Decreased Expression of Caveolin-1 Contributes to the Pathogenesis of Psoriasiform Dermatitis in Mice

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Psoriasis is a chronic inflammatory skin disease characterized by excessive proliferation and abnormal keratinocyte development, in which T helper type 17 cells and signal transducer and activator of transcription 3 (STAT3) activation have pivotal roles. Moreover, caveolin-1 (CAV-1) has been implicated in the regulation of signal transduction, and aberrant CAV-1 expression is involved in a variety of diseases. However, whether CAV-1 is involved in psoriasis is unknown. Here we examined CAV-1 expression in the psoriatic epidermis and investigated its role in the pathogenesis of psoriasis. CAV-1 was markedly reduced in lesional epidermis of psoriasis patients. *CAV1* silencing in keratinocytes *in vitro* revealed significant activation of STAT3, leading to increased expression of keratin 16 and several cytokine/chemokines, such as IL-6, C-X-C chemokine ligand 8 (CXCL8), CXCL9, and C-C chemokine ligand 20. In addition, psoriasis-related cytokines, including tumor necrosis factor-α (TNF-α), decreased CAV-1 expression in keratinocytes. Finally, administration of CAV-1 scaffolding domain peptide in a murine model of psoriasis-like skin inflammation induced by imiquimod improved the skin phenotype and reduced epidermal thickness and infiltrating cell counts. Furthermore, expression of TNF-α, IL-17A, and IL-23 was significantly suppressed by this treatment. Collectively, our study indicated that CAV-1 participates in the pathogenesis of psoriasis by regulating the STAT3 pathway and cytokine networks.

Journal of Investigative Dermatology (2015) **135,** 2764–2774; doi:10.1038/jid.2015.249; published online 20 August 2015

INTRODUCTION

Psoriasis vulgaris is a chronic immune-mediated inflammatory skin disease characterized by scaly papulosquamous plaque lesions (Griffiths and Barker, 2007). Innate effector cells present in psoriatic lesions, including dendritic cells and T helper type 17 cells, have key roles in the initial stages and also in the later amplifying inflammatory stages of psoriasis (Lowes *et al*, 2007; Nestle *et al*, 2009). In the chronic phase, various cytokines produced by infiltrated dendritic cells and T cells, such as tumor necrosis factor (TNF)-α, IL-17, and IL-22, induce hyperproliferation and keratinocyte activation via phosphorylation of signal transducer and activator of transcription 3 (STAT3). Activated keratinocytes also produce growth factors and cytokines/chemokines, causing further inflammation, epithelial–stromal hyperplasia, and immune cell mobilization (Nograles *et al*., 2010). Thus, a vicious cycle of inflammatory cytokines/chemokines triggering leukocyte infiltration, activation, and keratinocyte proliferation is produced, ultimately resulting in chronic skin lesion formation (Nickoloff and Nestle, 2004).

Caveolin-1 (CAV-1) is a 22-kDa membrane protein that is essential for the formation of flask-shaped membrane invaginations known as caveolae (Yamada, 1955). Caveolae have a distinctive composition of lipids, including cholesterol and sphingolipids, and participate in trafficking, endocytosis, and signaling (Schlegel *et al*, 2005; Galbiati *et al*, 2001; Parton and Richards, 2003). CAV-1 regulates a variety of signaling molecules and receptors, including SRC, SMAD/the transforming growth factor (TGF)-β receptor, the insulin receptor, AKT, and mitogen-activated protein kinase (MAPK); these molecules are able to bind to the CAV-1 scaffolding domain (CSD; amino acids 82–101) (Razani *et al*, 2001; Stan, 2005; Tourkina *et al*, 2005). CAV-1 has been implicated in various human diseases, including diabetes, cancer, cardiovascular disease, systemic sclerosis, and pulmonary fibrosis (Wang *et al*, 2006; Galdo *et al*, 2008; Strålfors, 2012; Yamaguchi *et al*, 2013; Núñez-Wehinger *et al*, 2014), in which it is a direct inhibitor of different plasma-membrane-initiated signaling cascades (Okamoto *et al*, 1998).

Campbell *et al* (2002) proposed participation of CAV-1 in psoriasis after showing that CAV-1 is downregulated in chronic plaque psoriasis. On the basis of these initial findings, we examined whether CAV-1 is explicitly involved in the pathogenesis of psoriasis and present evidence indicating that CAV-1 indeed has an important role in the pathogenesis of chronic psoriatic inflammation.

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Abbreviations: CAV-1, caveolin-1; CSD, caveolin-1 scaffolding domain; IMQ, imiquimod; JAK, Janus kinase; KRT16, keratin 16; MAPK, mitogen-activated protein kinase; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TNF, tumor necrosis factor

Received 25 January 2015; revised 2 June 2015; accepted 12 June 2015; accepted article preview online 2 July 2015; published online 20 August 2015

RESULTS

Reduced CAV-1 expression in the epidermis of psoriasis patients and in a murine model of psoriasis-like skin inflammation

We first evaluated CAV-1 levels in psoriatic skin. Immunohistochemical analysis indicated a marked decrease in CAV-1 expression in the epidermis of psoriasis patients compared with healthy control subjects (Figure 1a). *CAV1* mRNA and protein levels were also examined in the separated epidermis of skin samples from patients' plaque psoriasis lesions and from healthy control subjects, using semi-quantitative and quantitative PCR (qPCR) and western blotting. CAV-1 expression was clearly reduced in the lesions of psoriasis patients (Figure 1b and c). Consecutive skin biopsy samples containing both lesional and non-lesional areas indicated that only lesional skin had reduced CAV-1 levels (Figure 1d).

We then evaluated CAV-1 levels in the epidermis of a mouse model of psoriasis-like skin inflammation, induced using imiquimod (IMQ), as previously described (van der Fits *et al*, 2009). Real-time qPCR and immunohistochemistry indicated that CAV-1 levels were also reduced in the epidermis of these mice (Figure 1e and f).

Activation of the Janus kinase 2 (JAK2)/STAT3 pathway in CAV-1-silenced human keratinocytes

Recent studies have shown that CAV-1 negatively modulates signal transduction by directly binding to signaling molecules, suggesting that altered CAV-1 expression in different diseases could contribute to the disease pathogenesis. As direct interaction of CAV-1 and STAT3 has previously been reported in different cell types (Medina *et al*, 2006; Yuan *et al*, 2011),

