Induction of Regulatory T Cells and Correction of Cytokine Disbalance by Short-Chain Fatty Acids: Implications for Psoriasis Therapy

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Commensal microbes modulate the immune system in the colon through short-chain fatty acids, which induce regulatory T cells (Treg). Accordingly, the short-chain fatty acid sodium butyrate (SB) suppressed allergic contact dermatitis in mice through the activation of Treg. There is evidence that Treg exert the capacity to control inflammation in psoriasis. Thus, we were interested in studying the effect of SB in psoriasis, utilizing the imiquimod-induced psoriasis-like skin inflammation model. Topical application of imiquimod induced thickening of the skin, scales, and inflammation. This was associated with an upregulation of IL-17 and downregulation of IL-10 and FOXP3. Topically applied SB reduced imiquimod-induced inflammation and downregulated IL-17 and induced IL-10 and FOXP3 transcripts. The mitigating effect of SB was due to Treg because it was lost upon depletion of Treg in the depletion of regulatory T cell mice. Treg isolated from the blood of patients with psoriasis were reduced in their suppressive activity, which was normalized by SB. The fewer Treg numbers in the biopsies of psoriatic lesions as well as enhanced IL-17— and IL-6—expression levels and reduced IL-10— and FOXP3—expression levels were restored by SB. These data indicate that psoriasis is associated with an impairment of Treg and an altered cytokine milieu. Short-chain fatty acids appear to restore these alterations, thereby harboring a therapeutic potential for psoriasis.


INTRODUCTION

The intestinal microbiome is known to modulate inflammatory reactions. This is achieved through the expansion and activation of regulatory T cells (Treg) (Arpaia et al., 2013; Furusawa et al., 2013; Nagano et al., 2012; Trompette et al., 2014). The regulatory effects of commensal gut bacteria are not only confined to colitis but also influence inflammatory reactions of other organs like the central nervous and the airway systems (Ochoa-Repáraz et al., 2009; Trompette et al., 2014).

It was discovered that the intestinal microbiome utilizes short-chain fatty acids (SCFA) to exert these effects. SCFA, microbiota-derived bacterial fermentation products including butyrate, propionate, and acetate, were found to modulate Treg homeostasis in the gut (Maslowski et al., 2009; Smith et al., 2013). Accordingly, oral administration of SCFA obtained from the stool of healthy volunteers increased the number of Foxp3⁺ Treg in the gut of mice (Atarashi et al., 2013).

However, commensals regulate inflammation not only in the gut but also in the respiratory and urogenital tract, the oral cavity, and the skin (Belkaid and Naik, 2013). Because skin commensals also produce SCFAs (Christensen and Brüggemann, 2014), we postulated that SCFA might also exert a similar regulatory function in the skin. We surmised that SCFA produced by commensal skin bacteria may also stimulate Treg in the skin. According to this line, sodium butyrate (SB) applied topically suppressed allergic contact dermatitis in mice (Schwarz et al., 2017). This effect was associated with the induction and/or activation of Treg.

Psoriasis is a chronic inflammatory dermatosis with complex pathogenesis. Several pathways appear to be involved and contribute to the broad clinical appearance (Rendon and Schäkel, 2019). Psoriasis is regarded as a kind of autoimmune dermatosis (Prinz, 2017); several potential autoantigens were identified including ADAMTS-like protein 5 (Arakawa et al., 2015) or LL-37 (Lande et al., 2014). There is clinical and experimental evidence that Treg in psoriasis are reduced in number and impaired in their activity and thus, lose their capacity to control the inflammatory response (Owczarczyk-Saczonek et al., 2018; Stockenhuber et al., 2018). In contrast, other studies found FOXP3⁺ Treg rather increased in the skin and blood of patients with psoriasis (Zhang et al., 2010) and imiquimod (IMQ)-treated mice (Hartwig et al., 2018). The increase of Treg upon IMQ administration appears to have a mitigating effect on the inflammatory response because this was exaggerated upon depletion of Treg (Hartwig et al., 2018). However, it has not been elucidated yet whether activation of Treg can reduce psoriatic inflammation.

