PNAS RESEARCH ARTICLE | IMMUNOLOGY AND INFLAMMATION

Up-regulation of BTN3A1 on CD14$^+$ cells promotes Vγ9Vδ2 T cell activation in psoriasis

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Edited by Philippa Marrack, National Jewish Health, Denver, CO; received September 25, 2021; accepted September 20, 2022

Vγ9Vδ2 T cells play an important role in the development and progression of psoriasis vulgaris (PV), but how they promote skin inflammation and the molecular mechanisms underlying Vγ9Vδ2 T cell dysfunction are poorly understood. Here, we show that circulating Vγ9Vδ2 T cells are decreased and exhibit enhanced proliferation and increased production of IFN-γ and TNF-α in PV patients. Monocytes from PV patients express higher levels of the phosphoantigen sensor butyrophilin 3A1 (BTN3A1) than monocytes from healthy controls. Blockade of BTN3A1 suppresses Vγ9Vδ2 T cell activation and abolishes the difference in Vγ9Vδ2 T cell activation between PV patients and healthy controls. The CD14$^+$ cells in PV skin lesions highly express BTN3A1 and juxtapose to Vδ2 T cells. In addition, IFN-γ induces the up-regulation of BTN3A1 on monocytes. Collectively, our results demonstrate a crucial role of BTN3A1 on monocytes in regulating Vγ9Vδ2 T cell activation and highlight BTN3A1 as a potential therapeutic target for psoriasis.

BTN3A1 | psoriasis | Vγ9Vδ2 T cells | monocytes

Significance

Our study reveals that BTN3A1 up-regulation on monocytes in psoriasis patients can result in the hyperactivation of Vγ9Vδ2 T cells. This work also indicates that IFN-γ may amplify Vγ9Vδ2 T cell activation through the up-regulation of BTN3A1 on monocytes via a positive feedback loop. More important, our work provides insight into the molecular mechanisms of Vγ9Vδ2 T cell activation and highlights BTN3A1 as a potential therapeutic target for psoriasis. We believe that these findings could be of interest to the fields of both clinical medicine and basic immunology.

Psoriasis vulgaris (PV) is a common chronic inflammatory skin disease characterized by the hyperproliferation of keratinocytes and massive skin infiltration of immune cells (1). The interleukin (IL)-23/IL-17 axis is thought to be essential in the pathogenesis of PV (2, 3), reflected by the therapeutic efficacy of monoclonal antibodies (mAbs) against IL-17 or IL-23 (4). Although T helper cell (Th)17 cells were originally considered to be the major source of IL-17, recent reports have shown that gamma-delta (γδ) T cells are also capable of producing IL-17 and are implicated in the maintenance of chronic psoriatic inflammation (5–8). In IL-23- and imiquimod-induced psoriasis models, T cell receptor (TCR)δ$^{-/-}$ mice developed less skin inflammation than wild-type control mice, suggesting a pathogenic role for γδ T cells in psoriasis (5–7). Further studies reveal that dermal γδ T cells are the major IL-17 producers in the skin, not the dendritic epithelial T cells (5, 7). In mice, both Vγ4$^+$ γδ T cells and Vγ6$^+$ γδ T cells are capable of secreting IL-17 during skin inflammation (9, 10). In humans, most circulating γδ T cells express Vγ9Vδ2$^+$ TCRs. Studies have shown that circulating cutaneous lymphocyte-associated antigen-positive (CLA$^+$) Vγ9Vδ2 T cells are the important IL-17-producing γδ T cells during psoriasis (11). The frequency of CLA$^+$ Vγ9Vδ2 T cells is decreased in peripheral blood (PB), suggesting that they have been recruited to inflamed skin lesions to promote local tissue inflammation in PV (11). In addition to IL-17, γδ T cells are able to produce large amounts of interferon (IFN)-γ and tumpr necrosis factor (TNF)-α. However, whether human γδ T cells secrete IFN-γ and TNF-α to promote skin inflammation and the molecular mechanisms underlying γδ T cell dysfunction is poorly understood.

Unlike conventional αβ T cells that recognize peptide antigens presented by major histocompatibility complex molecules, human Vγ9Vδ2 T cells respond to small phosphorylated nonpeptide antigens known as phosphoantigens (pAgs). These pAgs are produced either by microbes via nonmevalonate pathways, such as (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP), or by tumor cells via mevalonate pathways, such as isopentenyl pyrophosphate (12–14). pAg recognition by γδ TCRs is mediated by butyrophilin 3A1 (BTN3A1) on antigen-presenting cells (APCs), which is one of the B7 family members of co-stimulatory receptors (15, 16). Binding of the intracellular B30.2 domain of BTN3A1 to pAgs is a critical step for Vγ9Vδ2 T cell activation (17–19). A recent study showed that BTN3A1 expression level and cellular redistribution are both associated with the efficiency of pAg-mediated Vγ9Vδ2 T cell activation (20).

BTN3A1 is widely expressed in a variety of immune cells, including monocytes, T cells, and neutrophils (21). Of note, monocytes serve as accessory cells to activate Vγ9Vδ2 T cells through BTN3A1 binding to pAgs or to an agonistic BTN3A1 antibody

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Author contributions: J. Zhou and J. Zhang performed the experiments and analyzed the data; L.T. and K.P. contributed to blood samples collection; Q.Z. and J.P. performed the immunofluorescence staining, image acquisition and data analysis; K.Y. and J.L. contributed to skin biopsies collection; X.S. and J.S. performed the flow cytometry and data analysis; J. Zhou, J. Zhang, and L.S. wrote the paper; L.S. and Z.Z. conceived, designed, and supervised the project.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2117523119/-/DCSupplemental.

Published October 26, 2022.

(clone 20.1) (22). Whether BTN3A1 expression on monocytes is dysregulated in PV remains unknown, as does the role of BTN3A1 in Vγ9Vδ2 T cell activation. In this study, we show that Vγ9Vδ2 T cells exhibit hyperactivation in PV patients due to increased BTN3A1 expression on monocytes that, in turn, likely results from excessive amounts of IFN-γ. Thus, our study provides evidence that up-regulation of BTN3A1 on monocytes can lead to Vγ9Vδ2 T cell hyperactivation, and highlights BTN3A1 as a potential therapeutic target for psoriasis.

Results

Peripheral Vγ9Vδ2 T Cells Are Decreased and Exhibit an Inflammatory Phenotype in PV Patients. Previous studies have demonstrated that γδ T cells play critical roles in regulating skin inflammation in psoriasis (5–7). To investigate γδ T cells in PV, we analyzed the frequency of these cells in PB. Human peripheral γδ T cells consist mainly of two subsets: Vγ9Vδ2 and Vδ1 T cells. Intriguingly, both the frequency and the absolute number of Vγ9Vδ2 T cells were decreased dramatically in PV patients, compared to healthy controls (HCs) (Fig. 1*A*). Moreover, the frequency of Vγ9Vδ2 T cells negatively correlated with disease severity, as reflected by the psoriasis area and severity index (PASI) score (Fig. 1*B*). By contrast, the frequency and absolute number of total Vδ1 T cells did not show any differences between PV patients and HCs, and the frequency of Vδ1 T cells had no correlation with PASI score (Fig. 1 *A* and *B*). We also analyzed the percentages of $CD3^+$, $CD4^+$, and $CD8^+$ T cells and none exhibited statistically significant differences between the two groups (*SI Appendix*, Fig. S1 *A–C*). These data point to Vγ9Vδ2 T cells as the subset of γδ T cells associated with psoriasis. The reduction of Vγ9Vδ2 T cells in PB is consistent with a previous report (11) and presumably reflects the migration of Vγ9Vδ2 T cells to inflamed skin.

