Deficient Cutaneous Antibacterial Competence in Cutaneous T-Cell Lymphomas: Role of Th2-Mediated Biased Th17 Function

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Abstract

Purpose: Primary cutaneous T-cell lymphomas (CTCL) are neoplastic disorders of skin-homing T cells. Affected skin areas show morphologic similarities with alterations in other T-cell–mediated dermatoses. Furthermore, as in atopic dermatitis but in contrast with psoriasis, patients with CTCL 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 patients with CTCL.

Experimental Design: Skin samples from CTCL, psoriasis, and atopic dermatitis 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 IHC.

Results: CTCL lesions compared with psoriatic lesions presented an impaired upregulation of antibacterial proteins (ABPs), with levels even below those in atopic dermatitis. This was associated with a relative deficiency of the ABP-inducing cytokine IL-17 and a strong presence of the ABP-downregulating 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 about 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.

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Introduction

Primary cutaneous T-cell lymphomas (CTCL) are neoplastic lymphoproliferative disorders that are primarily localized in the skin. Mycosis fungoides and Sézary syndrome are two common CTCL forms, accounting for approximately 67% of cases. For the United States, 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 mycosis fungoides varies over time and includes early patches, plaques with more pronounced infiltration, and the advanced tumor stage. In Sézary syndrome, 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, mycosis fungoides resembles lesions of T-cell–mediated dermatoses such as psoriasis or chronic atopic dermatitis, in which mediators of mixed T-cell...
infiltrates, by altering skin cell biology, and cause changes of skin architecture, including acanthosis and hyperkeratosis (4, 5). Only in later stages, the mycosis fungoides diagnosis is facilitated by the profound intraepidermal 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 mycosis fungoides/Sézary syndrome remains unknown. It has been hypothesized that mycosis fungoides cells are derived from skin-infiltrated, chronically activated Th cells (6, 7). In fact, they are generally CLA+/αβ TCR+, CD4+, CD45RO+, and CD26−/−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 mycosis fungoides lesions show an impaired skin barrier function. This, in turn, is associated with bacterial skin infections, for example, with 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 Sézary syndrome, they may become life threatening (12, 13). Importantly, an impaired skin barrier function is also found in patients with psoriasis and atopic dermatitis (14, 15). However, only atopic dermatitis skin shows an increased incidence of bacterial infections, which is mainly due to the limited upregulation of antibacterial proteins (ABP) by the keratinocytes of these patients (16). In contrast, in psoriasis, the antimicrobial 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 (TNF-α and IL-1β) and by T cells (IL-17A, IFN-γ, and IL-22; refs. 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 atopic dermatitis.

In this study, we aimed to shed light on the mechanisms underlying the increased bacterial infection risk of the skin in patients with mycosis fungoides.

**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), patients with mycosis fungoides (5 with patch stage mycosis fungoides, 2 with plaque stage mycosis fungoides, 3 with tumor stage mycosis fungoides), and patients with atopic dermatitis (100% moderate to severe disease). Of the patients with mycosis fungoides (age: from 52 to 89 years, 80% male), 8 had no therapy at the time of study, one got topical steroids and one had undergone PUVA therapy. For IHC analysis, skin biopsies were obtained from patients with mycosis fungoides, patients with psoriasis, and healthy volunteers (3 from each group). Samples were approved by the clinical institutional review boards of the Charité University Hospital (Berlin, Germany) or the Rockefeller University (New York, NY) 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 hours.

Peripheral blood mononuclear cells (PBMC) were separated from citrated venous blood of healthy volunteers and patients with Sézary syndrome by standard density gradient centrifugation using LeucoSep tubes (Greiner) and Ficoll–Paque Plus (Amersham and Biochrom). Th1, Th2, and Th17 were isolated from PBMC by flow-cytometric sorting as described below and were stimulated via CD3/CD28 for 2 days. 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 93.8 ±0.44%). Cells were stimulated for 8 days with Dynabeads Human T-activator CD3/CD28 beads (Invitrogen; 1 bead/1 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-
h) carbazole (Enzo Life Sciences; Th22), or IL-10, TGF-β1, 2, anti-IL-4 mAb, anti-IFN-γ mAb, and all-trans-retinoic 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 days, 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; 1 bead/T cell) for 2 days. 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 FACSAria. 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 naive CD4⁺ T-cell isolations, cells were stained with fluorescence-labeled Ab clones against the following molecules: CD3 (SK7), CD56 (NCAM16.2), CD45RA (HI100), CD45RO (UCHL1; all from BD Bioscience), CD4 (13B8.2), CD8 (B9.11), CD14 (RMO52), CD19 (J4.119), CD16 (3G8; 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 Invitrogen RNA Kit II (Invitek/Stratec molecular GmbH). Reverse transcription of mRNA was performed as described previously (27). qPCR 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, IL22, IL26, and matching hyposaxinine 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.

