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Inhibition of CtBP-regulated proinflammatory gene transcription attenuates psoriatic skin inflammation

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AUTHOR CONTRIBUTIONS

Conceptualization: MH
Data curation: BF, CG, CY, HD, HL, LB, MB, YD;
Formal analysis: CG, HL;
Funding acquisition: DN, MF, MH, RZ, XW;
Investigation: CG, HL, LB;
Methodology: CG, HL, LB;
Project administration: MH;
Resources: MF, WH;
Supervision: MH;
Validation: CG, HL, LB;
Visualization: CG, HL, MH;
Writing – original draft preparation: MH;
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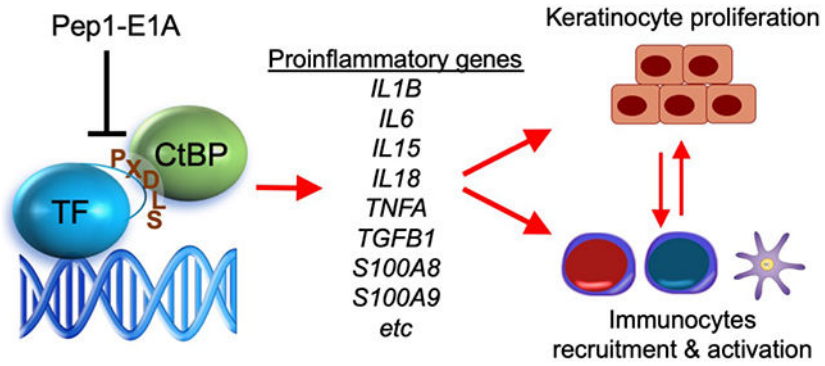
CONFLICT OF INTEREST

The authors state no conflict of interest.

Abstract

Psoriasis is a chronic immune-mediated disease characterized by excessive proliferation of epidermal keratinocytes and increased immune cell infiltration to the skin. Although it is well known that psoriasis pathogenesis is driven by aberrant production of proinflammatory cytokines, the mechanisms underlying the imbalance between proinflammatory and anti-inflammatory cytokine expression are incompletely understood. Here we report that the transcriptional coregulators C-terminal-binding protein (CtBP) 1 and 2 can transactivate a common set of proinflammatory genes both in the skin of imiquimod-induced mouse psoriasis model and in human keratinocytes and macrophages stimulated by imiquimod. We find that mice overexpressing CtBP1 in epidermal keratinocytes display severe skin inflammation phenotypes with increased expression of Th1 and Th17 cytokines. We also find that the expression of CtBPs and CtBP target genes is elevated both in human psoriatic lesions and in the mouse imiquimod psoriasis model. Moreover, we were able to demonstrate that topical treatment with a peptidic inhibitor of CtBP effectively suppresses the CtBP-regulated proinflammatory gene expression and thus attenuates psoriatic inflammation in the imiquimod mouse model. Together, our findings suggest new strategies for therapeutic modulation of the immune response in inflammatory skin diseases.

Graphical Abstract



Keywords

psoriasis; inflammation; proinflammatory genes; transactivation; C-terminal binding protein

INTRODUCTION

Psoriasis is a relapsing and remitting autoimmune disease of the skin and joint that is influenced by both genetic and environmental risk factors (Nestle et al., 2009). Plaque psoriasis, accounting for 90% of the disease cases, affects 6.7 million people in the United States (Helmick et al., 2014). This chronic inflammatory condition also contributes to a significantly higher risk of serious comorbidities often associated with an overactive immune response, such as arthritis, inflammatory bowel disease and metabolic syndrome (Takeshita et al., 2017). While the etiology of psoriasis remains unclear, progression and persistence of the disease occurs through the interplay among infiltrating leukocytes,

keratinocytes and other skin-resident cells, and is characterized by abnormal proliferation and differentiation of keratinocytes and overproduction of proinflammatory cytokines and chemokines (Baliwag et al., 2015, Li et al., 2014, Swindell et al., 2015). In particular, TNFα and cytokines of the IL-17/IL-23 axis emerge as central players in psoriasis pathogenesis in both human and mouse models (Chiricozzi et al., 2011, Ha et al., 2014, Swindell et al., 2011, van der Fits et al., 2009, Wohn et al., 2013), and their neutralizing antibodies have proved effective in disease treatment (Alexander and Nestle, 2017).

Although aberrant cytokine production is considered crucial for the development and persistence of psoriasis, the mechanism for the imbalance in gene expression between pro- and anti-inflammatory cytokines is not fully understood (Medzhitov and Horng, 2009, Smale, 2010). Several transcription factors that regulate the inducible expression of inflammatory genes, such as STAT3, AP-1 and NF-κB, have been implicated in inflammatory skin disorders including psoriasis. Epithelial activation of the signaling pathways upstream of these transcription factors (e.g., JAK, MAPK and TRAF6) have been demonstrated to be critical in initiating psoriatic inflammation (Matsumoto et al., 2018, Sakurai et al., 2019, Works et al., 2014). Increased activation of NF-κB by gain-of-function mutations in its activator *CARD14* in human or a constitutively active *p65* in transgenic mice leads to increased psoriasis susceptibility (Jordan et al., 2012, Poligone et al., 2013). Conversely, removal of the *Il36/IκBζ* axis of the NF-κB pathway protect mice from imiquimod (IMQ) induced psoriatic dermatitis (Muller et al., 2018, Tortola et al., 2012). Similarly, epithelial expression of a constitutively active Stat3 also promotes psoriasis-like skin phenotypes in transgenic mice (Sano et al., 2005). Despite their physical and pathological significance, no clinically useful inhibitors of STAT-3 or NF-κB are available for treating skin inflammation.