Hence, we studied whether the reduced activity of Treg can be restored by the administration of SCFA. To address this issue, we utilized IMQ-induced inflammation, a murine model of psoriasis-like skin inflammation (van der Fits et al., 2009), and samples from patients with psoriasis.
RESULTS

The inflammatory response in the IMQ-induced psoriasis-like skin inflammation model is reduced by SB

Mice were treated with IMQ cream and received a daily dose of 62.5 mg on the shaved backs and ears for 10 days. One group of the IMQ-treated mice received SB topically on the back skin and ears for 3 days. IMQ-treated mice developed thickening of the skin, erythema, and scales. The inflammatory response was reduced upon administration of SB, whereas the vehicle had no effect (Figure 1a). This was also confirmed by measuring the ear and back skin thickness (Figure 1b). Administration of SB on normal control mice did not have an effect (Supplementary Figure S1). Biopsies were taken, and paraffin sections stained with H&E. IMQ treatment induced acanthosis, hyperkeratosis, and an inflammatory infiltrate. These changes were almost completely reversed by SB but not by the vehicle (Figure 1c).

Because IMQ, though only topically applied, appears to induce also systemic immunologic alterations (van der Fits et al., 2009), mice were killed 14 days after initiation of IMQ treatment and spleens obtained. As described previously, the spleens of IMQ-treated animals were remarkably enlarged. Splenomegaly was much less pronounced upon the topical application of SB (Figure 1d).

SB downregulates the inflammatory response

Total RNA was isolated from the back skin and ears and RT-PCR for the transcription of Il-17 performed. Il-17 transcripts were significantly upregulated by IMQ (Figure 2a), which is in accordance with previous observations (van der Fits et al., 2009). SB downregulated Il-17 transcripts. In turn, transcription of Il-10 was remarkably induced by SB as observed in contact hypersensitivity (Schwarz et al., 2017). We surmise Treg as the primary source of Il-10, which was supported by the upregulation of the mRNA of the Treg-specific transcription factor Foxp3. These data support the assumption that SB might activate cutaneous Treg and thereby contribute to the downregulation of the inflammatory response.

To get an idea about the cause of the splenomegaly and to identify whether Treg are the primary sources of IL-10 and IL-17, cells were obtained from the spleens and lymph nodes and subjected to FACS analysis. Cells were gated for CD25 and double-positive cells for FOXP3 and IL-10 and FOXP3 and IL-17, respectively, were analyzed. The number of FOXP3+ and IL-17-expressing cells was enhanced in IMQ-treated mice, implying that the splenomegaly might be due in part to the infiltration and/or expansion of IL-17–expressing lymphocytes. This effect was prevented by SB. In addition, fewer cells expressing FOXP3 and IL-10 were detected after IMQ administration, suggesting the downregulation of IL-10 producing FOXP3+ Treg. This was also almost completely reversed by SB (Figure 2b).

To determine whether SB-induced and/or -activated Tregs express CD69, CD73, and FR4, CD25-gated cells were double-stained with FOXP3 for those markers (Figure 2c). CD69, which regulates the differentiation of Treg as well as the secretion of IFN-γ, IL-17, and IL-22 (Cibrian et al., 2017), was upregulated by SB. A similar induction was observed on FOXP3+ cells for CD73, an ecto-5’-nucleotidase that contributes to the inhibitory function of Treg by generating adenosine (Ring et al., 2011), and for FR4, which is required for the activity of Treg (Kinoshita et al., 2012).

The anti-inflammatory effect of SB is dependent on Treg

To prove whether the effect of SB is mediated through Treg, we utilized the depletion of regulatory T cells, which express a diphtheria toxin (DT) receptor-enhanced GFP under the control of the Foxp3 gene (Lahl et al., 2007). Thus, injection of DT resulted in the selective depletion of Foxp3+ cells, which included Treg. Depletion of regulatory T cells mice were treated with IMQ for 10 days and SB for 3 days. Two groups received 1 µg DT on 3 consecutive days before IMQ treatment and on day 10. IMQ-treated mice developed thickening of the skin, erythema, and scales (Figure 3a). The depletion of Treg with DT enhanced the inflammatory response. This is in accordance with recently published findings that Foxp3+ Treg control inflammation severity in the IMQ model (Hartwig et al., 2018; Stockenhuber et al., 2018). SB downregulated IMQ-induced inflammation, but this effect disappeared upon the injection of DT, implying that the anti-inflammatory effect of SB is critically dependent on Treg. These observations were confirmed by measuring the ear swelling and skin thickness (Figure 3b).

