INTRODUCTION

Psoriasis vulgaris is a common inflammatory skin disease affecting 2–3% of the population worldwide; however, the pathogenesis of psoriasis remains unclear (Nestle et al., 2009; Perera et al., 2012). Recently, much attention has been paid to the role of keratinocytes in the pathogenesis of psoriasis (Lowes et al., 2014). Keratinocytes can sense the damage-associated molecular pattern molecules and/or pathogen-associated molecular pattern molecules, which activate the inflammasome, produce IL-1β and IL-18, and elicit the inflammatory responses in psoriasis (Lowes et al., 2014). Activation of NF-κB in keratinocytes has been reported in psoriasis lesions, which results in the production of multiple immune-related proteins such as CCL-20, CCR-6, CXCL-1, CXCL-9–11, and IL-36 and leads to further inflammatory responses (Homey et al., 2000; Laggner et al., 2011). Thus, activation of keratinocytes would directly or indirectly contribute to the major histological features of psoriasis, including epidermal hyperplasia, parakeratosis, and skin inflammation, constituting a vicious circle to sustain the inflammation of psoriatic skin (Lowes et al., 2014). However, the exact role of keratinocytes in psoriasis still remains to be elucidated.

CD100 belongs to the fourth group of the semaphorin family and was initially identified as an evolutionarily conserved chemorepellent protein that regulates axonal guidance in the developing nervous system (Kolodkin, 1996). CD100 was the first semaphorin described to have immune functions; it stimulates tumor necrosis factor-α and IL-6 production by peripheral blood mononuclear cells (Yoshida et al., 2015). sCD100 is elevated in the sera of several autoimmune diseases, such as multiple sclerosis (Smith et al., 2015) and rheumatoid arthritis (Yoshida et al., 2015), and it stimulates tumor necrosis factor-α and IL-6 production by peripheral blood mononuclear cells (Yoshida et al., 2015). However, the role of CD100 in psoriasis is not clear yet.

Plexins constitute a family of transmembrane proteins that serve as receptors for semaphorins (Roney et al., 2013; Tamagnone et al., 1999). Activation of B-plexins by...
semaphorin ligands results in activation of the RhoGTPase nucleotide exchange factor proteins and subsequent activation of RhoA and RhoC (Aurandt et al., 2002; Perrot et al., 2002; Swiercz et al., 2002; Worzfeld et al., 2012). However, PlxnB2-mediated function is far less recognized. PlxnB2 was originally found to modulate neuronal migration and patterning of the developing nervous system (Deng et al., 2007). Interaction of PlxnB2 and CD100 can guide T-cell recruitment and function in the germinal center (Yan et al., 2017). CD100-PlxnB2 interaction mediates the adherence between monocytes and endothelial cells of the blood vessels (Luque et al., 2015). Witherden et al. (2012) reported that PlxnB2 is expressed in mouse keratinocytes and is involved in the epithelial repair process through its interaction with CD100, and PlxnB2 has effects on CD100-mediated γδ T-cell morphology. Thus, the role of PlxnB2 in skin diseases deserves further exploration.

In this study, we explored the role of PlxnB2 in the pathogenesis of psoriasis. We found that there was elevated expression of PlxnB2 on keratinocytes of psoriatic lesions and imiquimod (IMQ)-induced psoriatic dermatisms of mice. CD100 was found to be increased significantly in the sera of psoriasis patients and on keratinocytes of psoriatic skin. CD100-PlxnB2 activated the NLRP3 inflammasome in keratinocytes through activation of the RhoA/Rac1–NF-κB signaling pathway and promoted the production of various proinflammatory cytokines and chemokines. Thus, our data show that CD100-PlxnB2 interaction is involved in the pathogenesis of psoriasis.

