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REVIEW

Regulatory T cells and immunodeficiency in mycosis fungoides and Sézary syndrome

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Cutaneous T-cell lymphoma (CTCL) is the term for diseases characterized by primary accumulation of malignant T cells in the skin. Patients with the two predominant clinical forms of CTCL called mycosis fungoides (MF) and Sézary syndrome (SS) characteristically develop severe immunodeficiency during disease progression and consequently patients with advanced disease frequently die of infections and not from the tumor burden. For decades, it has been suspected that the malignant T cells actively drive the evolving immunodeficiency to avoid antitumor immunity, yet, the underlying mechanisms remain unclear. The identification of a subset of highly immunosuppressive regulatory T cells (Tregs) triggered a variety of studies investigating if MF and SS are malignant proliferations of Tregs but seemingly discordant findings have been reported. Here, we review the literature to clarify the role of Tregs in MF and SS and discuss the potential mechanisms driving the immunodeficiency.
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Introduction

Cutaneous T-cell lymphoma (CTCL) is the designation for a heterogeneous group of diseases characterized by clonal expansion of malignant T cells in the skin. The two predominant clinical forms of CTCL are mycosis fungoides (MF) and Sézary syndrome (SS).[1] MF initially presents as flat erythematous patches or plaques covering a limited area of the skin and bear great resemblance to non-malignant skin disorders like chronic eczema, allergic contact dermatitis and large-plaque parapsoriasis. In these early stages, MF typically exhibits an indolent clinical behavior and the disease can remain stable for many years. However, at some point the skin lesions may progress and large intradermal tumors can develop. In advanced disease, the malignant T cells occasionally disseminate to the lymph nodes, peripheral blood and internal organs, which is associated with a highly unfavorable prognosis.[2–4] Sometimes a substantial proportion of the malignant T cells become enlarged—a phenomenon referred to as large cell transformation. The transformation occurs in about 10% of MF patients and is associated with a mean survival of only 22 months.[5] The risk of disease progression is significantly higher in more advanced clinical stages demonstrating a change in the nature of the disease from indolent to more aggressive.[6] SS is an aggressive leukemic variant of CTCL with a median survival of 3 years that is characterized by generalized erythroderma, lymphadenopathy and the presence of high numbers of malignant T cells with atypical cerebriform nuclei (Sézary cells) in the peripheral blood.[6,7] As MF and SS will be the main focus of this review, in the following the term CTCL will exclusively refer to these two clinical variants.

The malignant T cells in both MF and SS typically exhibit the phenotype of mature $CD4^+$ memory T cells.[2–4] Besides the malignant T cells, the skin lesions also contain a large proportion of non-malignant inflammatory cells. In early stages of MF, the infiltrate primarily consist of non-malignant T helper 1 (Th1) cells and cytotoxic $CD8^+$ T cells, which to some degree seem to control the malignant T cells by secreting cytokines as interferon (IFN)-α and IFN-γ and cytotoxicity directed against malignant T cells expressing tumor-associated antigens.[8–12] Indeed, it has been shown that high numbers of infiltrating $CD8^+$ lymphocytes are associated with improved survival.[13] As the disease progresses, however, the malignant T cells accumulate concomitant with a decrease in the number of non-malignant immune cells.[2,13] In particular, there is a stage-dependent decrease in the number and function of activated $CD8^+$ T cells and natural killer cells resulting in suppression of the hosts' cellular immunity.[2,14–16] Consequently, a hallmark of CTCL patients is that they develop severe immunodeficiency during disease progression and in fact patients with advanced disease frequently die of infections rather than complications from the tumor burden.[17] But, what are the mechanisms responsible for the evolving immunodeficiency? Are the malignant T cells immunosuppressive or is the immunodeficiency simply the outcome of a competitive malignant replacement of benign $CD4^+$ and $CD8^+$ T cells? These are questions that have attracted much attention in CTCL research during the last decades.

Early studies on suppressor cells in SS and MF

In 1976, Broder *et al.*[18] published their seminal paper showing that T cells isolated from the peripheral blood of SS patients were helper T cells. However, shortly after two studies provided evidence that the malignant T cells from some SS patients had suppressive activity.[19,20] This finding lead Kansu *et al.*[19] to propose that SS could develop as an expansion of different T-cell subsets and that malignant T cells from individual patients therefore could act as helper T cells, suppressor T cells or cells performing other functions characteristic of T-cell subsets. This hypothesis was supported by a study published in 1984, which showed that $CD4^+$ T cells isolated from the peripheral blood of one SS patient were suppressive whereas the $CD4^+$ T cells from two other SS patients possessed neither T helper nor suppressive

