Staphylococcal enterotoxins stimulate lymphoma-associated immune dysregulation

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Patients with cutaneous T-cell lymphoma (CTCL) are frequently colonized with Staphylococcus aureus (SA). Eradication of SA is, importantly, associated with significant clinical improvement, suggesting that SA promotes the disease activity, but the underlying mechanisms remain poorly characterized. Here, we show that SA isolates from involved skin express staphylococcal enterotoxins (SEs) that induce crosstalk between malignant and benign T cells leading to Stat3-mediated interleukin-10 (IL-10) production by the malignant T cells. The SEs did not stimulate the malignant T cells directly. Instead, SEs triggered a cascade of events involving cell-cell and asymmetric cytokine interactions between malignant and benign T cells, which stimulated the malignant T cells to express high levels of IL-10. Much evidence supports that malignant activation of the Stat3/IL-10 axis plays a key role in driving the immune dysregulation and severe immunodeficiency that characteristically develops in CTCL patients. The present findings thereby establish a novel link between SEs and immune dysregulation in CTCL, strengthening the rationale for antibiotic treatment of colonized patients with severe or progressive disease. (Blood. 2014;124(5):761-770)

Introduction

Cutaneous T-cell lymphomas (CTCLs) are a group of neoplastic diseases characterized by expansion of malignant T cells in the skin. The 2 predominant clinical forms of CTCL are mycosis fungoides (MF) and Sézary syndrome (SS). MF typically presents as patch, plaque, or tumor lesions, whereas SS is characterized by erythroderma and the presence of malignant T cells in the skin and blood.1–4 During disease progression, there is a decline in the number and activity of benign immune cells leading to suppression of cell-mediated immunity and ultimately severe immunodeficiency.5–16 Much evidence supports that malignant T cells drive the immune dysregulation to impede antitumor immunity and that suppression of the patient’s cellular immunity is associated with increased disease activity.17–22 Two factors that seem to hold a central position in driving the immune dysregulation are signal transducer and activator of transcription 3 (Stat3) and the immunoregulatory cytokine interleukin-10 (IL-10). In keeping, the malignant activity of Stat3 and the expression of IL-10 increase during disease progression in parallel with the evolving immune dysregulation.23–26 Activation of Stat3 in malignant cells can lead to secretion of soluble mediators facilitating activation of Stat3 in infiltrating benign immune cells, thereby suppressing cell-mediated cytotoxicity and promoting accumulation of immunosuppressive regulatory T cells. Furthermore, aberrant activation of Stat3 in malignant cells can induce expression of immunoregulatory factors including IL-10.27 IL-10 possesses strong immunosuppressive capacities and can dampen immune responses by several means. Among these, IL-10 promotes accumulation of tolerogenic macrophages and dendritic cells and represses Th1-mediated immune responses while favoring differentiation of anergic and immunosuppressive T cells.28–30 IL-10 can, accordingly, suppress the antibacterial immune defense and increase the risk of septicemia.31 Supporting that IL-10 also plays an important role in cancer-associated immunosuppression, blocking IL-10 activity in combination with immunostimulatory agents can restore antitumor immune responses in animal models with resulting tumor inhibition or regression.28–30 Indeed, IL-10 represses the expression of Th1 cytokines from CTCL cells, and malignant CTCL cells inhibit dendritic cell maturation as well as activation of benign T cells in an IL-10–dependent manner.32–35 More
importantly, as in many other forms of cancer, high levels of IL-10 have been associated with progressive disease and resistance to therapy in CTCL. As a consequence of the compromised skin barrier and evolving immune dysfunction, CTCL patients very frequently acquire bacterial infections, which comprise a major clinical problem. In particular, there is a high prevalence of *Staphylococcus aureus* (SA), which is present in approximately 40% of the patients. Intriguingly, eradication of SA by antibiotics is associated with significant clinical improvement in colonized patients, including a reduced involved body-surface area as well as decreased redness and pruritus of the skin. It has further been reported that staphylococcal sepsis in SS patients is accompanied by increased disease activity often in absence of fever. Therefore, it has been suspected for decades that SA fosters the disease activity in CTCL, but the underlying mechanisms remain poorly characterized, and it is not common practice to initiate antibiotic treatment of colonized patients. One of the central means by which SA
promotes immune dysregulation in CTCL.