RESULTS
Elevated expression of PlxnB2 on keratinocytes of psoriasis patients and a mouse model of psoriasis-like dermatitis

It has been reported that PlxnB2 is expressed on keratinocytes of mouse epidermis and that the expression level is increased during the process of wound healing (Withenden et al., 2012). Here, we found that PlxnB2 was also expressed on keratinocytes in healthy human epidermis, and the expression level was increased greatly in the lesional skin of psoriasis patients, as evidenced by immunohistochemistry, quantitative real-time reverse transcriptase–PCR (qRT-PCR) and Western blot (Figure 1a–c; see also Supplementary Figure S1 online). There was only a marginal increase in the expression of PlxnB2 in the lesions of atopic dermatitis (AD) patients (Figure 1a–c). The expression of PlxnB2 in normal skin of psoriasis patients was similar to that of healthy individuals (see Supplementary Figure S2 online). In a mouse model of IMQ-induced psoriasis-like dermatitis, the expression level of PlxnB2 was increased in CD45-negative cells from epidermis of psoriatic dermatitis, as indicated by flow cytometry and mean fluorescence intensity of PlxnB2 staining (Figure 1d–f). Results of qRT-PCR were consistent with the flow cytometry data (Figure 1g). However, in an MC903-induced AD mouse model, there was no increase in the expression of PlxnB2 on keratinocytes (Figure 1d–g).

Next, we cultured primary human keratinocytes and analyzed the expression of PlxnB2 upon stimulation with IL-17A, IL-22, IFN-γ, IL-4, or IL-13. The result of Western blot showed that there was significantly increased expression of PlxnB2 in keratinocytes upon stimulation with IL-17A, IL-22, and IFN-γ, but not IL-4 and IL-13 (Figure 1h), which was confirmed by the result of immunofluorescence staining (Figure 1i) and qRT-PCR (see Supplementary Figure S3 online). Combination of IL-17A, IL-22, and IFN-γ further increased the expression of PlxnB2 (see Supplementary Figure S3). These results indicated that T helper (Th) type 1 and Th17 cytokines promoted that expression of PlxnB2.

In vivo silencing of PlxnB2 inhibited IMQ-induced psoriatic dermatitis

We painted PlxnB2-specific small interfering RNA (siRNA) (mixed siRNA that were functionally verified by Qiagen [Hilden, Germany]) that was pre-incubated with HiPerfect Transfection Reagent (Qiagen) onto the mouse ears and subsequently applied IMQ to the ears to produce psoriatic dermatitis. The permeation of siRNA into the inflammatory epidermis was confirmed using AF488-conjugated control siRNA, and the fluorescence was detected in the whole epidermis of IMQ-treated ears (see Supplementary Figure S4 online), indicating that siRNA could penetrate into the inflammatory epidermis efficiently. Application of PlxnB2-specific siRNA inhibited the increased expression of PlxnB2 in the epidermis after IMQ painting (Figure 2a and b). During the process of IMQ application, the mice pre-treated with PlxnB2-specific siRNA showed alleviated dermatitis compared with the mice pre-treated with control siRNA, as evidenced by gross morphology, ear thickness, histology, and epidermis thickness (Figure 2c–f).

The number of γδ TCR-low T cells, the major IL-17-producing cells in IMQ dermatitis, in the epidermis of inflammatory skin of the PlxnB2-specific siRNA group was significantly lower than that of the control siRNA group (Figure 2g and h). The relative numbers of T cells, macrophages, and neutrophils in skin were also lower in the group of PlxnB2-specific siRNA than in the control siRNA group (see Supplementary Figure S5 online). The expressions of CCL-20, CXCL-1, and IL-36 were significantly lower in the group of PlxnB2-specific siRNA than in the control siRNA group (Figure 2i). Expressions of IL-17, IL-22, and IL-23 were also decreased in the group of PlxnB2-specific siRNA, but without statistic significance. Collectively, our data show that in vivo silencing of PlxnB2 alleviated IMQ-induced psoriatic inflammation in the mouse model, indicating that PlxnB2 might be an important regulator in the inflammation of psoriasis.

