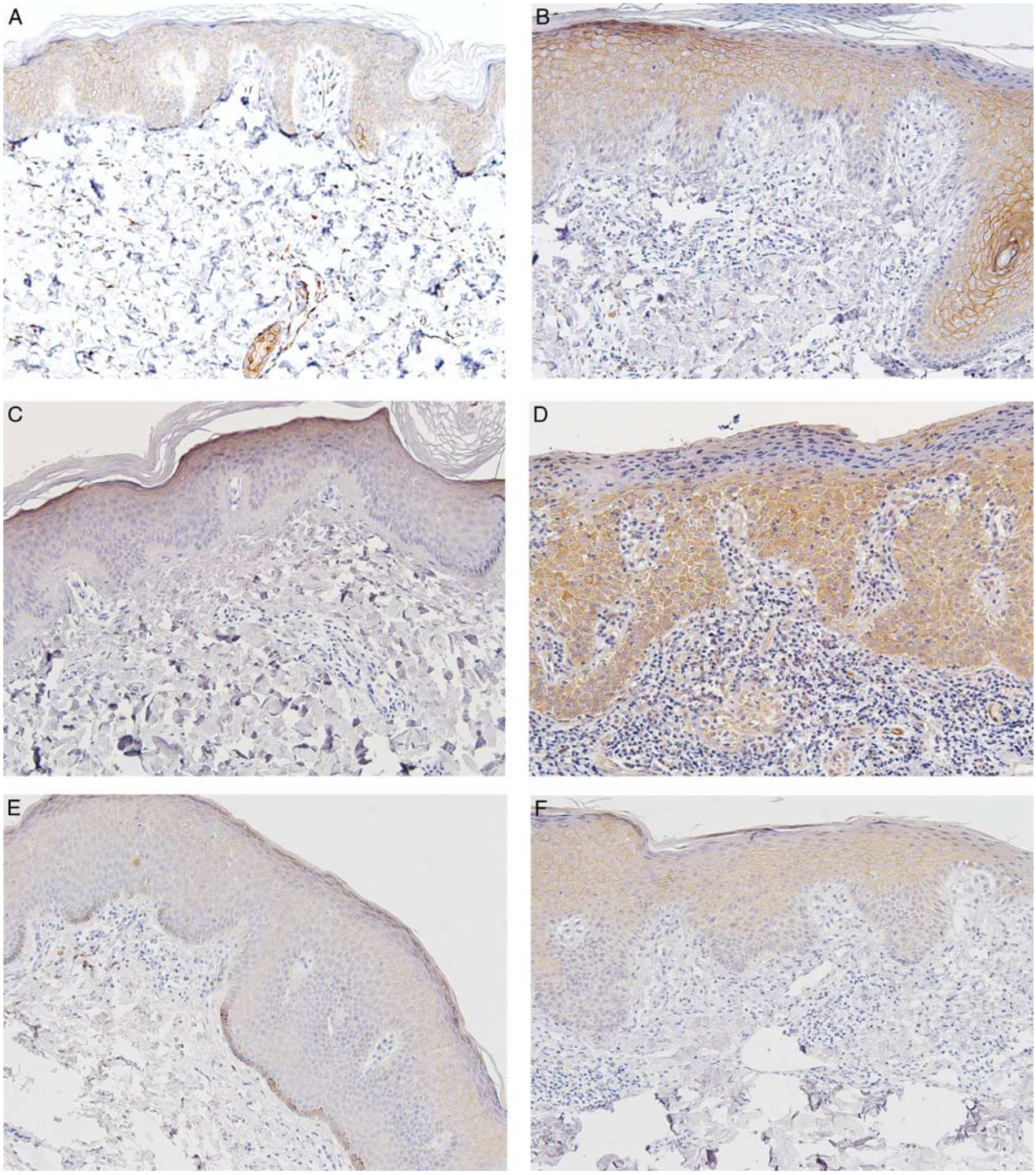

FIGURE 4. Expression of periplakin, epiplakin, and envoplakin in normal skin and psoriatic lesion. A, (Periplakin ×200) In normal skin, positive periplakin immunoreactivity with a membranous pattern was found in keratinocytes in the epidermis. B, (Periplakin ×200) In psoriatic lesion, a relatively strong and diffuse periplakin immunoreactivity with a membranous pattern was exhibited in keratinocytes in the epidermis. C, (Epiplakin ×200) In normal skin, weak positive epiplakin immunoreactivity was found in keratinocytes in the epidermis. D, (Epiplakin ×200) In psoriatic lesion, potent and diffuse epiplakin immunoreactivity was shown in keratinocytes in the epidermis. E, (Envoplakin ×200) In normal skin, weak positive envoplakin immunoreactivity was found in keratinocytes in the epidermis. F, (Envoplakin ×200) In psoriatic lesion, a similar weak positive envoplakin immunoreactivity was detected in keratinocytes in the epidermis. full color online