A novel mechanistic explanation of how SA colonization may lead to robust Janus kinase 3 (Jak3)/Stat3-mediated expression of IL-10 by the malignant T cells. The present data thereby provide independent crosstalk between malignant and benign T cells, which by modulating the inflammatory environment.

Figure 2. SEA induces expression of IL-10 from primary malignant T cells. (A) PBMCs were isolated from 7 SS patients and cultured with vehicle (PBS) or SEA (200 ng/mL). After 24 hours, the concentrations of IL-10 in the cell culture supernatants were determined by ELISA. Error bars represent SEM. (B) Flow cytometric analysis of IL-10 expressing malignant (CD4+CD26−) T cells, benign (CD4+CD26+) T cells, and CD4-negative cells (CD4−) in SS PBMCs cultured with vehicle (PBS) or SEA (200 ng/mL) for 24 hours. (C) Representative flow cytometric analysis of MHC-II expression on malignant (CD4+CD26−) and benign (CD4+CD26+) T cells in PBMCs isolated from the blood of an SS patient. Dashed lines represent isotype control staining and solid lines with fill MHC-II staining. Flow cytometric analysis of MHC-II expression on malignant and benign T cells from 5 SS patients are summarized in supplemental Figure 7.

Methods

Reagents

Western blotting antibodies against Jak3 and Erk1/2 were from Santa Cruz Biotechnology (Santa Cruz, CA) and the antibody against Stat3 from Cell Signaling Technology (Beverly, MA). Antibodies against MHC-I and MHC-II molecules were from Leinco Technologies (St. Louis, MO), and the IL-2 neutralizing antibody and isotype control antibodies were from R&D Systems (Minneapolis, MN). Fluorochrome-conjugated antibodies against CD4, CD7, CD26, the IL-10 receptor (IL-10R), MHC-II, and the respective fluorochrome-conjugated isotype control antibodies used for flow cytometry and fluorescence-activated cell sorting (FACS) were from R&D Systems, Biolegend (San Diego, CA), BD Biosciences (Franklin Lakes, NJ), and Leinco Technologies. The pan Jak inhibitor (Jaki/P6) was from Calbiochem (San Diego, CA), and the Jak3 inhibitor Tofacitinib (CP-690550) was from Selleck Chemicals (Houston, TX). Finally, SEs and biotin-labeled SEA were from Toxin Technology (Sarasota, FL), and dimethylsulfoxide was from Sigma-Aldrich (St. Louis, MO).