Increased expression of CD100 in psoriasis patients

We then analyzed the expression of the ligand of PlxnB2, sCD100 and membrane-bound CD100 (mCD100), in the blood and lesional skin of psoriasis patients. The results showed that the level of sCD100 in the sera of psoriasis patients was about 5-fold higher than in healthy individuals (Figure 3a), and there was linear correlation between the level of sCD100 and the Psoriasis Area and Severity Index of patients ($r^2 = 0.5171$, $P < 0.0001$) (Figure 3b). The expression of mCD100 on T cells, monocytes, and platelets, but not B cells, from blood mononuclear cells of psoriasis patients was lower than that of healthy control subjects, as evidenced by mean fluorescence intensity of CD100 staining (Figure 3c). Immunohistochemistry data showed that there was increased expression of mCD100 in the epidermis of psoriatic lesion (Figure 3d), which was confirmed by the results of qRT-PCR.
and Western blot (Figure 3e and f). The expression of Sema4A, another ligand of PlxnB2, was also analyzed in lesions of psoriasis patients, and the results of qRT-PCR showed that the expression was lower in psoriasis patients than in healthy control subjects (see Supplementary Figure S6 online).

**CD100-PlxnB2 Promotes Inflammation in Psoriasis**

To explore the role of CD100-PlxnB2 interaction in psoriasis, we analyzed the effects of sCD100 on the production of chemokines and cytokines by primary keratinocytes in an in vitro cell culture system. The results showed that there were significantly increased expressions of CXCL-1 and CCL-20 in sCD100-treated cells, as shown by qRT-PCR, ELISA, and Western blot (Figure 4a–c). After transfection with PlxnB2-specific siRNA, the increased expression of CXCL-1 and CCL-20 was significantly abrogated (Figure 4a–c), indicating that the effects of sCD100 were mediated by PlxnB2. The confirmation of the knockdown of PlxnB2-specific siRNA was shown in Supplementary Figure S7a and b online. A second PlxnB2-specific siRNA was also used, and the results of the two siRNAs were similar (data not shown). However, there were no changes in the production of IL-36 by keratinocytes after the stimulation with sCD100 (Figure 4a–c). The effects of sCD100 on the production of CXCL-1 and CCL-20 were verified when HaCaT cells were used (see Supplementary Figure S8 online).

**CD100-PlxnB2 Promotes the Formation of the NLRP3 Inflammasome in Keratinocytes**

We further analyzed the effects of sCD100 on NLRP3 inflammasome activation in keratinocytes in an in vitro cell culture system. The result showed that there was increased transcription of NLRP3, caspase-1, IL-1β, and IL-18 in keratinocytes.
after stimulation with sCD100, as indicated by qRT-PCR (Figure 4a). ELISA data were consistent with the qRT-PCR result that the expressions of caspase-1, IL-1β, and IL-18 in the supernatant of cell culture were increased after stimulation with sCD100 (Figure 4b). The Western blot result showed that the expression of NLRP3, active form of caspase-1 (p20), mature type of IL-1β, and IL-18 were all increased in keratinocytes after incubation with sCD100 (Figure 4d and e). When the expression of PlxnB2 was silenced by PlxnB2-specific siRNA, the increased expression of these molecules was abrogated (Figure 4a, b, d, and e). The effects of the second PlxnB2-specific siRNA were similar (data not shown). And the effects of sCD100 on the production of NLRP3, caspase-1, IL-1β, and IL-18 were verified when HaCaT cells were used (see Supplementary Figure S8 online). The expression of NLRP1 and NLRC4 in psoriatic lesions was also slightly increased; however, sCD100 stimulation could only up-regulate the expression of NLRP3, but not NLRP1 and NLRC4, in cultured keratinocytes (see Supplementary Figure S9 online), and the CD100-mediated release of IL-1β/IL-18 was NLRP3-dependent (see Supplementary Figure S10 online). Thus, our study results suggest that sCD100 activated the NLRP3 inflammasome in keratinocytes through binding to PlxnB2.

sCD100 activated the small GTPase–NF-κB signaling pathway in keratinocytes through PlxnB2

