Deficient cutaneous antibacterial competence in cutaneous T-cell lymphomas: role of Th2-mediated biased Th17-function


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Translational Relevance

Cutaneous bacterial infections, CTCL patients frequently suffer from, support lymphoma cell survival and may be life-threatening when the T-cell receptor repertoire becomes limited. In the context of the increased skin infection risk, we demonstrated a strongly limited up-regulation of ABPs in CTCL lesions, which was associated with specific deficiency of IL-17. The presence of other ABP-inducing cytokines did not compensate the IL-17 absence, demonstrating that synergistic action of several mediators is necessary for effective cutaneous anti-bacterial defense. IL-17 deficiency seems to be caused by specific inhibition of its production in Th17-cells by IL-4Rα ligands. Our results suggest that therapeutic IL-4Rα antagonizing (e.g., by blocking antibodies) may improve the prognosis of CTCL patients.
Abstract

Purpose: Primary cutaneous T-cell lymphomas (CTCL) are neoplastic disorders of skin-homing T-cells. Affected skin areas show morphological similarities with alterations in other T-cell-mediated dermatoses. Furthermore, like in atopic dermatitis (AD) but in contrast to psoriasis, CTCL patients are frequently afflicted by cutaneous bacterial infections that support the survival of lymphoma cells. Our aim was to investigate the mechanisms of elevated susceptibility to cutaneous infections in CTCL patients.

Experimental Design: Skin samples from CTCL, psoriasis, and AD patients were used to illuminate the antibacterial competence status and the presence of its modulating cytokines. For substantiation of findings, 3-dimensional epidermis models, isolated and in vitro generated Th-subpopulations were applied. Parameters were analyzed via qPCR and immunohistochemistry.

Results: CTCL lesions compared to psoriatic lesions presented an impaired up-regulation of antibacterial proteins (ABPs), with levels even below those in AD. This was associated with a relative deficiency of the ABP-inducing cytokine interleukin(IL)-17 and a strong presence of the ABP-down-regulating cytokine IL-13. The simultaneous presence of the Th17-cell cytokine IL-26 indicated that IL-17 deficiency in CTCL lesions results from functional deviation of Th17-cells. Accordingly, IL-17 but not IL-26 production by Th17-cells in vitro was inhibited by IL-4Rα ligand. Levels of other ABP inducers were comparable between CTCL and psoriasis lesions. The same was true regarding IL-22/TNF-α targets, including the keratinocyte hyper-regeneration marker K16 and the matrix-degrading enzyme MMP1.

Conclusion: Our results suggest that the cutaneous bacterial infections in CTCL are caused by impaired ABP induction as consequence of Th2-mediated biased Th17-cell function.
Primary cutaneous T-cell lymphomas (CTCL) are neoplastic lymphoproliferative disorders that are primarily localized in the skin. Mycosis fungoides (MF) and Sézary syndrome (SS) are two common CTCL forms, accounting for approximately 67% of cases. For the U.S.A. their incidence has been estimated to be approximately 3 to 8 cases per million inhabitants per year (1-3). Affected individuals are mostly older than 50 years. The clinical presentation of MF varies over time and includes early patches, plaques with more pronounced infiltration, and the advanced tumor stage. In SS the malignant cells are also found in the peripheral blood and lymph nodes; this condition is much more aggressive and associated with generalized pruritic erythroderma.

In its early stage, MF resembles lesions of T-cell-mediated dermatoses such as psoriasis or chronic atopic dermatitis (AD), in which mediators of mixed T-cell infiltrates, by altering skin cell biology, cause changes of skin architecture, including acanthosis and hyperkeratosis (4, 5). Only in later stages the MF diagnosis is facilitated by the profound intra-epidermal accumulations of lymphoma cells in the form of so-called Pautrier's microabscesses and by the occurrence of atypical nuclei of the lymphoma cells (2, 3). The etiology of MF/SS remains unknown. It has been hypothesized that MF cells are derived from skin-infiltrated, chronically activated Th-cells (6, 7). In fact, they are generally \(\text{CLA}^+ \alpha/\beta \text{TCR}^+, \text{CD4}^+, \text{CD45RO}^+, \text{and CD26}^{\text{dim}}\). However, the antigen(s) that are responsible for their primary stimulation has not been found, yet.