The transcriptional co-regulators C-terminal binding protein (CtBP) 1 and 2 are known to repress genes of diverse biological pathways by direct binding to transcription factors and recruiting chromatin remodeling proteins to their target gene promoters (Chinnadurai, 2009, Dcona et al., 2017). In addition, these two proteins have been postulated to function as context-dependent transcriptional coactivators of KLF4 in inducing epithelial differentiation genes and of NeuroD1 in inducing endocrine genes (Boxer et al., 2014, Ray et al., 2014). CtBPl and CtBP2 share ~80% amino acid sequence identity overall and both proteins exhibit a remarkable structural similarity to D2-hydroxyacid dehydrogenases including a central NAD(H)-binding domain (Kumar et al., 2002). NAD(H) promotes CtBPs dimerization and potentiates their transcriptional co-regulator activity (Balasubramanian et al., 2003, Madison et al., 2013). CtBP-mediated transcription plays a crucial role in epithelial cell fate specification both in development and in disease, especially in wound healing, through response to various upstream signals (e.g., TGFB1, JNK, ERK and JAK) and association with different transcription factors (e.g., ZNF750, ZEB1 and Smads) (Boxer et al., 2014, Han et al., 2013, Ichikawa et al., 2015, Shirakihara et al., 2011, Worley et al., 2018). Emerging evidence also implicates CtBP-regulated transcription in immune and inflammatory response. For example, CtBPs are required to repress *IL2* expression in $CD4^+$ regulatory T cells (Pan et al., 2009, Wang et al., 2009), to repress lipopolysaccharides (LPS)-induced *IL23* expression in glia cells treated with estrogen receptor agonists (Saijo et al., 2011), and to repress *HMOX1* in fibroblasts under hypoxia (Li et al., 2016). We

have recently shown that CtBPs are required for LPS-induction of proinflammatory genes (e.g., *IL1B, IL6, TNFA,* and *S100A8*) in microglia and macrophages (Li et al., 2020). In this report, we demonstrate that CtBP1 and CtBP2 can transactivate proinflammatory cytokine expression in cultured keratinocytes and macrophages upon stimulation with the TLR7/8 ligand IMQ and that elevated expression of the CtBPs and CtBP-regulated proinflammatory cytokines is a common characteristic observed in both human psoriatic lesions and the mouse IMQ psoriasis model. Furthermore, we find that a specific PXDLS-containing peptidic inhibitor of the CtBPs is able to attenuate cytokine induction and alleviate symptoms of skin inflammation in the IMQ mouse model, thus demonstrating the potential utility of CtBP-targeting therapeutics in treating psoriatic skin inflammation.

RESULTS AND DISCUSSION

CtBP1 overexpression in mouse epidermal keratinocytes causes a skin inflammation phenotype with increased Th1 and Th17 cytokine gene expression

The K5.CtBP1 transgenic mice have previously been shown to exhibit hair loss and epidermal hyperplasia, phenotypes that are often associated with psoriatic skin lesions (Deng et al., 2014). By three weeks of age, the transgenic skin displayed an elevated level of CtBP1 protein expression, thickened stratum corneum with retained nuclei (parakeratosis) and occasional formation of microabscesses (Fig. 1a-c). In addition, the volar skin of the K5.CtBP1 mice exhibited thickened epidermis with downgrowth (rete ridges, Fig. 1d), similar to those seen in human psoriasis. Immunofluorescence (IF) staining with the pan-leukocyte marker CD45 revealed the presence of a large number of leukocytes in the transgenic dermis and epidermis while only a minimal signal in the nontransgenic skin (Fig. 1e). Further IF analysis using Ly-6G and CD4 antibodies also confirmed an infiltration of granulocytes and T lymphocytes, respectively, in the transgenic skin (Fig. 1e). Moreover, there was an increase of $CD31^+$ microvessels in the transgenic skin, indicating enhanced angiogenesis (Fig. 1e). Consistent with an inflammatory skin phenotype, we found that the K5.CtBP1 skin contained significantly higher mRNA levels of several proinflammatory cytokine genes, including those of the Th1 (*Ifng*, *Tnfa*, *Lta/Tnfb* and *Tgfb1*) and Th17 types (*Il17a*, *Il17f* and *Il22*) (Korn et al., 2009) (Fig. 1f). These cytokines have been shown to act as the immunological driving force for psoriasis (Di Cesare et al., 2009, Hirahara et al., 2010, van der Fits et al., 2009). We also detected increased expression of cytokines *Il1b*, *Il6*, *Il15*, *Il18* and *Il23a*, and alarmins *S100a8* and *S100a9* in the K5.CtBP1 skin, all of which have been associated with inflammatory skin conditions (Bouchaud et al., 2013, Grossman et al., 1989, Li et al., 2004, Novick et al., 2013, Vogl et al., 2014). In contrast, there is no change in the Th2 type cytokines *Il4*, *Il5* and *Il13*, and only a two-fold increase in *Il10* (Fig. 1f). Taken together, these results indicate that an elevated expression of *CtBP1* in the transgenic mouse epidermis specifically activates proinflammatory cytokine expression, thereby resulting in skin abnormalities reminiscent of psoriasis-like inflammation.

Human psoriatic skin lesions contain higher levels of CtBP1 and CtBP2 proteins

To investigate whether abnormal *CtBP* expression is associated with human psoriasis, we assessed and compared protein levels of CtBP1 and CtBP2 in 12 psoriasis and six healthy skin biopsies by immunohistochemistry (IHC) analysis. All 12 psoriasis samples displayed

significantly elevated nuclear staining of both CtBP1 and CtBP2 in the epidermis relative to the controls (Fig. 2), suggesting that CtBP overexpression is a common phenomenon in human psoriasis. In line with previous studies (Han et al., 2010, Li et al., 2004), we found higher levels of TGFβ1 protein in the psoriatic epidermis (Fig. 2). Moreover, there is a significant positive correlation between the protein expression levels of CtBP1, CtBP2 and TGFβ1 (Table S1). Further comparison of CtBP1 and CtBP2 IHC signals between samples collected for this study and nine randomly chosen normal dermic tissues of the human tissue array SK2081 (Biomax) also confirmed that both proteins are significantly overexpressed in the psoriatic skin lesions (Fig. S1). Our findings suggest that CtBPs may play a role in transactivating cytokine expression in human psoriatic inflammation.

CtBP1 and CtBP2 are required for the transactivation of proinflammatory genes in imiquimod-stimulated keratinocytes and macrophages

