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TIGIT and Helios are highly expressed on CD4+ T cells in Sezary syndrome patients

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To The Editor

Cutaneous T cell lymphoma (CTCL) is commonly manifested as skin-restricted mycosis fungoides (MF) or Sézary syndrome (SS), a leukemic variant characterized by erythroderma and circulating malignant $CD4^+$ T cells with features of Th2 cells (Kim *et al.*, 2005).

TIGIT (T cell immunoreceptor with Ig and ITIM domains) is a recently identified co-inhibitory receptor expressed on the cell surface of activated or regulatory T cells, and NK cells. Emerging data suggests that TIGIT plays an inhibitory role by downregulating Th1 and Th17 while enhancing Th2 immune responses (Johnston *et al.*, 2015; Joller *et al.*, 2014; Kourepini *et al.*, 2016; Kurtulus *et al.*, 2015; Zhang *et al.*, 2016). Helios is a transcription factor in the *Ikaros* family present in regulatory T cells. Like TIGIT, Helios has inhibitory

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Conflict of Interest

The authors state no conflict of interest

activity and plays a role in anti-tumor immunity (Khaitan *et al.*, 2016; Muto *et al.*, 2015; Sebastian *et al.*, 2016). The expression and role of Helios and TIGIT in SS has not been explored, but given the immunosuppressive nature of the disease, both molecules may potentially contribute to this process.

We reported high expression of FCRL3 on $CD26^{-}$ $CD4^{+}$T cells in SS patients with high blood tumor burden. A microarray analysis of global gene expression that identified FCRL3, also showed significantly increased expression of Helios in $CD4^{+}$ cells from SS patients with high tumor burden (data not shown) (Wysocka *et al.*, 2014). A recent report linked the expression of FCRL3 with the expression of TIGIT and Helios (Bin Dhuban *et al.*, 2015).

Our data show significantly increased percentages of $TIGIT^{+}$ and $Helios^{+}$ $CD4^{+}$ T cells in patients with SS (mean: 33.6%, p=0.02, mean: 35.7%, p=0.0001, respectively) when compared to patients with MF and healthy donors (HDs) (Figure 1a,b,). TIGIT and Helios were also highly expressed in the skin of SS and MF patients compared with atopic dermatitis (AD), psoriasis (PS) and control skin (HD) (Figure 1c–e). TIGIT mRNA in skin from SS and MF patients was 327 and 110 fold increased, respectively, compared with levels in AD patients or control skin (Figure 1c). Similarly, Helios mRNA was increased in the skin of SS and MF patients, 77 and 13.6-fold, respectively, (Figure 1d), along with FCRL3, 765 and 68.3-fold for SS and MF, respectively, when compared to HD, (Figure 1e).

The data presented in Figure 1a, distinguishes patients with a high and low percentage of $TIGIT^{+}$ $CD4^{+}$ T cells. A thorough analysis of patients with high TIGIT expression (mean: 79.3%, SD: 9.7, Figure 2a) revealed that patients' $CD4^{+}$ T cells also demonstrate a high percentage of CD26 negative cells (mean: 87.1%, SD: 9.6), expresses a single TCRVβ (mean: 85.9%, SD: 11.3), highly express Helios (mean: 46.7%, SD: 19.4), FCRL3 (mean: 64.9% SD: 25.7), and CD164 (mean: 56.7%, SD: 26.6), but low PD1 (mean: 22.1%, SD: 15.8). Importantly, TIGIT expression correlated positively and significantly with CD26 negativity and with a single TCRVβ, (Figure 2b, c) but not with the expression of Helios, FCRL3 and CD164 due to the variability between patients.

Patients with low expression of TIGIT on $CD4^{+}$ T cells (mean: 7.9 %, SD: 5.12, Figure 2d) also demonstrate a low percentage of CD26 negativity (mean: 28.5%, SD: 18.7), no detectable single TCRVβ, low expression of Helios (mean: 29.6%, SD: 26.1), FCRL3 (mean: 11.9%, SD: 13.5), CD164 (mean: 21.5%, SD: 22.7) and PD1 (mean: 10.0%, SD: 12.6). This group of patients lacks a well defined cell surface malignant phenotype with the percentages of $CD4^{+}$ T cells expressing the aforementioned molecules being significantly lower compared to high TIGIT expressing patients ($p<0.05$ for all tested molecules), implying that low TIGIT expressing patients have a low circulating tumor burden. We analyzed the clinical differences between the high and low TIGIT/Helios groups in terms of tumor burden in the blood, stage, and LDH and found that all patients in the high TIGIT group (n=9) were diagnosed with advanced B2 disease, whereas among 16 patients with low TIGIT expression, 10 patients were diagnosed as B0 (62.5%) and 6 patients were diagnosed as B1 (37.5%).