Histologic analysis of biopsies confirmed these results (Figure 3c). IMQ-induced acanthosis, hyperkeratosis, and inflammation were reduced by SB, but this effect was lost upon the depletion of Treg. The systemic alterations were influenced in an identical fashion. IMQ-induced splenomegaly was reduced by SB but only in the presence of Treg (Figure 3d).

The suppressive activity of human psoriatic Treg is diminished compared with that of the healthy controls, and SB restores this effect

To clarify whether similar observations can be made in the human system, Treg (CD4+CD25+) were isolated from the human PBMC of patients with psoriasis and healthy controls, and an in vitro suppression assay was performed. Treg isolated from the PBMC obtained from patients with psoriasis revealed reduced suppressive activity when compared with Treg obtained from healthy controls, which is in accordance with previous observations (Sugiyama et al., 2005). Treatment of psoriatic and normal Treg with SB resulted in a moderate enhancement of their activity (Figure 4a), suggesting that SB exerts the capacity to restore the reduced suppressive activity of the Treg obtained from patients with psoriasis at least partially.

The expression of FOXP3 on the Treg obtained from patients with psoriasis was lower than that on the Treg obtained from healthy controls, as demonstrated by FACS analysis (Figure 4b); stimulation with SB enhanced the expression of FOXP3.

To evaluate the expression of FOXP3 in the skin, qRT-PCR was performed. Biopsies were taken from controls, lesional, and nonlesional psoriatic skin and placed into a 24-well plate. After, the dissection half of each biopsy was treated topically with SB or vehicle. The expression of FOXP3 was
reduced in lesional skin in comparison with that of the healthy controls but upregulated upon treatment with SB. The expression of FOXP3 in the nonlesional skin was also downregulated in comparison with that of the controls but not as pronounced as in lesional psoriatic skin. Upon stimulation with SB, the expression of FOXP3 in nonlesional skin was also upregulated (Figure 4c).

The induction of cutaneous Treg by SB was also confirmed in situ by immunofluorescence analysis. In lesional and nonlesional psoriatic skin, a lower number of
Figure 2. SB contributes to the downregulation of the inflammatory response and, thereby, might activate cutaneous Treg. (a) For quantitative real-time PCR, RNA was extracted from the skin of control mice, mice treated with IMQ, and in addition with either vehicle or SB. Relative gene expression was normalized to β-actin and Hprt1 according to the delta Ct method. Foxp3 *P < 0.02 IMQ versus IMQ + SB, P_{Anova} = 1.96 \times 10^{-4}, II-17 *P < 0.02 IMQ versus IMQ + SB, P_{Anova} = 6.04 \times 10^{-7}; II-10 *P < 0.005 IMQ versus IMQ + SB, P_{Anova} = 2.44 \times 10^{-7}. (b) Cells were obtained from the spleens and lymph nodes and subjected to FACS analysis. Cells were gated for CD25, and the double-positive FOXP3/IL-10 and FOXP3/IL-17 cell populations were analyzed. As negative controls served isotype control (IgG Co) or cells that were CD25^+ only (Neg Co). (c) Lymph node cells and splenocytes were gated for CD25 and the double-positive FOXP3/CD69, FOXP3/CD73, FOXP3/FR4 cells were analyzed. Each group contained four animals. Co, healthy control; IMQ, imiquimod; Neg Co, negative control; SB, sodium butyrate; Treg, regulatory T cell.
FOXP3⁺ cells was detected than in healthy controls. SB treatment resulted in an increased number of FOXP3⁺ cells in skin explants (Figure 4d). To characterize FOXP3⁺ cells more precisely, double staining with CD25 was performed, revealing that the majority of FOXP3⁺ cells were also CD25⁺ (Figure 4e). Likewise, double staining with CD3 indicated that the majority of FOXP3⁺ cells also expressed CD3 (Supplementary Figure S2).
Suppressive activity of psoriatic Treg is diminished compared with healthy controls and SB restores this effect. (a) Human PBMC obtained from Psor and Co were separated into CD4⁺CD25⁻ (responder cells) and CD4⁺CD25⁺ (Treg) cells. Treg were incubated with 200 μM SB or left untreated. Treg and responder cells were mixed at the ratios 1:2 and 1:4. Responder cells were activated with anti-Biotin MACS/Bead particles preloaded with biotinylated anti-CD2, anti-CD3, and anti-CD28 antibodies. After 4 days, cell proliferation was measured using Cell Counting Kit-8. Data are presented as percent suppression from one of three independent experiments. *P < 0.001. (b) Treg obtained from PBMC and stimulated with SB were stained with a PE-conjugated anti-human Foxp3 antibody. Histograms show one representative of two experiments. (c) Punch biopsies from Co, L and NL psoriatic skin (n = 3) were placed into 24-well plates on gaza saturated with DermaLife Medium. Half of each biopsy was treated topically with SB or vehicle. After 24 hours, RNA was extracted and qRT-PCR for FOXP3 was performed. Relative gene expression was normalized to 36b4 and GAPDH according to the delta cycle threshold method. Transcription of FOXP3 from controls was set as 1. *P < 0.02 L versus L + SB; P = 3.09 x 10⁻¹¹. (d) Skin biopsies obtained from controls and patients with psoriasis (n = 5) were analyzed by immunofluorescence. Foxp3-positive cells were visualized by staining with anti-human Foxp3 antibody. Bar = 100 μm. (e) Paraffin sections were stained with anti-human Foxp3 (green) and anti-human CD25 (red) antibody. Nuclear staining was performed with DAPI (blue). Bar = 10 μm. Co, healthy control; L, lesional; NL, nonlesional; Psor, patients with psoriasis; SB, sodium butyrate; Treg, regulatory T cell.
Acetylation of H3 histones is significantly decreased in Treg of patients with psoriasis