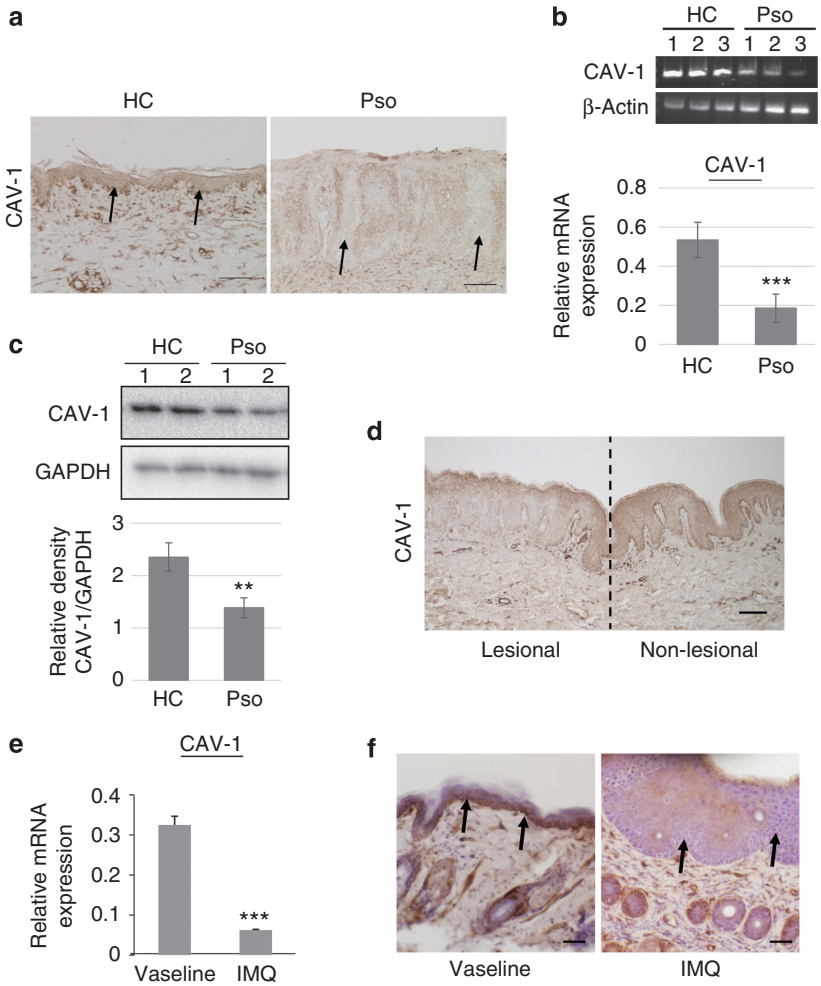

Figure 1. Decreased caveolin-1 (CAV-1) expression in the epidermis of psoriasis. (**a**) Representative images of immunohistochemical analysis of CAV-1 in the skin from a psoriasis patient (Pso) and a healthy subject (HC). Scale bar = 100 μm. (**b** and **c**) CAV-1 expression was evaluated using semi-quantitative and quantitative PCR (**b**) and western blotting (**c**). (**b**) Representative semi-quantitative PCR images from three patients and healthy controls are shown. Five individual samples were subjected to quantitative PCR in duplicate, $^{***}P < 0.001$. (**c**) Representative images of immunoblotting were shown. The CAV-1 protein levels were quantified using densitometry and expressed as the ratio of CAV-1 to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in **c**. $n = 5$; $^{**}P < 0.01$. (**d**) Skin tissue, which shows adjacent psoriatic lesions and normal skin, was stained with an anti-CAV-1 antibody. Scale bar = 300 μm. (**e**) CAV-1 expression levels in the epidermis of imiquimod (IMQ)-treated mice were determined by quantitative PCR. $n = 6$; $^{***}P < 0.001$. (**f**) Immunohistochemical analysis of CAV-1 in IMQ-treated mice skin. Scale bar = 50 μm.

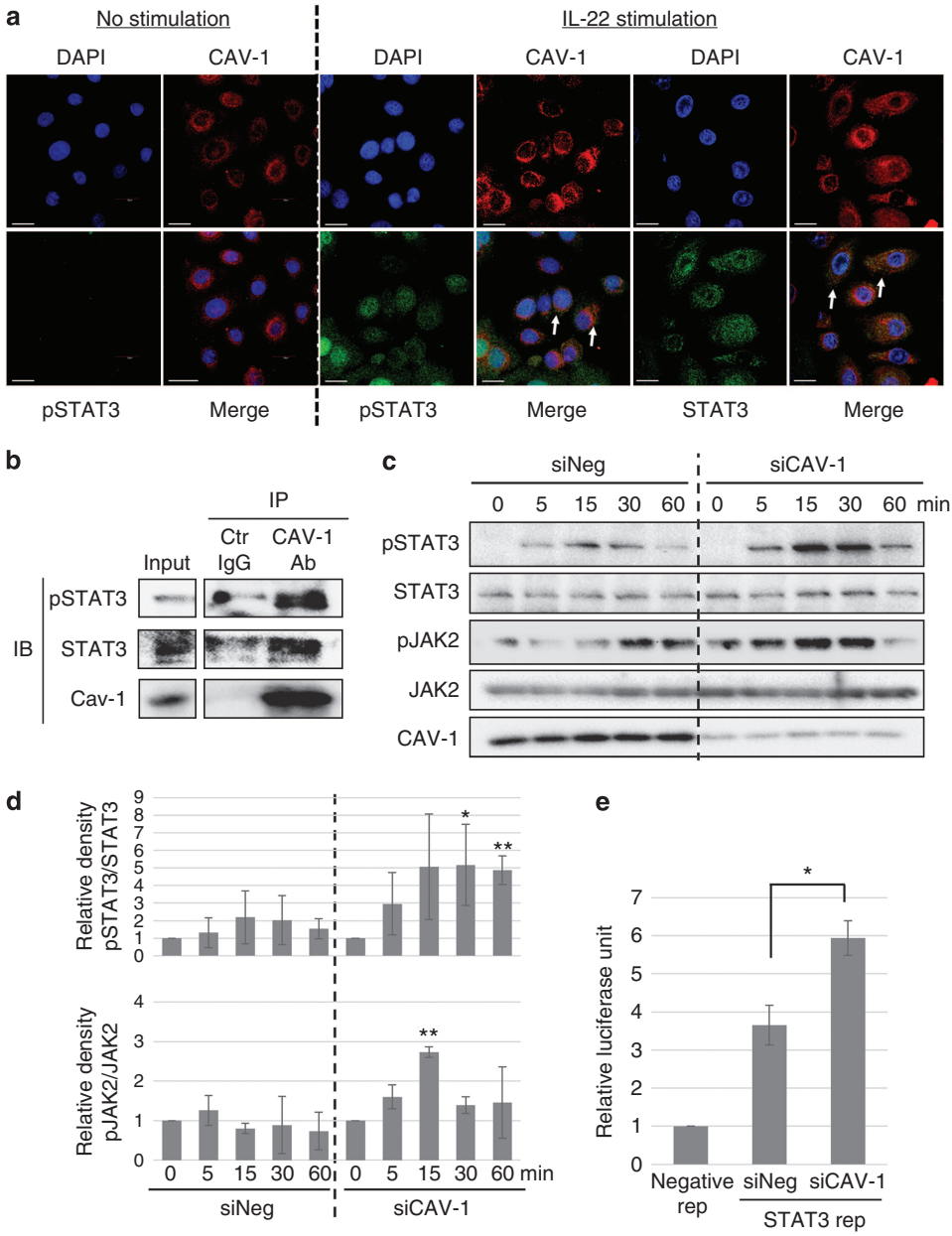

Figure 2. Activation of the signal transducer and activator of transcription 3 (STAT3) pathway in caveolin-1 (CAV-1)-silenced keratinocytes. (**a**) CAV-1 and phosphorylated STAT3 (pSTAT3)/STAT3 localization was determined by immunocytochemical staining. Human keratinocytes with or without prior IL-22 stimulation were stained for pSTAT3/STAT3 (green) and CAV-1 (red). DAPI (4,6-diamidino-2-phenylindole; blue) was used to identify nuclei. Scale bars = 20 μm. (**b**) Cell lysates from human keratinocytes were immunoprecipitated (IP) with an anti-CAV-1 antibody or control immunoglobulin G (IgG). Coprecipitation of pSTAT3 or STAT3 was examined by immunoblotting (IB). Non-precipitated sample was loaded and shown as input. Experiments were repeated three times. (**c**) CAV-1 was silenced using RNAi. pSTAT3 and pJAK2 were evaluated at the indicated time points after IL-22 stimulation in CAV-1-silenced (siCAV-1) or control siRNA (siNeg)-treated keratinocytes. Total STAT3 or JAK2 was quantified as controls. Experiments were performed on three independent occasions. (**d**) Protein levels shown in **c** were quantified with densitometry and expressed as the ratio of pSTAT3 to STAT3 and pJAK2 to JAK2. The level of each protein at time 0 was set to 1 arbitrary units. Statistical analysis was performed between siNeg versus siCAV-1 at the each same time point, $n = 3$; $^{*}P < 0.05$ and $^{**}P < 0.01$. (**e**) Transcriptional activity of STAT3 was detected in siCAV-1 or control keratinocytes (siNeg) with IL-22 stimulation using a luciferase STAT3 promoter assay. Negative promoter was used as control. Three independent experiments were performed. $^{*}P < 0.05$.

we confirmed colocalization and binding of CAV-1 and STAT3 proteins in human keratinocytes. CAV-1 colocalized with both STAT3 and phosphorylated STAT3 (pSTAT3) (Figure 2a), and direct interaction was confirmed by coimmunoprecipitation (Figure 2b), suggesting that CAV-1 may influence STAT3 signaling.