TIGIT is a known activation marker and is expressed on chronically activated, exhausted $CD4^+$T cells (Chew *et al.*, 2016; Le Mercier *et al.*, 2015; Pauken and Wherry, 2014). Furthermore, as we demonstrate, it is highly expressed on $CD26^-$ $TCRV\beta^+$ $CD4^+$T cells, defined as the malignant population in SS. We observed significantly decreased production of IFN-γ and IL-2 by $TIGIT^+$ compared with $TIGIT^-$ $CD4^+$T cells from patients with high TIGIT expression and advanced B2 disease (Figure 2e,f). This is consistent with the reported function of TIGIT positive, exhausted T cells and is strongly suggestive that TIGIT is associated with an immunosuppressive phenotype typically observed in advanced SS by being expressed on both exhausted and malignant $CD4^+$ T cells (Joller *et al.*, 2014; Kourepini *et al.*, 2016; Kurtulus *et al.*, 2015). In contrast to patients with high TIGIT expression, $TIGIT^+$ $CD4^+$ T cells from patients with low TIGIT expression produce IFN-γ and IL-2 at levels comparable to their TIGIT negative cells (Figure 2g, h). These results suggest that low TIGIT expression in these patients may be linked to activation status of CD4 T cells rather than to an exhausted phenotype.

The precise pathogenic nature of cells expressing TIGIT and Helios molecules in CTCL currently remains unknown. $TIGIT^+$ and/or $Helios^+$ $CD4^+$ T-cells may represent: malignant cells, exhausted cells or non-conventional $CD4^+$T regulatory cells, lacking Foxp3 and CD25 (data not shown). Malignant $Foxp3^+CD25^-$ $CD4^+$ T cells with suppressive activity have been identified in SS patients, further suggesting that the typical phenotype of T regulatory cells may be challenged in the context of CTCL (Heid *et al.*, 2009).

High expression of TIGIT and Helios identifies $CD4^+$ T cells with impaired immunological functions, primarily among patients with an advanced stage of Sézary syndrome, suggesting that high expression of these molecules may correlate with a poor prognosis. However, further studies are needed to define in detail the functional and biological significance of TIGIT and Helios expressing $CD4^+$ T cells in SS and the potential use of these markers as therapeutic targets.

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Abbreviations

CTCL	Cutaneous T-cell Lymphoma
SS	Sezary Syndrome
MF	mycosis fungoides
AD	Atopic Dermatitis

PS	Psoriasis
HDs	healthy donors

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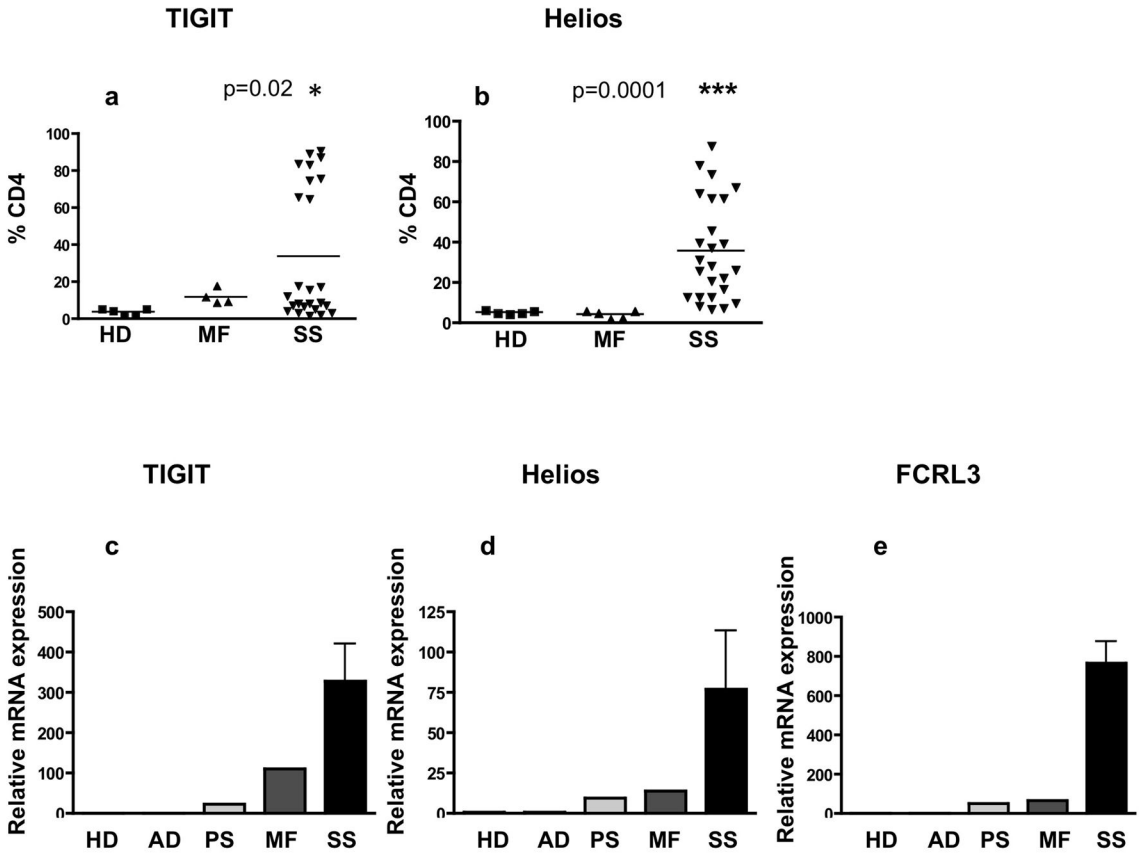