There is evidence that the suppressive activity of Treg is also under epigenetic control (Kitagawa and Sakaguchi, 2017). These epigenetic mechanisms include histone modifications and can cause alterations in gene expression and chromatin remodeling. To analyze the modification of histones, we isolated Treg from PBMC from healthy controls and patients with psoriasis. Histones were isolated and subjected to an antibody-based colorimetric assay for the measurement of the acetylation of H3 histones. Acetylation of H3 histones derived from Treg of patients with psoriasis was significantly decreased compared with the H3 histones obtained from the Treg of healthy controls (Figure 5a). Histone acetylation was upregulated by SB, implying that SB may act as a histone deacetylation inhibitor.

Disordered balance of cytokines in psoriatic skin is restored by SB

For the suppressive activity of Treg, IL-10 is essentially required, whereas IL-6 and IL-17 impede this process. To analyze the expression of these cytokines, skin biopsies were obtained from lesional and nonlesional psoriatic skin. After, the dissection half of each biopsy was treated topically with SB or vehicle. After 12 hours, RNA was isolated, and qRT-PCR was performed. As a control, cytokine transcription of biopsies of normal skin was used and set as 1. IL-6 and IL-17 transcripts were upregulated in lesional psoriatic skin but reduced by SB. In contrast, both cytokines were only minimally expressed in nonlesional psoriatic skin (Figure 5b and c). The expression of IL-10 was lower both in the lesional and nonlesional skin of patients with psoriasis but upregulated by SB (Figure 5d). Together, these findings imply that SB is able to correct the disturbed cytokine balance in psoriasis.

DISCUSSION

Psoriasis is driven by complex pathogenesis in which numerous cellular components and mediators are involved. Currently, the IL-23/T helper type 1/IL-17 axis is regarded as the most relevant player in the pathogenesis (Hawkes et al., 2018). Hence, many therapeutic strategies target this axis with impressive clinical results. However, it is unclear whether this axis is autonomously enhanced or whether downregulating mechanisms have lost their taming role. In
the latter scenario, Treg play an important role. Treg can be regarded as occasionally harmful components of the immune system because they can inhibit a protective immune response, as best demonstrated in tumor immunology where Treg suppress the immune reaction of the host against the tumor cells and thus support tumor growth (Jacobs et al., 2012). Accordingly, suppression of Treg activity, for example, by the application of checkpoint inhibitors has shown impressive antitumor responses (Ugurel et al., 2017). In turn, it has been recognized that Treg play an important physiological role in preventing autoimmune diseases and shutting off inflammatory reactions at the right time point. Accordingly, impairment of Treg has been demonstrated in a variety of autoimmune dermatoses (Loser and Beissert, 2012).

