IL-31 Is Produced by the Malignant T-Cell Population in Cutaneous T-Cell Lymphoma and Correlates with CTCL Pruritus

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TO THE EDITOR

Cutaneous T-cell lymphomas (CTCL) are a heterogenous group of non-Hodgkin's lymphomas of skin-trafficking T lymphocytes (Diamandidou *et al.,* 1996). CTCL patients often suffer from intense pruritus, which can cause significant morbidity and decreased quality of life (Sampogna *et al.,* 2009; Ahern *et al.,* 2012). At present, the underlying pathophysiologic mechanisms of itch in CTCL are not fully known, and therefore there is a significant unmet need for clinically effective treatments in reducing CTCL-associated itch (Ahern *et al.,* 2012).

IL-31, a short-chain 4-helix-bundle cytokine produced primarily by skin-homing CD4 T cells, was found to cause dermatitis, alopecia, and pruritus when overexpressed in transgenic mice (Dillon *et al.,* 2004). Moreover, IL-31 mRNA and protein levels are increased in pruritic human skin diseases including atopic dermatitis, allergic contact dermatitis, and prurigo nodularis, and serum IL-31 levels correlate with the severity of pruritus in atopic dermatitis (Sonkoly *et al.,* 2006; Raap *et al.,* 2008).

Recently, increased IL-31 protein was also reported in the serum of patients with CTCL, and, although its levels correlated with advanced CTCL disease stage, no data were presented correlating levels with pruritus severity (Miyagaki *et al.,* 2012; Ohmatsu *et al.,* 2012). Given the association of IL-31 with pruritic skin diseases and its recent discovery in CTCL, we investigated IL-31 in CTCL patients with mycosis fungoides or Sézary syndrome.

By using quantitative reverse transcriptase in real time, we tested the cells of 29 CTCL patients with leukemic involvement and 13 healthy, aged-matched controls for levels of IL-31 mRNA. The mean value of IL-31 mRNA from 29 patients was 24.73 with a median of 2.61 (interquartile range (IQR) 1.21, 10.56) when normalized to the mean value of 13 normal, aged-matched controls, assigned a value of 1.0.

We retrospectively reviewed physician notes to assess whether each patient complained of itch at that time of sample collection, and if present whether that itch had increased, decreased, or remained the same compared with the previous visit. On the basis of this retrospective chart review, IL-31 mRNA levels significantly correlated with patients experiencing marked pruritus at the time of sample collection (marked pruritus, mean 50.02, median 9.99 (IQR 5.86, 20.76); decreasing/absent pruritus, mean 3.94, median 1.83 (IQR 0.64, 3.25); Wilcoxon rank sum $P=0.006$) (Figure 1a).

We next tested the serum of 40 CTCL patients for the presence of IL-31 protein, 26 with pruritus at the time of sample collection and 14 without pruritus, and observed a statistically significant difference in IL-31 levels between pruritic and non-pruritic patients (pruritic, mean IL-31 value 88.32 pg ml^{-1}, median 9.71 (IQR 7.80, 29.62); non-pruritic, mean 11.47 pg ml^{-1}, median 7.80 (IQR 7.80, 7.80); Wilcoxon rank sum $P=0.008$; Figure 1b). There were no significant differences in age or gender between the marked pruritic and decreasing/absent pruritus patients, and there was also no correlation between IL-31 mRNA or protein and patient stage, as the majority of patients tested in our sampling were advanced stages 3A, 3B, or 4A (Supplementary Table S1 online).

The malignant cells in CTCL are typically CD3+ CD4+ CD26− T lymphocytes that may also express a single dominant Vβ TCR (Bernengo *et al.,* 2001; Jones *et al.,* 2001; Yawalkar *et al.,* 2003). Using flow cytometry, we tested 15 CTCL patients (two stage 3A, four stage 3B, nine stage 4A) and seven age-matched, healthy volunteers for intracellular IL-31 expression. We found that eight patients, all of whom were pruritic, expressed intracellular IL-31 by their stimulated CD4 T cells (Figure 2a). Among these eight patients, seven patients predominately expressed IL-31 within their CD3+ CD4+ CD26− T lymphocytes in distinction to their CD3+ CD4+ CD26+ lymphocytes (CD4+ CD26−: mean 3.22%, median 2.07 (IQR 0.72, 5.69); CD4+ CD26+: mean 0.744%, median 0.55 (IQR 0.11, 1.15), $P=0.0357$, Wilcoxon matched-pairs sign-ranked test, Supplementary Figure S1 online). We detected only minimal expression of IL-31 in two non-pruritic patients (representative patient, Figure 2b) and in healthy volunteer cells (<0.35%, data not shown).

In addition, three of eight IL-31-positive patients had a detectable single, dominant TCR Vβ clone, as determined by a panel of commercially available Vβ antibodies. We stained the cells of these patients with their specific anti-Vβ antibody and report that in all three cases CD4+ Vβ-positive cells, in comparison with CD4+ Vβ-negative cells, were the predominant producers of IL-31 (representative patient, Figure 2c).