We have identified a set of proinflammatory response genes, including cytokines (*IL1B*, *IL6*, *IL15*, *IL18*, *TNFA* and *TGFB1*) and alarmins (*S100A8* and *S100A9*) as potential transcriptional targets of the CtBP proteins through analysis of the CtBP ChIP-seq data obtained from unstimulated breast cancer cells (Di et al., 2013). To assess whether the inflammation-induced expression of these genes is dependent on the CtBPs, we evaluated the effects of siRNA-mediated knockdown of *CtBP1* and *CtBP2*, individually and in combination, on the mRNA expression levels of these candidate genes in three different cell lines, human keratinocyte HaCaT and monocyte/macrophage THP-1 and murine macrophage RAW264.7, in response to IMQ stimulation using the RT-qPCR method. As shown in Fig. 3a, IMQ treatment of HaCaT cells increases the mRNA expression of all eight genes (3- to 69-fold change). This IMQ-induced mRNA expression is attenuated markedly by silencing of *CtBP1* (20% to 39% reduction) or *CtBP2* (68% to 84% reduction), and is further attenuated by simultaneous knockdown of *CtBP1* and *CtBP2* (81% to 96% reduction; Fig. 3a). In addition, we note that the basal mRNA levels of these eight genes in unstimulated HaCaT cells are similarly reduced by depletion of *CtBP1* (25% to 44% decrease) or *CtBP2* (43% to 77% decrease), with simultaneous depletion of both genes exhibiting the strongest inhibitory effect (51% to 95% reduction; Fig. 3a). We also observed similar CtBP-dependency in both basal and IMQ-induced transcription of the eight genes in the THP-1 and RAW264.7 cells (Fig. S2). Moreover, we found that the expression of *CtBP2* is also induced by IMQ stimulation in all three cell lines (4- to 8-fold increase, Fig. 3a and Fig. S2). Together, these findings indicate that *CtBP1* and *CtBP2* have overlapping and nonredundant functions in the transcriptional activation of these eight target genes both under unstimulated conditions and in response to immune stimulation. Consistent with its transcriptional activation function, we find that a moderate overexpression of CtBP1 (4-fold increase in mRNA level, Fig. 3b) in HaCaT cells results in a significant increase in both the basal (3.6- to 8.5-fold change) and IMQ-stimulated (19- to 46-fold changes) mRNA expression levels of the above mentioned eight genes (Fig. 3b). Notably, *CtBP2* mRNA expression is not affected by *CtBP1* overexpression, (Fig. 3b).

To validate that the CtBPs are directly involved in transactivation, we performed ChIP experiments in the IMQ-stimulated HaCaT cells to measure CtBP1 binding to the promoter regions of the four strongly induced genes, *IL6*, *IL18*, *TGFB1* and *S100A8*. We found that

IMQ stimulation causes a markedly enhanced binding of CtBP1 (6- to 18-fold increase, Fig. 4a) to the promoter regions of these four genes, which is accompanied by significant induction of their mRNA expression (20- to 180-fold increase, Fig. 4b). We also found that the promoter recruitment of histone acetyltransferase (HAT) p300, a CtBP-interacting protein and a central component of multiple coactivator complexes, is intimately associated with CtBP1 occupancy at and transcriptional activation of the four target genes (Fig. 4 a and b).

Both *IL-18* and *TGFB1* have previously been shown to be transcriptional targets of AP-1 (Kim et al., 1989, Kim et al., 2000, Zhou et al., 2020). To investigate whether AP-1 is required for recruitment of CtBPs to these two target promoters, we compared the CtBP1 ChIP signals between HaCaT cells that were either untreated or pretreated with T-5224, a specific inhibitor of AP-1 (Tsuchida et al., 2004), before being stimulated IMQ. Pretreatment with T-5224 significantly suppressed the IMQ-induced recruitment of CtBP1 and p300/HAT (74% to 80% decrease) to the *IL-18* and *TGFB1* promoters, while having a much milder effect on their recruitment to the promoters of *IL-6* and *S100A8* (16% to 27% decrease, Fig. 4a). Consistent with the changes in CtBP1-p300 promoter occupancy, T-5224 treatment caused a more significant decrease (~70%) in the IMQ-induced transcription of *IL-18* and *TGFB1* relative to that of *IL-6* and *S100A8* (~30% decrease, Fig. 4b).

To further probe the cis- and trans-regulatory elements involved in CtBP-mediated transactivation, we performed luciferase reporter assays of the *TGFB1* promoter that contained mutations at the well-characterized binding sites (mt1-3) for AP-1 and Sp1 in non-stimulated human epithelial cell line FaDu (Weigert et al., 2000). The basal promoter activity is marked suppressed (80% decrease) by mt1 and not further reduced by silencing of CtBP1 and CtBP2 (Fig. 4c), suggesting that the CtBPs cooperate with AP-1 on the distal binding site to transactivate *TGFB1*.

Taken together, our findings indicate that CtBP1 and CtBP2 serve as transcriptional coactivators, likely through interaction with transcription factors such as AP-1 and p300/HAT, in both basal and IMQ-stimulated expression of these eight proinflammatory genes. In support of this model, CtBP2 was found to be in the same protein complex with c-FOS and c-JUN under overexpression conditions (Zhou et al., 2020).

Increased expression of the CtBP genes in a mouse IMQ psoriasis model

To assess whether the elevated CtBP expression in human psoriatic lesions is recapitulated in the mouse IMQ model of psoriasis, we characterized the mRNA or protein expression of *Ctbp1* and *Ctbp2* along with *Tnfa* and *Tgfb1*, two CtBP-regulated cytokine genes that are known to be induced by IMQ in vivo (Flutter and Nestle, 2013, Swindell et al., 2011), over a six-day time course of IMQ application (Fig. 5a). We observed a strong and time-dependent increase in mRNA expression of *Ctbp2* and a moderate increase in *Ctbp1*, *Tnfa* and *Tgfb1* in the IMQ-treated skin (Fig. 5b). In parallel, there is a gradual increase in CtBP2 protein expression in the IMQ-treated skin (Fig. 5c). Consistent with the continued increase in CtBP2 expression, we saw a gradual decrease in the protein expression of *Cdh1* (*i.e.*, E-cadherin, Fig. 5c), a gene that is transcriptionally repressed by the CtBPs (Lin et al., 2003). Besides the affected skin, we also found time-dependent increase in the mRNA

expression of *Ctbp2*, *Ctbp1*, *Tnfa* and *Tgfb1* in the peripheral blood leukocytes of the IMQ mice, indicating a systemic inflammatory response to topical IMQ treatment that is in agreement with previous publications (Griffith et al., 2018, Nerurkar et al., 2017). We note that the increase in *Ctbp1* and *Ctbp2* mRNA expression precedes that of *Tgfb1* in the treated skin (Fig. 5b), which is in accordance with the notion that the CtBPs transactivate *Tgfb1* in the IMQ-induced skin inflammatory response. A similar pattern was also observed in the microarray dataset from a previous comparison study between psoriasis patients and normal controls (Swindell et al., 2011). While all eight abovementioned CtBP target genes exhibit the highest level of expression in psoriatic lesions (PP) relative to non-lesional skins in psoriasis (PN) and normal skins (NN), the *CtBP2* mRNA level peaks in PN and exhibits no significant difference between PN and PP (Table S3).