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activity.[21] These early studies essentially highlighted the functional heterogeneity among malignant T-cell populations from SS patients and indicated that malignant T cells from some patients possessed suppressor activity. Yet, the functional activity of the lymphocytes was exclusively based on assays measuring T-cell-dependent immunoglobulin production by B cells and the phenotype of the suppressive T cells was poorly characterized. Consequently, these studies did not establish if malignant SS cells had the capability to suppress cell-mediated immunity. Owing to the lack of phenotypic markers and the difficulties in extracting the malignant T cells from the skin lesions, only a few studies addressed the functional activity of the malignant T cells in MF at that time. Interestingly, one study reported that tumor-infiltrating lymphocytes (TILs) but not malignant cells isolated from a MF lesion displayed suppressive properties suggesting that at least a sub-population of TILs may inhibit antitumor immunity in MF.[22]

The discovery of $CD4^+CD25^{Hi}FOXP3^+$ Tregs

The idea of a subset of specialized T cells with immunosuppressive activity had been of great scientific interest since the early 1970s but several factors including the failure to identify a phenotypically distinct subset of suppressive T cells lead to a general decline in the interest from the mid-1980s to the mid-1990s.[23] But then, Sakaguchi *et al*.[24] made the landmark discovery that BALB/c athymic nude mice spontaneously developed autoimmune diseases when inoculated with BALB/c splenic cell suspensions depleted of $CD4^+CD25^+$ T cells. Furthermore, autoimmunity was prevented by reconstitution of $CD4^+CD25^+$ cells within a limited period after transfer of the $CD4^+CD25^-$ cells indicating that a population of immunosuppressive T cells was present in the $CD4^+CD25^+$ subset.[24] Indeed, the existence of a subset of highly suppressive regulatory T cells (Tregs) characterized by high expression of CD25 and the transcription factor forkhead box P3 (FOXP3) was subsequently confirmed.[25–29]

It quickly became apparent that FOXP3 is not simply a marker of Tregs but that it has a central role in Treg biology. Loss of FOXP3 or its diminished expression in Treg cells leads to acquisition of effector T-cell properties and disruption of the *foxp3* gene elicits severe autoimmune diseases in mice and humans because of the failure to generate functional Tregs.[30–36] On the contrary, high and stable ectopic expression of FOXP3 in conventional T cells can confer a Treg phenotype and suppressive activity.[26,37] On the basis of these and other observations, FOXP3 is considered to be a 'master regulator' of Tregs. In humans, however, FOXP3 is expressed by most T cells on activation without conferring suppressive activity but the expression of FOXP3 in activated human T cells is only transient and lower than that observed in freshly isolated $CD4^+CD25^{Hi}FOXP3^+$ Tregs.[38,39] Therefore, although FOXP3 expression defines $CD4^+CD25^{Hi}$ Tregs, FOXP3-positivity alone is not a reliable marker of human Tregs. The differences in the stability of FOXP3 expression between Tregs and activated T cells, at least partly, rely on distinct epigenetic modifications in a region of the *foxp3* locus referred to as the Treg-cell-specific demethylated region (TSDR). Whereas CpG dinucleotides in the TSDR are fully demethylated in Tregs, they are methylated in naive $CD4^+CD25^-$ T cells and activated $CD4^+$ T cells.[40,41] Thus, a demethylated TSDR together with high and stable expression of FOXP3 currently seems to be the most reliable marker of Tregs.

Distinction between activated T cells and $FOXP3^+$ Tregs based on CD25 expression is difficult because there is no clear-cut threshold of CD25 expression that accurately separates the two subsets.[39] Besides high expression of CD25 and FOXP3, Tregs also typically display surface expression of cytotoxic T lymphocyte-associated 4 (CTLA-4) and glucocorticoid-induced tumor necrosis factor receptor-related gene and low expression of the interleukin (IL)-7 receptor (CD127) when compared with conventional T cells.[42–46] Current evidence suggest that Tregs can be divided into natural Tregs that develop in the thymus and inducible Tregs that develop from naïve $CD4^+CD25^-$ T cells in the periphery on activation in the presence of high levels of transforming growth factor (TGF)-β and IL-2. Despite their different origin, natural Tregs and inducible Tregs seem to be functionally and phenotypically similar in many aspects.[39,47] Notably, there exists at least two $FOXP3^-$ T-cell subsets with regulatory activity, namely, T regulatory 1 (Tr1) cells, which primarily mediate suppression by secreting high levels of IL-10 and Th3 cells, which produce large amounts of TGF-β.[48] In this review, the term Treg exclusively refers to $FOXP3^+$ Tregs.