Next, we explored the signaling pathway downstream of CD100-PlxnB2 using the HaCaT cell line. Stimulation with sCD100 induced increased expression of phospho-p65 within hours, which was inhibited by NF-κB inhibitor (Figure 5a). Stimulation with sCD100 also promoted the expression of phospho-p105 in HaCaT cells, and the expression was decreased when the cells were transfected with PlxnB2-specific siRNA, as determined by Western blot.
Figure 3. Increased expression of CD100 in psoriasis patients. (a) ELISA showing the concentrations of sCD100 in sera of psoriasis patients and healthy control subjects. \( n = 25, *** P < 0.001 \). (b) Correlation of sCD100 concentrations and PASI score of psoriasis patients. \( P < 0.0001 \). (c) MFI of mCD100 staining on different subpopulations of PBMCs. \( n = 10 \). (d) Immunohistochemistry showing the expression of CD100 in lesional skin. Original magnification \( \times 100 \). Scale bar = 200 \( \mu \)m. (e) qRT-PCR showing the expression of CD100 mRNA in epidermis from pooled skin biopsy samples. Data are shown as mean \pm standard deviation, \( n = 3 \). \( * P < 0.05 \), \( ** P < 0.01 \), \( *** P < 0.001 \). (f) Western blot showing the expression of CD100 in epidermis of skin biopsy samples. Data are representative of at least four independent experiments. mCD100, membrane-bound CD100; MFI, mean fluorescence intensity; PASI, Psoriasis Area and Severity Index; PBMC, peripheral blood mononuclear cells; sCD100, soluble CD100.

(Figure 5b), indicating that sCD100 activated the NF-κB signaling pathway through PlxnB2. Knockdown of PlxnB2 in HaCaT cells by PlxnB2-specific siRNA was confirmed by qRT-PCR and Western blot, as shown in Supplementary Figure S7c and d. Next, we found that sCD100 induced the activation of Rac1 and RhoA by using glutathione S-transferase pulldown assay (Figure 5c), and the effects of sCD10 were inhibited by PlxnB2-specific siRNA (Figure 5c). When RhoA inhibitor or Rac1 inhibitor was used, the sCD100-enhanced phosphorylation of p65 was significantly inhibited (Figure 5d and e). Blocking of RhoA and Rac1 signaling also decreased the expression of NLRP3 (Figure 5d and e). Our study suggested a new pathway for the NLRP3 inflammasome activation: CD100-PlxnB2 activates Rho GTPase, which induces the phosphorylation of NF-κB; then NF-κB primes NLRP3, and Rac1 activates the NLRP3 inflammasome as second signal (Figure 6).

DISCUSSION

In the past two decades, people paid extensive interest to the function and mechanism of T cells in the pathogenesis of psoriasis; however, keratinocytes have also been attracting more attention recently (Cai et al., 2012; Lowes et al., 2014; Perera et al., 2012). Various proteins expressed by keratinocytes (e.g., caspase recruitment domain family member 14, ZNF750, late cornified envelope proteins, and Act1) were shown closely associated with psoriasis (Bergboer et al., 2011; Birnbaum et al., 2011; Hobbs et al., 2017; Jordan et al., 2012), and integrated functional work also showed that keratinocytes actively participate in the initiation and maintenance of psoriasis by producing various proinflammatory cytokines and chemokines (Lowes et al., 2014). In this study, we showed the proinflammatory role of CD100-PlxnB2 in keratinocytes in the pathogenesis of psoriasis.

Recent studies report that mouse keratinocytes constitutively express substantial levels of PlxnB2 and that the expression increases during the process of wound healing (Witterden et al., 2012). Our data showed that PlxnB2 was also expressed in human keratinocytes, and under inflammatory conditions the expression of PlxnB2 was increased in the keratinocytes of psoriasis patients and in a mouse model of IMQ-induced psoriasis-like dermatitis. However, the expression of PlxnB2 in the lesions of AD patients was only slightly increased and was even decreased in MC903-induced AD mouse model. The difference of PlxnB2 expression between psoriasis and AD indicated that Th1 and/or Th17 inflammation, but not Th2 inflammation, promoted the expression of PlxnB2, which was confirmed by the finding that the expression of PlxnB2 was increased in primary keratinocytes stimulated with Th1 and Th17 cytokines in cell culture. The exact mechanism was not clear yet, and experiments were undertaken to elucidate the detailed mechanism of the modulation of Th1 and Th17 cytokines on the expression of PlxnB2. Previous studies report that PlxnB2 not only participates in the regulation of cell motility but also has immune functions (Holl et al., 2012; Yan et al., 2017). In contrast to previous studies that focused on hematopoietic cells, our study show that keratinocytes, a type of epithelial
cells, could be stimulated via PlxnB2 to produce proinflammatory cytokines and chemokines. Our data expand current knowledge on the immunological function of CD100-PlxnB2 from immune cells to epithelial cells. Considering the wide expression of PlxnB2 in the epithelial and endothelial systems, we assume that PlxnB2 would participate in various inflammatory diseases of multiple organs, including the gut, lung, and skin.