The CtBP inhibitor Pep1-E1A reduces IMQ-induced psoriasiform skin inflammation

We have previously developed a cell penetrating peptide, Pep1-E1A, which is able to disrupt the PXDLS-mediated interaction of the CtBPs with their transcriptional factor partners and block transcriptional repressor activities (Blevins et al., 2018). Both the wild-type (containing a PLDLS motif) and mutant (PLDEL) peptides internalized readily into HaCaT cells in culture (Fig. S3). To investigate whether Pep1-E1A can reduce CtBP-mediated inflammatory response, we assessed its therapeutic efficacy on psoriatic skin inflammation using the IMQ mouse model. The animals were randomized into four groups, one receiving sham cream (control) and the other three 5% IMQ cream daily on the dorsal skin for 6 days. Two of the IMQ groups also received topical treatment with Pep1-E1A (PLDLS) or Pep1-E1A^{Mut} (PLDEL) (2 mg/kg/day) starting on day 3 of IMQ application. An evaluation of skin inflammation was performed each day using a modified Psoriasis Area and Severity Index (PASI) scoring method (El Malki et al., 2013). Among the three IMQ groups, we found that the Pep1-E1A-treated animals exhibit reduced skin reddening (Fig. 6a) and significantly lower PASI scores in comparison to the other two groups starting from day 4 (Fig. 6b, $p < 0.001$). As expected, treatment with Pep1-E1A results in a marked decrease in mRNA expression of four of the eight CtBP target genes described above, *Il1b*, *Il6*, *Il18* and *Tnfa*, ranging from 42% to 85%, whereas Pep1-E1A^{Mut} has no effect (Fig. 6c). Moreover, expression of *Ifng* and four Th17-related cytokines, *Il17a*, *Il17f*, *Il22* and *Il23*, is also significantly suppressed by the Pep1-E1A treatment (43%–54% change, Fig. 6c). In accordance with the reduced inflammatory cytokine expression, the Pep1-E1A-treated skin exhibits much lower epidermal proliferation, as measured by BrdU incorporation (Fig. S4), and a remarkable decrease in the numbers of infiltrating immune cells (e.g., CD45$^+$ leukocytes and F4/80$^+$ macrophages) and CD31$^+$ cells in the epidermis and dermis (Fig. 6d), as revealed by IHC analysis. We also saw a markedly reduced level of TGFβ1 IHC signal in the epidermis of the Pep1-E1A-treated mice (Fig. 6d). Interestingly, Pep1-E1A causes a moderate decrease in the mRNA expression of *CtBP1* but has no such effect on *CtBP2* mRNA or protein expression (Fig. 6c), suggesting a that expression of *CtBP1* but not *CtBP2* is subject to CtBP-mediated transcriptional activation. Consistent with this notion, overexpression of *CtBP1* in HaCaT cells does not affect the basal or IMQ-induced expression levels of the *CtBP2* mRNA (Fig. 3b). Taken together, these results indicate that CtBP inhibition by Pep1-E1A can effectively suppress inflammatory cytokine expression and mitigate symptoms of psoriatic skin inflammation.

In summary, we demonstrate that CtBP1 and CtBP2 are required for the transactivation of select proinflammatory cytokine genes in IMQ-stimulated human keratinocytes and mouse skin, thus contributing to the initiation and progression of psoriasis-like skin phenotypes. Furthermore, we have shown that CtBP1 and p300/HAT are recruited to the *IL18* and *TGFB1* promoters via AP-1, a central transcription factor downstream of the MAPK pathway (Karin, 1995). Intriguingly, cutaneous activation of p38 MAPK has recently been shown to trigger psoriatic dermatitis, which can be reduced by a small-molecule inhibitor of p38 (Sakurai et al., 2019). These findings are consistent with the model that CtBPs can act as co-activators of AP-1 downstream of p38 to promote inflammatory genes expression in skin inflammation.

The CtBPs have emerged as important players in regulation of innate inflammatory response to tissue damage and infection. Both CtBP2 expression and CtBP-mediated transactivation are upregulated in traumatic brain injury, acute lung injury and chronic renal failure (Chen et al., 2020, Li et al., 2020, Zhou et al., 2020). The transcriptional co-regulator activity of CtBPs is sensitive to changes in cellular metabolic or redox state. NAD(H) binding has been shown to enhance oligomerization of CtBPs and their interaction with transcription factor partners (Balasubramanian et al., 2003, Bellesis et al., 2018, Jecrois et al., 2021). Consistent with this, the anti-inflammatory effect of 2-deoxy-D-glucose (2DG), a glycolysis inhibitor, has been attributed to its ability to lower cellular NADH levels (Li et al., 2016, Shen et al., 2017). Interestingly, it has been shown that keratinocytes at psoriatic lesions exhibit membrane enrichment of the glucose transporter SLC2A1/GLUT1 and that 2DG can suppress IMQ-induced epidermal hyperplasia in mice (Huang et al., 2019). Beside metabolic and redox sensitivity, the CtBP proteins also undergo dynamic posttranslational modifications that influence their stability and subcellular localization (Choi et al., 2010, Verger et al., 2006, Zhang et al., 2003). Our finding that human psoriatic lesions contain an elevated level of the CtBP1 protein but no change in its mRNA suggests an increased in CtBP1 protein stability in the inflammatory skin (Fig. 2 and Table S3).

These findings highlight CtBPs as a promising therapeutic target for immunomodulation. Here, we show that topical Pep1-E1A treatment suppresses the IMQ-induced proinflammatory cytokine production and skin inflammation. Similarly, Pep1-E1A administered via i.p. injection into mice after mild traumatic brain injury can reduce both systemic and brain inflammation resulting from the head trauma (Li et al., 2020). Intriguingly, the evolutionarily conserved and PIDLS-containing small protein MCRIP1 (97 amino acid residues in length), like Pep1-E1A, disrupts CtBP-mediated transcriptional repression by competitively inhibiting the PXDLS-mediated interaction of CtBPs with their transcription factor partner ZEB1 (Blevins et al., 2018, Ichikawa et al., 2015). Phosphorylation of MCRIP1 by ERK dissociates it from CtBP, thereby subjecting CtBP-mediated transcription pathways to modulation by upstream signal transduction (Ichikawa et al., 2015). Considering its limited skin penetrating ability and short half-life (< 2 h in cultured keratinocytes, data not shown), we surmise that Pep1-E1A exerts its effect primarily through the epidermal layer and by altering the transcriptional program in keratinocytes. Future research will define the contribution of CtBP-mediated transcriptional response to the interplay between keratinocytes and resident and infiltrating immunocytes in the psoriasis pathogenesis. Our findings suggest that selective modulation of CtBP-regulated

proinflammatory gene expression could potentially be a novel and effective strategy to alleviate skin inflammation. The advantage of a topical treatment for isolated acute inflammatory skin lesions warrants further preclinical and clinical studies.

