RESEARCH ARTICLE

Mechanism of anti-inflammatory effects of rifampicin in an ex vivo culture system of hidradenitis suppurativa

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Abstract

Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease of the hair follicles leading to painful lesions, associated with increased levels of pro-inflammatory cytokines. Numerous guidelines recommend antibiotics like clindamycin and rifampicin in combination, as first-line systemic therapy in moderate-to-severe forms of inflammation. HS has been proposed to be mainly an auto-inflammatory disease associated with but not initially provoked by bacteria. Therefore, it has to be assumed that the pro-inflammatory milieu previously observed in HS skin is not solely dampened by the bacteriostatic inhibition of DNA-dependent RNA polymerase. To further clarify the mechanism of anti-inflammatory effects of rifampicin, *ex vivo* explants of lesional HS from 8 HS patients were treated with rifampicin, and its effect on cytokine production, immune cells as well as the expression of Toll-like receptor 2 (TLR2) were investigated. Analysis of cell culture medium of rifampicin-treated HS explants revealed an anti-inflammatory effect of rifampicin that significantly inhibiting interleukin (IL)-1β, IL-6, IL-8, IL-10 and tumour necrosis factor (TNF)-α production. Immunohistochemistry of the rifampicin-treated explants suggested a tendency for it to reduce the expression of TLR2 while not affecting the number of immune cells.

KEYWORDS
anti-bacterial agents, anti-inflammatory agent, hidradenitis suppurativa, rifampin, therapeutics

1 | INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease caused by the obstruction of the hair follicle. It manifests in form of profound painful nodules and abscesses up to the formation of fistulas and sinus tracts. Areas involved in men and women are axillae, inguinal, gluteal and perianal whereby women can additionally be affected sub-mammary.[1] Many patients are smokers and suffer from comorbidities such as obesity, type 2-diabetes, chronic inflammatory bowel diseases and depression. This is associated with a considerable reduction of quality of life.[2,3] So far, the exact pathophysiological mechanisms underlying HS have not been understood completely. However, hyperkeratosis and perifolliculitis in the pilosebaceous-apocrine unit are assumed to precede the occlusion, inflammation and rupture of the hair follicle.[4–6] Subsequently, an increased inflammatory response due to the release of keratins,

damage-associated molecular patterns and bacteria[7] can lead to the formation of abscesses.[6] The inflammatory infiltrate found in HS lesions includes B cells,[8,9] T cells,[6,10–13] neutrophilic granulocytes, macrophages and dendritic cells.[10,14] Furthermore, pro-inflammatory cytokines such as Interleukin (IL)-1β, IL-6, IL-8, IL-12, IL-17, IL-23, macrophage colony-stimulating factor, interferon-γ, tumour necrosis factor (TNF)-α and anti-inflammatory IL-10 have been implicated to play an important role in the aberrant immune response of HS.[12,13,15–17] Besides, the inflammatory signalling pathways Notch, phosphoinositide-3-kinase/AKT, the inflammasome and the mechanistic target of rapamycin complex 1 show increased activity in HS.[14,18,19] Our limited understanding of the exact pathophysiological pathways contributing to the development and progression of HS, the treatment emphasizes the symptoms and the causes of the disease. The German National and European S1 guideline for the treatment of HS as well as the North American clinical management guideline recommend the combination of the antibiotics clindamycin and rifampicin as first-line systemic therapy for moderate-to-severe forms of HS.[20–22] This treatment is mainly given to suppress inflammatory processes, while it reduces bacterial colonization of the hair follicle,[20] which is often observed as a secondary infection.[23] There is an ongoing scientific controversy about the application of rifampicin and clindamycin in combination or rather as monotherapy in HS treatment.[24,25] Since rifampicin has already been shown to decrease clindamycin levels in patients by 90% through induction of p450 enzymes within approximately 10 days, it can be assumed that the effect of clindamycin and rifampicin in combination could be provoked by rifampicin completely.[26] Therefore, we examined the immunomodulatory properties of rifampicin in more detail. Rifampicin was used due to its inhibition of DNA-dependent RNA polymerase in bacteria.[27,28] Additionally, there is evidence that the drug initiates anti-inflammatory effects that have not been further elucidated. So far, a mechanism of action whereby rifampicin inhibits the lipopolysaccharide (LPS)-induced secretion of IL-1β and TNF-α has been described in cell lines of mononuclear cells, BV-2 microglial cells, RAW 264.7 macrophages as well as primary microglia and macrophages.[27,29,30] Furthermore, lower levels of IL-6 and IL-10 after rifampicin treatment were demonstrated in an *in vitro* model of Parkinson's disease and an *in vivo* model of endotoxin shock.[31,32] Also, upon rifampicin treatment, a reduction of the pattern recognition receptor Toll-like receptor (TLR) 2 expression was demonstrated in *in vitro* models of neurodegenerative disease, immune-regulatory models and animal model of multiple sclerosis (MS).[30,33–36] While TLR2 is expressed on both immune cells and keratinocytes,[37] it has also been reported to be elevated in HS.[10] TLR2 activation leads to the production of inflammatory cytokines, for example IL-1β or TNF-α. Upregulation of TLR2 expression is associated with inflammation and chronic wound healing disorder.[37] Treatment with rifampicin for 1 hour inhibits LPS-induced TLR2 and cytokine expression in monolayer cultures of RAW 264.7 and neutrophils.[35,36]

The aim of our investigation was to determine the anti-inflammatory potential of rifampicin in HS on molecular level, which was never investigated before. Therefore, we examined the effects of rifampicin on different immune cells and TLR2 by immunohistochemistry of the explants and cytokine analysis of the medium.