There are studies that have demonstrated a decrease in Treg frequencies in the peripheral blood and also in psoriatic lesions. In addition, some studies found dysfunction of Treg in psoriasis (Sugiyama et al., 2005; Wang et al., 2008). Bovenschen et al. (2011) observed that Treg from psoriatic lesions can differentiate toward T helper type 17 cells, which express Foxp3 but produce IL-17A and IL-22. Recently, it was shown in the IMQ model that Treg play an important role in taming the inflammatory response because depletion of Treg resulted in an exacerbation of the inflammation (Hartwig et al., 2018; Stockenhuber et al., 2018). Depletion of Treg induced IFN-1 and IFN-1–stimulated gene expression and caused accumulation of CD8+ T cells in lesional skin (Stockenhuber et al., 2018). In addition, upon Treg depletion, granulocyte-macrophage colony-stimulating factor producing CD4+ T cells migrated into lesional skin (Hartwig et al., 2018).

Although the latter two studies indicate a taming role of Treg in psoriasis, it was not studied whether activation of Treg can reduce psoriatic inflammation. Hence, we studied whether this can be achieved by SB. We did not only analyze skin and blood samples from patients with psoriasis and healthy controls but also utilized IMQ-induced inflammation (van der Fits et al., 2009). IMQ induced an inflammatory response characterized by thickening of the skin, erythema, and scales. Enhanced expression of IL-17 was detected as well as reduction of IL-10 and Foxp3, implying a reduction of Treg by IMQ. The topical application of IMQ even caused a systemic inflammatory response, which was associated with pronounced splenomegaly. As in the skin, a higher frequency of IL-17–expressing cells and lower frequencies of IL-10– and Foxp3–expressing cells were found.

Similar findings were observed in humans; a lower frequency of Foxp3–expressing cells was detected in the blood of patients with psoriasis than in that of controls. In addition, Treg isolated from patients with psoriasis were impaired in their suppressive activity. The same pattern was observed in the skin in situ. The number of Treg was decreased in lesional and, though to a lesser extent, nonlesional skin. This correlated with the cytokine expression levels, showing increased expression of IL-17 and IL-6 and reduced expression of IL-10. In this context, it is important to mention that IL-6 is regarded as a master switch dictating whether the immune response is dominated by pro-inflammatory T helper type 17 cells or protective Treg (Bettelli et al., 2006). Because the latter analysis was done with skin samples, we cannot discern whether the cytokine alterations observed, especially those observed upon administration of SB, are a direct effect of Treg or indirect through other cells. This is a limitation of the ex vivo approach.

Together, these data indicate an alteration both in the number and function of Treg. Thus, our findings join the group of studies that described the alteration of Treg in psoriasis. However, one has to be aware that this alteration is certainly not the only mechanism being involved and it is still unclear whether alteration of Treg is a cause or consequence of the many other pathophysiological events in psoriasis. For example, on one hand, diminished expression of IL-10 can result in a decreased frequency of Treg, and on the other hand, a reduced number of Treg can be responsible for the decreased expression of IL-10.

Irrespective of these uncertainties, we studied whether these alterations can be modulated. On the basis of our previous findings that SCFA, especially SB, can mitigate allergic contact dermatitis most likely by modulating Treg (Schwarz et al., 2017), we studied whether the alterations of Treg and the cytokine disbalance in psoriasis can be corrected by SB. The topical application of SB reduced IMQ-driven cutaneous inflammation. Surprisingly, though only topically applied, SB reduced also the systemic inflammatory response, demonstrated by the reduction of splenomegaly and IL-17 expression and induction of IL-10 and Foxp3 in the spleen. Because we have not done penetration studies, it is unclear whether topically applied SB reaches the spleen at relevant concentrations. If this is not the case, then the alterations in the spleen have to be a consequence of the processes in the skin induced by IMQ. Splenomegaly during an autoinflammatory process is a feature more typical for lupus erythematosus than for psoriasis. Hence, the IMQ model has also been suggested as a novel model of inducible systemic lupus erythematosus in wild-type mice, although in this setting, IMQ was applied for at least 4 weeks (Yokogawa et al., 2014). Thus, it is tempting to speculate to utilize SCFA as a therapeutic approach also in this model.

The effects of SB are critically dependent on Treg because they were lost upon depletion of Treg, as demonstrated in the depletion of regulatory T cell mice. SB appears to restore the reduced frequency of Treg in IMQ-treated skin, as demonstrated by in situ staining and by the enhanced expression of Foxp3. Similar observations were made in the human system. Number of Treg was enhanced upon treatment of skin explants obtained from patients with psoriasis with SB. However, SB does not only appear to induce the number of Treg but also to increase the activity of Treg obtained from PBMC, as demonstrated in an in vitro suppression assay.