MATERIALS & METHODS

Human specimens

Human skin samples were obtained from patients with written and informed consent under the protocol approved by Colorado Multiple Institutional Review Board (COMIRB). Clinical information of psoriasis patients was provided in Table S2.

Mice

All animal experiments were reviewed and approved by the University of Colorado Anschutz Medical Campus Institutional Animal Care and Use Committee. The K5.CtBP1 mice were previously described (Deng et al., 2014). Wild-type FVB 6 mice were purchased from Charles River Laboratories (Wilmington, MA).

IMQ-induced psoriasis mouse model

Gender-matched FVB mice at 16-20 weeks of age received a daily topical dose of 62.5 mg of IMQ cream (5%, Aldara, HealthWarehouse) or the vehicle control (Vaseline) on the shaved dorsal skin for up to six consecutive days. Sequences, expression and preparation of the of WT and mutant Pep1-E1A peptides were as described previously (Blevins et al., 2018). For peptide treatment, 60 μg (in 25 μL PBS) of Pep1-E1A or Pep1-E1A^{Mut} were applied to the depilated dorsal skin daily starting from day 3 of the IMQ time course. Severity of skin inflammation was assessed daily with a modified PASI scoring system consisting of parameters for erythema, thickness and scaling (each on a scale of 0 to 4) as described (Vinter et al., 2016). A cumulative score of all three parameters is shown.

Histology

Skin tissue samples were fixed in 4% formaldehyde, embedded in paraffin, and cut into 6-μm sections for hematoxylin and eosin (H&E) and immunohistochemistry staining as previously described (Wang et al., 1999). IHC signal intensities were quantitated using ImageJ software.

Antibodies

The primary antibodies used in this study included mouse anti-CtBP1, -CtBP2 (BD Biosciences) and -p300 (Santa Cruz), rabbit anti-mouse F4/80 and CD45 (Cell Signaling Technology), rabbit anti-CD31 (Abcam), -K14 (Fitzgerald) and -GAPDH (New England Biolab), rat anti-CDH1 (Sigma), -CD4 (BF Biosciences) and -Ly-6G (eBioscience), goat anti-mouse ALK1 and chicken anti-TGFβ1 (R&D Systems).

Cell culture, transfection and IMQ treatment

All cell lines used in this study were from ATCC. RAW264.7 and HaCaT cells were cultured in DMEM and THP-1 were in RPMI1640 medium (Corning), all supplemented

with 10% fetal bovine serum (GEMINI) and 0.1% penicillin/streptomycin (Corning). Cells were transfected with Lipofectamine 2000 (Life Technologies) in suspension with siRNAs (sequences listed in Table S4). The transfected cells were incubated in DMEM for 24 h, followed by treatment with 200 μg/ml of IMQ for 6 h, and harvested for RNA extraction.

Preparation of luciferase reporter constructs and luciferase activity assay

The *TGFα1*-luciferase reporter vector was generated by cloning a PCR-amplified 663 bp fragment of the *TGFβ1* promoter (−434 to +199) into the *Kpn* I and *Bgl* II sites of pGL4.26 (Promega). Mutations at the distal AP-1 site (mt1: TGACTCT to TGgtTCT), the proximal AP-1 site (mt2: TGTCTCA to gtTCTCA), and the SP1 site (mt3: GCCCGCC to GCCtaCC) were introduced by site-directed mutagenesis. An empty renilla luciferase vector (pGL4.79) was used for normalization. FaDu cells were transfected with the WT or mutant reporter construct individually or in combination with siRNAs and luciferase activity was measured 48 hours afterwards.

RNA isolation, reverse transcription and quantitative real-time PCR

Total RNA was extracted from freshly harvested cells or frozen tissues using TRIzol™ reagent (Life Technologies) and analyzed by RT-qPCR. First-strand cDNA was reverse transcribed from 1.0 μg total RNA with oligo (dT) primers using Verso cDNA synthesis kit (Thermo Fisher Scientific). Quantitative PCR with SYBR green detection (Applied Biosystems) was performed using 1% of the reversely transcribed cDNA mixture on a BioRad CFX96 real-time detection system. Relative expression of individual genes was normalized to *ACTB* (β-Actin) expression using the $2^{-\Delta\Delta Ct}$ method (Winer et al., 1999). The sequences of all RT-qPCR primers were provided in Table S4.

Chromatin immunoprecipitation

ChIP experiments were carried out as described (Li et al., 2020). For each antibody, relative ChIP signal was calculated as percent of input using the $2^{-\Delta\Delta Ct}$ method (Winer et al., 1999), and the ChIP signals of the IMQ-stimulated cells were normalized to that of the unstimulated control. The sequences of all ChIP-qPCR primers were listed in Table S4.

Western Blotting.

Total protein extracts were isolated from mouse skin tissues using 1x RIPA buffer containing 150 mM NaCl, 1% NP-40, 0.1% SDS, 25 mM Tris (pH 7.4), and 1 × complete protease inhibitor cocktail (Roche). Proteins were separated by 10-12% SDA-PAGE and transferred onto a PVDF membrane for immunodetection. Blots were developed using an enhanced chemiluminescence substrate (Millipore) and scanned with a ChemiDoc MP imager (Bio-Rad). Raw signal intensity for each band was measured using Image J software (4.0.1 version).

Statistical Analysis

Graphs was performed using GraphPad Prism version 8.0 for OSX (GraphPad Software). Data are represented as mean ± standard deviation (SD). Statistical differences were analyzed using two-tailed Student *t* test.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability Statement

Datasets related this article can be found at the at the NCBI Gene Expression Omnibus through https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE13355.

