LYMPHOID NEOPLASIA

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 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
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. 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...
manipulates the host’s immune system is by secreting staphylococcal enterotoxins (SEs). SEs (and SE-like toxins) constitute a large family of secreted proteins (SEA-SEE, SEG-SEJ, SELK-R, SEU, and TSST-1) that function as superantigens. Thus, SEs bypass the normal antigen-restricted activation of T cells by binding outside the antigen-binding groove of major histocompatibility complex class II (MHC-II) molecules on one cell and to the Vβ region of T-cell receptors (TCRs) on a T cell.45 Little is known about the functional differences of SEs, but each SE interacts with a restricted repertoire of MHC-II alleles and TCR Vβ segments47 and thereby targets specific T-cell subsets. The host’s reaction to SEs seems to be dependent on the site of exposure. Whereas SEs are best known for their ability to cause food poisoning after ingestion, cutaneous colonization with SA strains producing high levels of SEs is commonly observed in chronic inflammatory skin diseases as atopic dermatitis and psoriasis, where they are believed to exacerbate the disease.

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+CD26−) 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.1 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.51 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 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).
Cultured in human serum (HS) media (RPMI-1640 [Sigma-Aldrich], 2 mM L-glutamine [Sigma-Aldrich], and 100 mg/mL penicillin/streptomycin [Sigma-Aldrich]), and 10% HS [Blood Bank, State University Hospital, Copenhagen, Denmark]) supplemented with 10^5 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 CD4+ human T-cell lines from healthy donors have been described and characterized previously.54

Cell lines

The malignant T-cell line, SeAx, and the benign T-cell line, MF1850, were established from patients diagnosed with CTCL.52-54 The cell lines were cultured in human serum (HS) media (RPMI-1640 [Sigma-Aldrich], 2 mM L-glutamine [Sigma-Aldrich], and 100 mg/mL penicillin/streptomycin [Sigma-Aldrich], and 10% HS [Blood Bank, State University Hospital, Copenhagen, Denmark]) supplemented with 10^5 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 CD4+ human T-cell lines from healthy donors have been described and characterized previously.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 × 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.

Transfection

Transient transfections were essentially performed as described previously,53 using 0.25 μmol 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.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). 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 SA. Thus, SEE induced an almost identical IL-10 response (supplemental Figure 5A). SEE 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.

Figure 4. SEA induces IL-10 expression via a mechanism that is dependent on cell-cell contacts between malignant and benign T cells as well as secretion of IL-2 by benign T cells. (A) Malignant (SeAx) and benign (MF1850) T cells were mono- and cocultured with vehicle (PBS) or SEA (50 ng/mL). In cocultures, the malignant and benign T cells were either cultured in transwells where the 2 subsets are separated by a cell-impermeable filter (indicated by a line) or together without a cell-impermeable filter (no line). The supernatants were harvested after 24 hours and the concentrations of IL-10 determined by ELISA. Error bars represent SEM of 3 independent experiments. (B) Malignant (SeAx) and benign (MF1850) T cells were cultured alone and together with SEA for different periods of time. At each given time point, the cells were harvested and the cocultured cells were sorted into pure populations of malignant and benign T cells by FACS. Finally, the relative levels of IL-2, IL-10, and GAPDH mRNA in each sample were analyzed by qPCR. The levels of IL-2 and IL-10 mRNA were normalized to that of GAPDH and 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). 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 SA. Thus, SEE induced an almost identical IL-10 response (supplemental Figure 5A). SEE 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.

Figure 4 (continued) depicted as fold change when compared with benign and malignant T cells respectively at time point zero. Malign. (Benign) indicates cytokine expression in malignant T cells that had been cocultured with benign T cells, and vice versa for Benign (Malign.). Data are representative of 2 independent experiments. (C) Malignant (SeAx) and benign (MF1850) T cells were mono- and cocultured in the presence of SEA (100 ng/mL) together with an IL-2 neutralizing antibody (Anti-IL-2, 1 mg/mL), an isotype control antibody (1 mg/mL), or vehicle (PBS) for 24 hours before the concentrations of IL-10 in the cell culture supernatants were determined by ELISA. Error bars represent SEM of 3 independent experiments.
the addition of SEA (Figure 3A). Importantly, SEA induced a marked
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detectable or very low levels of spontaneous IL-10 expression in
puri-
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that the response required the presence of benign T cells. To con-
SEs induced expression of IL-10 in primary malignant T cells but
in the patients were responsive to SEA (data not shown). Concordant
Figure 5. SEA induces IL-10 via a mechanism that is dependent on the Jak3/Stat3 pathway in malignant but not benign T cells. (A-B) Malignant (SeAx) and benign
(MF1850) T cells were mono- and cocultured in the presence of SEA (50 ng/mL) together with vehicle (dimethylsulfoxide), (A) a pan Jak inhibitor (JAKI, 1 ÌM) or (B) a Jak3
inhibitor (Tofacitinib, 0.3 ÌM). After 24 hours of culture, the concentrations of IL-10 in the cell culture supernatants were determined by ELISA. Error bars represent SEM of 3
independent experiments. (C) Representative western blot of Jak3 and Erk1/2 expression after transient transfection of malignant (SeAx) and benign (MF1850) T cells with
Jak3 or nontargeting (NT) siRNA. (D) Malignant (SeAx) and benign (MF1850) T cells were transiently transfected with NT or Jak3-specific siRNA and monocultured for
24 hours. Then, the transfected cells were washed and cocultured in the presence of SEA (50 ng/mL) for another 24 hours before the concentrations of IL-10 in the cell culture
supernatants were determined by ELISA. Shown is the percent IL-10 expression relative to cocultures of malignant and benign T cells transfected with NT siRNA. Error bars
represent SEM of 3 independent experiments. (E) Representative western blot of Stat3 and Erk1/2 expression after transient transfection of malignant (SeAx) and benign
(MF1850) T cells with Stat3 or NT siRNA. (F) Malignant (SeAx) and benign (MF1850) T cells were transiently transfected with NT or Stat3-specific siRNA and monocultured
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bars represent SEM of 3 independent experiments. DMSO, dimethylsulfoxide.

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
in the SE-mediated induction of IL-10.59 Therefore, mono- and
cocultured malignant and benign T cells generally exhibited no
suppressor activity (Figure 4A). The SEA-induced expression of IL-10
in 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 expres-
sion 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 ex-
pression. SEs have previously been shown to induce secretion of IL-2
from benign CD4 T cells.58 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-10.59 Therefore, mono- and
cocultured malignant and benign T cells were treated with SEA and

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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, it was investigated if SEs mediate tumor growth suppression. Both a pan-Jak inhibitor and a Jak3 inhibitor efficiently blocked the SE-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 SE-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 SE-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 malignant T cells can suppress 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. 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. 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. 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 family 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. 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 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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