Tregs can induce immunosuppression by influencing a variety of cell types such as $CD4^+$ and $CD8^+$ T cells, natural killer cells and antigen-presenting cells. They can exert their suppressive activity by an arsenal of mechanisms including IL-2 deprivation, production of the immunosuppressive cytokines IL-10 and TGF-β, cytolysis, metabolic disruption and modulation of the function of antigen-presenting cells.[49] It is now widely accepted that Tregs are crucial for maintaining peripheral tolerance and preventing inappropriate or excessive immune reactions. However, as a two-edged sword Tregs can also be exploited by tumor cells to suppress antitumor reactions. Accordingly, a high proportion of Tregs in the tumor microenvironment is associated with poor prognosis in a number of solid malignancies.[50] In T-cell lymphomas, the picture seems more complicated as the tumor cells are of lymphoid origin and, therefore, have the potential to be suppressed by Tregs.

The identification of $CD4^+CD25^{Hi}FOXP3^+$ Tregs resurrected the theory that malignant CTCL cells are derived from of a subset of immunosuppressive T cells and using an *in vitro* model Berger *et al*.[51] showed that after co-culture with immature dendritic cells loaded with apoptotic T cells, CTCL cells adopted Treg phenotype and functions. Thus, the CTCL cells upregulated the expression of FOXP3, CD25, CTLA-4, IL-10 and TGF-β and suppressed normal T-cell antigen-driven secretion of IL-2 and IFN-γ. These findings lead the investigators to propose that CTCL is a malignant proliferation of Tregs and that this could explain the immunosuppressive nature of the disease.[51]

Tregs in MF

Arguing against that MF is a malignant proliferation of Tregs, three independent studies exclusively detected FOXP3 expression in TILs and not in the malignant T cells by immunohistochemical analyzes of collectively 44 skin lesions.[52–54] Similarly, a study using the controversial hFOXY anti-FOXP3 antibody found that four patients with non-transformed MF were FOXP3-negative but that the malignant T cells in four of five patients with transformed MF expressed FOXP3 indicating that transformation of MF to a high-grade lymphoma is associated with adoption of a Treg phenotype.[55] However, $FOXP3^+$ malignant T cells were not detected when sections from the same patients were subsequently stained with three distinct FOXP3-specific antibodies.[56] In a larger study, Gjerdrum *et al*.[57] did observe a sub-population of $FOXP3^+$ malignant T cells in 6 of 86 patients with CTCL of which 69 had MF and 17 CTCL unspecified. Furthermore, two independent groups recently reported malignant

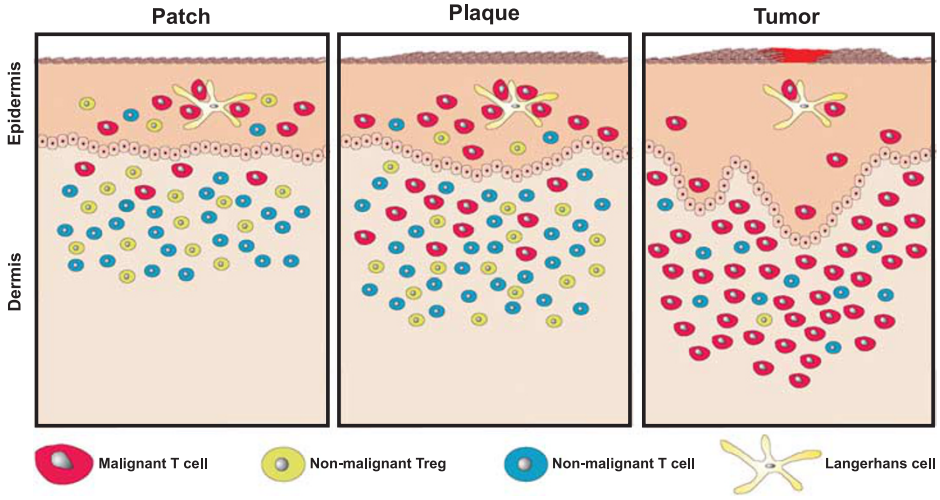

Figure 1 Schematic illustrating changes in the numbers of malignant and non-malignant T cells in MF skin lesions during disease progression. In patch stage lesions, there is a small population of malignant T cells that primarily are located in the epidermis and an infiltrate of non-malignant T cells that preferentially are located in the upper dermis. Importantly, the benign lymphocytic infiltrate contains a relatively large proportion of Tregs that potentially suppress infiltrating immune cells as well as the malignant T cells. In the plaque stage, both the number of malignant and non-malignant T cells increases but the proportion of Tregs in the benign lymphocytic infiltrate remains fairly constant. However, in the tumor lesions, there is a decrease in the number of infiltrating non-malignant T cells. Especially, there is a steep decrease in the proportion of Tregs within the benign lymphocytic infiltrate and a large increase in the number of malignant T, which are now primarily found in the dermal compartment. In plaque and patch stage lesions, a population of the malignant T cells might express low levels of FOXP3 but this expression seems to be almost absent in tumor stage lesions.