We hypothesized that reduced CAV-1 activity could influence JAK/STAT signal transduction and thereby

contribute to psoriasis pathogenesis. CAV-1 was knocked down in human keratinocytes using RNA interference, and cells were stimulated with IL-22 to induce STAT3 activation. About 63% reduction in CAV-1 protein was achieved (Supplementary Figure S1 online). Interestingly, pSTAT3 levels were significantly enhanced in CAV-1-silenced keratinocytes compared with those in controls (Figure 2c and d). Similar augmentation was also observed for pJAK2, an upstream signaling molecule of STAT3.

We further performed STAT3-dependent luciferase reporter gene assays to prove the transcriptional activity of STAT3. Consistent with the immunoblotting data, luciferase activation was enhanced in CAV-1-silenced keratinocytes compared with that in the control after IL-22 stimulation (Figure 2e). Thus, reduction in CAV-1 activity in keratinocytes, as is observed in psoriatic inflammation, activates STAT3. Therefore, alterations in CAV-1 may exert pathogenic effects in psoriasis patients.

Increased expression of a proliferation marker, inflammatory cytokines, and chemokines in CAV-1-silenced keratinocytes

Because STAT3 is involved in keratinocyte proliferation and affects the expression of several cytokines, we hypothesized that enhanced STAT3 activity in CAV-1-reduced keratinocytes could further enhance proliferation and production of inflammatory molecules in keratinocytes. To this end, we determined the levels of keratin 16 (KRT16), a marker of keratinocyte hyperproliferation, in CAV-1-silenced keratinocytes following IL-22 stimulation. *KRT16* mRNA levels were markedly elevated in CAV-1-reduced keratinocytes compared with controls (Figure 3a).

Next, we determined the quantity of psoriasis-related cytokines and chemokines, such as C-X-C chemokine ligand 8 (CXCL8), CXCL9, C-C chemokine ligand 20 (CCL20), and IL-6, released from keratinocytes; reduced CAV-1 levels resulted in a marked increase in the quantity of cytokines and chemokines secreted. Thus, the levels of pSTAT3-associated cytokine mRNA were shown to be increased, at least by qPCR (Figure 3b).

Psoriasis-related inflammatory cytokines reduce CAV-1 expression in human keratinocytes

Diminished CAV-1 levels in psoriatic keratinocytes likely contribute to augmentation or maintenance of pathogenic inflammation. However, the trigger for reduced CAV-1 levels is unclear. Given that reduced CAV-1 levels are only observed in lesional skin, it is possible that psoriatic inflammation itself causes the reduction in CAV-1 levels. To investigate this, we examined the effect that psoriasis-related cytokines exert on CAV-1 expression levels in cultured keratinocytes treated with IL-17A, IL-22, TNF-α, TGF-β, epidermal growth factor, or IL-1β. *CAV1* mRNA expression was significantly suppressed by the indicated psoriasis-related cytokines, especially TNF-α, suggesting the possibility that psoriatic inflammation itself may decrease CAV-1 expression (Figure 3c).

CSD peptide improves the skin phenotype in an IMQ-induced murine model of psoriasis-like skin inflammation

Having verified the importance of CAV-1 in the chronic inflammation observed in psoriasis, we investigated the effect of restoring CAV-1 function using a CSD peptide. This is a membrane-permeable peptide based on the scaffolding domain of CAV-1, which has previously been shown to mimic the inhibitory effect of full-length CAV-1 on cellular signaling (Bucci *et al*, 2000). We intradermally administered CSD peptide daily for 7 days, following each IMQ administration, and evaluated the skin phenotype. The skin phenotype of IMQ-treated mice was markedly suppressed by administration of the CSD peptide, as compared with the control peptide–treated mice (Figure 4a). The clinical skin scores for erythema, induration, and scale were notably improved by CSD treatment (Figure 4b). In addition, histological analysis indicated that the epidermal thickness and the number of infiltrating cells were strikingly reduced in the CSD peptide–treated mice (Figure 4c–e). In particular, the number of infiltrated $CD4^+$ cells was significantly decreased by CSD treatment, and $CD11c^+$ cells also tended to be suppressed, although this was not statistically significant (Figure 5a and b).

We also evaluated the expression levels of psoriasis-related cytokines in the dermis, such as TNF-α, IL-23p19, IL-17A, and IFN-α. IMQ treatment significantly increased the expression levels of TNF-α, IL-23p19, and IL-17A, which was inhibited by concomitant CSD treatment (Figure 5c). There was no difference in IFN-α expression.

Furthermore, pSTAT3-positive cells were clearly diminished in CSD peptide–treated mice (Figure 5d). Thus these results demonstrate that administration of the CSD peptide provides a remarkable degree of protection against IMQ-induced psoriasis-like skin inflammation. Notably, *CAV1* mRNA levels were significantly higher in CSD peptide–treated mice than in control peptide–treated mice (Figure 6a). CAV-1 protein levels also tended to be increased by CSD peptide treatment (Figure 6b). Taken together, CAV-1 exerts an important role in the chronic inflammation of psoriasis (Figure 6c). Thus, modifying CAV-1 levels might help combat the vicious cycle of chronic inflammation in psoriasis.

DISCUSSION

Our goal was to evaluate CAV-1 levels in psoriatic skin and to investigate its role in the pathogenesis of psoriasis. We observed a significant reduction in CAV-1 in the epidermis of psoriasis patients only in the lesional skin, not in non-lesional areas, suggesting that reduced CAV-1 may not be a fundamental trigger of psoriasis but could be directly involved in the formation or maintenance of psoriatic skin lesions. Our immunohistological staining consistently showed a significant decrease of CAV-1 within hyperproliferative regions, including the basal cell layers but not particularly in the upper granular layers in psoriasis patients, in agreement with the findings by Campbell *et al* (2002). Further investigation of the effect of CAV-1 reduction on STAT3 activation and cytokine/chemokine production in keratinocytes revealed some pathogenic mechanisms associated with psoriatic