2 | MATERIAL AND METHODS

2.1 | Materials

Both rifampicin and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich (Darmstadt, Germany). Rifampicin was dissolved in 100% DMSO and adjusted to a final concentration ranging from 0.05 to 1.2 mM by diluting it with culture media.

2.2 | Ethics

Lesional skin was obtained from excised skin after radical surgery of clinically confirmed HS at the Department of Dermatology, Venerology and Allergology of the University Hospital in Frankfurt am Main, Germany, from September 2018 to January 2021. The examined tissue stems from 8 donors from a total of 10 surgeries with active inflammatory lesions. All work on this tissue was carried out in accordance with the Declaration of Helsinki and in agreement with the ethics committee of the University Hospital Frankfurt am Main (Local Ethics Commission/institutional review board). The surgical material stems from anonymous donors so that no patient-related data were collected.

2.3 | Ex-vivo skin culture treatment

Six millimetre punch biopsies were taken from actively inflamed HS lesions. The biopsies were instantly transferred into a transwell system (ThinCert™; Greiner bio-one, Kremsmünster, Austria) and placed into an insert of a 24-well plate with the epidermis pointing upwards in the air–liquid interface. The *ex vivo* explants were cultured for 2 or 24 h at 37°C in the presence of 5% CO_2 in a humidified atmosphere. The culture media consisted of Iscove's Modified Dulbecco's medium (Gibco, Carlsbad, USA) supplemented with 0.5% human AB serum (TCS Bioscience)[17] in the presence or absence of rifampicin (0 –1.2 mM) or DMSO, respectively, and was pipetted below the insert. After treatment, the biopsies were fixed in 4% formalin and the culture medium was collected, frozen and stored at −20°C until further investigation.

2.4 | Immunohistological staining and analysis

Fixed biopsies were embedded in paraffin, sectioned at 4 μM and stained with haematoxylin (Merck, Darmstadt, Germany) and eosin (Microm International, Dreieich, Germany). Immunohistological staining was performed using anti-cCasp3 (Cell Signaling Technology Cat# 9664, RRID: AB_2070042), anti-CD3 (BioGenex

Cat# AM322-5 M RRID: AB_2893301), anti-IL-1β (Novus Cat# NB600-633, RRID: AB_10001060), anti-IL-8 (Santa Cruz Cat# sc-376750 RRID: AB_2891256), anti-Ki-67 (DCS Cat# KI681, RRID: AB_2722785), anti-MPO (Cell Signaling Technology Cat# 14569, RRID: AB_2798516), anti-TLR2 (Thermo Fisher Scientific Cat# PA5-20020, RRID: AB_11151892) and anti-TNF-α (Abcam Cat# ab6671 RRID: AB_305641). As a negative control, the same biopsy was stained with an isotype antibody of irrelevant specificity. Immunostaining was visualized using the alkaline phosphatase method and by haematoxylin cell nucleus staining. To quantify the CD3, interleukin (IL)-1β, IL-8, myeloperoxidase (MPO), Toll-like receptor (TLR) 2 and tumour necrosis factor (TNF)-α staining, three randomly selected fields per biopsy were counted at 40-fold magnification with an Olympus CKX41 microscope. CD3, IL-1β, IL-8, MPO, TLR2 and TNF-α-positive cells were then normalized to total counted cells, and the mean was calculated.

2.5 | Cytokine assays

To assess the influence of rifampicin treatment on the cytokine profile of HS, ex vivo explants, IL-1β and TNF-α were measured in the culture medium after treatment using enzyme-linked immunosorbent assay (R&D systems, Minneapolis, USA). In addition, a cytometric bead assay (BD Bioscience) was performed to simultaneously measure further cytokines (IL-1β, IL-6, IL-8, IL-10 and TNF-α). Both assays were used according to the manufacturer's instructions.

2.6 | Statistical analysis

Column statistics were performed to calculate the mean and standard deviation (SD). The Kruskal–Wallis test followed by Dunn's multiple comparison test was used to evaluate statistical significance of the cytokine assays; a p value ≤.05 was considered significant. The Wilcoxon–Mann–Whitney U-test was performed to evaluate statistical significance of immunohistochemically quantification; a p value ≤.05 was considered significant. Box plots were used to visualize the biological variance within the group. For statistical calculation, GraphPad Prism version 5 (GraphPad Software, Inc.) was used.