expression of FOXP3 as judged from immunohistochemical examination of MF lesions[58] and by flow cytometric analysis of primary malignant T cells *ex vivo*.[59] The former study found that approximately 40% of the malignant T cells were FOXP3$^+$ in 16 patch and plaque stage lesions and that the frequency declined to about 5% in tumor lesions.[58] In agreement, the latter study provided quantitative data showing that the majority of the malignant T cells in early lesions expressed FOXP3 but that the level of expression on an average was significantly lower than in non-malignant Tregs. The malignant T cells not only expressed low levels of FOXP3 but they also expressed relatively low levels of CD4 suggesting a phenotype of chronically activated T cells rather than *bone fide* Tregs.[59] Studies addressing the methylation pattern of the *foxp3* locus as well as the suppressive capacity of malignant T cells freshly isolated from MF skin lesions are needed to establish if they exhibit a Treg-like phenotype and function. The reported heterogeneous expression of FOXP3 in a clonal malignant population could seem counter-intuitive. Providing a possible explanation, Wasik *et al*.[58] found that the expression of FOXP3 by CTCL cells is influenced by IL-2-type cytokines and proposed that the phenotype and functionality of the malignant T cells is modulated by the local microenvironments within the CTCL lesions.

So far, all the studies conducted concur that MF skin lesions contain FOXP3$^+$-infiltrating non-malignant T cells.[52–54,57,58] Supporting that the infiltrating FOXP3$^+$ T cells are functional Tregs, benign suppressive TILs have been isolated from MF lesions.[22] Furthermore, the percentages of CD4$^+$CD25^{Hi} and FOXP3$^+$ cells are similar in the peripheral blood of MF patients and healthy individuals and CD4$^+$CD25$^+$ cells isolated from the blood of MF patients produce immunosuppressive cytokines on activation and are extremely potent in suppressing the proliferation of activated CD4$^+$CD25$^-$ cells.[60–62] In early skin lesions, the percentage of non-malignant FOXP3$^+$ TILs seems to be slightly lower or similar to that observed in benign inflammatory skin diseases but their numbers decrease significantly in advanced lesions and, importantly, a high frequency of FOXP3$^+$ TILs has been shown to be associated with improved survival[53,57,58,63,64] (Figure 1). This suggests that benign tumor-infiltrating Tregs might suppress the propagation of the neoplastic T cells and imply that evolvement of mechanisms, which make the malignant T cells resistant to this suppression or impair the function and numbers of infiltrating Tregs can promote disease progression. If this is the case, special consideration should be taken when introducing new modalities that could influence the number or function of Tregs for treatment of patients with MF, especially in early stages. Noteworthy, several treatment options that are already being used for MF therapy have the potential to modulate Tregs. However, bexarotene was not observed to influence the peripheral frequency of Tregs or their suppressive capacity whereas extracorporael photochemotherapy induced a small decrease in the percentage of circulating Tregs and a slight increase in serum levels of TGF-β.[62,65,66] It was proposed that the changes during extracorporael photochemotherapy therapy could represent an effect of extracorporael photochemotherapy towards normalization in the patients.[65] Nevertheless, a convincing link between modulation of Treg function and CTCL therapy has not been established so far.

Tregs in SS

Supporting that the malignant T cells in some SS patients are Tregs, Capriotti *et al*.[67] detected FOXP3 mRNA in peripheral blood mononuclear cells from 6 of 25 SS patients with high tumor burdens in their peripheral blood and found that FOXP3$^+$ patients had a significantly worse prognosis than those lacking FOXP3 mRNA. In keeping with this dichotomy, Klemke *et al*.[53] reported that some SS patients had significantly higher and some have significantly lower percentages of FOXP3$^+$ cells in their peripheral blood when compared with MF and psoriasis patients as well as healthy donors.[61] Importantly, we and others shortly after showed that FOXP3 protein is expressed in malignant