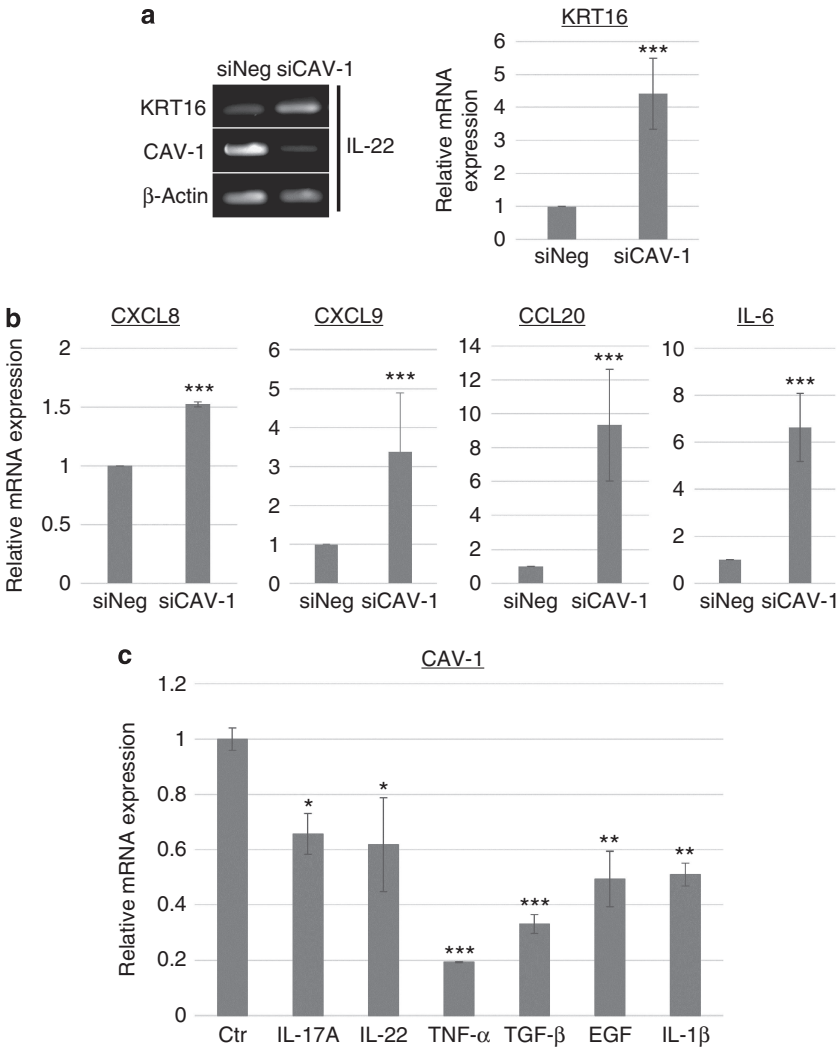

Figure 3. Enhanced proliferation and chemokine production in caveolin-1 (CAV-1)-decreased keratinocytes. (**a**) The level of keratin 16 (KRT16) expression in CAV-1-silenced (siCAV-1) and control siRNA (siNeg) keratinocytes stimulated with IL-22 for 24 hours. Results of representative semi-quantitative PCR (left panel) and quantitative PCR (right panel) are shown. Experiments were performed on three independent occasions in duplicate. ***$P<0.001$. (**b**) The relative levels of C-X-C chemokine ligand 8 (CXCL8), CXCL9, C-C chemokine ligand 20 (CCL20), and IL-6 gene expression were determined by quantitative PCR in same samples used in **a**. ***$P<0.001$. (**c**) Psoriasis-related cytokines may reduce CAV-1 expression in human keratinocytes. Cultured human keratinocytes were stimulated with IL-17A, IL-22, tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β, epidermal growth factor (EGF), or IL-1β. After 48-hour incubation, CAV-1 gene expression levels were determined by quantitative PCR. Experiments were performed on three independent occasions in duplicate. Phosphate-buffered saline was used as control (Ctr). One-way analysis of variance (*post hoc* Dunnett), *$P<0.05$; **$P<0.01$; ***$P<0.001$, versus control.

inflammation. More specifically, elevated KRT16, IL-6, CXCL8, CXCL9, and CCL20 expression in CAV-1-reduced keratinocytes would lead to keratinocyte hyperproliferation and mobilization of neutrophils, T lymphocytes, and dendritic cells. Furthermore, psoriasis-related cytokines, including TNF-α, IL-17A, and IL-22, secreted by activated immune cells and keratinocytes, might trigger further CAV-1 downregulation in keratinocytes. This finding is partially supported by previous studies which indicate that TNF-α and TGF-β suppress CAV-1 expression in other cell types, such as fibroblasts, alveolar macrophages, and monocytes (Galdo *et al*, 2008; Fakhrzadeh *et al*, 2008; Tourkina *et al*, 2010). Therefore, altered CAV-1 expression appears to be responsible for maintaining or enhancing the vicious cycle of chronic inflammation in psoriasis.

STAT proteins, in particular STAT3, have been implicated in psoriasis. Epidermal keratinocytes in psoriatic lesions are characterized by STAT3 activation, and increased levels of cytokines and growth factors promote STAT3 activation (Sano *et al*, 2005). For instance, IL-22, an effector cytokine expressed by T helper type 17 cells, mediates acanthosis through the activation of STAT3 (Zheng *et al*, 2007). K5. Stat3C transgenic mice, in which keratinocytes express a constitutively active form of STAT3, develop psoriasis-like skin lesions, indicating that STAT3 activation is critical for the development of psoriasis (Sano *et al*, 2005). Given our finding