According to the altered skin architecture, already early stage MF lesions show an impaired skin barrier function. This, in turn, is associated with bacterial skin infections, e.g. with \(\text{Staphylococcus aureus}\) (8-10). Those infections support survival and expansion of lymphoma cells (11). In line with this, antibiotic treatment has been shown to lead to clinical improvement of lesions (8-10). Moreover, if those local infections spread systemically in connection with the impaired systemic T-cell repertoire in SS, they may become life-threatening (12, 13). Importantly, an impaired skin barrier function is also found in patients with psoriasis and AD (14, 15). However, only AD skin shows an increased incidence of bacterial infections, which is mainly due to the limited up-regulation of antibacterial proteins (ABPs) by the keratinocytes of these patients (16). In contrast, in psoriasis, the anti-microbial
competence of the disturbed skin is increased to a level that almost perfectly prevents cutaneous infection (16, 17).

ABP expression in the skin is known to be upregulated by specific cytokines, including those produced preferentially by monocytes/macrophages and keratinocytes [tumor necrosis factor(TNF)-α, interleukin(IL)-1β] and by T-cells [IL-17A, interferon(IFN)-γ, IL-22] (18-22). Moreover, mediators of Th2-cells including IL-4 and IL-13 reduce keratinocyte ABP levels (16, 23, 24). Previous studies demonstrated that the relative deficiency of the Th17-cell cytokine IL-17 and the overproduction of Th2-cell cytokines are responsible for the limited cutaneous ABP production in AD.

In this study, we aimed to shed light on the mechanisms underlying the increased bacterial infection risk of the skin in patients with MF.
Materials and methods

Patients

For analysis of mRNA expression, skin samples were obtained from control donors, psoriasis vulgaris patients (65.2% moderate to severe disease), MF patients (5 with patch stage MF, 2 with plaque stage MF, 3 with tumor stage MF), and AD patients (100% moderate to severe disease). Of the MF patients (age: from 52 to 89 years, 80% male), eight had no therapy at the time of study, one got topical steroids and one had undergone PUVA therapy. For immunohistochemistry analysis, skin biopsies were obtained from MF patients, psoriasis patients, and healthy volunteers (3 from each group). Samples were approved by the clinical institutional review boards of the Charité University Hospital, Berlin, or the Rockefeller University, New York, N.Y., and informed consent was obtained from each subject.

Cell culture

Underdeveloped EpiDerm-201™ reconstituted human epidermis (RHE; MatTek Corp.) was cultured as described previously (25) and stimulated or not (control) with a cytokine mix containing recombinant human IL-22 (1 ng/ml), IL-17A (1 ng/ml), IFN-γ (0.1 ng/ml), IL-20 (5 ng/ml), and TNF-α (0.1 ng/ml) or with the mix lacking one of the T-cell cytokines for 72 h.

Peripheral blood mononuclear cells (PBMC) were separated from citrated venous blood of healthy volunteers and SS patients by standard density gradient centrifugation using LeucoSep® tubes (Greiner) and Ficoll Paque Plus (Amersham and Biochrom). Th1, Th2, Th17 were isolated from PBMC by flow-cytometric sorting as described below and were stimulated via CD3/CD28 for 2 d. Naive CD4 T-cells were isolated from PBMC of healthy donors by the MACS™ system using the Naive CD4 T cell isolation kit II (Miltenyi Biotec)(mean purity 95.8 ±0.44%). Cells were stimulated for 8 d with Dynabeads® Human T-activator CD3/CD28 beads (Invitrogen; 1 bead/T-cell) in the presence of either IL-12 and anti-IL-4 mAb (Th1), IL-4 and anti-IFN-γ mAb (Th2), IL-1β, IL-6, IL-23, TGF-β1.2, anti-IL-4 mAb, and anti-IFN-γ mAb (Th17), IL-6, TNF-α, anti-IL 4 mAb, anti-IFN-γ mAb, anti-TGF-β1.2 mAb, and 6-formylindolo[3,2-b] carbazole (Enzo Life Sciences) (Th22), or IL-
10, TGF-β1.2, anti-IL-4 mAb, anti-IFN-γ mAb, anti-TGF-β1.2 mAb, and all-trans-retinoid acid (Sigma-Aldrich) (regulatory Th cells), as previously described (26). In further experiments, Th17-cell differentiation was carried out in the absence of IL-1β, IL-6, IL-23, or TGF-β or in the presence of 10 ng/ml IL-10. Finally, Th17-cells, generated during 8 d were stimulated for another day with anti-CD3/anti-CD28 Ab-coated Dynabeads in the presence and absence of 10 ng/ml IFN-γ or 20 ng/ml IL-4. Th1-, Th2-, and Th17-cells isolated from PBMC of healthy donors by FACS cell sorting (see below) were left unstimulated or were stimulated with anti-CD3/anti-CD28 Ab-coated Dynabeads (Invitrogen) for 2 d.