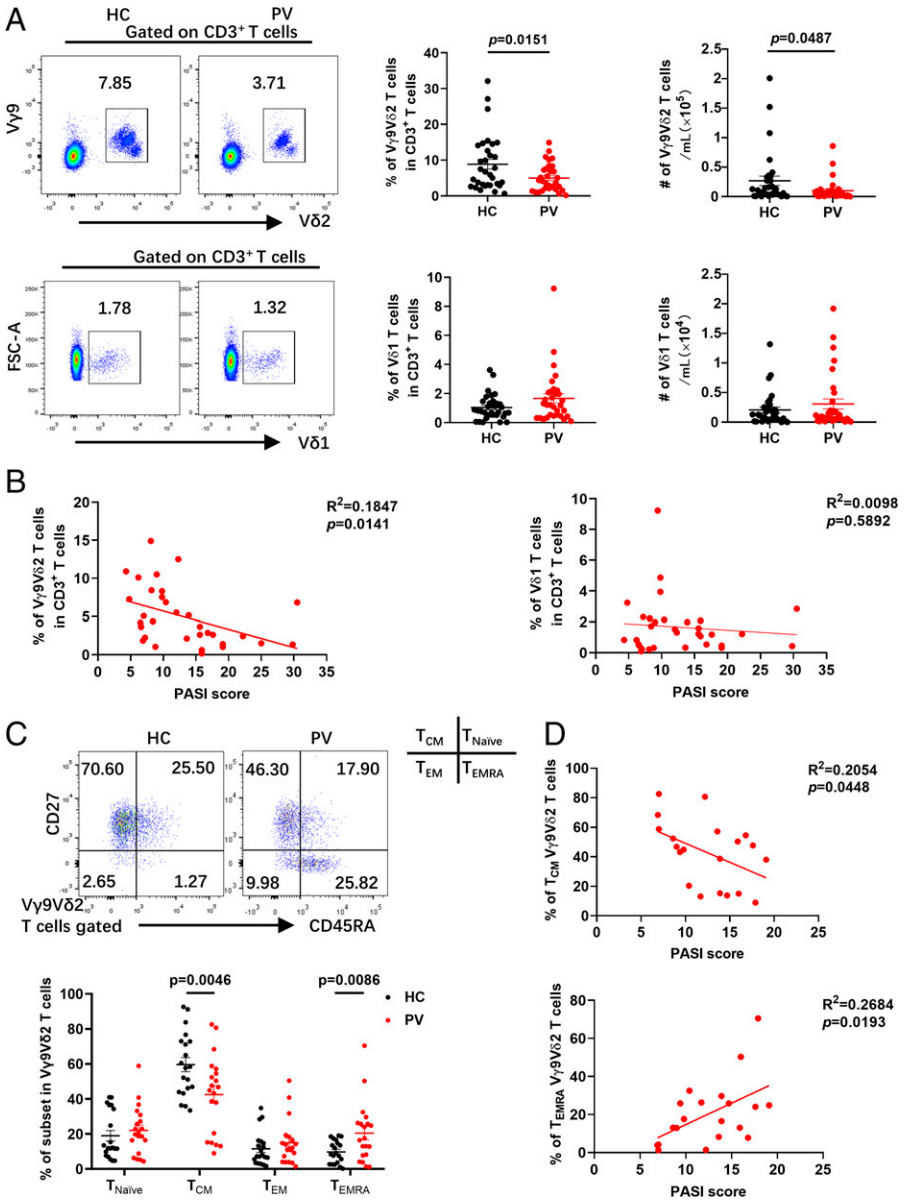

Fig. 1. Peripheral Vγ9Vδ2 T cells are decreased and exhibit an inflammatory phenotype in PV patients. (*A*) The frequencies and absolute numbers of Vγ9Vδ2 T cells and Vδ1 T cells in HC and PV ($n = 32$ for each group). (*B*) Correlations between the frequency of Vγ9Vδ2 T cells or Vδ1 T cells and PASI score in the PV group. (*C* and *D*) Peripheral Vγ9Vδ2 T cells were divided into $T_{Naïve}$, T_{CM}, T_{EM}, and T_{EMRA} subsets according to CD27, CD45RA expression. (*C*) The frequency of each subset in HC and PV ($n = 20$ for each group). (*D*) Correlations between the frequency of T_{CM} or T_{EMRA} and PASI score in the PV group. Each symbol represents an independent donor (*A–D*). Results are presented as means ± SEMs and are analyzed with two-tailed unpaired Student's *t* test (**A**-Vγ9Vδ2 T cells) or two-tailed Welch's *t* test (**A**-Vδ1 T cells). Correlation analysis was performed with Spearman's rank correlation test (*B* and *D*).

Next, we investigated the functional status of Vγ9Vδ2 T cells in PV. Based on their differentiation status, human Vγ9Vδ2 T cells can be divided into four subsets: naïve cells ($T_{Naïve}$, $CD45RA^+CD27^+$), central-memory cells (T_{CM}, $CD45RA^-CD27^+$), effector-memory cells (T_{EM}, $CD45RA^-CD27^-$), and terminally differentiated cells (T_{EMRA}, $CD45RA^+CD27^-$). Among these subsets, $T_{Naïve}$ and T_{CM} are present in secondary lymphoid organs and lack immediate effector function, while T_{EM} and T_{EMRA} reside in inflammatory sites and display immediate effector functions by releasing cytokines (23). As shown in Fig. 1*C*, T_{CM} was the predominant subset of Vγ9Vδ2 T cells in the PB of both PV patients and HCs. However, the T_{CM} subset was dramatically decreased in PV patients by comparison to the control group and the proportion of T_{CM} negatively correlated with PASI score (Fig. 1*D*). By contrast, T_{EMRA} were increased significantly in PV patients and the proportion of T_{EMRA} positively correlated with PASI score (Fig. 1 *C* and *D*). These results demonstrate that circulating Vγ9Vδ2 T cells in PV patients have a skewed terminally differentiated effector memory phenotype that correlates with clinical severity, indicating that T_{EMRA} may contribute to skin inflammation. Taken together, these data suggest that homeostasis in the Vγ9Vδ2 T cell compartment is disrupted in psoriasis.

Vγ9Vδ2 T Cells from PV Patients Display Enhanced Proliferation and Proinflammatory Cytokine Production. To investigate the functional status of peripheral Vγ9Vδ2 T cells in PV patients, we assessed their proliferation and cytokine production in response to defined stimuli. PB mononuclear cells (PBMCs) were labeled with 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE) and stimulated with pAg HMBPP in the presence of IL-2. In the absence of HMBPP, Vγ9Vδ2 T cells from both HCs and PV patients exhibited a spontaneous proliferation rate of approximately 21%, which may be due to the effect of IL-2 (Fig. 2*A*). When stimulated with HMBPP, Vγ9Vδ2 T cells increased their proliferation dramatically and reached 60 to 80% of the division rate in total cells (Fig. 2*A*). Interestingly, Vγ9Vδ2 T cells from PV patients displayed stronger proliferation than those from HCs (Fig. 2*A*). Next, we examined the production of proinflammatory cytokines, including IFN-γ, TNF-α, and IL-17A, by Vγ9Vδ2 T cells. PBMCs were stimulated with phorbol 12-myristate 13-acetate (PMA)/ionomycin (PI), HMBPP, and anti-BTN3A mAb clone 20.1, respectively. Clone 20.1 is an agonistic antibody that has been demonstrated to activate Vγ9Vδ2 T cells (15). HMBPP and clone 20.1 mimic physiological TCR stimulation of Vγ9Vδ2 T cells, while PI directly activates intracellular signaling in these cells. PI stimulation led to a much greater production of IFN-γ and TNF-α by Vγ9Vδ2 T cells than HMBPP or clone 20.1 (Fig. 2*B*). However, there were no differences in IFN-γ and TNF-α production between PV patients and HCs when the cells were stimulated with PI (Fig. 2*B*). Intriguingly, upon stimulation with HMBPP or clone 20.1, Vγ9Vδ2 T cells from PV patients produced significantly higher levels of IFN-γ and TNF-α than HCs (Fig. 2*B*). Although IL-17A is a crucial pathogenic cytokine in psoriasis, its expression by circulating Vδ2 T cells was relatively low compared to IFN-γ and TNF-α (Fig. 2*B*). Nonetheless, consistent with the changes in IFN-γ and TNF-α, IL-17A production by Vδ2 T cells from PV patients exceeded those from HCs upon stimulation with HMBPP or clone 20.1 (Fig. 2*B*). Taken together, these data suggest that Vγ9Vδ2 T cells from PV patients are hyperactivated.