Increased activity of inflammasome in psoriasis lesion has been reported by several groups (Dombrowski et al., 2011; Tervaniemi et al., 2016), and IL-1β has been identified as a key player in the pathogenesis of psoriasis (Nestle et al., 2009) and promotes the IL-23–triggered development of Th17 cells and production of various cytokines (Ghoreschi et al., 2010). Studies have also reported that semaphorin-plexin signaling activates the NF-κB pathway (Catalano et al., 2009; Yang et al., 2011; Zielonka et al., 2014). Our study shows that CD100-PlxnB2 activated the NLRP3 inflammasome and promoted the production of IL-1β. The stimulators for inflammasome activation in psoriasis were supposed to include infection and injury, and our study added the semaphorin-plexin-mediated signaling as a possible stimulator for inflammasome activation. Activation of the NLRP3 inflammasome is under tight control, and CD100-PlxnB2 may activate the NLRP3 inflammasome by inducing both the primary transcription signal and secondary activating signal: NF-κB signaling regulates the transcription of NLRP3, and Rac1 GTPase activates the NLRP3 inflammasome (Catalano et al., 2009; Yang et al., 2011; Zielonka et al., 2014).

Figure 4. CD100 promoted the production of proinflammatory cytokines and chemokines and activated the inflammasome in keratinocytes via PlxnB2. Primary keratinocytes were incubated with sCD100 and/or with PlxnB2-specific siRNA or control siRNA and were cultured for 48 hours. (a) qRT-PCR showing the mRNA levels of various molecules from cultured keratinocytes. Data are shown as mean ± standard deviation. *P < 0.05, **P < 0.01, ***P < 0.001. (b) ELISA results showing the concentration of various molecules in the supernatant of keratinocyte culture. Data are shown as mean ± standard deviation. *P < 0.05, **P < 0.01, ***P < 0.001. (c) Western blot showing the expressions of CXCL-1, CCL-20, IL-36, pre–IL-1β, mature IL-1β, pre–IL-18, mature IL-18, NLRP3, pre–caspase-1 and mature caspase-1 in the lysates of cultured keratinocytes. Densitometry quantifies the expression levels of various molecules relative to actin or tubulin. Data shown are representatives of at least five independent experiments. Ctrl, control; ns, not significant; qRT-PCR, quantitative real-time reverse transcriptase-PCR; sCD100, soluble CD100; siRNA, small interfering RNA.
Several studies report that the GTPases and some of their guanine nucleotide exchange factors can activate NF-κB, and PlxnB2 was recently shown to activate the small GTPase family RhoA and Rac1 and promote invasive growth of malignant glioma (Le et al., 2015). It was reported that Rho GTPase are involved in the pathogenesis of psoriasis. Rac1 is highly activated in human psoriatic lesional skin (Winge et al., 2016), and Ras activity is also elevated in psoriatic lesions (Gunderson et al., 2013). RhoA is activated under Th17 conditions and can regulate the production of IL-17, IL-21, and IL-22 (Ricker et al., 2016). In this study, we found that sCD100 activated the RhogTPase RhoA and Rac1 in keratinocytes through PlxnB2, and sCD100 also induced the activation of NF-κB and the NLRP3 inflammasome, which was inhibited by RhoA and Rac1 blocker. Thus, we propose a new pathway for the activation of the NLRP3 inflammasome: CD100-PlxnB2 activates RhoA and Rac1, which induces the phosphorylation of NF-κB; then NF-κB induces the priming of NLRP3, and Rac1 acts as a second signal to activate the NLRP3 inflammasome (Figure 6).