There is recent evidence that the suppressive activity of Treg is under epigenetic control (Kitagawa and Sakaguchi, 2017). Acetylation of H3 histones was found to be associated with the activity of Treg. Accordingly, the acetylation of H3 histones derived from the Treg of patients with psoriasis was significantly decreased compared with H3 histones obtained from healthy controls. Histone acetylation was upregulated by SB, implying that SB may act as a histone deacetylation inhibitor. This activity of SB was already
described in other systems by demonstrating that SB regulates intestinal macrophage function through histone deacetylase inhibition (Chang et al., 2014). Very recently, it was shown that butyrate fosters the antimicrobial defense by driving differentiation from monocytes to macrophages through histone deacetylase 3 inhibition (Schulthess et al., 2019).

Together, these data indicate that psoriasis is associated with an alteration of Treg both in frequency and in function. This may be due to or at least associated with an altered cytokine milieu. SCFA appear to restore these alterations. Hence, SCFA may harbor a therapeutic potential for psoriasis. Whether this is the case has to be proven in clinical studies addressing the effect of topically applied SCFA in psoriasis. The utilization of SCFA for therapeutic purposes appears to be attractive because SCFA are natural compounds that lack toxicity.

**MATERIALS AND METHODS**

**Mice**

The 8–9-week-old female C57BL/6j mice (Janvier Labs, Le Genest Saint Isle, France) were housed in the central animal facilities of the University Clinics Schleswig-Holstein (Kiel, Germany). Animal care was utilized by expert personnel under specific pathogen-free conditions in compliance with relevant laws and institutional guidelines. Experiments were approved by the Institutional Review Board. C57BL/6j mice were treated with IMQ cream (Aldara) and received 62.5 mg on the shaved backs and ears for 10 days. One group of IMQ-treated mice received topically SB (1 mM in a 7:3 mixture of 1,2-propandiol:isopropyl alcohol) on the back skin and ears for 3 days. Furthermore, we utilized the depletion of Treg mice, which express a DT receptor-enhanced GFP under the control of the Foxp3 gene (Lahl et al., 2007).

**Real-time qPCR**

RNA was extracted by using peqGOLD TriFastTM (VWR, Darmstadt, Germany), treated with Dnase I, and converted to complementary DNA by reverse transcription. Quantitative detection of Foxp3, IL17, IL10, as well as human FOXP3 was performed with StepOnePlus system (Applied Biosystems, Life Technologies, Darmstadt, Germany) using a SYBR Green Master Mix. Primer sequences are available upon request. Relative gene expression was normalized to ACTB and HPRT1 according to the delta Ct method.

**FACS analysis**

For intracellular cytokine staining, cell suspension was prepared from lymph nodes and spleens and stimulated with phorbol 12-myristate 13-acetate and ionomycin in the presence of brefeldin and monensin (Cell Stimulating Cocktail with Protein Transport Inhibitors [eBioscience, Frankfurt, Germany]) for 4 hours. After stimulation, cells were stained with FITC-conjugated anti-mouse CD25 (BioLegend, Fell, Germany), followed by fixation and permeabilization (Tru-Nuclear Transcription Factor, BioLegend). Finally, cells were stained for allophycocyanin-conjugated anti-mouse Foxp3 (eBioscience) and PE-conjugated anti-mouse IL-10 and IL-17A (BioLegend). For activation marker, the following antibodies were used: anti-mouse PE-CD69, PE-CD73, and PE-FR4 (BioLegend). Cells were stained for FITC-conjugated anti-mouse CD25 and activation markers, followed by intracellular staining for Foxp3. For analysis of human Treg, Tru-Nuclear Transcription Factor System was also used. Finally, cells were stained with PE-conjugated anti-human Foxp3 (BioLegend). Cells were analyzed using a CytoFlex (Beckman Coulter, Krefeld, Germany).

**Immunofluorescence**

For ex-vivo analysis, 6-mm-diameter punch biopsies were obtained from lesional and nonlesional skin of patients with untreated psoriasis. Institutional approval of experiments and written informed patient’s consent were obtained. Nonpsoriatic individuals presenting with basal cell carcinoma gave informed consent for using healthy excess tissue after surgery to provide control skin samples. The biopsy tissue was cut into two pieces and placed into a 12-well plate on sterile gauze saturated with DermaLife Medium. One half was treated topically with SB; the other half left untreated. After incubation for 24 hours, 5-mm thick paraffin-embedded tissue sections were stained with a polyclonal anti-human Foxp3 antibody (Affinity Bioscience, Cincinnati, OH), followed by secondary antibody (anti-rabbit Alexa 488; Life Technologies). Double-staining immunofluorescence analysis was conducted using a polyclonal anti-human Foxp3 antibody. The double-positive cells were identified by staining with anti-human CD25 antibody (eBioscience), followed by anti-mouse Alexa 594 (BioLegend). Cell nuclei were stained with DAPI (BioLegend). Sections were analyzed with an immunofluorescence microscope (Axiovert 40 CFL; Carl Zeiss, Hamburg, Germany).