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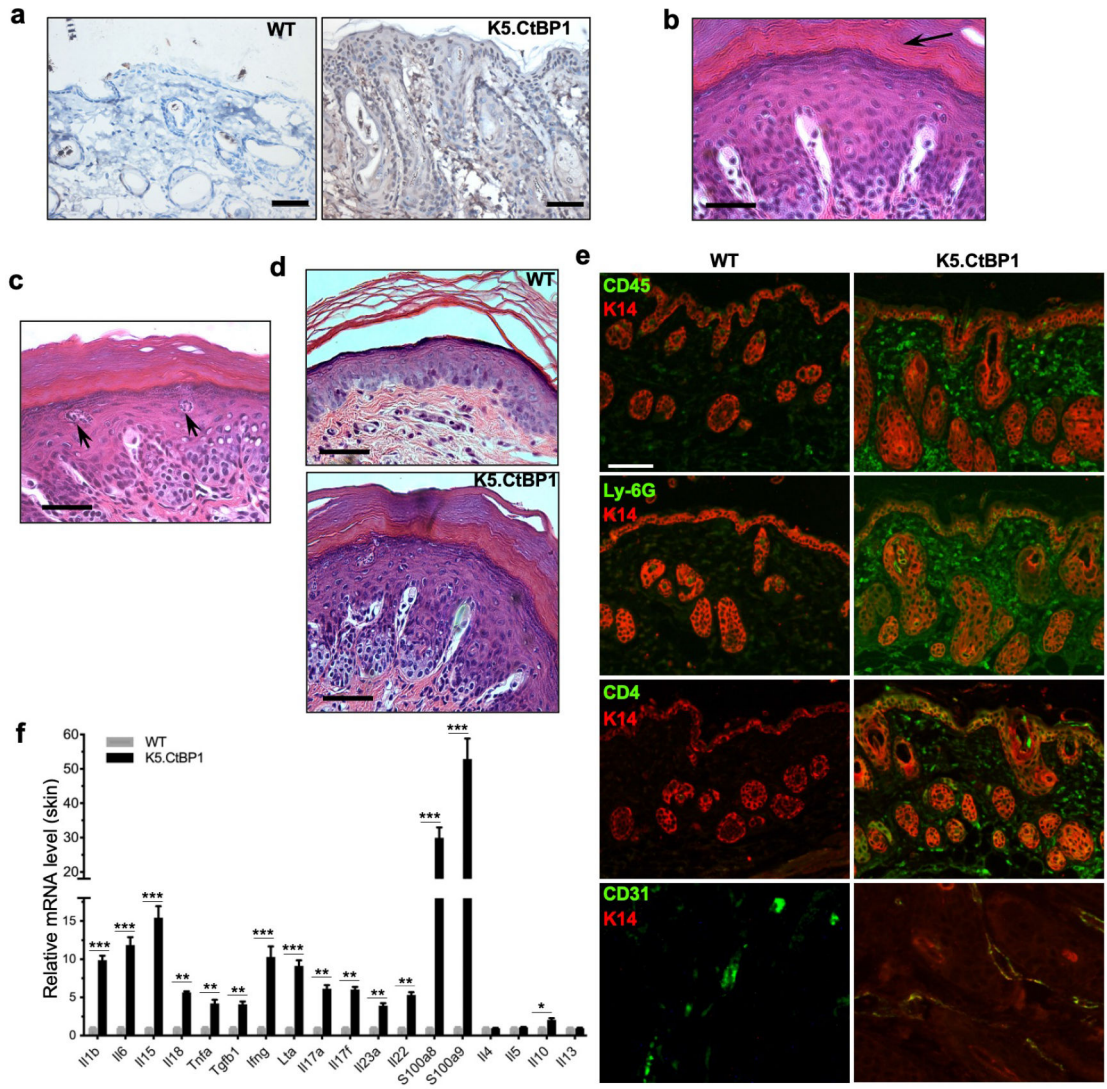

Figure 1. The K5.CtBP1 mice exhibited a skin inflammation phenotype with elevated Th1 and Th17 cytokine expression.

(**a**) Representative images of CtBP1 immunohistochemistry analysis of the wild type (WT) and transgenic skin ($n = 3$). Scale bar = 40 μm.

(**b**-**d**) H&E staining of the transgenic skin showing parakeratosis (**b**) and subcorneal microabscesses (black arrows) (**c**), and of the volar skin showing epidermal downgrowth in the K5.CtBP1 mice (**d**). Scale bar = 40 μm.

(**e**) Immunofluorescence staining of the WT and transgenic skin sections for CD45, Ly-6G and CD4 (green) with K14 counterstaining (red) and for CD31 (green) with ALK1 counterstaining (red). Scale bar = 50 μm.

(**f**) Relative mRNA expression of the indicated genes (mean ± SD) in the WT and K5.CtBP1 skin ($n = 4$). *, $p<0.05$, **$p<0.01$, ***$p<0.001$.

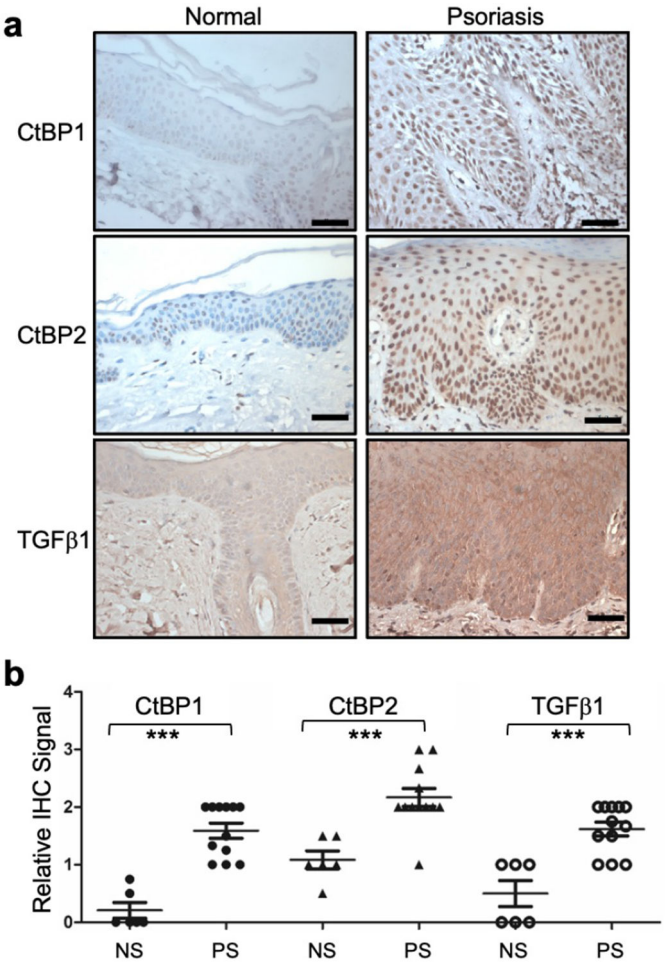

Figure 2. Enhanced CtBP1 and CtBP2 expression in human psoriatic skin lesions.

(**a**) Representative IHC images of CtBP1, CtBP2 and TGFβ1 in human normal skin (NS) and psoriatic skin lesions (PS). Scale bar = 20 μm.

(**b**) Scatter plots show the distribution of total IHC scores of CtBP1, CtBP2 and TGFβ1 among normal ($n = 6$) and psoriatic skin samples ($n = 12$), ***$p < 0.001$.