To gain insights into the molecular mechanisms responsible for this hyperactivation, we performed RNA sequencing (RNA-seq) analysis of Vγ9Vδ2 T cells isolated from PB to compare the global transcriptome between PV patients and HCs. Gene expression analysis identified 2,516 significantly changed genes (fold change >1.5, $P < 0.05$) out of 17,606 expressed protein coding genes in Vγ9Vδ2 T cells from PV patients. Among them, 1,016 were up-regulated and 1,500 were down-regulated. The genes associated with Vγ9Vδ2 T cell activation and function were broadly up-regulated in PV patients (Fig. 2*C*). Gene Ontology (GO) analysis of the genes increased in PV patients showed enrichment in the inflammatory response, the TNF-mediated signaling pathway, T cell activation, and T cell proliferation (Fig. 2*D*). Gene set enrichment analysis (GSEA) showed enrichment in TNF-α signaling via the nuclear factor κB, IFN-γ response (Fig. 2*E*). Together, these data suggest that multiple signaling pathways and programs are involved in regulating the proliferation and activation of Vγ9Vδ2 T cells in PV patients.

Monocytes Are Required for Vγ9Vδ2 T Cells to Sense pAgs. Previous studies have shown that expression of the butyrophilin surface protein BTN3A1 on APCs is required for Vγ9Vδ2 T cell recognition of pAgs and activation (19). Because monocytes are key APCs in PBMCs and express BTN3A1, we hypothesized that activation of Vγ9Vδ2 T cells in PBMCs is dependent on monocytes. To test this hypothesis, monocytes were depleted from PBMCs using CD14 microbeads. Strikingly, both IFN-γ and TNF-α production by Vγ9Vδ2 T cells were markedly decreased when monocytes were removed, suggesting that these cells are required for Vγ9Vδ2 T cell activation in response to HMBPP (Fig. 3*A*). To confirm the ability of monocytes to activate Vγ9Vδ2 T cells, sorted Vγ9Vδ2 T cells were cultured with purified $CD14^+$ monocytes in the presence or absence of HMBPP. Without $CD14^+$ monocytes, Vγ9Vδ2 T cells displayed a low level of spontaneous production of IFN-γ and TNF-α, reflecting the self-activation of Vγ9Vδ2 T cells that was possibly due to the surface expression of BTN3A1 on these cells (Fig. 3*B*). By contrast, IFN-γ and TNF-α production by Vγ9Vδ2 T cells was substantially increased upon the addition of $CD14^+$ monocytes to the culture (Fig. 3*B*). These results indicate that monocytes are required to sense pAgs for Vγ9Vδ2 T cell activation in PBMCs.

Next, we sought to determine whether the hyperactivation of Vγ9Vδ2 T cells that we observed in PBMCs from PV patients is dependent on interactions between monocytes and Vγ9Vδ2 T cells. We evaluated the proliferation of Vγ9Vδ2 T cells in coculture with $CD14^+$ monocytes. Upon the addition of HMBPP, the proliferation rate of Vγ9Vδ2 T cells was enhanced dramatically in both PV patients and HCs, but even more so in PV patients (Fig. 3*C*). Consistent with the proliferation data, the levels of IFN-γ and TNF-α produced by Vγ9Vδ2 T cells were significantly higher in PV patients compared to HCs (Fig. 3*D*). Notably, Vγ9Vδ2 T cells from PV patients also produced more IFN-γ and TNF-α in response to the agonistic anti-BTN3A antibody (clone 20.1) (*SI Appendix*, Fig. S2 *A* and *B*). Together, these data indicate that the hyperactivation of Vγ9Vδ2 T cells in PV patients is dependent on interaction with monocytes.

Up-regulation of BTN3A1 on Monocytes Results in the Hyperactivation of Vγ9Vδ2 T Cells in PV Patients. Given that the enhanced activation of Vγ9Vδ2 T cells in PV was dependent on interaction with monocytes, we asked whether monocytes are altered in PV patients. Remarkably, the frequency of $CD14^+$ monocytes was significantly increased in PV patients compared to HCs (Fig. 4*A*). In addition, the frequency of $CD14^+$ monocytes