If not indicated otherwise, antibodies and recombinant cytokines were purchased from R&D Systems.

**Flow cytometry**

Cell sorting was done starting with PBMC by means of FACS Aria. Fluorescence-labeled Ab clones against the following molecules were used: CD4 (SK3; BD Biosciences), CXCR3 (49801; R&D Systems), CCR4 (255410; R&D Systems), CCR6 (11A9; BD Biosciences), and CCR10 (314305; R&D Systems). Th-cells were defined as CD4+ CXCR3+ CCR6- cells (Th1), CD4+ CCR6- CCR4+ cells (Th2), and CD4+ CCR6+ CCR4+ CCR10- cells (Th17).

To assess the purity of total and naïve CD4+ T-cell isolations, cells were stained with fluorescence-labeled Ab clones against the following molecules: CD3 (SK7), CD16 (3G8), CD45RA (HI100), CD45RO (UCHL1) (all from BD Bioscience), CD4 (13B8.2), CD8 (B9.11), CD14 (RMO52), CD19 (J4.119), CD56 (NCAM16.2) (all from Beckmann Coulter).

**Quantitative gene expression analysis**

Snap frozen skin biopsies and surgically excised skin areas were homogenized during thawing in lysing solution from the Invisorb® RNA kit II (Invitek/Stratec molecular GmbH) by means of the Omni labor homogenizer (Südlabor). Isolation of total cellular RNA from homogenized cells and tissues was performed using Invisorb® RNA kit II (Invitek/Stratec molecular GmbH). Reverse transcription of mRNA was performed as described previously (27). Quantitative PCR on reverse transcribed mRNA (qPCR) was performed in triplicate assays using universal master mix (Applied
Biosystems) or the Maxima™ Probe/ROX qPCR Master Mix (Thermo Fisher Scientific/Fermentas), sequence systems with double-labeled fluorescent probes and comparable amplification efficiency, and either the Stepone plus or the ABI Prism™ 7700 Sequence Detection System (both from Applied Biosystems). Oligonucleotide sequences used for expression analysis of β-defensin 2, β-defensin 3, IL-22, IL-26 and matching hypoxanthine phosphoribosyl-transferase 1 (HPRT) were published previously (18, 28). All other detection systems were purchased from Applied Biosystems. Expression levels were calculated relative to those of HPRT, which was analyzed in parallel with the matching analysis system.

Immunohistochemistry

Immunohistochemistry analysis was performed on frozen sections of human MF, psoriasis vulgaris, and normal skin samples (n=3 for each). Antibodies used in this study were anti-DEFB2 (Perpro Tech, goat polyclonal, diluted 1/20), anti-Lipocalin 2 (Abcam, mouse monoclonal, clone HYB211-01, diluted 1/100), and anti-MMP1 (Abcam, mouse monoclonal, clone SB12e, diluted 1/100). Sections were incubated with the primary antibody over night at 4°C. The staining signal was amplified using avidin-biotin complex (Vector Laboratories) for 30 min at room temperature and developed using chromogen 3-amino-9-ethylcarbazole. The microscope used was a Nikon Eclipse 50i with a Nikon DS-Fi1 camera.