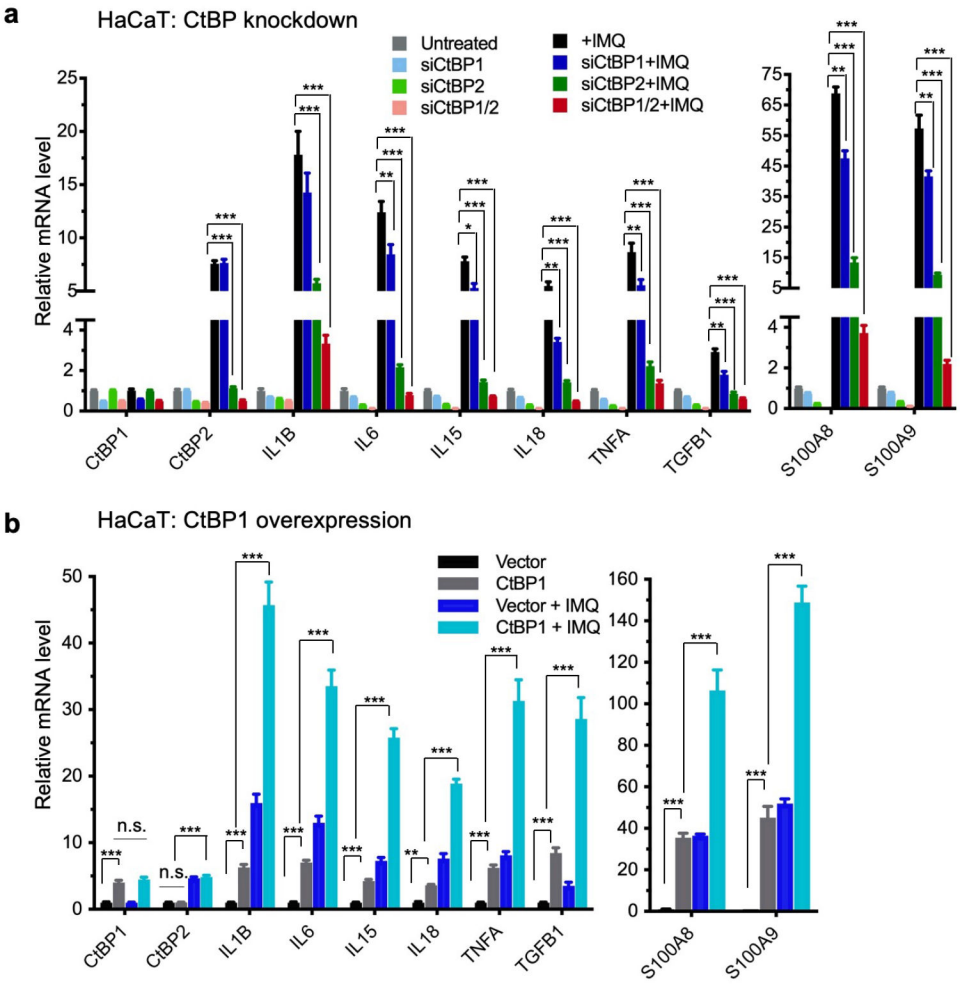

Figure 3. CtBP1 and CtBP2 are required for IMQ-induced proinflammatory gene expression in human keratinocytes.

(**a**) Silencing of *CtBP1* and *CtBP2*, individually or in combination, suppresses mRNA expression of proinflammatory genes in IMQ-stimulated HaCaT cells. Relative mRNA expression was analyzed by RT-qPCR and depicted as fold change (mean ± SD, in triplicates and two independent experiments) versus the non-stimulated control (Untreated). *p<0.05, **p<0.01, ***p<0.001.

(**b**) Stably transfected HaCaT cells harboring a *CtBP1*-expressing plasmid or the empty vector were stimulated with IMQ and analyzed for cytokine mRNA expression as in (**a**).

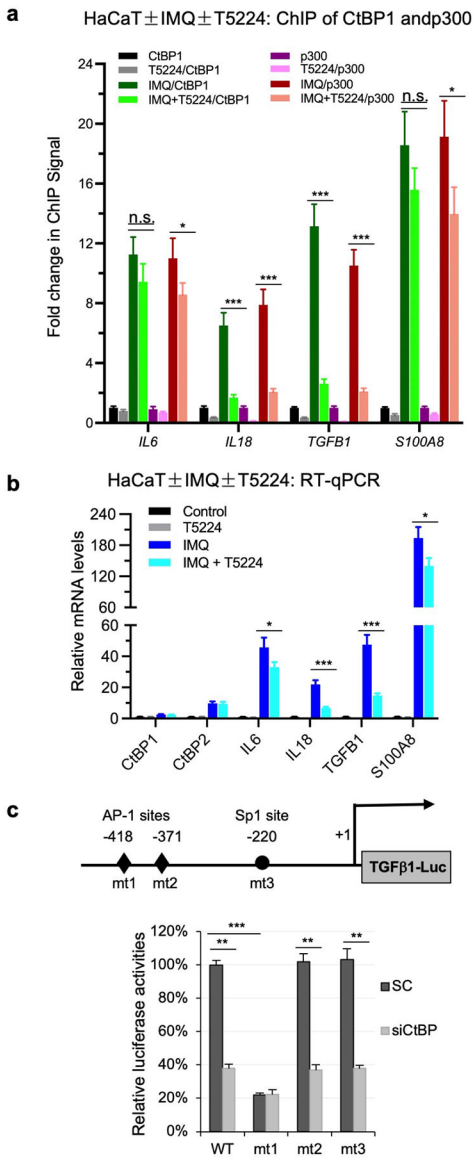

Figure 4. IMQ-induced recruitment of CtBP1 and p300 to the *IL-18* and *TGFB1* promoters is dependent on AP-1.

(**a**) IMQ induces AP-1-dependent recruitment of CtBP1 and p300 to the *IL18* and *TGFB1* promoters. Chromatin fractions from control and IMQ-stimulated HaCaT cells that were untreated or pretreated with 10 μM T-5224 were precipitated with antibodies specific to CtBP1 and p300. Bars represent fold changes of relative ChIP signals normalized to the respective controls (mean ± SD, in triplicates and two independent experiments).

(**b**) Relative mRNA expression of the indicated genes in cells from (a) (mean ± SD).

(**c**) Relative luciferase activity (mean ± SD, in triplicates and three independent experiments) of FaDu cells transfected with WT or mutant *TGFB1*-luciferase reporters individually or in combination with scrambled siRNAs (SC) or siRNAs for CtBP1 and CtBP2.