IHC

IHC analysis was performed on frozen sections of human mycosis fungoides, 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 overnight at 4°C. The staining signal was amplified using avidin–biotin complex (Vector Laboratories) for 30 minutes at room temperature and developed using chromogen 3-amino-9-ethylcarbazole. The microscope used was a Nikon Eclipse 50i with a Nikon DS-Fil 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 mycosis fungoides patients and control patients/participants were analyzed using the Mann–Whitney U test (two tailored). Correlations were analyzed on the basis of the Spearman 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 atopic dermatitis but in contrast with patients with psoriasis, patients with CTCL have an increased susceptibility to cutaneous bacterial infections. We hypothesized that, like in patients with atopic dermatitis, an attenuated increase of ABPs may be responsible. To investigate this hypothesis, mRNA expression of different ABPs including lipocalin 2, β-defensins, RNase 7, IL37, and S100 proteins was quantified by qPCR in skin lesions of patients with mycosis fungoides (carrying patch stage, plaque stage, tumor stage lesions). For comparison, analyses of healthy skin from control donors and lesional skin from patients suffering from psoriasis and from atopic dermatitis were included. As demonstrated in Fig. 1A, the expression of most ABPs was upregulated in the patient groups compared with healthy skin. However, as it is the case for atopic dermatitis, the upregulation in CTCL was strongly limited, with significantly lower levels of all ABPs in CTCL compared with those observed in psoriatic skin. A clear difference between CTCL and psoriasis was also found upon IHC analyses of skin sections performed for lipocalin-2 and β-defensin 2 (Fig. 1B). In case of β-defensin 2 and 3, IL37, S100A7, and S100A9, the expression in CTCL was even lower than that in atopic dermatitis lesions (Fig. 1A). The relative deficiency in ABP expression may therefore account for the increased susceptibility to bacterial infections in patients with CTCL.
CTCL lesions show reduced upregulation 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 down-regulate (IL-13) epidermal ABP expression. As demonstrated by qPCR data, most of the analyzed cytokines were expressed at similar levels in CTCL compared with psoriatic lesions (Fig. 2A). However, two were not: IL-17A was found having a significantly lower expression, whereas IL-13 expression was higher compared with psoriasis. Hence, the cytokine expression pattern in CTCL highly resembled that found in atopic dermatitis 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 with psoriasis lesions are 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 Table 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 (Table 1).

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 atopic dermatitis (n = 10) were analyzed for ABP mRNA expression by qPCR. Data are given as mean ± SEM relative to HPRT expression. Significances compared with 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 IHC. Representative pictures (magnification: 200-fold) are presented.
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 CTCL 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 TNFα 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 IHC 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 are 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 Materials and 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 have 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