Figure 1. Helios, TIGIT and FCRL3 are highly expressed in skin and PBMC of Sézary syndrome patients

(a,b) The cell surface expression of TIGIT (**a**) and intracellular expression of Helios (**b**) was assessed by flow cytometry in $CD4^+$ T cells from SS patients (n=25) MF patients (n=5) and healthy donors HDs (n=5). Results are expressed with the mean. (**c,d,e**) Skin samples from 11 SS patients, 7 MF patients, 4 atopic dermatitis (AD), 3 psoriasis (PS) and 8 normal controls (HDs, pooled samples) were examined for mRNA expression of TIGIT (**c**), Helios (**d**) and FCRL3 (**e**) by qRT-PCR.

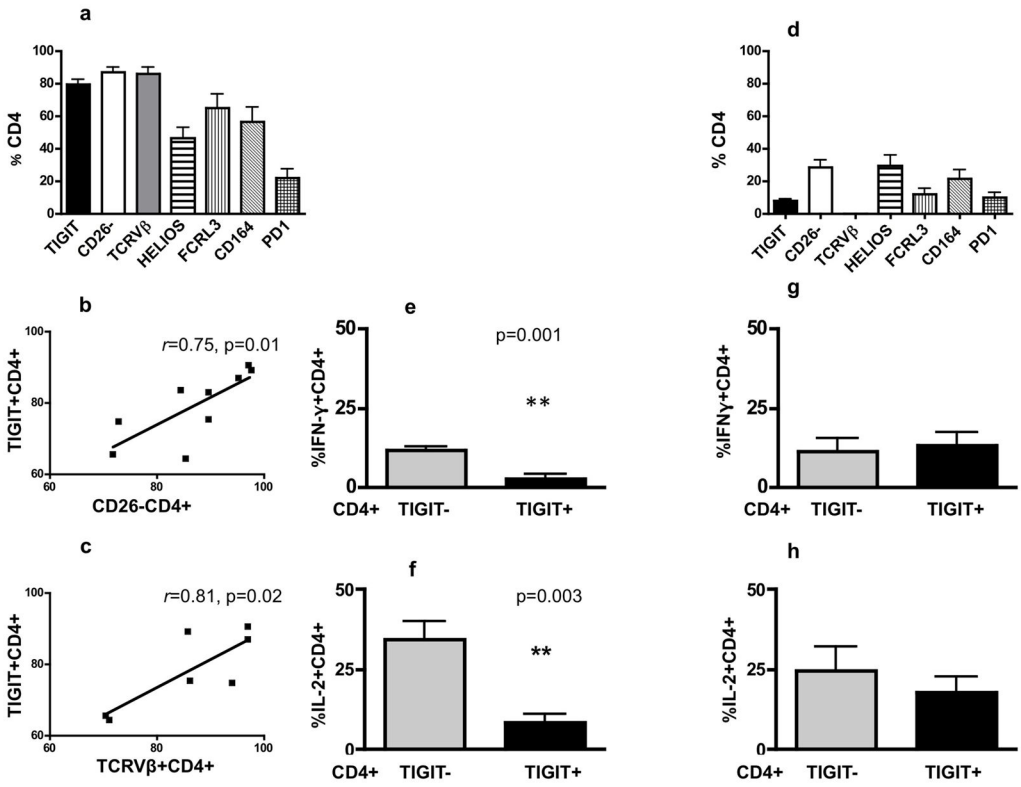

Figure 2. High TIGIT expression correlates with the high percentage single TCRVβ$^+$CD4$^+$ T cells, loss of CD26 expression and is associated with impaired cytokines production

(**a**) Peripheral blood mononuclear cells (PBMC) from patients with high TIGIT expression (n=9 including 7 patients with defined TCRVβ) were analyzed by flow cytometry to assess the expression of molecules on CD4$^+$ T cells. (**b,c**) High TIGIT expression in CD4$^+$ T cells correlates significantly with high percentage of CD26 negative (**b**, r=0.75, p=0.01, n=9) and single TCRVβ positive CD4$^+$ T cells (**c**, r=0.81, p=0.02, n=7). (**d**) Flow cytometry analysis of PBMC from patients with low TIGIT expression to assess the expression of molecules on patients' CD4$^+$ T cells. In this group of patients, there is a significant decrease in the percentages of CD4$^+$ T cells expressing tested molecules compared to percentages for corresponding molecules in patients with high TIGIT expression (p<0.05). (**e,f**) TIGIT$^+$CD4$^+$ T cells from patients with high TIGIT expression produce significantly less IFN-γ (**e**, p=0.001, n=6) and IL-2 (**f**, p=0.003, n=6) compared with TIGIT$^-$CD4$^+$ T cells from these patients. (**g,h**) In patients with low TIGIT expression there were no differences in levels of IFN-γ (**g**) and IL-2 (**h**) production between TIGIT$^+$ and TIGIT$^-$ CD4$^+$ T cells (n=6).