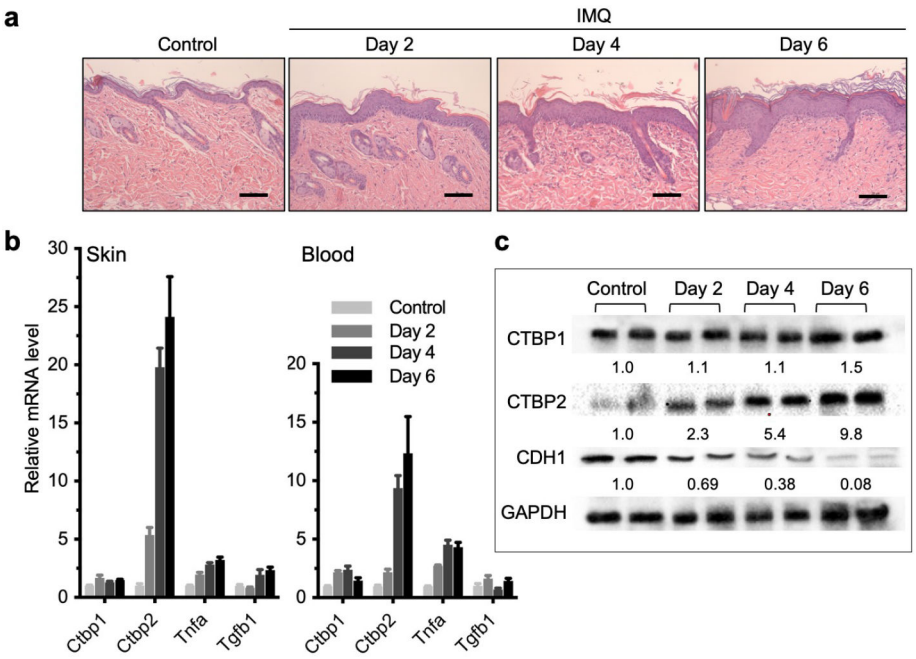

Figure 5. Increased CtBP1 and CtBP2 expression in IMQ-treated mouse skin.

Mice were analyzed after receiving daily topical IMQ treatment for 2, 4 or 6 days ($n = 4$ per group).

(**a**) Representative images of H&E staining of control and IMQ-treated skin sections. Scale bar = 50 μm.

(**b**) Relative mRNA levels of indicated genes (mean ± SD) in the skin and circulating leukocytes.

(**c**) Western blot analysis of CtBP1, CtBP2 and CDH1 in the skin samples. All signals were normalized to the level of GAPDH.

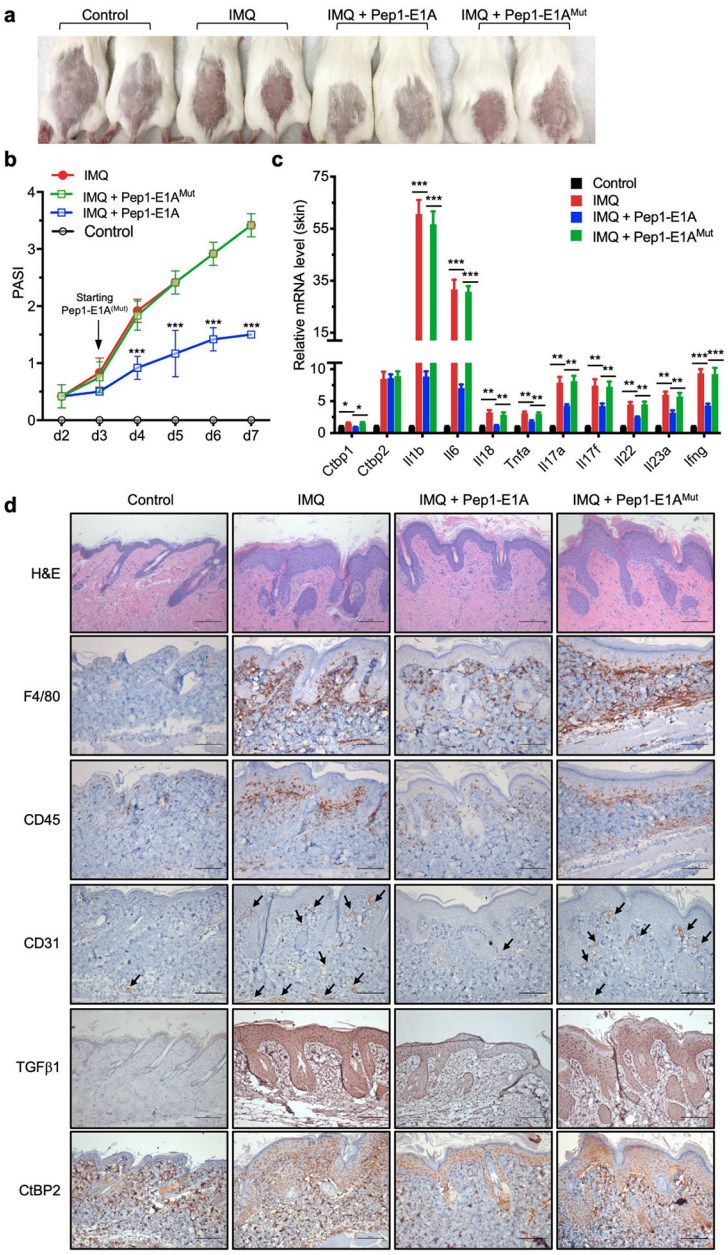

Figure 6. Pep1-E1A reduces CtBP-dependent proinflammatory gene expression and ameliorates skin inflammation in the mouse IMQ psoriasis model.

Mice received sham cream (Control) or 5% IMQ cream daily for 6 days. Pep1-E1A or Pep1-E1A^{Mut} were topically applied to the IMQ-treated skin starting on day 3. All animals were collected for histological and RT-qPCR analyses on day 7.

(**a**) Phenotypical presentation of mouse dorsal skin from each group on day 7 of IMQ treatment.

(**b**) Comparison of PASI scores on day 4 through day 7. Data are presented as mean ± SD and were analyzed between the IMQ and IMQ + Pep1-E1A groups at indicated time points by multiple t-tests. $n = 5$ per group, ***$p<0.001$.

(**c**) Relative mRNA expression of the indicated genes in the skin samples were normalized to sham control (mean ± SD). $n = 5$; *$p<0.05$, **$p<0.01$, ***$p<0.001$.
(**d**) Representative images of H&E-stained skin sections (three sections from two animals for each group) and IHC staining for F4/80, CD45, CD31, TGFβ1 and CtBP2. Scale bar = 50 μm.