Patients and SA isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of 9 patients diagnosed with SS in accordance with the World Health Organization-European Organization for Research and Treatment of Cancer classification. A characteristic of malignant T cells in SS patients is that they typically lack expression of the cell-surface markers CD7 and/or CD26 and often display reduced expression of CD4 when compared with benign T cells. As exemplified in supplemental Figure 1 (available at the Blood Web site), all SS patients included in the study displayed a clear abnormal accumulation of CD4−CD7− and/or CD4+CD26− T cells in their PBMCs. Accordingly, malignant (CD4+CD7−, CD4+CD26−) and benign (CD4+CD7+, CD4+CD26+) T-cell populations were defined on the basis of CD4, CD7, and/or CD26 surface expression. The malignant TCR Vβ subtype usage was characterized with the IOTest Beta Mark kit (Beckman Coulter, Indianapolis, IN). In 4 patients, the malignant population expressed a single TCR Vβ subtype, whereas the malignant population expressed high levels of several TCR Vβ subtypes in 1 patient. We did not identify a predominant malignant TCR Vβ subtype in the remaining patients, which is probably because the IOTest Beta Mark kit only covers approximately 70% of the TCR Vβ repertoire. SA bacteria were isolated from 2 patients diagnosed with MF and 1 patient diagnosed with SS. In brief, bacteriologic samples were collected from involved and uninvolved skin surfaces using sterile cotton swabs wetted with 0.1% Triton X-100 in 0.075 M phosphate buffer (pH 7.9) and transferred to Stuart’s transport medium. Subsequently, the bacteriologic samples were cultivated on blood agar and incubated overnight at 37°C in air supplemented with 5% CO2. Representative colonies of the 10 most dominant colony types were isolated, subcultivated, and identified by matrix-assisted laser desorption/ionization time-of-flight spectroscopy. The SA isolates were examined for production of SEs (SEA-SEE and TSST-1) using the latex agglutination kits SET-RPLA and TST-RPLA (Oxoid, Basingstoke, Hampshire, United Kingdom) and the RIDASCREEN SET A.B.C.D.E kit (R-Biopharm AG, Darmstadt, Germany). In accordance with the Declaration of Helsinki, the samples were obtained with informed consent and after approval by the Committee on Health Research Ethics (#22559).
The malignant T-cell line, SeAx, and the benign T-cell line, MF1850, were established from patients diagnosed with CTCL.\textsuperscript{52-54} The cell lines were cultured in human serum (HS) media (RPMI-1640 [Sigma-Aldrich], 2 mM L-glutamine [Sigma-Aldrich], 100 mg/mL penicillin/streptomycin [Sigma-Aldrich], and 10% HS [Blood Bank, State University Hospital, Copenhagen, Denmark]) supplemented with 10\(^3\) U/mL IL-2 (Proleukin) from Chiron (Emeryville, CA). Prior to experimental setup, the cell lines were starved overnight in HS media (without IL-2), and, likewise, all experiments were performed in HS media (without IL-2). Alloantigen-specific CD\(^+\) human T-cell lines from healthy donors have been described and characterized previously.\textsuperscript{54}

Figure 3. SE-mediated expression of IL-10 by primary malignant T cells requires the presence of benign T cells. (A-C) Malignant T cells were purified from the blood of SS patients and cultured for 24 hours with vehicle (PBS) or SEA (200 ng/mL) as well as (A) benign T cells purified from the blood of SS patients, (B) the benign CTCL T-cell line MF1850, or (C) a CD4 T-cell line established from the blood of a healthy donor (HD). Depicted is the concentration of IL-10 in the cell culture supernatants per 1 \times 10^6 malignant T cells as measured by ELISA. Malignant T cells from 3 (A,C) and 4 (B) different patients were analyzed. Error bars represent SEM.

**Cell lines**

The malignant T-cell line, SeAx, and the benign T-cell line, MF1850, were established from patients diagnosed with CTCL.\textsuperscript{52-54} The cell lines were cultured in human serum (HS) media (RPMI-1640 [Sigma-Aldrich], 2 mM L-glutamine [Sigma-Aldrich], 100 mg/mL penicillin/streptomycin [Sigma-Aldrich], and 10% HS [Blood Bank, State University Hospital, Copenhagen, Denmark]) supplemented with 10^3 U/mL IL-2 (Proleukin) from Chiron (Emeryville, CA). Prior to experimental setup, the cell lines were starved overnight in HS media (without IL-2), and, likewise, all experiments were performed in HS media (without IL-2). Alloantigen-specific CD\(^+\) human T-cell lines from healthy donors have been described and characterized previously.\textsuperscript{54}