CD100 is the most recognized ligand for PlxnB2. A large amount of sCD100 is detectable in the sera of autoimmune-prone MRL/lpr mice (Wang et al., 2001) and in the sera of systemic sclerosis patients (Besliu et al., 2011) and rheumatoid arthritis patients (Yoshida et al., 2015). The increased level of sera sCD100 in psoriasis patients is similar to the autoimmune disease mentioned earlier. Surface CD100 expressing on T cells is the major source of sCD100 after shedding by certain matrix metalloproteinases (Maleki et al., 2016). In our study, the levels of mCD100 were decreased on T cells, monocytes, and platelets of psoriasis patients; however, the mCD100 level on B cells was increased. The mechanism for the different expression of mCD100 on different cells in peripheral blood mononuclear cells awaits further exploration, which might clarify the origin of the elevated serum sCD100. Besides the effects of sCD100 and/
or mCD100 on keratinocytes, there may also be interaction between keratinocytes and T cells via PlxnB2 and mCD100. Havran’s group reports that PlxnB2 expressed on keratinocytes induces the cellular rounding of CD100-expressing γδ T cells (Witherden et al., 2012). Because of the important role of γδ T cells in the pathogenesis of psoriasis, the interaction between keratinocytes and T cells via PlxnB2 and CD100 deserves further exploration. Because CD100 can act as a receptor for PlxnB2, our data cannot exclude the possibility that the increased inflammation mediated by CD100-PlxnB2 interaction might be through mCD100. Future work using CD100-knockout mice would give more information.

CD100-PlxnB2 targeting might be a potential strategy for the treatment of psoriasis. Currently, several anti-CD100 antibodies are under investigation at different stages of clinical trials for the treatment of several tumors, multiple sclerosis, and Huntington disease (Maleki et al., 2016). Some of the trials showed good results, and psoriasis might be the future indication of anti-CD100 treatment.

MATERIALS AND METHODS

We provide a brief description of the methods here, with additional details in Supplementary Materials and Methods online.

Human skin samples

Patients with a dermatologist-confirmed diagnosis of chronic plaque psoriasis or AD and healthy individuals were recruited in this study. All participants provided written, informed consent for their participation. The study protocol was designed and carried out according to the principles of the Declaration of Helsinki and was approved by the ethics review board of the Xijing Hospital, Fourth Military Medical University. See also in the Supplementary Materials and Methods.

Mice and treatment

The animal experiments were performed in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals of the Fourth Military Medical University. The detailed procedures for the IMQ-induced psoriasis mouse model (Tortola et al., 2012), MC903-induced AD model (Leyva-Castillo et al., 2013), and topical siRNA application (Ritprajak et al., 2008) are described in the Supplementary Materials and Methods.

Cell culture

Primary keratinocytes were prepared according to standard protocol, and the detailed procedure is described in the Supplementary Materials and Methods.

siRNA preparation and cell transfection

The procedure is described in the Supplementary Materials and Methods.

qRT-PCR

The procedure and the primers are described in the Supplementary Materials and Methods.

Western blot

The procedure and the antibodies used are described in the Supplementary Materials and Methods.

Immunohistochemistry

The procedure and the antibodies used are described in the Supplementary Materials and Methods.

ELISAs

An in-house optimized sandwich ELISA was established to detect sCD100 in patient serum as reported previously (Liu et al., 2013). The procedure is described in the Supplementary Materials and Methods.

Flow cytometry

The procedure and the antibodies used are described in the Supplementary Materials and Methods.

Immunofluorescence analysis

The procedure and the antibodies used are described in the Supplementary Materials and Methods.

Statistics

The procedure is described in the Supplementary Materials and Methods.

CONFLICT OF INTEREST

The authors state no conflict of interest.
ACKNOWLEDGMENTS
This work was supported by the National Natural Science Foundation of China (grant numbers 81472885, 81573038, 81400073, 81371738, 31400782, 81573045, and 81371735).

AUTHOR CONTRIBUTIONS
CZ, CX, ED, and JC performed all experiments and analyzed data. CZ, XY, and WL wrote the paper. MF and BJ statistically analyzed data and reviewed the paper. MF and ZZ helped with technical experiments and analyzed data. BJ, XL, and GW reviewed the paper. CZ and WL designed the research, analyzed data, and wrote the paper.

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of this paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2017.09.005.

REFERENCES