3 | RESULTS

HS explants were cultivated with 0.05 and 1.2 mM rifampicin and examined by immunohistochemistry. Rifampicin concentrations used were based on therapeutic doses in HS. Haematoxylin–eosin (HE) staining revealed no effect on the morphology of the epidermis and dermis when compared to untreated controls (Figure 1). Staining of cCasp3 suggested no apoptotic activity while Ki-67 staining indicated that rifampicin treatment did not affect the ability of keratinocytes to proliferate (quantification data not shown). DMSO controls

did not show any effect compared to untreated controls (data not shown).

To investigate the influence of rifampicin on inflammatory processes, cytokine release into the medium of the ex vivo explants after treatment with different concentrations of rifampicin was determined. Treatment with 1.2 mM rifampicin for 24 h leads to a significant reduction of TNF-α and IL-1β secretion measured by enzyme-linked immunosorbent assay (Figure 2A and B) and cytometric bead assay (Figure 2C and D). In addition, treatment with 1.2 mM rifampicin for 24 h resulted in the reduction of both pro-inflammatory IL-6 and IL-8 and the anti-inflammatory IL-10 in the medium of explants as measured by cytometric bead assay (Figure 2E to G). The immunohistochemical staining of TNF-α and IL-1β of the rifampicin-treated explants also showed an inhibitory trend, which was not statistically significant (Figure S1A and B). Treatment of explants with 1.2 mM rifampicin resulted in significantly decrease of IL-8(+) cells (Figure S1C). DMSO controls did not show any effect compared to untreated controls (data not shown).