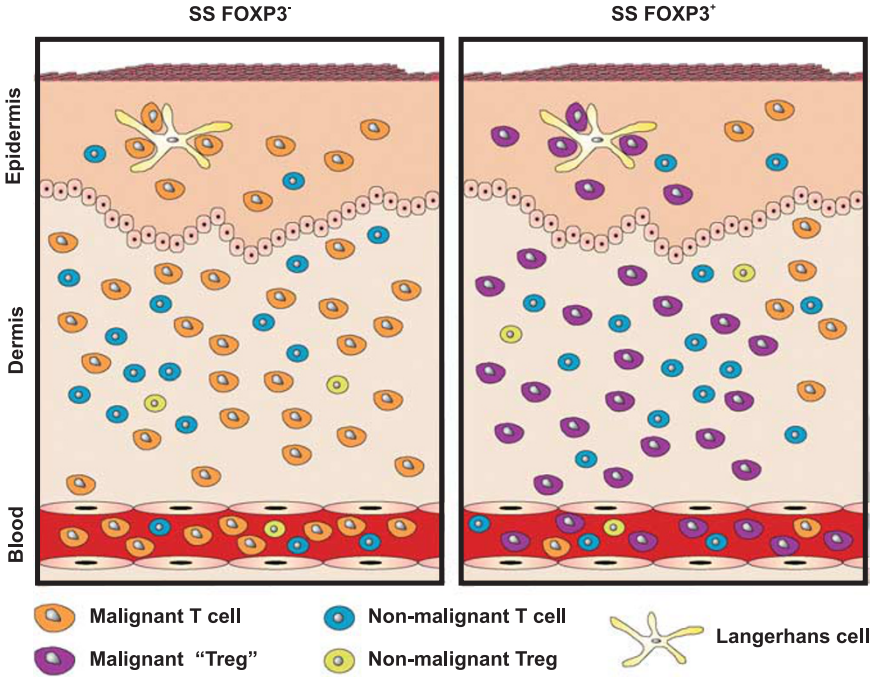

Figure 2 Schematic illustrating the dichotomy of SS patients with regard to the malignant expression of FOXP3. SS patients have low frequencies of benign Tregs in their skin and blood but can be subdivided into one population of patients where the malignant T cells lack expression of FOXP3 (SS FOXP3$^-$) and one population of patients where a proportion of the malignant T cells express FOXP3 and exhibit a Treg phenotype (SS FOXP3$^+$). Some of the malignant T cells expressing FOXP3 display a full-fledged Treg phenotype whereas the majority only exhibits a Treg-like phenotype. The dichotomy of SS patients as well as variations in the Treg phenotype could arise because of differences in the origin of the malignant T cells and in the tumor-microenvironment.

T cells in both the blood and skin of approximately 35–40% of SS patients at levels comparable to normal Tregs.[58,61,68] The percentage of malignant T cells identified as FOXP3$^+$ is variable in these patients. In some patients, practically all of the malignant T cells express FOXP3 whereas FOXP3 is only expressed in a sub-population of malignant T cells in other patients indicating heterogeneity in the malignant population. Taken together, these studies suggest that SS patients in general have a low frequency of benign Tregs but that the malignant T cells in some SS patients express FOXP3 and that these patients accordingly have relatively high numbers of FOXP3$^+$ cells in their skin and blood (Figure 2). As SS is a particular aggressive clinical form of CTCL, the paucity of normal Tregs is in line with the observation from MF that aggressive disease is associated with decreased numbers of Tregs.

A central issue is whether the FOXP3$^+$ malignant T cells are indeed Tregs or if the expression of FOXP3 simply reflects that they are chronically activated. Besides expressing high levels of FOXP3, several observations support that the malignant T cells are Tregs. Accordingly, it has been shown that purified FOXP3$^+$ malignant T cells isolated from SS patients suppress T-cell receptor (TCR)-induced cytokine expression in conventional T cells to a similar extent as Tregs from healthy donors whereas FOXP3$^-$ malignant T cells are not able to suppress TCR-induced cytokine expression. Furthermore, purified FOXP3$^+$ malignant T cells either have a highly demethylated TSDR region similar to Treg cells from healthy donors or a partially demethylated TSDR region that is less demethylated than in Tregs but more than in conventional T cells. On the contrary, the TSDR region in FOXP3$^-$ malignant T cells is methylated as in conventional T cells.[61] Finally, FOXP3$^+$ malignant T cells isolated from SS patients express CTLA-4 together with the immunosuppressive cytokines IL-10 and TGF-β and malignant SS cell lines suppress the proliferation of activated non-malignant T cells *in vitro*.[58,68,69] However, in other aspects the FOXP3$^+$ malignant T cells do not resemble *bona fide* Tregs. For instance, although the protein level of FOXP3 in the malignant T cells seems to be similar to that in normal Tregs, the level of FOXP3 mRNA in the malignant T cells is lower than in unstimulated Tregs from healthy donors. Instead, the level of FOXP3 mRNA is approximately equal to that of Tregs expanded *in vitro* and conventional T cells stimulated with α-CD3/αCD-28 antibodies overnight.[61] Moreover, it appears that the FOXP3$^+$ malignant T cells in most patients do not express CD25 on their surface.[61,67] It is also well established that malignant SS cells typically produce Th2-related cytokines and that they are responsive to IL-7 indicating surface expression of CD127.[8,70–73] Interestingly, it was recently reported that a large proportion of skin resident FOXP3$^+$ Tregs in fact express high levels of CD127 and that Tregs upregulate CD127 expression during activation.[74] In conclusion, the FOXP3$^+$ malignant T cells in SS are suppressive and display phenotypic characteristics of Tregs. However, although the malignant T cells do resemble classical Tregs in some cases, they do not exhibit a full-fledged Treg phenotype in most FOXP3$^+$ patients.