**qPCR**

Total cellular messenger RNA (mRNA) was purified and reverse transcribed into complementary DNA as described previously.\textsuperscript{55} Quantitative polymerase chain reaction (qPCR) was subsequently performed using the Brilliant II SYBR green qPCR kit from Stratagene (La Jolla, CA) and the samples analyzed on a Mx3000P (Stratagene). For amplification, 0.2 \(\mu\)M of the following primers was used: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward, 5'-AAGGTGAAGGTCGGAGTCAA-3'; GAPDH reverse, 5'-CAAGAAGGGGTCAATGGTG-3'; IL-10 forward, 5'-CTGGGG GGAAGACTGAAG-3'; IL-10 reverse, 5'-TGTCCTTTAGATGCTTT TCC-3'; IL-2 forward, 5'-CAAGAAGGCACAGAACTGA-3'; and IL-2 reverse, 5'-GCTGCTCTACGCATATTCACA-3'.

**Flow cytometry**

For surface staining, cells were stained with antibodies for 30 minutes at 4°C and washed before flow cytometric analysis or FACS. IL-10 staining was performed using the IL-10 Secretion Assay Detection Kit from Miltenyi Biotech (Bergisch Gladbach, Germany). Data acquisition and flow cytometric analysis were done on LSRII and Fortessa flow cytometers (BD Biosciences) using FlowJo software (Tree Star, Ashland, OR).

**Cell isolation and sorting**

PBMCs were isolated from the blood of SS patients by Lymphoprep density gradient centrifugation (Axis-Shield, Oslo, Norway). For some experiments, the PBMCs were either used directly for flow cytometric analyses or cultured in HS media with PBS or SEA. For other experiments, the PBMCs were sorted into separate populations of malignant and benign T cells on the basis of CD4, CD7, and/or CD26 surface expression and then mono- and cocultured in HS media with PBS or SEA. For isolation of the malignant and benign T cells, the PBMCs were stained with fluorochrome-conjugated antibodies against CD4, CD7, and CD26 and subjected to FACS using a FACSARia (BD Biosciences). The purity of the sorted malignant and benign T cells was higher than 99% and 95%, respectively. Flow cytometric analysis of a representative purification is shown in supplemental Figure 2A.

In experiments where cocultured SeAx and MF1850 cells were sorted, the SeAx cells were stained prior culture with 5 \(\mu\)M carboxyfluorescein diacetate succinimidyl ester as previously described.\textsuperscript{56} Subsequently, the carboxyfluorescein diacetate succinimidyl ester–positive and negative cells were sorted by FACSARia, resulting in a purity of >98%. Flow cytometric analysis of a representative purification is shown in supplemental Figure 2B.

**Transfection**

Transient transfections were essentially performed as described previously\textsuperscript{23} using 0.25 \(\mu\)M Jak3, Stat3, or nontargeting ON-TARGETplus SMARTpool small interfering RNA (siRNA) (Dharmacon, Chicago, IL).

**Western blotting**

Protein extraction and western blotting were performed as described earlier.\textsuperscript{57}

**Results**

SE-producing SA from infected CTCL lesions induces expression of IL-10 in cocultures of malignant and benign T cells