As effects of rifampicin on immune cells have previously been reported using immunohistochemistry, we analysed whether rifampicin also has an impact on the immune cell content of HS explants. Quantification of immune cells after 2 and 24 h of rifampicin treatment showed no effect on the number of CD3(+) T cells and neutrophils (Figure 3A and B). The mean values and standard deviations of the quantification are listed in Table 1. DMSO controls did not show any effect compared to untreated controls (data not shown).

Furthermore, we examined whether the anti-inflammatory effect on cytokine release in HS explants after rifampicin treatment is due to TLR2 signalling. Therefore, the explants were stained with an anti-TLR2 antibody, and TLR2 expression was quantified. As TLR2 is expressed on both keratinocytes and immune cells, it was differentiated between epidermal and dermal TLR2 expression. Treatment with rifampicin for 2 and 24 h did not significantly reduce the number of TLR2(+) cells in the Stratum papillare of the dermis (Figure 4A and B). The mean values and standard deviations of the quantification are listed in Table 2. In addition, rifampicin treatment had no statistically significant effect on TLR2 expression on the epidermis. DMSO controls did not show any effect compared to untreated controls (data not shown).

4 | DISCUSSION

The present investigation highlights the potential of rifampicin, an antibiotic and first-line medication in the treatment of moderate-to-severe HS, to significantly reduce the release of IL-1β, IL-6, IL8, IL-10 and TNF-α in stable HS lesional explant cultures upon 24 h of treatment, while immunohistochemistry did not show a significant reduction of IL-1β(+) and TNF-α(+) cells. We assume that this difference is due to the fact that the concentration of cytokines in the tissue was not high enough in contrast to the concentration in the medium to detect a significant effect. However, in this context, we are the first to take a closer look at the mechanism of rifampicin.
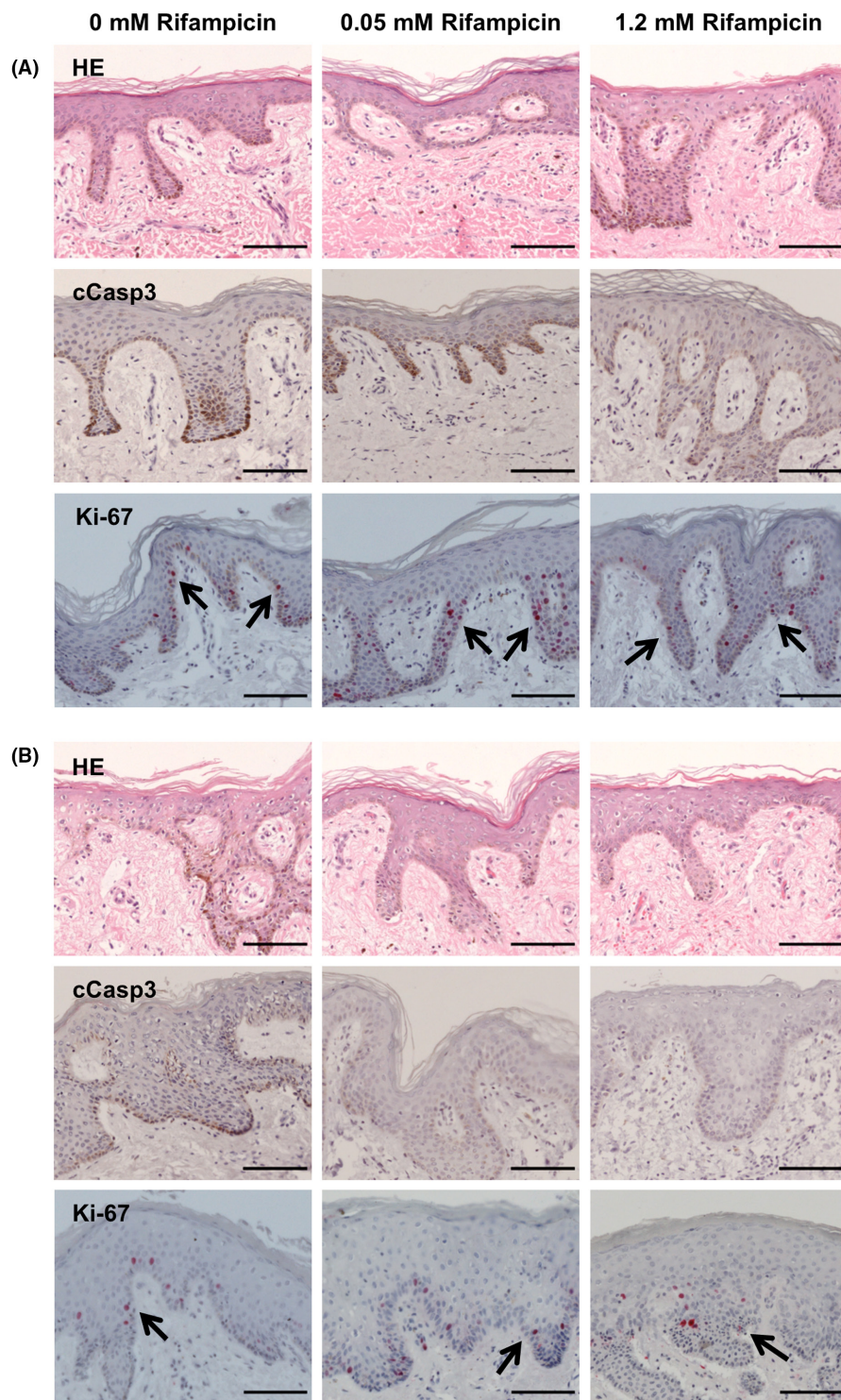
0 mM Rifampicin **0.05 mM Rifampicin** **1.2 mM Rifampicin**