**Isolation of human lymphocytes**

Human PBMC were isolated from the peripheral blood of healthy and psoriatic donors by Ficoll density gradient centrifugation (Lymphocytes Separation Media, Capricorn Scientific GmbH, Ebdsdorfergrund, Germany). Cells were separated in CD4+CD25+ and CD4+CD25− T cells by magnetic bead separation (CD4+CD25+ Regulatory T Cell Isolation Kit, Miltenyi Biotec, Gladbach, Germany).

**In vitro suppression assay**

CD4+CD25+ responder T cells (5 × 10^5 cells/ml) obtained from patients with psoriasis and healthy volunteers were seeded into 96-well plates and mixed with CD4+CD25− Treg. Treg and responder cells were mixed at the ratios of 1:2 and 1:4. CD4+CD25− cells alone served as a control. Anti-biotin MACSibead particles pre-loaded with biotinylated anti-CD2, anti-CD3, and anti-CD28 antibodies were added for T-cell stimulation (Miltenyi Biotec). After 4 days, cell proliferation was measured using Cell Counting Kit-8 (Sigma-Aldrich, Taufkirchen, Germany). Data are presented as percentage suppression.

**Acetylation assay**

The acetylation assay was performed according to the manufacturers’ instructions (ab115102-Histone H3 Acetylation Assay Kit, Abcam, Cambridge, UK). Histone acetylation of SB-treated T cells is presented as percentage change compared with that of untreated T cells.

**Statistical analysis**

Statistical analysis was performed using Student’s t-test. Values of P < 0.05 were considered statistically significant. Unless otherwise stated, data show one representative of three independently performed experiments. For multiple comparisons, we carried out one-way ANOVA to assess whether at least two groups significantly differ. For statistical testing of suppression assay, we log-transformed the data in order to stabilize variances and applied a one-sided t-test for independent samples on the log-transformed values.
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**Data availability statement**
No datasets were generated or analyzed during this study.

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**CONFLICT OF INTEREST**
The authors state no conflict of interest.

**AUTHOR CONTRIBUTIONS**
Conceptualization: AS, TS; Formal Analysis: AS; Funding Acquisition: AS, TS; Investigation: AS, RP; Methodology: AS; Resources: TS; Supervision: AS, TS; Validation: AS; Visualization: AS, RP; Writing - Original Draft Preparation: AS; Writing - Review and Editing: TS

**SUPPLEMENTARY MATERIAL**
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2020.04.031.

**REFERENCES**
Supplementary Figure S1. Administration of SB on the untreated skin has no effect. To study whether simple application of SB does affect the untreated skin, mice were topically treated with 100 μl SB (1 mM in a 7:3 mix of 1,2-propandiol:isopropyl alcohol) for 3 days. Some groups obtained IMQ or IMQ and SB (see results). (a) Back skin thickness and (b) ear swelling were measured. (d) In addition, biopsies were taken and paraffin-embedded sections stained with H&E. (c) Application of SB on untreated control skin did not cause modification of the skin, whereas IMQ-induced acanthosis, hyperkeratosis, and an inflammatory infiltrate were completely reversed by SB. The vehicle had not such an effect. (a) *P < 2.3 × 10^{-4} versus Co; **P < 2.57 × 10^{-7} versus IMQ, P_{Anova} = 3.1 × 10^{-7}
(b) *P < 7.0 × 10^{-5}; **P < 0.02; P_{Anova} = 9.4 × 10^{-7} (d) Bar = 100 μm. Co, healthy control; IMQ, imiquimod; SB, sodium butyrate.
Supplementary Figure S2. Co-expression of Foxp3 and CD3. Double-color immunofluorescence analysis with anti-human Foxp3 (green) and anti-human CD3 (red) antibody revealed that the majority of Foxp3$^+$ cells also express CD3. Bar = 10 μm.