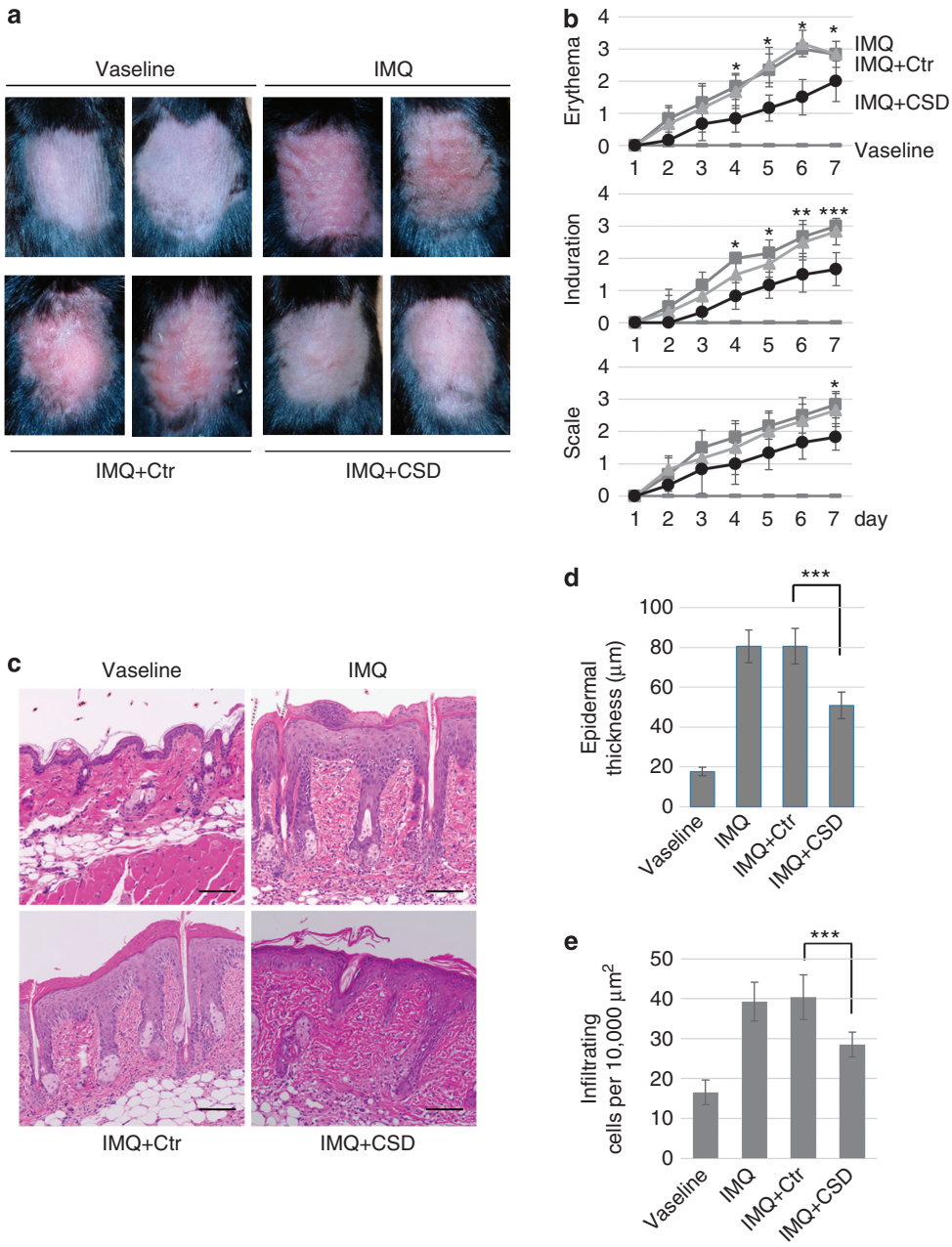

Figure 4. The phenotype of imiquimod (IMQ)-induced murine model of psoriasis-like skin inflammation. (**a**) Mice were treated with IMQ alone, IMQ and the CAV-1 scaffolding domain (CSD) peptide (IMQ+CSD), IMQ with a control peptide (IMQ+Ctr), or Vaseline for 7 days. Representative images of mice are shown. For each group, $n = 6$. (**b**) Skin scores represent the erythema, induration, and scales scores in each group: Vaseline (lines); IMQ alone (squares); IMQ+CSD (circles); and IMQ+Ctr (triangles). Graphs indicate the mean ± SD of each group ($n = 6$). (**c**) Representative hematoxylin and eosin staining of murine skin. Scale bar = 100 μm. (**d**) The epidermal thickness and (**e**) the number of infiltrating cells were analyzed. Graphs indicate the mean ± SD of each group ($n = 6$). One-way analysis of variance (*post hoc* Tukey), $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (IMQ+CSD vs. IMQ+Ctr).

of aberrant downregulation of CAV-1 in psoriatic epidermis, we investigated the cross-regulation between CAV-1 and STAT3 in psoriasis. Previous studies have shown that CAV-1 regulates cellular signaling through its CSD, and CAV-1 binding can inhibit the nuclear translocation of various signaling molecules (Engelman *et al*, 1998; Galdo *et al*, 2008). In addition, binding of CAV-1 to phosphotyrosine residues of cytokine receptors can prevent the binding of JAKs and STAT proteins to the receptor; this suggests that CAV-1 is homologous to the SOCS pseudosubstrates and functions as a negative regulator of STAT signaling (Park *et al*, 2002). We have demonstrated increased JAK2 and STAT3 phosphorylation in CAV-1-silenced keratinocytes. In addition, CAV-1 reduction upregulated transcriptional activity of STAT3 in a luciferase reporter assay, revealing that activation of the JAK/STAT pathway results from CAV-1 downregulation in

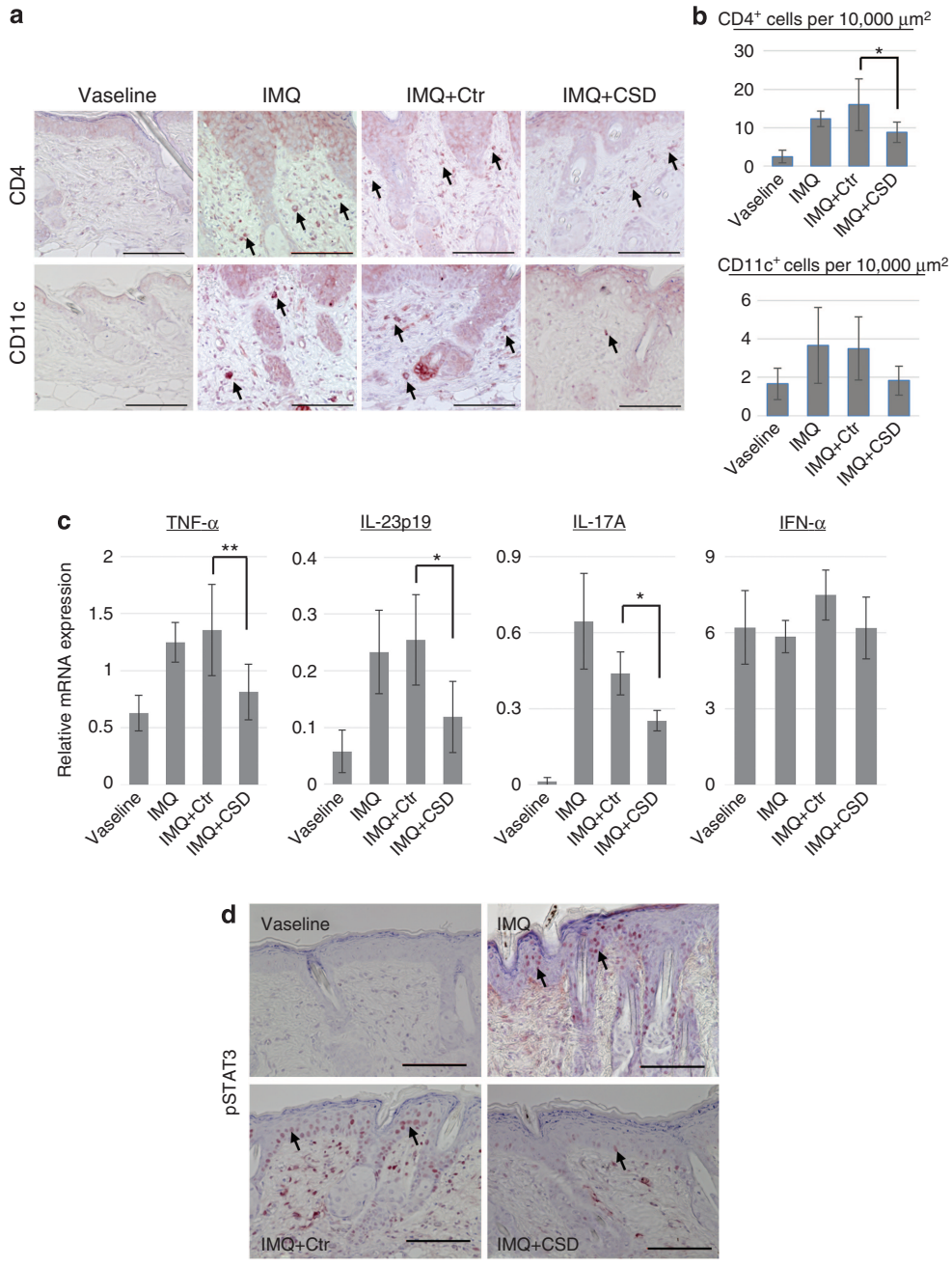

Figure 5. Suppressed inflammation in mice treated with imiquimod (IMQ) and CAV-1 scaffolding domain (CSD) peptide. (**a**) Skin sections obtained from mice were stained with antibodies against CD4 or CD11c, and (**b**) the number of $CD4^+$ or $CD11c^+$ infiltrated cells was analyzed. Scale bar = 100 μm. (**c**) The relative levels of tumor necrosis factor (TNF)-α, IL-23p19, IL-17A, and IFN-α gene expression in the dermis of mice were determined by quantitative PCR. Graphs indicate the mean ± SD of each group ($n = 6$). One-way analysis of variance (*post hoc* Tukey), $^*P < 0.05$; $^{**}P < 0.01$ (IMQ+CSD vs. IMQ+Ctr). (**d**) Skin sections were stained with anti-pSTAT3 (anti-phosphorylated signal transducer and activator of transcription 3) antibody. Arrows represent pSTAT3-positive cells. Scale bar = 100 μm.

keratinocytes. Moreover, CAV-1-reduced keratinocytes had increased levels of a hyperproliferation marker and psoriasis-related cytokine/chemokines, supporting a recent report. Yuan *et al* (2011) showed that STAT3 activation in CAV-1-deficient mice resulted in an enhanced inflammatory response to *Pseudomonas aeruginosa* respiratory infection. In that model, infected CAV-1 knockout mice expressed higher levels of inflammatory cytokines, such as IL-6, TNF-α, and IL-12a. In addition to the JAK/STAT3 signaling pathway, other signaling cascades, such as the RAS/MAPK and phosphoinositide-3/AKT pathways, are also activated by psoriasis-related cytokines and growth factors. Given that CAV-1 can also negatively regulate the RAS/MAPK and phosphoinositide-3/AKT pathways, impaired CAV-1