FIGURE 1 Staining of explants treated with rifampicin. Explants of hidradenitis suppurativa tissue were fixed in formalin after treatment with different concentrations of rifampicin for (A) 2 and (B) 24 h. The biopsies were stained with haematoxylin–eosin (HE), anti-cleaved-Caspase 3 (cCasp3) and anti-Ki-67 antibody, visualized with the permanent AP Red kit and imaged using a Nikon Eclipse Ni microscope. Representative sections are shown (A) $n = 5$ from three donors; (B) $n = 9$ from five donors; scale bar = 100 μM)

Previous clinical trials indicated an improvement of HS under combined systemic therapy using clindamycin and rifampicin.[26,38–40] Additionally, rifampicin demonstrated anti-inflammatory effects in the context of other immune-mediated chronic diseases such as psoriasis.[28] Ziglam et al. studied LPS-induced cytokine levels following rifampicin treatment of primary monocytes in an *in vitro* setting and showed reduced release of IL-1β and TNF-α, which was consistent with our findings. However, in contrast to our observations, the authors described increased levels of both IL-6 and IL-10.[27] This

could be explained by the different cell types. While our observations stem from heterogeneous HS immune cell infiltrate, Ziglam et al. investigated the effects on primary monocytes only.[41] Another difference is the lower drug concentration used by Ziglam et al. Instead of concentrations ranging between of 5 μg/ml and 20 μg/ml, our observations were made at rifampicin concentrations of 41 and 988 μg/ml (0.05 and 1.2 mM) respectively. They were much higher than the actual serum concentrations (5–13 μg/ml) seen in patients 2 h after the administration of 450 mg rifampicin.[42] Based on the
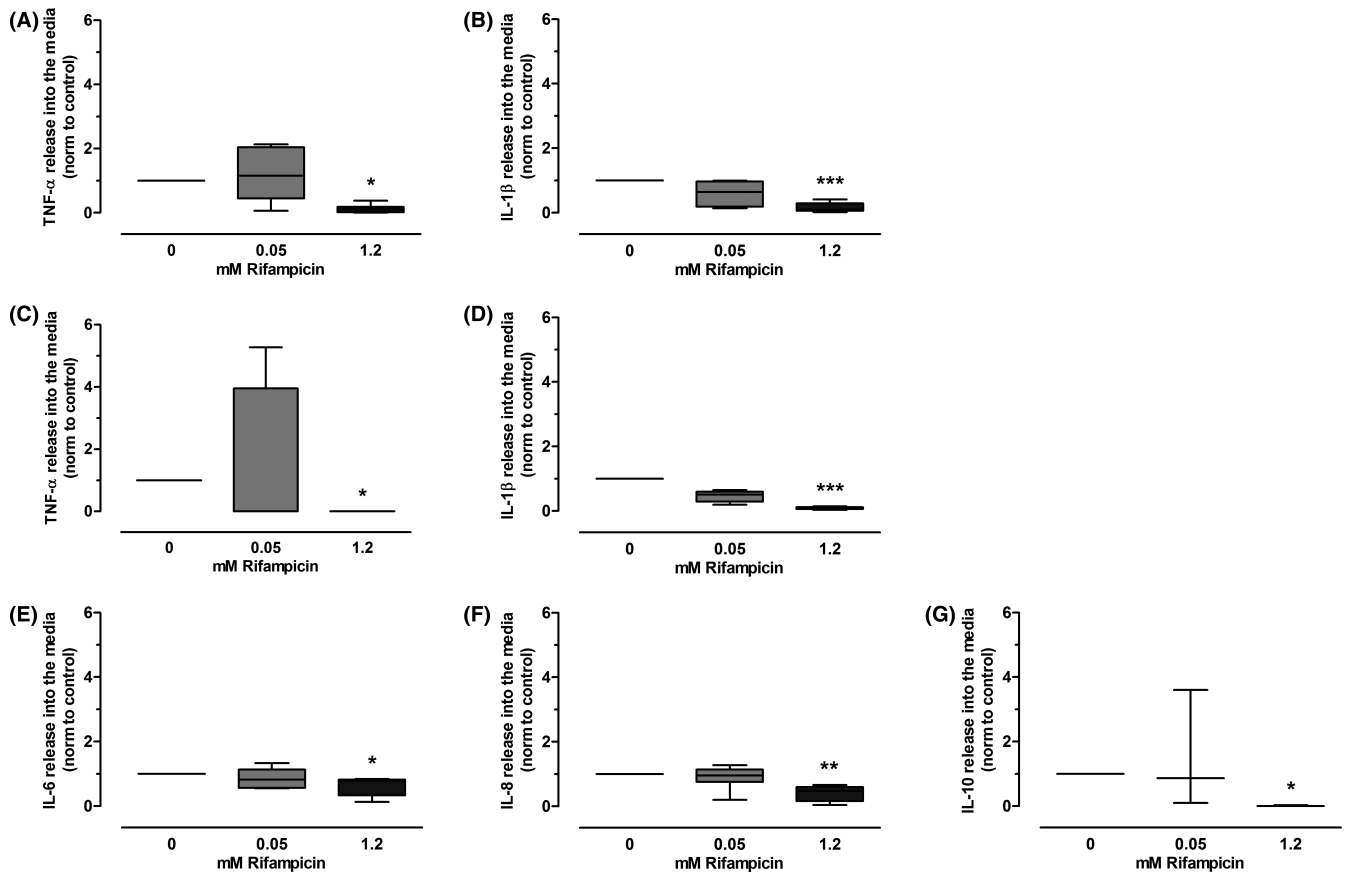
FIGURE 2 Cytokine release of the explants after rifampicin treatment. Explants of hidradenitis suppurativa tissue were treated with different concentrations of rifampicin for 24 h. The cytokines in the medium were measured by (A) and (B) enzyme-linked immunosorbent assay and (C) to (G) cytometric bead assay. The values were normalized to 0 mM Rifampicin and illustrated as whisker plot (A) $n = 6$ from three donors; (B) $n = 6$ from five donors; (C) $n = 4$ from two donors; (D) $n = 5$ from two donors; (E) $n = 6$ from three donors; (F) $n = 6$ from three donors; (G) $n = 3$ from two donors). P-values were calculated using Kruskal–Wallis test followed by Dunn's multiple comparison, $p^*<.05$, $p^{**} <.01$, $p^{***} <.001$

common dosing schemes applied in the treatment of HS patients, higher tissue concentrations can be assumed. The LPS-induced releases of IL-1β and TNF-α were not observed in HS explants only but also in BV-2 cells and RAW 264.7 macrophages. LPS-induced reduction of TNF-α was reported to occur in rifampicin-treated primary microglia, astrocytes and macrophages previously.[29] In contrast, rifampicin did not inhibit the LPS-induced upregulation of IL-1β and TNF-α mRNA levels in polymorphonuclear leukocytes but instead reduced the mRNA level of the IL-8 receptor.[36] In an *in vivo* model of MS, treatment with rifampicin resulted in a decreased serum level of IL-6 and IL-17A.[33] These findings *in vitro* and *in vivo* concur with our observations and indicate the potential of rifampicin to mediate anti-inflammatory responses also in patients affected by HS.