To investigate the potential role of SA in CTCL immune dysregulation, we initially isolated SA from the skin of CTCL
patients (Figure 1A) and examined their impact on IL-10 expression by malignant and benign CTCL T-cell lines. Importantly, SA isolates from involved (lesional) skin stimulated vigorous production of IL-10 in cocultures of malignant (SeAx) and benign (MF1850) T cells (Figure 1B-D), whereas SA isolates from uninvolved skin did not (Figure 1B-C). Separate cultures of malignant and benign T cells were unresponsive or responded weakly to SA isolates (Figure 1B-D), indicating that a vigorous IL-10 response depended on crosstalk between the malignant and benign T cells. Supernatant from SA obtained from involved skin also triggered IL-10 production in cocultures of malignant and benign T cells, demonstrating that the induction of IL-10 expression was at least partly mediated by soluble factors (Figure 1E). Both the malignant and benign T-cell lines expressed MHC-II molecules (Figure 1F), which are high-affinity receptors for SEs and are required for SE-mediated activation of T cells. Antibodies directed against MHC-II molecules profoundly inhibited the binding of SEA to the malignant and benign T cells (supplemental Figure 3) and the supernatant-induced IL-10 production (Figure 1E), strongly suggesting that the response was induced by SEs. Indeed, SEs were detected in the SA isolates from involved skin but not in the SA isolates from uninvolved skin (supplemental Table 1). Because all of the SA isolates from involved skin expressed SEA, we next examined the effect of the purified toxin on the T-cell lines (supplemental Table 1). Purified SEA mimicked the effect of SA and SA supernatant by inducing a potent increase in IL-10 mRNA and protein in cocultures, but not in monocultures, of malignant and benign T cells (Figure 1G-H). The response was not restricted to this particular set of T-cell lines, as analogous results were obtained with other combinations of malignant and benign T-cell lines (supplemental Figure 4A-B). SEA at concentrations as low as 0.5 ng/mL induced IL-10 production in cocultures, indicating a high potency of SEA to activate cellular crosstalk (supplemental Figure 4C). Moreover, the ability to induce an IL-10 response in cocultures was not restricted to SEA. Thus, SEE induced an almost identical IL-10 response (supplemental Figure 5A). SEB did not induce IL-10 production (supplemental Figure 5A), an expected result given the inability of the T-cell lines to respond to this type of SE. Only the benign T cells proliferated in response to SEA and SEE stimulation in monocultures, indicating that SEs had limited or no direct influence on the malignant T cells (supplemental Figure 5B). Consequently, it was important to establish if IL-10 was produced by the benign T cells, the malignant T cells, or both. We therefore sorted malignant and benign T cells after coculture with or without SEA and determined their relative expression of IL-10. Whereas SEA strongly increased the level of IL-10 mRNA in cocultured malignant T cells, the level of IL-10 mRNA was essentially unaffected in cocultured benign T cells (Figure 1I). These results provided evidence that SEs can induce high expression of IL-10 by malignant CTCL T-cell lines via a mechanism that requires the presence of benign T cells.
SEA induces secretion of IL-10 from primary malignant T cells in the presence of benign T cells

Having established that SEs induce expression of IL-10 by malignant T-cell lines when cocultured with benign T-cell lines, we next addressed whether SEA induced a similar response in primary CTCL cells. Indeed, SEA induced variable but potent secretion of IL-10 from primary malignant T cells (supplemental Figure 6). In line with the observations from the cell lines, the malignant T cells were the principal contributors to the SEA-mediated expression of IL-10 (Figure 2B), although SEA had no direct effect on the expression of IL-10 by purified primary malignant T cells (supplemental Figure 6). MHC-II molecules were expressed on the primary malignant and benign T cells, demonstrating that they had the capacity to bind SEs (Figure 2C and supplemental Figure 7). Substantiating that the induction of IL-10 was not mediated by direct stimulation of the malignant T cells, none of the malignant TCR βζ subtypes identified in the patients were responsive to SEA (data not shown). Concordant with the findings made with the T-cell lines, these results indicated that SEs induced expression of IL-10 in primary malignant T cells but that the response required the presence of benign T cells. To confirm this conclusion, we purified primary malignant and benign T cells from SS patients and cultured them with or without SEA. The purified malignant and benign CTCL T cells generally exhibited no detectable or very low levels of spontaneous IL-10 expression in monolayer cultures, and the expression was not significantly influenced by the addition of SEA (Figure 3A). Importantly, SEA induced a marked increase in the expression of IL-10 when the primary malignant T cells were cultured with benign SS T cells (Figure 3A). Likewise, SEA mediated a potent induction of IL-10 when primary malignant T cells were cultured together with the benign CTCL T-cell line MF1850 and benign CD4 T cells from healthy donors (Figure 3B–C). The SEA-induced secretion of IL-10 from primary malignant T cells was thus dependent on the presence of benign T cells but did not rely on a specific nature of benign T cells derived from CTCL patients.