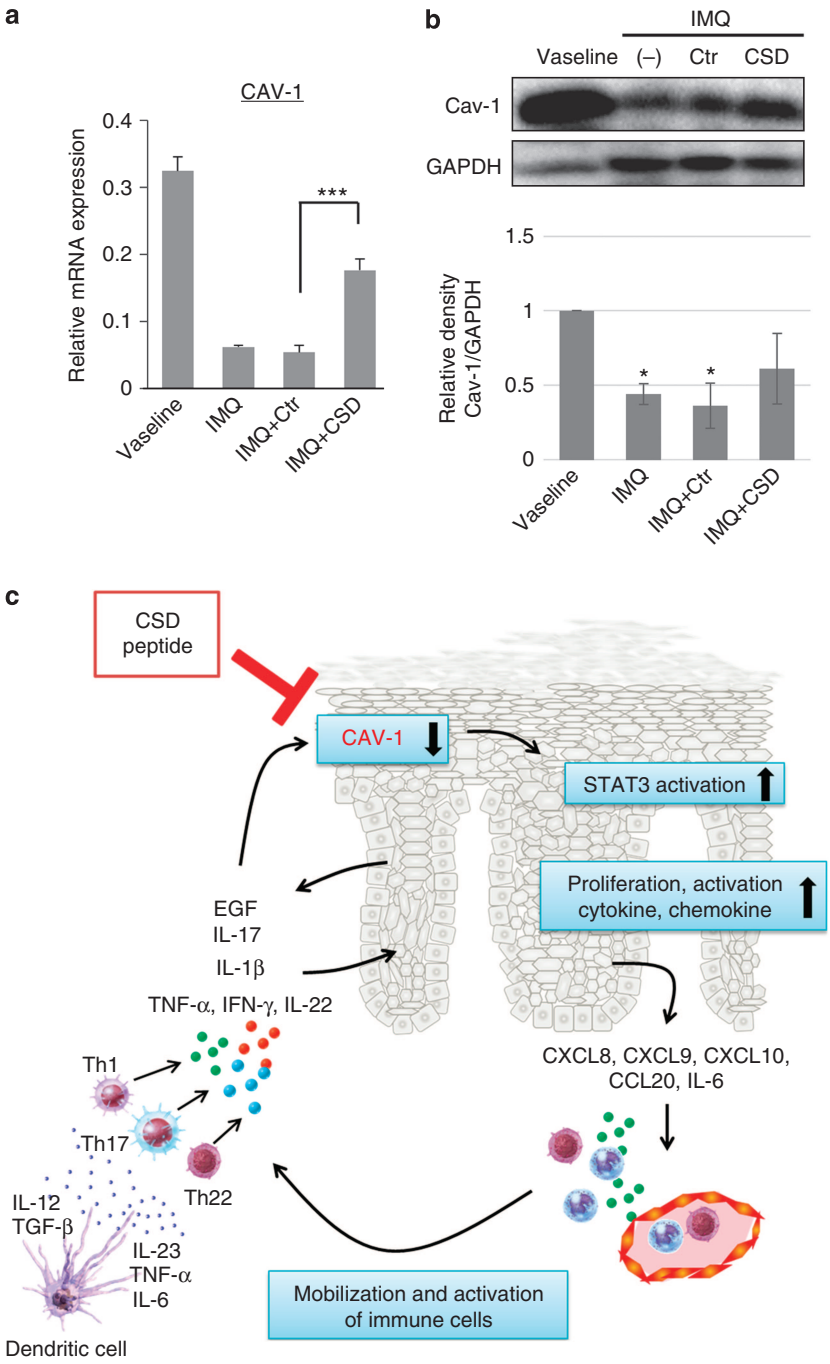

Figure 6. The expression of CAV-1 in mice treated with imiquimod (IMQ) and CAV-1 scaffolding domain (CSD) peptide. (**a**) The relative levels of caveolin-1 (CAV-1) gene expression in the epidermis of mice were determined by quantitative PCR, $n = 6$. One-way analysis of variance (ANOVA; *post hoc* Tukey), $^{***}P < 0.001$ (imiquimod+CAV-1 scaffolding domain (IMQ+CSD) vs. IMQ+Ctr). (**b**) CAV-1 protein levels in the epidermis of mice were analyzed by western blotting. The CAV-1 protein levels were quantified using densitometry and expressed as the ratio of CAV-1 to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). $n = 3$. One-way ANOVA (*post hoc* Tukey), $^{*}P < 0.05$ versus Vaseline. (**c**) Schematic representing the continuous psoriatic inflammation loop related to reduced CAV-1 expression. Decreased levels of CAV-1 in psoriatic keratinocytes enhance signal transducer and activator of transcription 3 (STAT3) activation, which induces keratinocyte activation and proliferation. Various keratinocyte-produced cytokines and chemokines lead to further mobilization of activated immune cells, which in turn produce more psoriasis-related cytokines and trigger even more CAV-1 downregulation in keratinocytes. Modulating CAV-1 function might interrupt this vicious cycle of psoriasis-related chronic inflammation.

expression in psoriasis may enhance signaling in these pathways in addition to affecting STAT3 signaling.

Given the link between reduced CAV-1 expression and psoriatic inflammation in human keratinocytes, we investigated the effect of modulating CAV-1 function *in vivo*. Because of the lack of good animal models for psoriasis, we used the IMQ-induced murine model of psoriasis-like skin inflammation. These mice are often used

as a psoriasis-like model because of their psoriasis-like skin phenotype, including the histology, cell types, and cytokine expression (Flutter and Nestle, 2013); however, it is not a true psoriasis model but rather an acute skin inflammation model. Nevertheless, we also observed a significant CAV-1 reduction within the epidermal hyperplasia of these mice, similar to that seen in psoriasis patients; therefore, we used these mice to analyze CAV-1 function using the CSD peptide. It has previously been shown that CSD peptide can cross the plasma membrane as it is synthesized as a fusion with the antennapedia internalization sequence (Bucci *et al*, 2000). CSD peptide can bind to, and inhibit, different CAV-1-target signaling molecules (Hua *et al*, 2003; Oka *et al*, 1997), and restoration of CAV-1 function using a CSD peptide has been suggested as a potential therapeutic strategy in fibroproliferative disorders, in which CAV-1 inhibits TGF-β signaling by promoting TGF-β receptor degradation and preventing SMAD2 phosphorylation (Razani *et al*, 2001; Tourkina *et al*, 2008). We found that administration of CSD peptide markedly reduced the clinical and histological signs of psoriasis in our model mice. Furthermore, CSD peptide–treated mice had fewer pSTAT3-positive cells in the epidermis and had significantly reduced expression of inflammatory cytokines in the dermis, indicating that the CSD peptide exerts a protective function against IMQ-induced inflammation, although it is not clear whether it can reverse established inflammation, as we administered treatment from the onset of IMQ treatment. When we postponed the onset of CSD treatment until day 3, when histological features of IMQ treatment became evident (Supplementary Figure S2a online), clinical features were also reduced and epidermal thickness was significantly suppressed. Further study is required to evaluate the potential of CSD peptide as a therapeutic agent.

Interestingly, CSD treatment resulted in higher expression of CAV-1 compared with control peptide treatment (Figure 6). Previously, CSD peptide was not found to affect the expression of CAV-1 *per se*, suggesting that CSD peptide alters the expression of proteins downstream from CAV-1 (Tourkina *et al*, 2008). CSD peptide itself was not detected, as it is only a short functional domain of CAV-1 and only mimics CAV-1 function by interacting with signaling molecules.