Compared to healthy skin, there are elevated amounts of CD3(+) T cells in the skin of HS patients.[10,11] To investigate whether treatment with rifampicin affects CD3(+) T cells in HS, we quantified CD3(+) T cells in explants of HS skin after treatment with rifampicin. To our knowledge, the influence of rifampicin on CD3(+) T cells has not yet been investigated. In an *in vivo* model of MS, treatment with rifampicin resulted in an inhibition of the differentiation of Th17 cells in the central nervous system. MS is characterized by

demyelination and neuro-inflammatory infiltrate, which primarily involves CD4(+) T cells.[33] It is known that IL-17 expressing CD4(+) T cells also play a role in maintaining inflammatory processes in HS.[6,12,13] Treatment with rifampicin did not affect the number of CD3(+) T cells in the explants but might modify their inflammatory expression profile resulting in decreased inflammation. Rifampicin is known to regulate cytochrome p450 enzymes via the pregnane X receptor (PXR). The PXR belongs to the nuclear receptor family and is expressed in the liver and intestine. In addition, B and T cells have also been shown to express the PXR. In this context, Dubrac et al. showed *in vitro* that agonists of the PXR, such as rifampicin and RU-486, a glucocorticoid receptor antagonist, inhibit T-cell functions as well as the synthesis and production of interferon-γ.[43] This mechanism may also be responsible for the anti-inflammatory effect of rifampicin in HS explants. Besides CD3(+) T cells, neutrophilic granulocytes are increased in HS.[8,14] Neither 2 h nor 24 h of rifampicin treatment affected the number of neutrophils in the HS explant cultures. Neutrophils are involved in acute inflammation through a variety of functions. They are activated by different cytokines like IL-8 and growth factors, which attract them to the site of inflammation.[44] *In vitro* studies showed an inhibited chemotaxis
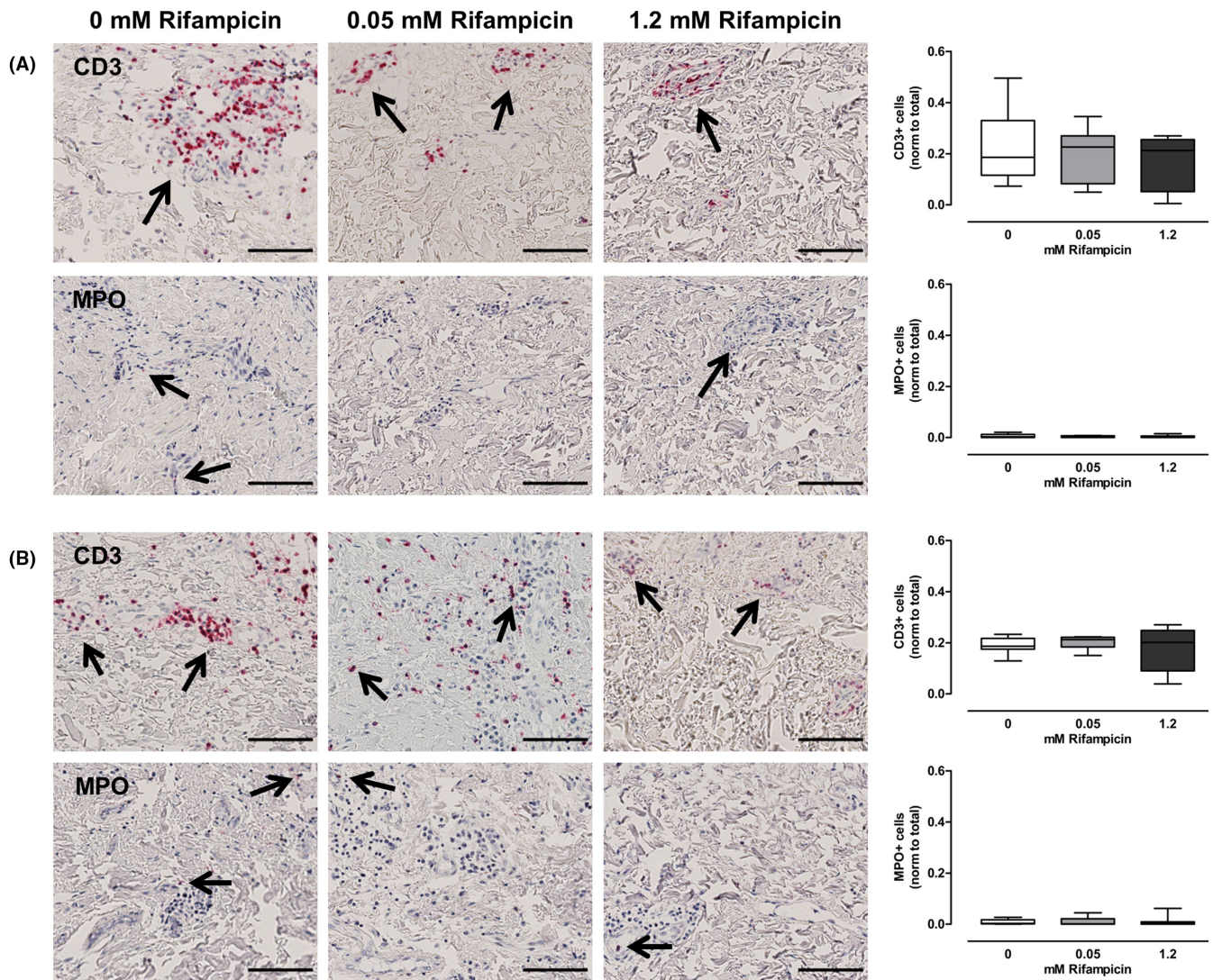
FIGURE 3 Immunohistological detection and quantification of CD3(+) T cells and neutrophil granulocytes (MPO+) in the *Stratum papillare* of the dermis in explants treated with rifampicin. Explants of hidradenitis suppurativa tissue were fixed in formalin after treatment with different concentrations of rifampicin for (A) 2 and (B) 24 h. The biopsies were stained with anti-CD3 and anti-MPO antibodies, a marker for neutrophile granulocytes, visualized with the permanent AP Red kit and images were acquired by a Nikon Eclipse Ni microscope. The number of positive cells was normalized to total cells in three randomly selected fields. Representative sections are shown, and the quantification is illustrated as whisker plot (A) n CD3(+) =7 from four donors, n MPO(+) =7 from four donors; (B) n CD3(+) =7 from 5 donors, n MPO(+) =7 from six donors; scale bar = 100 µM; black arrows = CD3(+) and MPO(+) cells respectively). *p*-values were calculated using Wilcoxon–Mann–Whitney *U*-test, which were not statistically significant