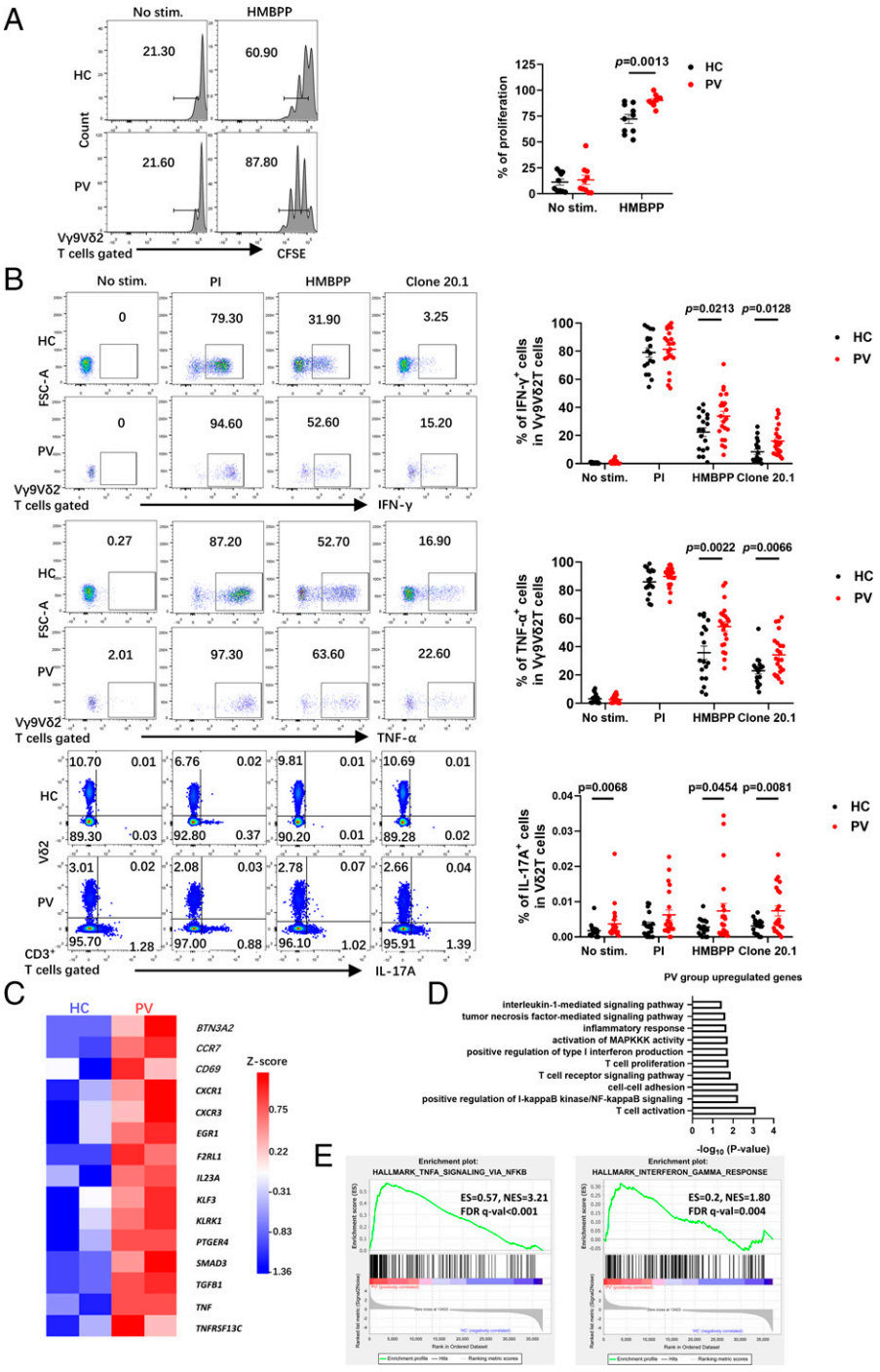

Fig. 2. Vγ9Vδ2 T cells from PV patients display enhanced proliferation and cytokine production. (*A*) CFSE-labeled PBMCs from HC and PV were cultured with HMBPP for 3 d ($n = 10$ for each group). CFSE dilution in Vγ9Vδ2 T cells was analyzed by flow cytometry. (*B*) PBMCs were stimulated with PI, HMBPP, and clone 20.1 for 5 h ($n = 18$ in HC, $n = 22$ in PV). Representative cytokine staining for IFN-γ and TNF-α produced by Vγ9Vδ2 T cells and IL-17A produced by Vδ2 T cells. Statistical analysis of the percentages of IFN-γ- and TNF-α-producing Vγ9Vδ2 T cells and IL-17A-producing Vδ2 T cells. (*C*–*E*) RNA-seq of Vγ9Vδ2 T cells was performed in HC and PV ($n = 2$ for each group). (*C*) Heatmap of genes associated with Vγ9Vδ2 T cell activation in HC and PV. (*D*) GO analysis was performed using DAVID. (*E*) GSEA analysis was performed using the web tool from the Broad Institute. ES, enrichment score; FDR, false discovery rate; NES, normalized enrichment score. Each symbol represents an independent donor (*A* and *B*). Results are presented as means ± SEMs and are analyzed with two-tailed unpaired Student's *t* test (*A* and *B*).

positively correlated with the PASI score, suggesting that monocytes may play a pathogenic role in the development of psoriasis (Fig. 4*B*). Because BTN3A1 expression on APCs is required for pAg recognition and activation of Vγ9Vδ2 T cells, we next asked whether BTN3A1 expression was altered in PV patients. Intriguingly, we found that BTN3A1 expression on $CD14^+$ monocytes was up-regulated in the PV group and also showed a positive correlation with PASI score (Fig. 4 *C* and *D*). We also compared the expression of BTN3A1 on other immune cells, including $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, Vδ1 T cells, and Vγ9Vδ2 T cells between PV and HC groups. However, none showed statistically significant differences between the two groups, except for Vγ9Vδ2 T cells (*SI Appendix*, Fig. S3 *A*–*E*). Together, these data suggest that the monocyte compartment is altered in psoriasis.

Because BTN3A1 is necessary for pAg-mediated activation of Vγ9Vδ2 T cells, we hypothesized that enhanced Vγ9Vδ2 T cell activation in psoriasis patients is caused by increased BTN3A1 expression on monocytes. To test this hypothesis, we