Statistical analysis

Data are presented as the mean ± SEM. For further analyses, SPSS 14.0 software (SPSS Inc.) was used. Comparison of results between MF patients and control patients / participants were analyzed using the Mann-Whitney U-test (two-tailed). Correlations were analyzed based on the Spearman’s correlation coefficient. Results on paired in vitro treated cell cultures were tested using the Wilcoxon matched-pairs signed-rank test (two-tailed).
Results

CTCL skin lesions show a relative deficiency in ABP production

Similar to patients suffering from AD but in contrast to patients with psoriasis, CTCL patients have an increased susceptibility to cutaneous bacterial infections. We hypothesized that, like in AD patients, an attenuated increase of ABPs may be responsible. To investigate this hypothesis, mRNA expression of different ABPs including lipocalin 2, β-defensins, RNase 7, LL37, and S100 proteins was quantified by qPCR in skin lesions of MF patients (carrying patch stage, plaque stage, and tumor stage lesions). For comparison, analyses of healthy skin from control donors and lesional skin from patients suffering from psoriasis and from AD were included. As demonstrated in Fig. 1A, the expression of most ABPs was up-regulated in the patient groups compared to healthy skin. However, as it is the case for AD, the up-regulation in CTCL was strongly limited, with significantly lower levels of all ABPs in CTCL compared to those observed in psoriatic skin. A clear difference between CTCL and psoriasis was also found upon immunohistochemistry analyses of skin sections performed for lipocalin-2 and β-defensin 2 (Fig. 1B). In case of β-defensin 2 and 3, LL37, S100A7, and S100A9, the expression in CTCL was even lower than in AD lesions (Figure 1A). The relative deficiency in ABP expression may therefore account for the increased susceptibility to bacterial infections in CTCL patients.

CTCL lesions show reduced up-regulation of IL-17A expression and high levels of IL-13

We then analyzed the levels of cytokines known to induce (TNF-α, IL-1β, IL-17A, IFN-γ, IL-22) or downregulate (IL-13) epidermal ABP expression. As demonstrated by qPCR data, most of the analyzed cytokines were expressed at similar levels in CTCL compared to psoriatic lesions (Fig. 2A). However, two were not: IL-17A was found having a significantly lower expression while IL-13 expression was higher compared to psoriasis. Hence, the cytokine expression pattern in CTCL highly resembled that found in AD lesions (Fig. 2A).
The limited cutaneous IL-17A production may account for the low keratinocyte ABP levels in CTCL

We then asked how the differential cytokine expression patterns in CTCL compared to psoriasis lesions is related to the limited ABP expression. We first investigated the statistical relationship between lesional cytokine and ABP expression in both patient groups. As demonstrated by Tab. 1, the positive statistical correlation between IL-17A and most of the analyzed ABP expressions indeed supported the idea of a causal relationship of deficient upregulation of IL-17A expression and low ABP expression in CTCL versus psoriasis. No correlation was found between IL-13 and ABP expression (Tab. 1). We additionally performed in vitro studies with 3-dimensional epidermis models composed of stratified human keratinocytes. Using a mix of different cytokines imitating the inflammatory condition in psoriasis (including IL-17A, IL-22, IFN-γ, TNF-α, IL-20), strongly induced ABP expressions were found after 3 days of stimulation (Fig. 2B, here shown for lipocalin-2). In parallel, the mix with one of the T-cell cytokines omitted at each time was tested. Omitting IL-17A had the clearest limiting effect on the induction of lipocalin 2. These data indicate that IL-17A deficiency limits the cutaneous upregulation of ABPs even in the presence of other ABP inducers and demonstrate the importance of this observation for the cutaneous situation in CTCL.

IL-22/TNF-α-dependent epidermal functions are similarly altered in CTLC and psoriasis.

Next, we asked whether, apart from the antimicrobial competence, CTCL differs from psoriasis regarding further keratinocyte alterations. Using qPCR, we analyzed respective skin samples for a marker of the keratinocyte hyperproliferation/regeneration (K16) as well as for the matrix-degrading enzyme MMP1. Both parameters are essentially regulated by IL-22, especially in combination with TNF-α, but not by IL-17A (29, 30). In line with the comparable expression of IL-22 and TNF-α in psoriasis versus CTCL (Fig. 2A), the expression of K16 and MMP1 was similar in these diseases (Fig. 3A). Comparable epidermal MMP1 levels were also confirmed by immunohistochemistry analysis (Fig. 3B).

CTCL lesions do not demonstrate a general Th17 cytokine deficiency
IL-17A is mainly produced by Th17-cells. Apart from IL-17A, these cells have been described to produce IL-17F and IL-26. Analyzing cytokine expressions by qPCR revealed similar to IL-17A - only low IL-17F expression in CTCL lesions, with mean levels being approximately 50 times lower than in psoriasis lesions (Fig. 4A). Surprisingly however, IL-26 expression was much higher in CTCL versus healthy skin and did not differ between psoriasis and CTCL.