TABLE 1 Quantification of immunohistological detection of CD3(+) T cells and neutrophil granulocytes (MPO+) in the *Stratum papillare* of the dermis in explants treated with rifampicin (mean ± SD)

Time point	Immune cell	0 mM rifampicin mean ± SD	0.05 mM rifampicin mean ± SD	1.2 mM rifampicin mean ± SD
2 h	CD3(+)	0.2316 ± 0.1500	0.1956 ± 0.1049	0.1645 ± 0.1080
	MPO(+)	0.0069 ± 0.0078	0.0033 ± 0.0035	0.0035 ± 0.0055
24 h	CD3(+)	0.1891 ± 0.0335	0.2033 ± 0.0270	0.1683 ± 0.0867
	MPO(+)	0.0090 ± 0.0101	0.0100 ± 0.0171	0.0122 ± 0.0223

of rifampicin-treated human neutrophils.[36,45] It is possible that prolonged treatment, as in systemic therapy, may affect these cells. Furthermore, Spisani et al. demonstrated concentration-dependent inhibition of phorbol 12-myristate 13-acetate-induced superoxide in human neutrophils.[45] The *in vitro* data suggest that rifampicin affects neutrophil function, which we cannot comment on due to limitations in the experimental setup. We only examined the number of neutrophils after treatment with rifampicin.
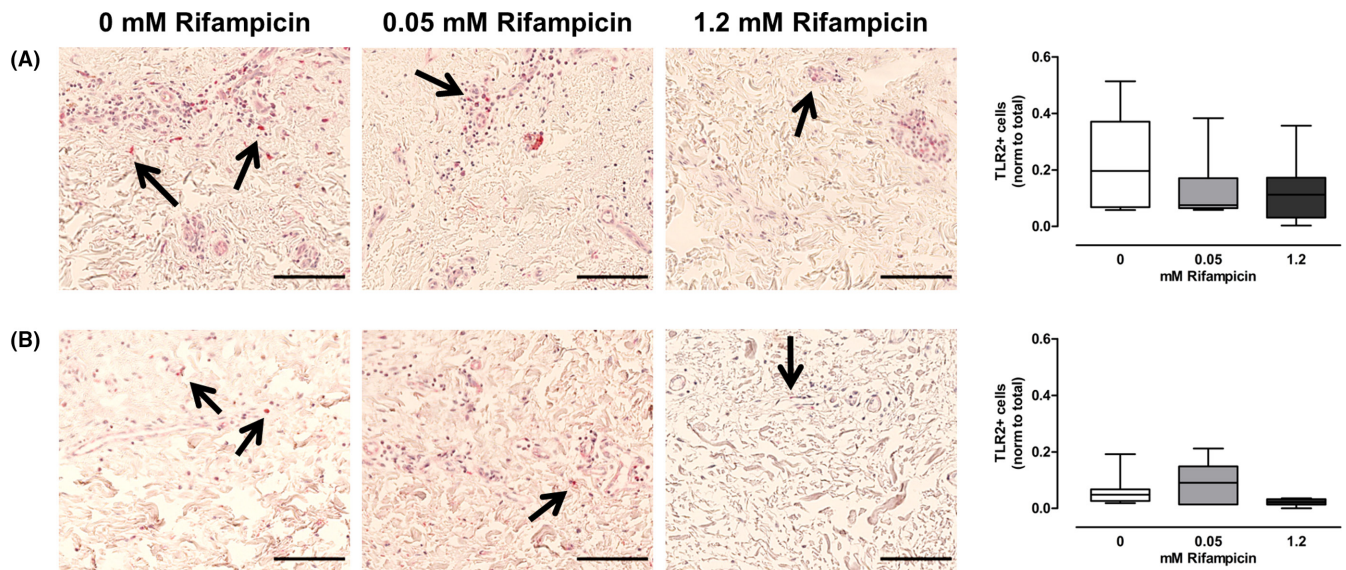
FIGURE 4 Immunohistological detection and quantification of Toll-like receptor (TLR) 2+ cells in the *Stratum papillare* of the dermis in explants treated with rifampicin. Explants of hidradenitis suppurativa tissue were fixed in formalin after treatment with different concentrations of rifampicin for (A) 2 and (B) 24 h. The biopsies were stained with anti-TLR2 antibody, visualized with the permanent AP Red kit and images were acquired by a Nikon Eclipse Ni microscope. Counted positive cells were normalized to total cells in three randomly selected fields. Representative sections are shown, and the quantification is illustrated as whisker plot (A) $n = 7$ from 4 donors; (B) $n = 7$ from four donors; scale bar = 100 μM). *p*-values were calculated using Wilcoxon–Mann–Whitney U-test, which were not statistically significant