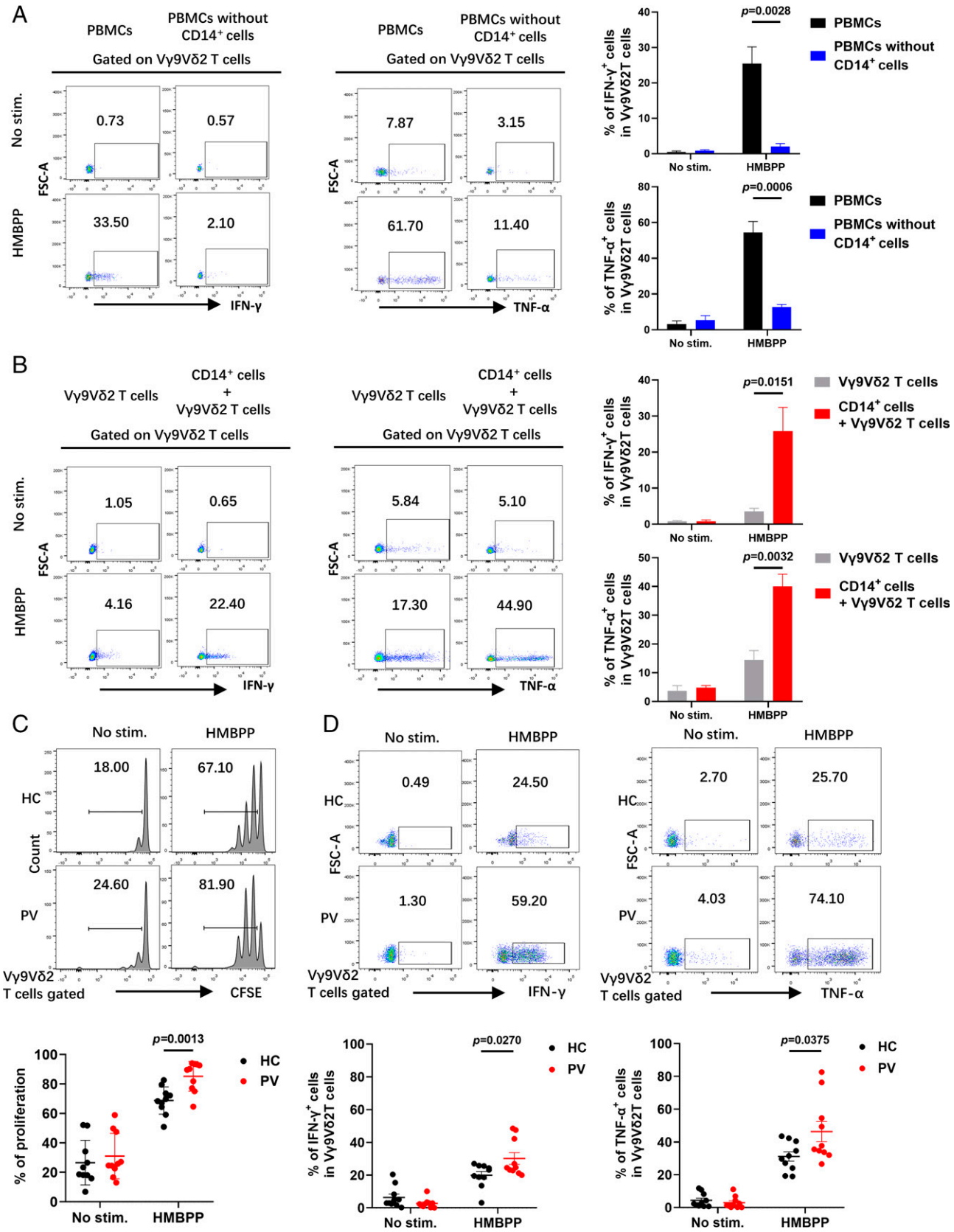

Fig. 3. Monocytes are required for Vγ9Vδ2 T cell activation in PBMCs. (*A*) PBMCs with or PBMCs without monocytes from the HC group ($n = 4$) were stimulated with HMBPP for 5 h. Representative cytokine staining for IFN-γ and TNF-α production by Vγ9Vδ2 T cells and statistical analysis of the percentages of IFN-γ- and TNF-α-producing Vγ9Vδ2 T cells are shown. (*B–D*) $CD14^+$ monocytes were cocultured with Vγ9Vδ2 T cells at a ratio of 4:1 in the presence of HMBPP. (*B*) Analysis of IFN-γ and TNF-α production by Vγ9Vδ2 T cells from the HC group after 5 h culture ($n = 4$). (*C*) The proliferation rate of CFSE-labeled Vγ9Vδ2 T cells was determined by flow cytometry after 3 d culture ($n = 10$ for each group). (*D*) Analysis of IFN-γ and TNF-α production by Vγ9Vδ2 T cells after 5 h of culture ($n = 10$ for each group). Each symbol represents an independent donor (*C* and *D*). Results are presented as means ± SEMs and are analyzed with two-tailed unpaired Student's *t* test (*A–D*).

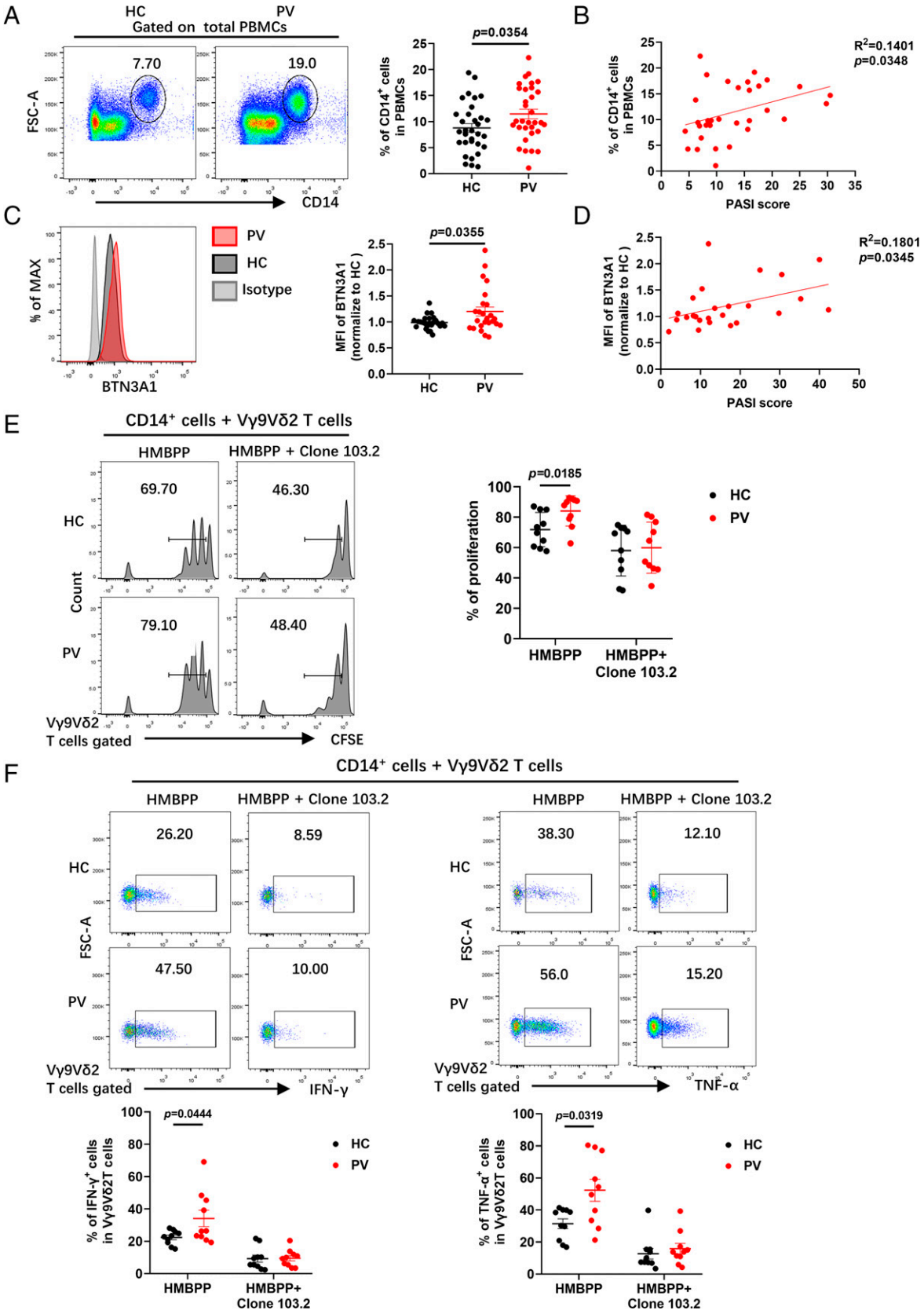

Fig. 4. The increased expression of BTN3A1 on monocytes results in the hyperactivation of Vγ9Vδ2 T cells in PV patients. (*A*) Analysis of the frequency of CD14$^+$ monocytes in HC and PV (n = 32 for each group). (*B*) Correlation between the frequency of CD14$^+$ monocytes and PASI score in the PV group. (*C*) Flow cytometry analysis of BTN3A1 expression on CD14$^+$ monocytes (n = 25 for each group). (*D*) Correlation between BTN3A1 expression and PASI score in the PV group. (*E* and *F*) CD14$^+$ monocytes were cocultured with CFSE-labeled Vγ9Vδ2 T cells at a ratio of 4:1 in the presence of HMBPP. Clone 103.2 was used for blocking BTN3A1, which was added at a level calibrated to only partially block stimulation of the Vγ9Vδ2 T cells (n = 10 for each group). (*E*) CFSE dilution in Vγ9Vδ2 T cells was detected by flow cytometry after 3 d of culture. (*F*) Analysis of IFN-γ and TNF-α production by Vγ9Vδ2 T cells after 5 h of culture. Each symbol represents an independent donor (*A*–*F*). Results are presented as means ± SEMs and are analyzed with two-tailed unpaired Student's t test (*A*, *C*, *E*, and *F*). Correlation was performed with Spearman's rank correlation test (*B* and *D*). FSC-A, forward scatter area; MFI, mean fluorescence intensity.

added the BTN3A1 blocking antibody clone 103.2 to the cultures of CD14$^+$ monocytes and Vγ9Vδ2 T cells. Upon HMBPP stimulation, Vγ9Vδ2 T cells from PV patients displayed stronger proliferation than Vγ9Vδ2 T cells from HCs (Fig. 4*E*), and the enhanced proliferation in PV patients was abolished by clone 103.2, suggesting a critical role of BTN3A1 in mediating the hyperactivation of Vγ9Vδ2 T cells in PV patients (Fig. 4*E*). Similar to the cell proliferation findings, the expression of IFN-γ and TNF-α by Vγ9Vδ2 T cells from both PV and HC groups was decreased significantly in the presence of clone 103.2. In addition, the increased production of IFN-γ and TNF-α by Vγ9Vδ2 T cells in PV patients was diminished when clone 103.2 was added to the cocultures (Fig. 4*F*). The inhibitory effects of clone 103.2 on the proliferation and cytokine production of Vγ9Vδ2 T cells were observed in the PBMC culture system as well (*SI Appendix*, Fig. S4 *A* and *B*). Together, these data indicate that increased BTN3A1 leads to Vγ9Vδ2 T cell hyperactivation in PV patients.