To rule out that Th-cells other than of the Th17 lineage a main IL-26 producers, we investigated Th-cell populations that were either isolated from the peripheral blood by flow-cytometric sorting based on their chemokine expression patterns or were generated from naive T-cells in vitro (see Methods section). As demonstrated by qPCR data shown in Fig. 4B and C, activated Th17-cells (stimulated via CD3 and CD28) were indeed the strongest producers not only of IL-17F but also of IL-26. These data suggest that the production of individual cytokines is differently regulated in Th17-cells, explaining the selective relative IL-17A/F deficiency in CTCL.

We therefore looked for conditions that may cause a differential regulation of IL-17 versus IL-26 production by Th17-cells. The generation and maintenance of Th17-cells has been described to depend on IL-1β, IL-6, IL-23, and TGF-β. When omitting one of these cytokines during the in vitro Th17-cell generation period, we found that IL-17F expression was highly dependent on the presence of TGF-β, whereas IL-26 was not (Fig. 5A). We therefore analyzed TGF-β expression in the patients’ skin using qPCR. However, no TGF-β deficiency was found in CTCL lesions compared to psoriatic lesions that would explain the differential expression of IL-17 versus IL-26 in these samples (Fig. 5B). Another candidate of a regulator of differential Th17-cytokine expressions in CTCL is IL-10, whose expression was more highly expressed in CTCL compared to psoriatic skin (Fig. 5C). However, when performing Th17-cell differentiation in the presence of IL-10 had no influence on the IL-17F or IL-26 expression of these cells (Fig. 5D). Further mediators, whose expression highly differs between CTCL versus psoriatic lesions, are IL-13 (Fig. 2A) and IL-4 (31, 32). Both cytokines share the IL-4Rα subunit and have been shown to inhibit the IL-17A production by Th17-cells (26, 33). When exposing in vitro generated human Th17-cells to IL-4 and, as comparison, to IFN-γ, IL-4 indeed inhibited IL-17 expression but had no influence on IL-26 expression of these cells (Fig. 5E).
The differential presence of IL-4Rα ligands in CTCL versus psoriasis skin may therefore account for the differential expression among Th17-cell cytokines in these patients.
Discussion

The skin forms the major outer barrier of the organism against infections (15, 34). Among the different mechanisms to achieve this protection, the production of ABPs such as β-defensins, RNase-7, S100 proteins, lipocalin-2, and cathelicidin/LL37 by keratinocytes is of high importance (17, 35). ABPs directly inhibit the growth of microbes (17, 35). β-defensins, for example, are very small, highly positively charged proteins that kill microbes by destabilizing their membranes. S100A7 acts through zinc sequestration and also through permeabilization of the bacterial membrane. Some ABPs are constitutively produced, though high ABP levels are attained by the presence of cytokines (18-22). The up-regulation of cutaneous ABP levels appears to be especially important in situations with disturbed skin barrier function. This is the case not only after wounding but also in chronic-inflammatory skin diseases. In psoriasis, the cutaneous ABP production is increased to a dimension that effectively prevents cutaneous infection (16, 17). This seems to be achieved by the synergistic action of different mediators of the local cytokine milieu, including the Th-cell cytokines IL-17 and IL-22 and also proinflammatory cytokines derived from activated dendritic cells / macrophages and tissue cells (e.g., TNF-α, IL-24, IL-20) (36). In contrast, relative IL-17 deficiency and overproduction of ABP-down-regulating Th2-cytokines do not allow effective ABP induction in AD (16, 23, 24, 37), while deficient production of IL-22 and its downstream mediator IL-20 prevents sufficient ABP up-regulation in Hidradenitis suppurativa (acne inversa) (36). In addition to AD and Hidradenitis suppurativa lesions, CTCL lesions are known for their increased susceptibility towards bacterial infections.