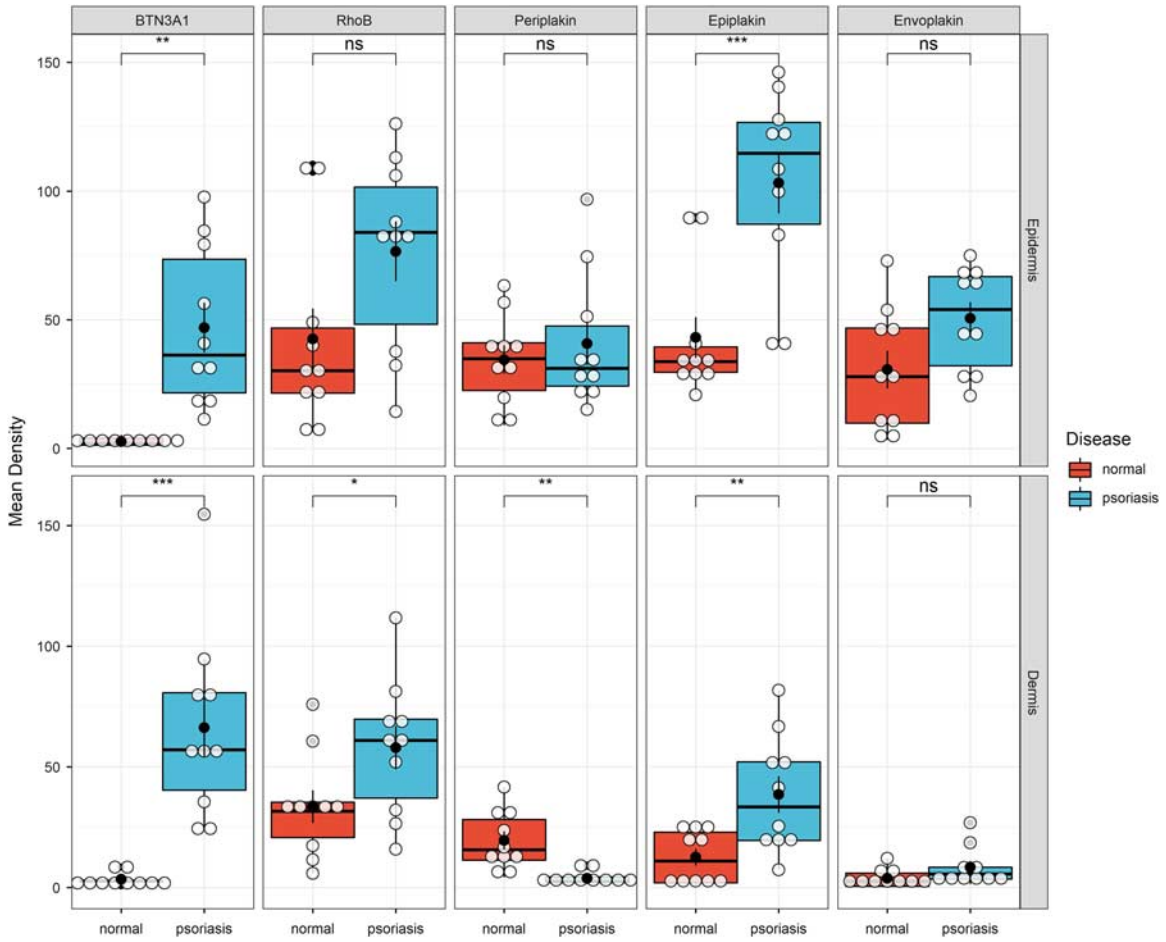

FIGURE 5. Quantitative evaluation of the immunohistochemical staining. The graph shows the expression levels of butyrophilin 3A1 (BTN3A1), RhoB, periplakin, epiplakin, and envoplakin in the psoriatic lesions and normal skin. Statistically enhanced expression of BTN3A1 was found between the psoriatic lesions and normal skin in both the epidermis and the dermis. A statistically enhanced expression of RhoB in the psoriatic lesions as compared with the normal skin was found in the dermis. A remarkable increase in the epidermal immunostaining intensity was detected in the psoriatic lesions when compared with the normal skin. A significant downregulation of periplakin was found in the dermis of psoriasis lesion and a statistically enhanced expression of epiplakin was found in the psoriatic lesions compared with normal skin. Statistical analysis was performed by the Student *t* test. Values that are statistically significant are indicated by $*P<0.05$; $**P<0.01$; $***P<0.001$. ns indicates no significance. full color online

filaments.[17] Besides, epiplakin has been reported to accelerate keratin bundling in proliferating keratinocytes during wound healing and might contribute to the reinforcement of keratin networks under mechanical stress.[18] These studies suggested a role for epiplakin in skin repair though it has been reported to be dispensable for the skin barrier function in mice.[19] Despite epiplakin was identified as an antigen in autoimmune blistering disease,[20] no evidence was shown to relate epiplakin to the pathology of psoriasis or the activation of Vγ9Vδ2 T cells. Hence, the precise function of epiplakin and envoplakin in psoriasis remains to be determined.

Because of the limited sample size and the instability of the immunohistochemical staining, the result should be interpreted carefully. Further targeted study is needed for elucidating the relation of BTN3A1, RhoB, and the plakin protein family toward γδ T-cell responses in skin and their characters in psoriasis pathology.

CONCLUSION

Our data suggest that BTN3A1 and RhoB might participate in the pathogenesis of psoriasis through Vγ9Vδ2 T-cell responses. In addition, a downregulation of periplakin and an upregulation of epiplakin were found, indicating a potential involvement for them in the pathology of psoriasis. Further investigations to disclose their correlation with the activation of γδ T cells and their precise characters in psoriasis pathology are required.

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