It is not clear why CAV-1 levels were increased by CSD treatment in our study; however, having observed decreased pSTAT3-positive cells and reduced cytokine levels in CSD peptide–treated mice, it is reasonable that reduction of inflammation inhibited CAV-1 downregulation. Thus CSD peptide may modulate cellular signaling, inhibit keratinocyte activation, inhibit immune cell mobilization, and consequently suppress the production of cytokines that trigger CAV-1 downregulation. Thus the efficacy of CSD peptide treatment may result from an interruption of the vicious cycle of chronic inflammation.

In summary, we have demonstrated that CAV-1 participates in the pathogenesis of chronic psoriatic inflammation. Significant reduction of CAV-1 in the epidermis of psoriasis patients may enhance STAT3 activation, keratinocyte hyperproliferation, and cytokine/chemokine production. Mimicking CAV-1 function with a CSD peptide inhibited cell migration, decreased psoriasis-related cytokines in the dermis, and ameliorated the psoriatic phenotype in an IMQ-induced murine model. Further studies may clarify whether the restoration of CAV-1 function represents a therapeutic approach for inhibiting chronic inflammation in psoriasis.

MATERIALS AND METHODS

Preparation of skin samples

Skin biopsy samples were collected from 18 psoriasis patients and 6 healthy volunteers. Where necessary, human or murine skin was incubated in 3 mg ml^{-1} dispase II overnight at 4 °C (Roche Applied Science, Tokyo, Japan) to separate the epidermis and dermis. Cell suspensions were prepared for western blotting and PCR analyses as appropriate. All samples were obtained after obtaining written informed consent, in accordance with the Declaration of Helsinki. The study was approved by the Institutional Review Board of Yokohama City University (Approval No: B120705021).

Cell culture and stimulation

Normal human epidermal keratinocytes (NHEKs) were purchased from Takara Bio (Shiga, Japan) and cultured in Keratinocyte Basal Medium (KBM) supplemented with growth factors (KGM-Gold SingleQuots; Lonza, Walkersville, MD) or KBM alone prior to experiments. Briefly, NHEKs were cultured in KBM alone overnight prior to stimulation with various human recombinant proteins (R&D Systems, Minneapolis, MN): rIL-22 (200 ng ml^{-1}), recombinant epidermal growth factor (100 ng ml^{-1}), recombinant IL-17A (50 ng ml^{-1}), recombinant TNF-α (25 ng ml^{-1}), recombinant TGF-β (10 ng ml^{-1}), and recombinant IL-1β (25 ng ml^{-1}). Cells were harvested 24 or 48 hours after stimulation as appropriate.

Immunohistochemistry

Skin samples were embedded in paraffin and sectioned. Immunohistochemical analysis was performed as previously described (Yamaguchi *et al*, 2013). Briefly, sections were incubated with antibodies against CAV-1 (BD Transduction Laboratories, Lexington, KY), CD4 (Bioworld Technology, Louis Park, MN), CD11c (Abcam, Cambridge, UK), pSTAT3 (Tyr705, Cell Signaling Technology, Danvers, MA), or isotype controls. The antigen was detected using EnVision Dual Link System-HRP or LSAB2 system-HRP, and then the DAB chromogen or AEC+ high sensitivity substrate chromogen was applied (Dako, Carpinteria, CA).

Semi-quantitative and qPCR

Total RNA was extracted using TRIzol and the Illustra RNAspin Mini RNA Isolation Kit (GE Healthcare, Uppsala, Sweden). First-strand cDNA was synthesized using the High Capacity RNA-to-cDNA Kit in accordance with the manufacturer's instructions (Applied Biosystems, Carlsbad, CA). qPCR was performed in duplicate or triplicate using TaqMan gene expression assays or the Thunderbird SYBR qPCR Mix (Toyobo, Osaka, Japan) and an ABI Prism 7900 sequence detection system in accordance with the manufacturers' protocols. Gene expression levels were normalized to β-actin or *GAPDH* as appropriate and compared using the $2^{-\Delta\Delta Ct}$ method. TaqMan probes for human *KRT16*, *IL6*, *CXCL8*, *CXCL9*, *CCL20*, and β-actin were obtained from Applied Biosystems. The primer sets used for semi-quantitative PCR and SYBR qPCR are shown in Supplementary Table S1 online.

Western blotting analysis

Samples were prepared using RIPA buffer containing a protease inhibitor cocktail as previously described (Yamaguchi *et al*, 2011). Equal quantities of protein were analyzed by western blotting using one of the following antibodies: CAV-1 (BD Transduction), pSTAT3 (Tyr705), STAT3, pJAK2, JAK2, GAPDH (glyceraldehyde-3-phosphate dehydrogenase; Cell Signaling), and mouse GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA). Signals were detected using horseradish peroxidase–conjugated secondary antibody and ECL Prime Western Blotting Detection Reagent (GE Healthcare).

Immunocytostaining

NHEKs were cultured in chamber slides in KBM alone overnight prior to stimulation with human rIL-22. After 15-minute stimulation, cells were fixed and then permeabilized with 0.1% Triton X-100. After blocking, cells were incubated with anti-CAV-1 (Thermo Fisher Scientific, Rockford, IL) and anti-pSTAT3 or anti-STAT3 antibodies (Cell Signaling), followed by Alexa Flour 488- or 633-conjugated secondary IgG (Invitrogen, Grand Island, NY). Appropriate isotype IgG was used as a control. Prolong Gold antifade reagent with DAPI (4,6-diamidino-2-phenylindole; Life Technology, Carlsbad, CA) was used for nuclear identification and mounting. Images were taken using an Olympus Fluoview 1000 microscope (Olympus America, Melville, NY) and fixed camera settings.

Coimmunoprecipitation

A total of 5×10^5 NHEKs were cultured and treated with rIL-22 for 10 minutes prior to collection by scraping with RIPA buffer containing protease inhibitors. Untreated cells were used as a control. Equal quantities of protein lysate were incubated with 2.5 μg of CAV-1 antibody (BD Transduction) or control IgG and rotated at 4 °C overnight. Protein complexes were precipitated using Protein A/G PLUS-Agarose Immunoprecipitation Reagent (Santa Cruz Biotechnology), which was added for an additional 4 hours of rotation. Bound proteins were analyzed by western blotting.

CAV-1 silencing

CAV1-specific small-interfering RNA (Stealth RNAi) was purchased from Invitrogen. Control RNAi was used as a negative control. For reverse transfection, 100 pM of each RNAi was added to 500 μl Opti-MEM, and 5 μl Lipofectamine RNAiMAX2000 (Invitrogen) was added to each well. After 20 minutes, 8×10^4 NHEKs were seeded in KBM containing growth factors but lacking antibiotics. Cells were cultured for 48 hours prior to harvesting or stimulation with IL-22. The medium was changed to KBM without growth factors 16 hours before stimulation.

Luciferase reporter gene assay

Transcriptional activity of STAT3 was measured using the Cignal STAT3 Reporter Kit (SA Biosciences, Valencia, CA) according to the manufacturer's instructions. NHEKs (8×10^6 cells per well) were co-transfected with a STAT3-responsive reporter and with CAV-1 RNAi or control RNAi. The Cignal negative reporter was also used as control. After 48 hours, the cells were treated with 200 ng ml^{-1} of IL-22 for 1 hour. Cell lysates were subjected to luciferase activity analysis using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI).

Murine model of psoriasis-like skin inflammation

For all *in vivo* experiments, 6-week-old C57BL/6J female mice (Oriental Yeast, Tokyo, Japan) were used. IMQ was used to induce skin inflammation as previously described (van der Fits *et al*, 2009). A daily dose of 62.5 mg of 5% IMQ cream (Beselna cream; Mochida Pharmaceutical, Tokyo, Japan) was applied to the back skin of mice for 7 days. Vaseline was used as control. Animal experiments were performed in accordance with the Yokohama City University Institutional Animal Care and Use Committee guidelines (Approval No: F-A-13-051).