IFN-γ Induces Up-Regulation of BTN3A1 on Monocytes. Since previous studies have shown that IFN-γ or TNF-α up-regulates BTN3 expression on endothelial cells (21), we hypothesized that the enhanced expression of BTN3A1 on monocytes in PV patients may be due to the actions of these inflammatory cytokines. In accordance with previous reports of higher levels of IFN-γ and TNF-α in the serum of PV patients (24), the expression of both IFN-γ and TNF-α was significantly increased in PV patients compared to HCs (Fig. 5*A*). To determine the impact of these cytokines on BTN3A1 expression on monocytes, PBMCs from HCs were treated with IFN-γ and TNF-α, respectively, and the BTN3A1 expression level of CD14$^+$ cells was determined by flow cytometry. IFN-γ induced the up-regulation of BTN3A1 on CD14$^+$ monocytes in a dose-dependent manner (Fig. 5*B*), but TNF-α had no effect (*SI Appendix*, Fig. S5). BTN3A1 on purified CD14$^+$ monocytes could also be up-regulated by IFN-γ, indicating a direct effect of IFN-γ on monocytes (Fig. 5*C*). Together, these data suggest that the enhanced BTN3A1 expression on monocytes in PV patients is likely due to increased proinflammatory signals.

CD14$^+$ Cells Highly Express BTN3A1 and Are Juxtaposed to Vδ2 T Cells in Psoriatic Skin Lesions. Because psoriasis is characterized by chronic skin inflammation, we asked whether the hyperactivation of Vγ9Vδ2 T cells observed in PB also existed in PV skin lesions. Skin biopsies were collected from both PV patients and HCs and subjected to immunofluorescence studies. Consistent with previous reports (5), we observed a higher frequency of Vδ2 T cells in skin lesions of PV patients compared to HCs, which may be attributed to the migration of PB Vδ2 T cells (Fig. 6 *A* and *B*). Interestingly, approximately 40% of the Vδ2 T cells expressed IL-17A in the skin lesions of psoriasis patients, while no IL-17A expression was detected in the

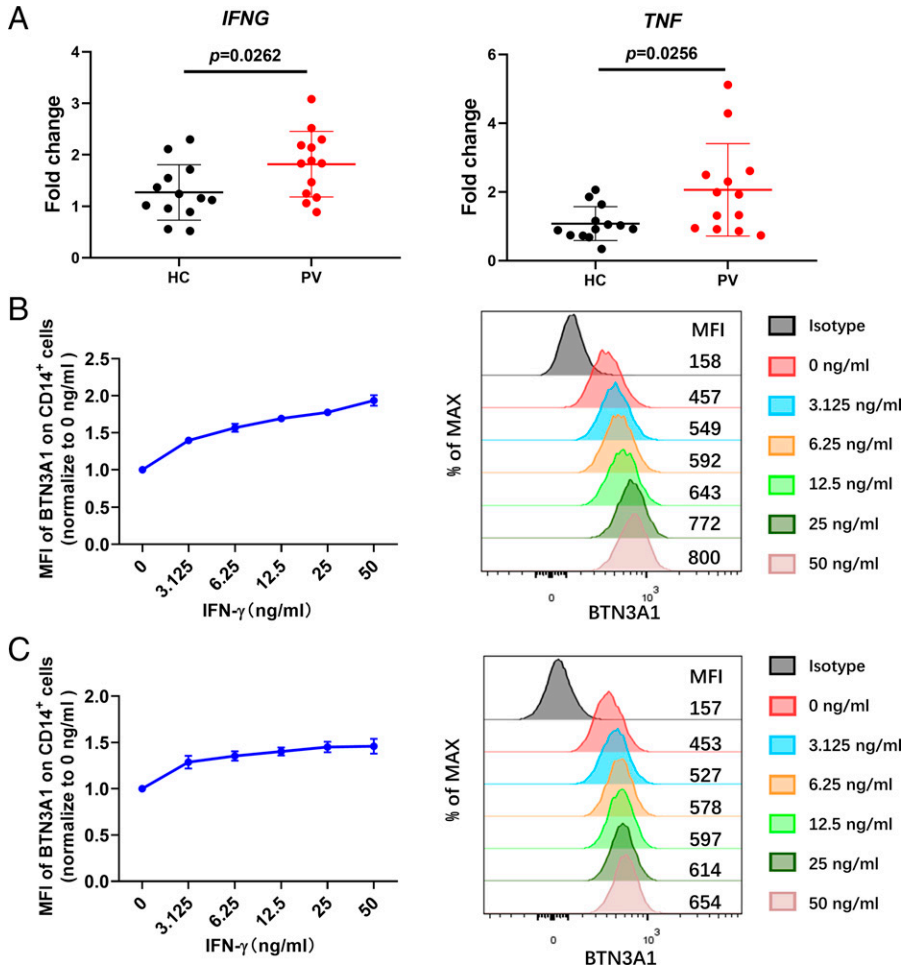

Fig. 5. IFN-γ induces up-regulation of BTN3A1 on monocytes. (*A*) Quantitative RT-PCR analysis of IFN-γ (*IFNG*) and *TNF* mRNA expression in PBMCs (n = 13 for each group). (*B* and *C*) PBMCs (*B*) or purified CD14$^+$ monocytes (*C*) from HC group (n = 3 to 4) were stimulated with IFN-γ at different concentrations, and the expression of BTN3A1 on CD14$^+$ monocytes was analyzed by flow cytometry. Each symbol represents an independent donor (*A*). Results are presented as means ± SEMs and are analyzed with two-tailed unpaired Student's t test (*A*).

skin sections from HCs (Fig. 6 *A* and *C*). Like activated T cells, monocytes are recruited to local tissue where they can differentiate into macrophages or dendritic cells in response to local environmental cues. We found dramatically increased $CD14^+$ cell infiltration in the skin lesions in PV patients (Fig. 6 *D* and *E*). The expression level of BTN3A1 on $CD14^+$ cells in PV patients was significantly higher than that in HCs (Fig. 6*D*). Notably, $CD14^+$ cells were juxtaposed to Vδ2 T cells, suggesting direct cell contact between monocytes and Vδ2 T cells in the skin lesions (Fig. 6*F*). Taken together, these data suggest that direct interactions between Vδ2 T cells and monocytes in the skin of psoriasis patients promote Vδ2 T cell activation and skin inflammation.

Discussion

A variety of studies have demonstrated that γδ T cells play a crucial role in the development and progression of psoriasis (25), but how human γδ T cells promote skin inflammation and the nature of the molecular mechanisms underlying γδ T cell dysfunction are poorly understood. Here, we have shown that circulating Vγ9Vδ2 T cells from PV patients produce more IFN-γ and TNF-α, which