SEA induces expression of IL-10 via a mechanism that is dependent on cell-cell contact and IL-2

To investigate the mechanisms underlying the SE-induced expression of IL-10, malignant and benign T cells were cocultured in wells with or without cell-impermeable filters that allow transport of soluble messengers but prevent cell-cell contacts. As shown in Figure 4A, the SEA-induced expression of IL-10 was strongly inhibited in transwell cocultures as compared with cocultures without cell-impermeable filters, suggesting that cell-cell contact between the 2 cell types is required to induce robust IL-10 expression. SEs have previously been shown to induce secretion of IL-2 from benign CD4 T cells. In agreement, SEA stimulated benign CTCL cells to express IL-2 mRNA and protein (Figure 4B and supplemental Figure 8). Because IL-2 can promote the expression of IL-10 by malignant T cells, we speculated that IL-2 was involved in the SE-mediated induction of IL-10. Therefore, mono- and cocultured malignant and benign T cells were treated with SEA and
the relative expression of IL-2 and IL-10 determined at different time points. SEA induced a quick and strong upregulation of IL-2 mRNA in the benign T cells in both mono- and cocultures, whereas SEA essentially had no effect on the expression of IL-2 in the malignant T cells (Figure 4B). Increased expression of IL-2 was already seen 1 hour after stimulation with SEA (Figure 4B). In parallel, 3 hours after stimulation with SEA, a steep increase in the level of IL-10 mRNA was observed in cocultured malignant T cells, while SEA had no significant impact on the IL-10 expression in monocultured malignant T cells (Figure 4B). The overlap in the expression of IL-2 and IL-10 prompted us to investigate the potential role of IL-2 in a more direct manner. To this end, malignant and benign T cells were cultured with SEA in the presence of an IL-2 neutralizing antibody, or vehicle. Importantly, the IL-2 neutralizing antibody almost completely blocked the SEA-induced expression of IL-10 in transwell cocultures and profoundly inhibited it in direct cocultures when compared with the isotype control and vehicle (Figure 4C).

These findings suggest that SEA promoted the malignant expression of IL-10 both by stimulating the benign T cells to produce IL-2 and by modulating cell-contact-dependent interactions between the malignant and benign T cells.

**SE-induced expression of IL-10 is dependent on the Jak3/Stat3 pathway in malignant T cells**

Because the Jak3/Stat3 pathway can promote the expression of IL-10 in malignant T cells, we investigated its possible involvement. Both a pan-Jak inhibitor (Figure 5A) and a Jak3 inhibitor (Figure 5B) efficiently blocked the SEA-mediated IL-10 expression. Likewise, transfection of the malignant T cells with Jak3-specific siRNA lead to efficient depletion of Jak3 (Figure 5C) and totally abrogated the SEA-induced expression of IL-10 in cocultures of malignant and benign T cells (Figure 5D). On the other hand, depleting Jak3 in the benign T cells had no effect on the expression of IL-10 (Figure 5C-D). Depletion of Stat3 in the malignant but not in the benign T cells also significantly inhibited the SEA-induced expression of IL-10 (Figure 5E-F). Altogether, these results demonstrate that the SE-induced expression of IL-10 was mediated via a Jak3/Stat3-dependent mechanism in malignant T cells.

**Expression of the IL-10 receptor is low or absent in malignant T cells**

It has previously been shown that malignant T cells can suppress the activation of benign T cells in an IL-10–dependent manner, raising the question whether IL-10 could also mediate autocrine suppression of the malignant T cells. Therefore, we analyzed surface expression of IL-10R on malignant and benign T cells. IL-10R was expressed in the benign T-cell line, MF1850, but not in the malignant T-cell line, SeAx (Figure 6A). In a similar manner, IL-10R expression was absent or strongly decreased on malignant T cells when compared with benign T cells in PBMCs isolated from the blood of SS patients (Figure 6B-C and data not shown), indicating that malignant T cells generally are resistant to IL-10 stimulation.