Although the expression of FOXP3 seems stable *in vivo*, it is clearly dependent on the IL-2 receptor β (IL-2Rβ)—signalling cytokines IL-2 or IL-15 *in vitro*, which induce the FOXP3 expression by activating Jak3 and Stat5.[58] Thus, the malignant T cells appear to be pre-primed *in vivo* to bypass the need for TCR stimulation *in vitro* to express FOXP3 in response to IL-2Rβ cytokines. In a similar fashion, the expression of IL-10 is induced by IL-2 in a Jak3-dependent manner but whereas the expression of FOXP3 is induced by Stat5 the expression of IL-10 is primarily

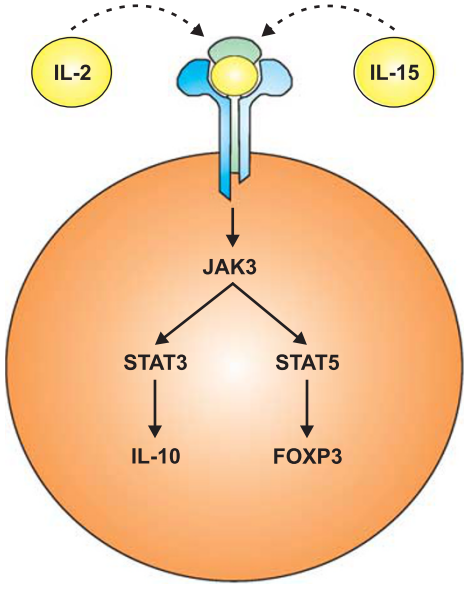

Figure 3 IL-2 and IL-15 induce expression of IL-10 and FOXP3 by the malignant T cells in a Jak3-dependent manner. The expression of IL-10 is primarily induced via activation of Stat3 whereas the expression of FOXP3 is induced via activation of Stat5.

induced by Stat3 [refs 58,68] (Figure 3). These findings imply that epigenetic alterations in concert with activation of the Jak/Stat pathway drive the Treg phenotype and promote the suppressive activity of the malignant T cells in FOXP3$^+$ SS patients.

Interestingly, the malignant T cells not only express full-length FOXP3 but also low molecular splice forms of FOXP3.[68] As these splice forms are functionally distinct from full-length FOXP3 in some aspects it seems likely that they have overlapping but also discrete roles in the disease pathogenesis.[68,75] Notably, in contrast to full-length FOXP3, the low molecular splice forms of FOXP3, exhibit very weak or no inhibitory effect on the activity of NF-κB, which is known to be constitutively activated and crucial for the proliferation and survival of the malignant T cells.[68,76–79] Yet, further studies are needed to clarify the potential contribution of the low molecular splice forms of FOXP3 to the propagation of SS.