CSD peptide treatment

Peptides corresponding to the CSD (DGIWKASFTTFTVTKYWFYR) and a scrambled control peptide (Ctr: WGIDKAFFTTSTVTYKWFRY), fused to the antennapedia internalization sequence (RQIKIWFQN RRMKWKK), were synthesized and dissolved in dimethyl sulfoxide.

For injection, peptides were diluted in phosphate-buffered saline, and 100 μl of a 0.1 mM solution of the CSD or Ctr peptide was injected intradermally into the back skin of mice following each IMQ treatment for 7 days. Mice were euthanized by CO_2 asphyxiation, and the skin surrounding the injection site was harvested at day 8 and used for analysis.

Measurement of skin scores, epidermal thickness, and the number of infiltrating cells

The clinical skin score of mice was determined from day 1 (the first day of treatment) and each treatment day until day 7 using the modified psoriasis severity index score. The degree of skin erythema, induration, and scale were classified as follows: 0, no symptoms; 1, mild; 2, moderate; 3, severe; or 4, very severe. Sections from paraffin-embedded mouse skin samples were stained with hematoxylin and eosin. Images were captured using a microscope (model BZ-9000; Keyence, Osaka, Japan). For each section, the thickness of the epidermis was measured from the stratum basale to the stratum granulosum using BZ-II Analyzer software (Keyence). The average value from 10 random fields of view was calculated for each mouse. Infiltrated cells were also counted from 10 random fields of view of 10,000 μm^2 for each mouse.

Statistical analysis

Statistical comparisons were performed using the unpaired Student's *t*-test. In some analyses, one-way analysis of variance (*post hoc* Tukey or Dunnett) was used as indicated.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by a grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology (to YY).

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Bucci M, Gratton JP, Rudic RD *et al.* (2000) In vivo delivery of the caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation. *Nat Med* 6:1362–7

Campbell L, Laidler P, Watson RE *et al.* (2002) Downregulation and altered spatial pattern of caveolin-1 in chronic plaque psoriasis. *Br J Dermatol* 147:701–9

Engelman JA, Chu C, Lin A *et al.* (1998) Caveolin-mediated regulation of signaling along the p42/44 MAP kinase cascade in vivo. A role for the caveolin scaffolding domain. *FEBS Lett* 428:205–11

Fakhrzadeh L, Laskin JD, Laskin DL (2008) Regulation of caveolin-1 expression, nitric oxide production and tissue injury by tumor necrosis factor-alpha following ozone inhalation. *Toxicol Appl Pharmacol* 227:380–9

Flutter B, Nestle FO (2013) TLRs to cytokines: mechanistic insights from the imiquimod mouse model of psoriasis. *Eur J Immunol* 43:3138–46

Galbiati F, Razani B, Lisanti MP (2001) Emerging themes in lipid rafts and caveolae. *Cell* 106:403–11

Galdo FD, Sotgia F, de Almeida CJ *et al.* (2008) Decreased expression of caveolin 1 in patients with systemic sclerosis: crucial role in the pathogenesis of tissue fibrosis. *Arthritis Rheum* 58:2854–65

Griffiths CE, Barker JN (2007) Pathogenesis and clinical features of psoriasis. *Lancet* 370:263–71

Hua H, Munk S, Whiteside CI (2003) Endothelin-1 activates mesangial cell ERK1/2 via EGF-receptor transactivation and caveolin-1 interaction. *Am J Physiol Renal Physiol* 284:F303–12

Lowes MA, Bowcock AM, Krueger JG (2007) Pathogenesis and therapy of psoriasis. *Nature* 445:866–73

Medina FA, de Almeida CJ, Dew E *et al.* (2006) Caveolin-1-deficient mice show defects in innate immunity and inflammatory immune response during *Salmonella enterica* serovar typhimurium infection. *Infect Immun* 74:6665–74

Nestle FO, Kaplan DH, Barker J (2009) Psoriasis. *N Engl J Med* 361:496–509

Nickoloff BJ, Nestle FO (2004) Recent insights into the immunopathogenesis of psoriasis provides new therapeutic opportunities. *J Clin Invest* 113: 1664–75

Nograles KE, Davidovici B, Krueger JG (2010) novel insights in the immunologic basis of psoriasis. *Semin Cutan Med Surg* 29:3–9

Núñez-Wehinger S, Ortiz RJ, Díaz N *et al.* (2014) Caveolin-1 in cell migration and metastasis. *Curr Mol Med* 14:255–74

Oka N, Yamamoto M, Schwencke C *et al.* (1997) Caveolin interaction with protein kinase C. *J Biol Chem* 272:33416–21

Okamoto T, Schlegel A, Scherer PE *et al.* (1998) Caveolins, a family of scaffolding proteins for organizing "preassembled signaling complexes" at the plasma membrane. *J Biol Chem* 273:5419–22

Park DS, Lee H, Frank PG *et al.* (2002) Caveolin-1-deficient mice show accelerated mammary gland development during pregnancy, premature lactation, and hyperactivation of the Jak-2/STAT5a signaling cascade. *Mol Biol Cell* 13:3416–30

Parton RG, Richards AA (2003) Lipid rafts and caveolae as portals for endocytosis: new insights and common mechanisms. *Traffic* 4:724–38

Razani B, Zhang XL, Bitzer M *et al.* (2001) Caveolin-1 regulates transforming growth factor (TGF)-β/Smad signaling through an interaction with TGF-β type I receptor. *J Biol Chem* 276:6727–38

Sano S, Chan KS, Carbajal S *et al.* (2005) Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. *Nat Med* 11:43–9

Schlegel A, Pestell RG, Lisanti MP (2005) Caveolins in cholesterol trafficking and signal transduction: implications for human disease. *Front Biosci* 5: D929–37

Stan RV (2005) Structure of caveolae. *Biochim Biophys Acta* 1746:334–48

Strålfors P (2012) Caveolins and caveolae, roles in insulin signaling and diabetes. *Adv Exp Med Biol* 729:111–26

Tourkina E, Gooz P, Pannu J *et al.* (2005) Opposing effects of protein kinase Cα and protein kinase Cε on collagen expression by human lung fibroblasts are mediated via MEK/ERK and caveolin-1 signaling. *J Biol Chem* 280: 13879–87

Tourkina E, Richard M, Göös P *et al.* (2008) Antifibrotic properties of caveolin-1 scaffolding domain in vitro and in vivo. *Am J Physiol Lung Cell Mol Physiol* 294:L843–61

Tourkina E, Richard M, Oates J *et al.* (2010) Caveolin-1 regulates leucocyte behaviour in fibrotic lung disease. *Ann Rheum Dis* 69:1220–6

van der Fits L, Mourits S, Voerman JS *et al.* (2009) Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol* 182:5836–45

Wang XM, Zhang Y, Kim HP *et al.* (2006) Caveolin-1: a critical regulator of lung fibrosis in idiopathic pulmonary fibrosis. *J Exp Med* 203:2895–906

Yamada E (1955) The fine structure of the gall bladder epithelium of the mouse. *J Biophys Biochem Cytol* 1:445–58

Yamaguchi Y, Yasuoka H, Stolz DB *et al.* (2011) Decreased caveolin-1 levels contribute to fibrosis and deposition of extracellular IGFBP-5. *J Cell Mol Med* 15:957–69

Yamaguchi Y, Ono J, Masuoka M *et al.* (2013) Serum periostin levels are correlated with progressive skin sclerosis in patients with systemic sclerosis. *Br J Dermatol* 168:717–25

Yuan K, Huang C, Fox J *et al.* (2011) Elevated inflammatory response in caveolin-1-deficient mice with *Pseudomonas aeruginosa* infection is mediated by STAT3 protein and nuclear factor kappaB (NF-kappaB). *J Biol Chem* 286:21814–25

Zheng Y, Danilenko DM, Valdez P *et al.* (2007) Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature* 445:648–51