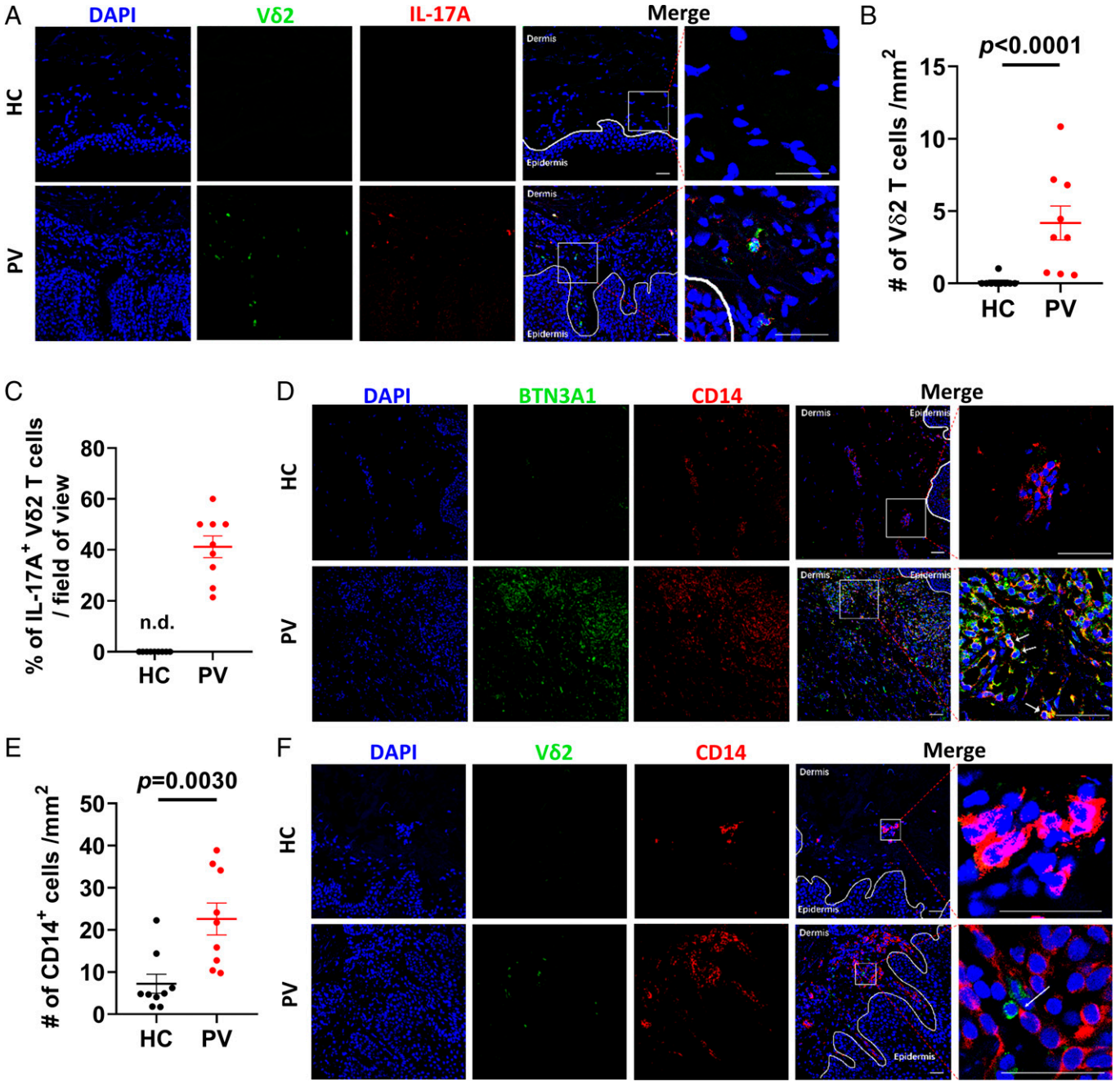

Fig. 6. $CD14^+$ cells highly express BTN3A1 and are juxtaposed to Vδ2 T cells in skin lesions of psoriasis patients. (*A*) Immunofluorescence staining of Vδ2 T cells in skin biopsies from HC and PV. Nuclei were stained with DAPI (blue), Vδ2 T cells were stained with anti-Vδ2 TCR mAb (green), and IL-17A was stained with anti-IL-17A mAb (red). The dermo-epidermal barrier is indicated with a white line. (*B*) Analysis of total number of Vδ2 T cells per square millimeter of skin ($n = 9$). (*C*) Analysis of the percentage of $IL\text{-}17^+$ Vδ2 T cells in total Vδ2 T cells per field of view ($n = 9$). (*D*) Immunofluorescence staining of $CD14^+$ cells and the expression of BTN3A1. $CD14^+$ cells were stained with anti-CD14 mAb (red) and BTN3A1 was stained with anti-CD277 mAb (green). (*E*) Analysis of total number of $CD14^+$ cells per square millimeter of skin ($n = 9$). (*F*) Immunofluorescence staining of Vδ2 T cells and $CD14^+$ cells. Scale bar, 50 μm (*A*, *D*, and *F*). Each symbol represents an independent donor (*B*, *C*, and *E*). Results are presented as means ± SEMs and are analyzed with two-tailed unpaired Student's *t* test (*B*, *C*, and *E*).

amplify Vγ9Vδ2 T cell activation by up-regulating the expression of BTN3A1 on monocytes in a positive feedback manner.

Previous studies have shown that γδ T cells infiltrating skin lesions of psoriatic patients can produce IL-17, which is considered a central factor in the pathogenesis of psoriasis (5). Despite the presence of resident dermal Vδ1 T cells, recent reports indicate Vγ9Vδ2 T cells recruited from the PB also produce IL-17, thereby contributing to the disease (11). Thus, we speculate that circulating Vγ9Vδ2 T cells from PV patients may produce more IL-17. Indeed, circulating Vγ9Vδ2 T cells from PV patients produced more IL-17 than Vγ9Vδ2 T cells from HCs, even though the expression of IL-17 was low. The low IL-17 expression by cultured blood Vγ9Vδ2 T cells may be due to the lack of aryl hydrocarbon receptor (AhR) agonist in the RPMI medium used in our study, which has been reported to promote Th17 differentiation (26). The enhanced IL-17 production by Vδ2 T cells was further confirmed in the skin lesions of PV patients by *in situ* microscopy. In addition to IL-17, γδ T cells produce other proinflammatory cytokines, including IFN-γ and TNF-α. Both IFN-γ and TNF-α are thought to contribute to the development of psoriasis because they can drive the production of IL-23 and IL-1β by dendritic cells and therefore promote IL-17 production by T cells or other innate lymphocytes such as ILC3 cells (27). The role of TNF-α in psoriasis has been confirmed by the remarkable clinical efficacy of anti-TNF-α mAbs (28). In the present study, the frequency of circulating TNF-α/IFN-γ–producing Vγ9Vδ2 T cells and the proliferation rate of Vγ9Vδ2 T cells were markedly increased in PV patients compared to those of HCs. Of note, most of the Vγ9Vδ2 T cells from HCs were distinct from those in PVs, because more T_{EMRA} and fewer T_{CM} cells were present in PV patients. Nonetheless, we observed reduced circulating Vγ9Vδ2 T cells in psoriasis patients, which could be explained by the increased migration of these cells into inflamed skin. Consistently, we observed more Vδ2 T cell infiltration in the skin lesions of PV patients. These data are in accord with previous reports (11).

Studies have shown that monocytes are required to serve as accessory cells for Vγ9Vδ2 T cell activation in response to HMBPP or agonistic anti-CD277 mAbs (22), because the depletion of $CD14^+$ monocytes from total leukocytes completely abolished the γδ T cell response to zoledronic acid, a nitrogen-bisphosphonates (*n*-BP) (29). Consistent with these data, we found that monocytes were required for HMBPP-mediated Vγ9Vδ2 T cell activation. Recent reports have shown that BTN3A1 is essential for the recognition of pAgs by Vγ9Vδ2 T cells (30). Given these data, we hypothesized that BTN3A1 may be involved in promoting Vγ9Vδ2 T cell activation in psoriasis. Consistent with this hypothesis, we found that the expression of BTN3A1 on monocytes, but not other immune cells, was increased in PV patients. Not only was BTN3A1 expression increased on circulating monocytes but also the monocyte-derived cells in PV skin lesions expressed higher BTN3A1 levels. Blockade of BTN3A1 with clone 103.2 mAb abolished the difference in Vγ9Vδ2 T cell activation between HCs and PV patients. These data suggest that the up-regulation of BTN3A1 on monocytes promotes the hyperactivation of Vγ9Vδ2 T cells in psoriasis. Interestingly, BTN3A1 was also up-regulated on Vγ9Vδ2 T cells in PV patients. This raised the possibility that Vγ9Vδ2 T cells exhibit self-activation by interacting with one another. Indeed, Vγ9Vδ2 T cells from psoriasis patients exhibited higher proinflammatory cytokine production when stimulated with HMBPP in vitro. Given the low frequency of Vγ9Vδ2 T cells in blood, the possibility of self-activation in vivo seems low.