TABLE 2 Quantification of immunohistological detection of Toll-like receptor (TLR) 2+ cells in the *Stratum papillare* of the dermis in explants treated with rifampicin (mean ± SD)

Time point	0 mM rifampicin mean ± SD	0.05 mM rifampicin mean ± SD	1.2 mM rifampicin mean ± SD
2 h	0.2128 ± 1.7220	0.1405 ± 0.1172	0.1276 ± 0.1183
24 h	0.0619 ± 0.0592	0.0856 ± 0.0747	0.0217 ± 0.0125

Additionally, rifampicin spontaneously oxidizes in aqueous environments to rifampicin quinone.[31] Therefore, we cannot conclude that the effects observed in this study are caused by rifampicin alone. Degradation increases lipophilicity, which potentially affects membrane permeability. In this context, previous studies reported microglial pre-treatment with rifampicin quinone to significantly reduce the inflammatory response and neuronal damage.[46] This specific effect of the oxidized compound could be due to the physicochemical properties, leading to enhanced intracellular availability of the drug.

Rifampicin is associated with immunomodulating effects via activation of the glucocorticoid receptor[47] and inflammatory signalling pathways, such as nuclear factor κ of activated B cells (NFκB),[29,35] Janus kinase-signal transducer, and activators of transcription[30] and TLR2.[35,36] Immune cells, for example monocytes, dendritic cells, B cells and T cells, as well as keratinocytes, express TLR2. The activation by its ligands lipoprotein or peptidoglycan can initiate mitogen-activated protein kinases or NFκB signalling pathways. This leads to increased gene transcription and production of the pro-inflammatory cytokines IL-1β, IL-6, IL-12 and TNF-α, the chemokine IL-8 and initiates subsequent immune responses.[48] Both mRNA and immunohistochemistry expression levels of TLR2 have been reported to be increased in HS.[10] Therefore, the effect of

rifampicin on TLR2 expression was examined. We observed a non-significant lower TLR2 expression after 2 h of treatment with rifampicin in the dermis of our explant cultures. This trend continued even after 24 h of treatment, while no differences in TLR2 expression were observed in the epidermal cells. Kim et al. and Mu et al. demonstrated inhibition of LPS-induced TLR2 mRNA level in a dose-dependent manner in a macrophage cell line and primary neutrophils.[35,36] Furthermore, Kim et al. reproduced this finding by stimulating macrophages with BAY 11–7085, an NFκB inhibitor, instead of rifampicin, indicating an NFκB suppressing activity and thus an anti-inflammatory effect. In addition, a concentration-dependent decrease of TNF-α mRNA expression, very similar to the reduced TLR2 mRNA expression, was observed.[35] Although we were not able to detect significantly reduced TLR2 expression, the findings of Kim et al. and Mu et al. support our theory, that rifampicin might inhibit the secretion of pro-inflammatory cytokines by reducing TLR2 expression on infiltrating cells. When looking at the results, it, however, has to be noted that the experimental setups differ. While Kim et al. and Mu et al. measured mRNA expression of TLR2, our findings are based on protein expression via immunohistochemistry. More likely for the discrepancy between the results could be the increased biological variance and complexity of the *ex vivo* explants compared to *in vitro* cells.

In conclusion, rifampicin reduces the release of pro-inflammatory cytokines in *ex vivo* explants of lesional HS skin, supporting the immunomodulatory effects of rifampicin observed in the clinical practice. For the first time, we demonstrated that the anti-inflammatory effect of rifampicin in HS explants may be explained by a tendency to lower TLR2 expression levels on infiltrating cells leading to a significant decrease of IL-1β, IL-6, IL-8, IL-10 and TNF-α but not in a decrease in the number of immune cells. This effect of rifampicin is presumably to alter the inflammatory expression pattern of immune cells resulting in impaired pro-inflammatory cytokines. Whether rifampicin has an anti-inflammatory effect in HS via downregulating TLR2 has to be investigated in subsequent studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

AP, AK, CB, RK and IH conceived, designed and supervised the study. IH and TI performed the experiments. SD supported in running the enzyme-linked immunosorbent assay. Cytometric Bead Assay was performed in the laboratory of SS. CMW and MGW contributed to evaluating the data in an advisory capacity. AP, AK and IH interpreted the data. IH wrote the draft. All authors read and commented on the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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Supplementary material