In our current study we demonstrate that, compared to psoriatic lesions, up-regulation of ABP production in CTCL lesions was highly impaired. Low levels of IL-17A and IL-17F in CTCL lesions may be responsible for this deficiency. Apart from our observation of very low IL-17A and IL-17F expression in CTCL lesions, which is in line with previous reports (31, 38, 39), we demonstrate that these expressions are significantly lower compared to those in psoriatic skin, and that IL-17 absence had a strong negative impact of the ABP induction even when other inducers were present. The presence of IL-4Rα ligands (IL-13 and IL-4) might further contribute to the relative ABP deficiency.
in these lesions. Apart from their known direct ABP down-regulating capacity, they seem to limit Th17-cell IL-17 production. The situation in CTCL lesions therefore resembles that in lesional skin of AD patients. Importantly, the lacking significant up-regulation of IL-17A and IL-17F in CTCL skin appears to be caused by the inhibition of their production rather than the absence of Th17-cells. This was indicated by high expression of IL-26, another Th17-cell cytokine, in CTCL lesions. Importantly, in contrast to IL-17A/F, IL-26 production by Th17-cells was not affected by IL-4Rα ligands.

Bacterial infections may be pathogenetically important for CTCL as they may initiate and/or trigger the disease course. It has been suggested that malignant T-cell transformation arises upon persistent stimulation of T-cells (7) and that even transformed cells do not necessarily show high autonomous proliferation but require external stimulation (40, 41). This is in line with observed increasing monoclonality of T-cell populations arising with progression of CTCL only. A role of bacteria in these processes is also in line with the clinical improvement of lesions due to antibiotic treatment of patients (8, 9). Pre-malignant/malignant cell stimulation by bacteria may occur either directly by bacterial superantigens (9, 10) or indirectly via induction of cytokines, that serve as growth factors for CTCL cells, in macrophages and dendritic cells (42), in non-malignant T-cells or in tissue cells (43-46). Furthermore, following presentation via MHC class II by macrophages and dendritic cells, microbial antigens matching the specificity of the transformed T-cells might play an important role in the activation of respective CTCL cells, as recently demonstrated for a subset of human B-cell lymphomas (47). Additionally, in the final stages of CTCL, when the T-cell repertoire in the body becomes restricted to that of the tumor cells, local infections may be origin of fatal systemic infections (12, 13).

Besides IL-17A/F, CTCL lesions show a cocktail of other different T-cell cytokines. It will be interesting to determine for each of these cytokines whether it is mainly derived from the lymphoma cells themselves or from infiltrating reactive immune cells especially present in the dermis of the CTCL lesions (48, 49). These cytokines may be responsible for specific epidermal alterations observed in CTCL lesions. In fact, the lesions often show tissue cell reactions, which are also known from non-malign chronic-inflammatory skin diseases. In this study we additionally demonstrate a psoriasis/AD-like increased expression of the hyper-regeneration marker K16 and the matrix-
degrading enzyme MMP1 in CTCL lesions. The fact that the expression of both parameters is particularly regulated by IL-22/TNF-α (50) might explain the similar levels of these parameters in the three investigated diseases. While K16 is a parameter histologically associated with acanthosis, MMP1 may be important for the infiltration of lymphoma and normal immune cells into the epidermis.

In summary, we demonstrated a relative deficiency in the antibacterial defense of CTCL lesions that might be due to the inhibition of Th17-cell IL-17A/F production caused by high abundance of IL-4Rα ligands.
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References


**Tables**

**Table 1**

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Figure and Table legends

**Table 1.** IL-17 is an important regulator of the keratinocyte ABP expression. Messenger RNA expression data were statistically correlated with ABP mRNA expression data obtained by qPCR analysis of lesional skin samples from CTCL (n=9) and psoriasis (n=13) patients. Correlation coefficients r, and, in parentheses, P-values as calculated by means of the Spearman's correlation test are indicated and, in case of significant relationships, are italicized.

**Figure 1.** CTCL skin lesions show a deficient induction of ABP expression.

A, Biopsies of healthy skin from control participants (n=9) and of skin lesions from patients with psoriasis (n=13), CTCL (n=9) and AD (n=10) were analyzed for ABP mRNA expression by qPCR. Data are given as the mean ± SEM relative to HPRT expression. Significances compared to the CTCL group are indicated. *P<0.05, **P<0.01, ***P≤0.001. B, Biopsies of healthy skin from control participants (n=3) and of skin lesions from patients with CTCL (n=3) and psoriasis (n=3) were analyzed for ABP expression by immunohistochemistry. Representative pictures (magnification: 200-fold) are presented.