An intriguing question is if the FOXP3$^+$ malignant T cells have acquired Treg phenotype and function or if they originally are derived from infiltrating or skin resident Tregs as previously suggested.[80] Favoring that the malignant T cells have acquired Treg properties, a high frequency of immature antigen-presenting cells are present in CTCL patients.[81] These cells could drive the malignant T cells toward a Treg phenotype as demonstrated in the study conducted by Berger *et al*.[51] On the other hand, recent studies have highlighted that human Tregs exhibit some degree of plasticity in that their phenotype and function can be modulated in specific inflammatory settings.[82] If the FOXP3$^+$ malignant T cells originate from Tregs, it could be that the deviations from classical Tregs observed in most patients (for example, lack of CD25 surface expression, expression of CD127 and intermediate FOXP3 mRNA levels) are due to their progressive expansion in a chronic inflammatory environment. As an alternative, a heterogeneity model where Tregs can be subdivided into a major population of committed Tregs that are stable and a minor population of uncommitted Tregs that are plastic and can acquire effector phenotype and functions under inflammatory conditions was recently proposed.[83] Interestingly, it seems that plastic Tregs are restricted to a fraction of FOXP3$^+$ Tregs that is enriched in the CD25-negative subset.[84] Therefore, it could be speculated that the FOXP3$^+$ malignant T cells showing only partial demethylation of the TSDR region, loss of CD25 expression and acquirement of effector functions represents plastic uncommitted Tregs whereas FOXP3$^+$ malignant T cells showing a fully demethylated TSDR region and CD25 expression represents committed Tregs. Both the hypotheses that the FOXP3$^+$ malignant T cells have acquired a Treg phenotype and that they are derived from uncommitted Tregs are consistent with the proposal by Wasik *et al*.[58] stating that CTCL cells are plastic and that their phenotype is modulated by the local microenvironment (Figure 4). Accordingly, the same malignant clone can have various functional subclones (for example, Treg, Th2, Tr1 and Th17) at any time depending on stimuli from the immediate local environment, which is in agreement with the observations that the Treg phenotype is lost after cytokine-starvation *in vitro*,[58] that a substantial proportion of SS cells typically produce Th2- and Th17-related cytokines[70,71,85] and that the fraction of FOXP3-expressing cells is highly variable among malignant T cells in SS.[58,61,68] Finally, it is possible that the regulatory phenotype of the malignant T cells in at least some cases is induced by external factors such as bacterial or viral infections as recently indicated.[86] Of notice, evidence suggests that bacterial infections can modulate the cross-talk between the malignant and non-malignant T cells in a manner that promotes malignant proliferation and administration of antibiotics to patients with bacterial infections can result in significant clinical improvement.[87,88] Thus, decreased immunity in MF and SS patients could pave the way for viral or bacterial infections that in turn promote tumor growth and immunodeficiency thereby creating a positive feedback loop.

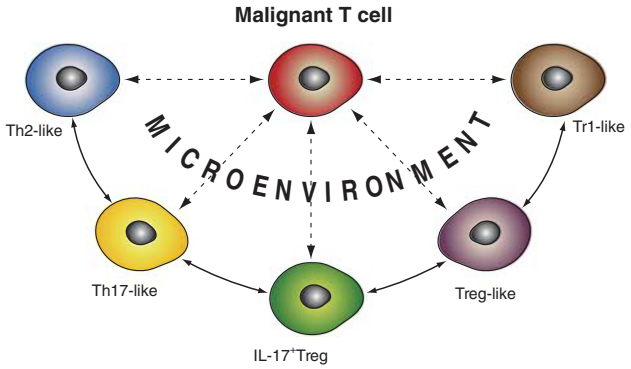

Figure 4 Schematic of the plasticity model where the phenotype of the malignant T cells is modulated by the local microenvironments in the lesional tissue and blood. Accordingly, the same malignant clone can have various subclones exhibiting phenotypes similar to that of different benign CD4 T-cell subsets. The malignant T cells can also assume intermediate phenotypes co-expressing markers characteristic of different CD4 T-cell subsets such as FOXP3 and IL-17 (unpublished observations).

Alternative mechanisms responsible for the impaired cellular immunity in CTCL

Collectively, the current data suggest that the malignant T cells in a substantial proportion of CTCL patients generally are not Tregs, in particular in the advanced stages of MF and in a subset of SS patients. So which mechanisms are contributing to the suppression of cell-mediated immunity in these patients? In this

section, some of the factors that could be involved will briefly be discussed.

It is well established that a hallmark of malignant CTCL cells is that they exhibit persistent activation of the Jak3/Stat3 pathway.[89–91] Interestingly, recent evidence indicates a central role of Stat3 in sustaining chronic inflammation while antagonizing antitumor immunity. Activation of Stat3 in the tumor cells can both promote malignant expression of immunosuppressive factors as well as the production of factors that can induce Stat3 activation in surrounding cells, which then in turn suppress antitumor immunity. For example, Stat3 is crucial for the immunosuppressive and tumor-promoting effects of myeloid-derived suppressor cells and tumor-associated macrophages.[92] In malignant CTCL cells, the Jak3/Stat3 pathway induces secretion of the two immunosuppressive cytokines IL-10 and TGF-β.[58,68,93] Based on the pleiotropic suppressive activities of these cytokines, it seems likely that they have an impact on the cellular immunity in CTCL patients. In accordance, IL-10 is important for the suppressive function of SS cell lines *in vitro*.[68] One could speculate that the expression of immunosuppressive cytokines would also repress the function and growth of the malignant T cells themselves. Although this probably occurs to some extent, it is likely that during tumor evolution such a selective pressure will drive the development of malignant resistance to the inhibitory factors. Indeed, it has been demonstrated that the expression of the TGF-β receptor is severely downregulated or lost on malignant T cells from SS patients thereby rendering them insensitive to growth inhibition by TGF-β.[94–96] Interestingly, aberrant activation of Stat3 has been shown to induce constitutive expression of suppressor of cytokine signaling-3, which protects the malignant T cells from IFN-mediated growth inhibition.[97,98] Thus, it seems that Stat3 not only induces secretion of immunosuppressive cytokines from the malignant T cells, but that it also induces the expression of factors, which protects them from suppressive cytokines.