The up-regulation of BTN3A1 expression on human vein endothelial cells has been reported to be induced by Th1-related inflammatory cytokines IFN-γ or TNF-α (21). Given the higher IFN-γ and TNF-α levels in psoriatic serum (24, 31), we propose that the up-regulation of BTN3A1 on monocytes likely also results from such signals. In our studies, IFN-γ induced the expression of BTN3A1 on monocytes, which could explain the enhanced BTN3A1 expression on monocytes in psoriasis. Our study provides insight into the molecular mechanisms of Vγ9Vδ2 T cell activation in psoriasis and highlights a potential role of BTN3A1 as a target for psoriasis treatment.

Materials and Methods

Patients and HCs. This study protocol was approved by the Scientific Ethical Committee of Fudan University. Written informed consent from all of the participants was obtained in accordance with the World Medical Association Declaration of Helsinki. A total of 88 PV patients (ages 18 to 70 y, 63 males and 25 females) with PASI scores (2.0 to 53.0) and 75 HCs (ages 23 to 68 y, 46 males and 29 females) were enrolled in this study (*SI Appendix*, Table S1). All PV patients were diagnosed by at least two experienced dermatologists based on clinical features and/or histological examinations. Patients who developed pustules or joint symptoms during the observation were excluded from the study. The PASI score was used as an indicator of the extent and severity of skin inflammation.

Cell Isolation. PBMCs were isolated from heparinized blood using Ficoll-Hypaque (Lymphoprep; Stemcell) by density gradient centrifugation. γδ T cells were enriched from PBMCs with human anti-TCR γδ MicroBead Kit (Stemcell), and then Vγ9Vδ2 T cells were sorted by FACSAria III (BD Biosciences). Monocytes were isolated from PBMCs using CD14 MicroBeads (Stemcell). The purity of Vγ9Vδ2 T cells and $CD14^+$ monocytes was assessed by flow cytometry. Cells were cultured in complete RPMI medium 1640 (Gibco) containing 10% fetal bovine serum (Gibco), 1% penicillin-streptomycin (Gibco), and 0.5% L-glutamine (Gibco).

Flow Cytometry. Dead cells were excluded by staining with fixable viability stain 620 (BD Biosciences). Nonspecific binding to Fc receptors was blocked by CD16/32 antibody (Biolegend) for 5 min at room temperature before surface staining (32). The antibodies used for flow cytometry are listed in *SI Appendix*, Table S2. Before cytokine staining, cells were stimulated with PMA (50 ng/mL; Sigma) and ionomycin (500 ng/mL; Enzo Life Sciences) or HMBPP (10 μM; Sigma-Aldrich) and agonistic anti-CD277 mAb clone 20.1 (5 μg/mL; ebioscience) for 5 h, respectively. Brefeldin A (10 μg/mL; Biolegend) was added for the last 3 h before cells were harvested for analysis. For antibody-blocking experiments, the optimal concentration of clone 103.2 was determined by titration assay, and 0.01 μg/mL was used to partially inhibit but not abrogate Vγ9Vδ2 T cell activation (*SI Appendix*, Fig. S6). Cells were collected on BD LSRFortessa flow cytometer, and data were analyzed with FlowJo software (Tree Star).

Proliferation Assays by CFSE. PBMCs or purified Vγ9Vδ2 T cells were labeled with 0.5 μM CFSE (Invitrogen) for 5 min at 37 °C and then cultured for 3 d. Cells were stimulated with HMBPP (10 nM, Sigma-Aldrich) in the presence or absence of clone 103.2 (0.01 μg/mL). All of the cells were cultured in complete RPMI containing IL-2 (50 U/mL, PeproTech). Cells were analyzed by fluorescence-activated cell sorting.

RNA-Seq. Purified Vγ9Vδ2 T cells (1×10^4) from PV patients and HCs were treated with the SMART-Seq HT Kit (Takara) according to the manufacturer's instructions to generate high-quality, full-length cDNA. Libraries were created using the TruePrep DNA Library Prep Kit V2 for Illumina (Vazyme) and sequenced with the Illumina Hiseq X Ten system (32). The raw reads were aligned to the human reference genome (hg19) using Hisat2 RNASeq alignment software. Differentially expressed genes (DEGs) between HCs and PV patients were identified by DESeq2 using two replicas in each condition. Deep analysis was further performed based on DEGs of protein coding genes (fold change >1.5, $P < 0.05$), including GO analysis using DAVID and GSEA (Broad Institute).

Quantitative RT-PCR. Total mRNA of PBMCs was isolated using TRIzol Reagent (Invitrogen) in accord with the manufacturer's protocol. cDNA was transcribed

using the PrimeScript RT master Kit (Takara). SYBR Select Master Mix reagent (Thermo Scientific) was used to quantify mRNA expression levels in a 384-well plate on a ViiA 7 real-time PCR system (32). The results were displayed as relative expression values normalized to *HPRT1* and calculated by the $2^{-\Delta\Delta Ct}$ method. The primers used for quantitative RT-PCR are listed in *SI Appendix*, Table S3.

Immunofluorescence Staining. Human skin samples were fixed with 4% paraformaldehyde and then permeabilized with 0.3% (vol/vol) Triton X-100. Cells were incubated with anti-Vδ2TCR (Biolegend) and anti-CD14 (Abcam) or anti-CD277 (Invitrogen) mAbs followed by donkey anti-rabbit secondary Ab and DAPI for nucleus. Images were collected by the Leica TCS SP8 confocal microscope system (20× magnification). Quantification of cells was performed using ImageJ2 Software (Fiji), with detection engines set for our purpose. Identical exposure times and threshold settings were used for each channel on all sections of similar experiments. The number of cells per dermal area (square millimeter) was calculated.

Statistical Analysis. Statistical analysis was performed with the two-tailed unpaired Student's *t* test or the two-tailed Welch's *t* test on independent groups using GraphPad Prism 8.0 program (GraphPad Software). Data are presented as mean values ± SEMs. Correlation was performed with Spearman's rank correlation test. Statistical significance was assumed at $P < 0.05$.

Data, Materials, and Software Availability. RNA-sequencing data reported in this paper have been deposited in Gene Expression Omnibus under the accession number GSE173387 (33). All of the study data are included in the article and/or supporting information.

ACKNOWLEDGMENTS. We thank the entire Lei Shen laboratory for help and suggestions and the Flow Cytometry Core and Image Core at the Shanghai Institute of Immunology for services and assistance. This study was supported by grant 2020YFA0509200 from the Ministry of Science and Technology of China, grant 81971487 (to L.S.), 81673073 and 81974471 (to Z.Z.) from the National Natural Science Foundation of China, grant SHDC2020CR6022 (to Z.Z.) from the Clinical Research plan of Shanghai Shenkang Hospital Development Center (SHDC), grants 20ZR1430200 and 20142202300 (to L.S.) from the Science and Technology Commission of Shanghai Municipality. We also thank the support from the Shanghai Frontiers Science Center of Cellular Homeostasis and Human Diseases (to L.S.).

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