**Discussion**

It has long been suspected that SEA via expression of SEs can influence the malignant expansion and evolving immune dysregulation in CTCL patients. Indeed, prior studies have reported that SEs in some cases can stimulate the proliferation of malignant T cells. However, a mechanistic connection between SEs and lymphoma-associated immune dysregulation has remained elusive. Here, we demonstrate that SEs can induce vigorous expression of the immunoregulatory cytokine IL-10 in cocultures of malignant and benign T cells, thereby providing a novel link between SEs and immune dysregulation in CTCL. Remarkably, SEs did not induce high expression of IL-10 in monocultured malignant or benign T cells. Instead, SEs modulated the interactions between malignant and benign T cells to induce high malignant expression of IL-10 (Figure 7A-C). Our results suggest a mechanism where SEs induce cell-cell contact–dependent crosstalk between malignant and benign T cells and stimulate the benign T cells to produce IL-2 (Figure 7B). These interactions in turn promote activation of the Jak3/Stat3 pathway in the malignant T cells, which induces expression of IL-10 and subsequently leads to the inhibition of cell-mediated immunity and antitumor responses (Figure 7C). Because both strong activation of Stat3 and high expression of IL-10 have been identified as markers of poor response to treatment in CTCL, the present data indicate that elimination of SA may not only lead to clinical improvement but also enhance the patient’s response to treatment with other therapeutics.
IL-10 has been reported to promote CTCL immune dysregulation by several means, including direct inhibition of benign T cells.\textsuperscript{17,32-35} It could thus be speculated that IL-10 might similarly suppress the proliferation and cytokine production of malignant T cells. We found that the expression of IL-10R was very low or completely absent on malignant T cells, indicating that they are protected against suppressive effects mediated by IL-10. Likewise, malignant CTCL cells express the immunosuppressive cytokine transforming growth factor β (TGF-β) but often lose surface expression of TGF-β receptor II.\textsuperscript{5,59,61-63} This suggests that the malignant T cells elegantly manipulate and use the immune system while escaping its control. Similar to IL-10R and TGF-β receptor II, malignant T cells from advanced disease typically display decreased surface expression of CD3 and/or exhibit resistance to TCR stimulation and apoptosis.\textsuperscript{51,64} By targeting the benign T cells, SEs may still influence the malignant T cells even as they become resistant to TCR stimulation. Moreover, the proposed indirect mechanism implies that the pathogenic role of SEs is not limited to cases of patients expressing a SE-responsive TCR Vβ on the malignant clone. On the contrary, patients harbor benign T cells with a wider repertoire of TCR Vβ families, making them vulnerable to this indirect mode of action.

Infections comprise a major clinical problem in CTCL, and patients with advanced disease frequently die of infections rather than complications from the tumor burden.\textsuperscript{37} We hypothesize that SEs are part of a vicious circle where initial deterioration of the skin barrier leads to colonization with SE-producing SA. The SEs then modulate the interactions between malignant and benign immune cells leading to enhanced suppression of the host’s cellular immunity and increased disease activity, thereby contributing to further deterioration of the skin barrier. In turn, weakening of the host’s defenses stabilizes the SA colonization, facilitates its propagation, and increases the susceptibility to secondary infections.

In conclusion, we identify a mechanistic link between colonization with SE-producing SA and immune dysregulation in CTCL, thus strengthening the rationale for antibiotic treatment of colonized patients with severe or progressive disease. Furthermore, the present study supports accumulating evidence that bacterial toxins may promote cancer\textsuperscript{65-70} and conceptually demonstrates how bacterial toxins may contribute to cancer by influencing the crosstalk between tumor and immune cells.

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Authorship


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