In early disease stages, the production of Th1-associated cytokines such as IFN-γ and IL-12 is comparable to that of healthy individuals. During disease progression, however, the expression of Th1 cytokines declines whereas there is an increase in the level of Th2 cytokines leading to a Th2 skewed condition.[70,71,99] Supporting that the shift to a Th2-like response impedes the cellular immunity, therapy of CTCL patients with IL-12 can induce lesional regression, which is associated with increased numbers of $CD8^+$ T cells in the resolving skin lesions.[9,100,101] As mentioned, a characteristic feature of malignant CTCL cells is that they display constitutive activation of the transcription factor NF-κB, which promotes their proliferation and survival.[76–79] In this context, it is interesting that Stat3 activation can antagonize NF-κB- and Stat-1-mediated expression of Th1 cytokines such as IL-12 and IFN-γ.[92]

Abnormal regulation of other proteins could also promote the immunodeficiency in CTCL. For instance, the malignant T cells express the immunosuppressive proteins PD-1/CD279 and PD-L1/CD274, which have been associated with evasion of tumor immunity.[102] Peripheral blood mononuclear cells from CTCL patients also express significantly increased levels of the immunosuppressive protein CTLA-4 on stimulation and there is a correlation between higher expression of CTLA-4 and higher disease grade.[69] Furthermore, a large fraction of the malignant T cells express high levels of Fas ligand (FasL) on their surface and the numbers of $CD8^+$ T cells are decreased in areas of skin lesions were FasL-positive tumor cells are abundant.[103] Oppositely, the malignant expression of Fas is decreased during disease progression suggesting that the malignant T cells develop resistance to FasL-mediated apoptosis but can kill infiltrating $CD8^+$ T cells in a FasL-dependent manner.[104] In addition, a loss of TCR repertoire complexity is observed in CTCL patients especially in late disease stages, which could increase the susceptibility to infections.[105] Finally, it is imperative that the competition between the malignant and benign T cells for survival factors and nutrients in advanced stages is likely to provide a substantial contribution to the depletion of the normal T-cell compartment and thereby the development of immunodeficiency.

Conclusion

When scrutinizing the literature, the observations made from early investigations are, fascinatingly, in line with the findings from more recent studies. Nevertheless, further studies are needed to fully establish if the malignant T cells in MF express low levels of FOXP3 and potentially if this reflects acquisition of Treg-like properties or merely chronic activation. There seem to be full-fledged benign Tregs in the early MF skin lesions where they may suppress the malignant expansion and progression to advanced disease stages is associated with a decrease in the number of infiltrating Tregs. SS patients exhibit a severe paucity of benign functional Tregs but the malignant T cells in a substantial fraction of SS patients display a Treg phenotype and suppressive function thereby possibly hampering antitumor responses and promoting systemic immunodeficiency. Yet, other mechanisms are likely to make a considerable contribution to the increasing inhibition of cellular immunity in CTCL patients. These mechanisms probably include constitutive activation of the Jak/Stat pathway, malignant secretion of immunosuppressive cytokines as TGF-β and IL-10, dysregulated expression of the immunoregulatory proteins as CTLA-4, PD-L1 and FasL, loss of TCR repertoire complexity and competitive replacement of benign T cells by malignant T cells. Thus, the evolving immunodeficiency underlying the increased susceptibility of CTCL patients to infections appears to be mediated by a complex network of factors that to some extend vary among subgroups of patients. Although the malignant expansion might indirectly add to the immunodeficiency, much evidence currently supports that this network is also actively orchestered by the malignant T cells. Accordingly, we hypothesize that blockage of tumor-associated immunosuppression might adjuvate antitumor therapies as well antibiotic treatment of bacterial infections, which is a major cause of death in patients with advanced disease.

Conflict of interest

The authors declare no conflict of interest.

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