**Figure 2.** The lesional expression of selected ABP-regulating cytokines differs between patients with CTCL and psoriasis patients.

A, Biopsies of healthy skin from control participants (n=9) and of skin lesions from patients with psoriasis (n=13), CTCL (n=9) and AD (n=10) were analyzed for cytokine mRNA expression by qPCR. Data are given as the mean ± SEM relative to HPRT expression. Significances compared to the CTCL group are indicated. *P<0.05, **P<0.01, ***P≤0.001. B, Epidermis models were stimulated or not (control) for 72 h with a cytokine mix described in the Material and Methods section or with the same mix with the exception of one of the T-cell cytokines lacking at each time. Lipocalin-2 mRNA expression was analyzed relative to HPRT expression by qPCR, and data from 3 experiments are given as the mean ± SEM as the percentage of the non-stimulated group.
**Figure 3.** The expression of parameters known to be regulated by IL-22/TNF-α are similarly regulated in CTCL and psoriasis.

A, Biopsies of healthy skin from control participants (n=9) and of skin lesions from patients with psoriasis (n=13) and CTCL (n=9) were analyzed for K16 and MMP1 mRNA expression by qPCR. Data are given as the mean ± SEM relative to HPRT expression. Significances compared to the CTCL group are indicated. ***P≤0.001. B, Biopsies of healthy skin from control participants (n=3) and of skin lesions from patients with CTCL (n=3) and psoriasis (n=3) were analyzed for MMP1 expression by immunohistochemistry. Representative pictures (magnification: 200-fold) are presented.

**Figure 4.** The cutaneous expression of IL-26, another Th17-cell cytokine, is not deficient in CTCL patients.

A, Biopsies of healthy skin from control participants (n=9) and of skin lesions from patients with psoriasis (n=13) and CTCL (n=9) were analyzed for cytokine mRNA expression by qPCR. Data are given as the mean ± SEM relative to HPRT expression. Significances compared to the CTCL group are indicated. ***P≤0.001. B, CD4+ CXCR3+ CCR6- cells (Th1), CD4+ CCR6- CCR4+ cells (Th2), and CD4+ CCR6+ CCR4+ CCR10- cells (Th17), isolated from peripheral blood, were stimulated via CD3/CD28 for 2 d. Cytokine expression was analyzed by qPCR. Mean data ±SEM from 3 donors are given as percentage of maximum expression. C, Naïve CD4+ T-cells were stimulated for 8 d via CD3 and CD28 in the presence of Th-subset-polarizing conditions (see Materials and Methods section) to generate Th1-, Th2-, Th17-, Treg- and Tr1-cells. Cytokine expression was analyzed by qPCR. Mean data ±SEM of 5 experiments are given as percentage of Th17-cell expression. Significances compared to the Th17-cells are indicated. *P<0.05.

**Figure 5.** Possible mechanisms underlying the cutaneous deficiency in the upregulation of IL-17 in CTCL patients.

A, Th17-cells were generated as described in Fig. 4C or with one of the polarizing cytokines omitted as indicated. Cytokine mRNA expression were analyzed by qPCR. Mean data ±SEM of 5 experiments are given as percentage of the Th17 full polarization group. Significances compared to the Th17 full
polarization group are indicated. *P<0.05. **Biopsies of skin lesions from patients with psoriasis (n=13) and CTCL (n=9) were analyzed for TGF-β mRNA expression by qPCR. Data are given as the mean ± SEM relative to HPRT expression. No significance was detected between both patient groups. **Biopsies of skin lesions from patients with psoriasis (n=13) and CTCL (n=9) were analyzed for IL-10 mRNA expression by qPCR. Data are given as the mean ± SEM relative to HPRT expression. Significance compared to the CTCL group is indicated. *P<0.05. **Th17-cells were generated as described in Fig. 4C in the presence or absence of IL-10. Cytokine mRNA expression were analyzed by qPCR. Mean data ±SEM of 5 experiments are given as percentage of the Th17 group without IL-10. **Th17-cells generated as described in Fig. 4C were treated or not (control) with IFN-γ or IL-4 from day 8 to day 9. Cytokine mRNA expression was analyzed by qPCR. Mean data ±SEM of 3 experiments are given as percentage of the control group.
Deficient cutaneous antibacterial competence in cutaneous T-cell lymphomas: role of Th2-mediated biased Th17-function

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