### Table 1. IL-17 is an important regulator of the keratinocyte ABP expression

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TNF-α</th>
<th>IL-1β</th>
<th>IL-13</th>
<th>IFN-γ</th>
<th>IL-17A</th>
<th>IL-22</th>
</tr>
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<tr>
<td>LCN2</td>
<td>0.14</td>
<td>0.548</td>
<td>-0.10</td>
<td>-0.60</td>
<td>0.68</td>
<td>0.37</td>
</tr>
<tr>
<td>β-defensin 2</td>
<td>0.00</td>
<td>0.547</td>
<td>0.06</td>
<td>0.056</td>
<td>0.50</td>
<td>0.00</td>
</tr>
<tr>
<td>β-defensin 3</td>
<td>0.15</td>
<td>0.67</td>
<td>-0.43</td>
<td>0.18</td>
<td>0.86</td>
<td>0.28</td>
</tr>
<tr>
<td>RNase 7</td>
<td>0.31</td>
<td>0.35</td>
<td>-0.10</td>
<td>-0.12</td>
<td>0.41</td>
<td>0.38</td>
</tr>
<tr>
<td>LL37</td>
<td>0.07</td>
<td>0.76</td>
<td>-0.21</td>
<td>0.12</td>
<td>0.50</td>
<td>0.18</td>
</tr>
<tr>
<td>S100A7</td>
<td>0.04</td>
<td>0.297</td>
<td>-0.16</td>
<td>0.02</td>
<td>0.72</td>
<td>0.44</td>
</tr>
<tr>
<td>S100A8</td>
<td>0.04</td>
<td>0.28</td>
<td>-0.21</td>
<td>0.25</td>
<td>0.81</td>
<td>0.33</td>
</tr>
<tr>
<td>S100A9</td>
<td>0.06</td>
<td>0.30</td>
<td>-0.15</td>
<td>0.05</td>
<td>0.68</td>
<td>0.42</td>
</tr>
</tbody>
</table>

**NOTE:** 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 correlation test are indicated and, in case of significant relationships, are italicized.
qPCR. However, no TGF-β deficiency was found in CTCL lesions compared with 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 with psoriatic skin (Fig. 5C). However, when performing Th17-cell differentiation, 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, with 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-cytokines in these patients.

Discussion

The skin forms the major outer barrier of organisms 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 upregulation of cutaneous ABP levels seems 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; ref. 36). In contrast, relative IL-17 deficiency and overproduction of ABP-downregulating Th2 cytokines do not allow effective ABP induction in atopic dermatitis (16, 23, 24, 37), whereas deficient production of IL-22 and its downstream mediator IL-20 prevents sufficient ABP upregulation in Hidradenitis suppurativa (acne inversa; ref. 36). In addition to atopic dermatitis and Hidradenitis suppurativa lesions, CTCL lesions are known for their increased susceptibility toward bacterial infections.

In our current study, we demonstrate that, compared with psoriatic lesions, upregulation of ABP production in CTCL lesions was highly impaired. Low levels of 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 with those in psoriatic skin, and that IL-17 absence had a strong negative impact of the ABP induction even when other inducers were present. Apart from their known direct ABP-downregulating capacity, they seem to limit Th17-cell IL-17 production. The situation in CTCL lesions therefore resembles that in lesional skin of patients with atopic dermatitis. Importantly, the lacking significant upregulation of IL-17A and IL-17F in CTCL skin seems 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 with IL-17A/F, IL-26 production by Th17 cells was not affected by IL-4Rα ligands.

Figure 5. Possible mechanisms underlying the cutaneous deficiency in the upregulation of IL-17 in patients with CTCL. A, Th17 cells were generated as described in Fig. 4C or with one of the polarizing cytokines omitted as indicated. Cytokine mRNA expressions were analyzed by qPCR. Mean data ±SEM of five experiments are given as percentage of the Th17 full polarization group. Significances compared with the Th17 full polarization group are indicated. *, P < 0.05. B, 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 mean ± SEM relative to HPRT expression. No significance was detected between both patient groups. C, 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 with the CTCL group is indicated. *, P < 0.05. D, Th17 cells were generated as described in Fig. 4C in the presence or absence of IL-10. Cytokine mRNA expressions were analyzed by qPCR. Mean data ±SEM of five experiments are given as percentage of the Th17 group without IL-10. E, 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 three experiments are given as percentage of the control group.
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). Premalignant/malignant cell stimulation by bacteria may occur either directly by bacterial superantigens (9, 10) or indirectly via induction of cytokines, in macrophages and dendritic cells (42), nonmalignant T cells, or tissue cells (43–46), which serve as growth factors for CTCL cells. 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). In addition, 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 nonmalign chronic-inflammatory skin diseases. In this study, we additionally demonstrate a psoriasis/atopic dermatitis-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. Although 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.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Mitsui, W. Sterry, R. Sabat
Study supervision: K. Wolk, H.-D. Volk, W. Sterry, R. Sabat
Other (editing and discussion of the manuscript): E. Witte
Other (partial funding of research): J.G. Krueger

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