We also investigated whether clinical resolution of pruritus correlates with declining IL-31 protein. Patient 1

Abbreviations: CTCL, cutaneous T-cell lymphoma; PBMC, peripheral blood mononuclear cell

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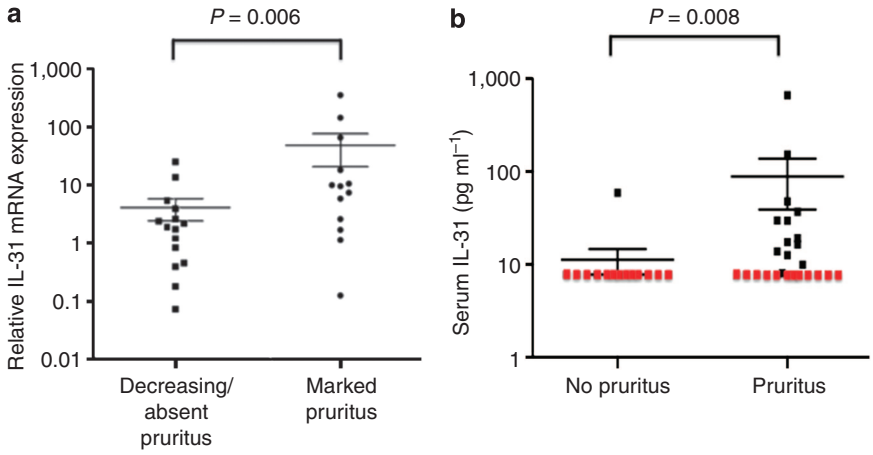

Figure 1. Peripheral blood mononuclear cell (PBMC) IL-31 mRNA and serum IL-31 correlates with marked pruritic symptoms in cutaneous T-cell lymphoma (CTCL) patients. (**a**) PBMCs were stimulated with phorbol 12-myristate 13-acetate (PMA)/ionomycin. IL-31 mRNA isolated from PBMCs of CTCL patients with decreasing or absent pruritus versus those with marked pruritus, $P = 0.006$. Note that all CTCL patient mRNA values were standardized relative to the average values from a selected pool of 13 aged-matched, normal controls. Error bars: mean ± SEM (*n*: decreasing/absent pruritus = 16; marked pruritus = 13). (**b**) Serum IL-31 levels in CTCL patients without pruritus ($n = 14$) versus those with pruritus ($n = 24$), $P = 0.0083$. Error bars: mean ± SEM (*n*: no pruritus = 14; pruritus = 26). Note that the linear range of the assay was 7.8 pg ml^{-1} and that all samples with a value below 7.8 pg ml^{-1} or those without any detectable IL-31 were assigned a value of 7.8 pg ml^{-1} and are colored in red.

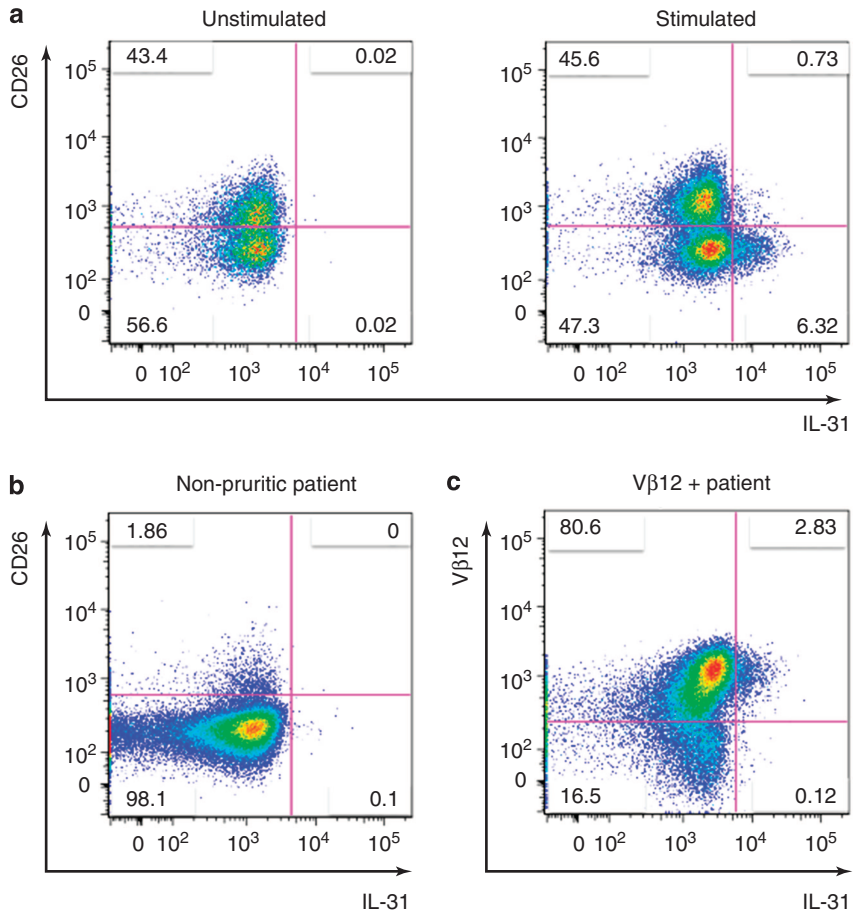

Figure 2. IL-31 is produced by malignant CD4+ CD26−/CD4+ Vβ+ CTCL cells. Fifteen cutaneous T-cell lymphoma (CTCL) patients' CD4 T cells, stimulated with phorbol 12-myristate 13-acetate (PMA)/ionomycin, were assessed for intracellular expression of IL-31 by flow cytometry. (**a**) Representative plots of IL-31 expression from cells without (left) and with (right) PMA/ionomycin stimulation. (**b**) Representative flow cytometric plot of IL-31 expression from a non-pruritic patient. (**c**) Representative plot of IL-31 expression from CD4+ Vβ+ CTCL cells.

initially presented with diffuse intractable pruritus, 17.4 pg ml^{-1} serum IL-31, and 6.32% IL-31-positive CD4+ CD26− cells. After multimodality therapy with photopheresis, IFN-α, and oral bexarotene, the patient had near resolution of pruritus, undetectable serum IL-31, and only 1.1% IL-31-positive CD4+ CD26− cells (Supplementary Figure S2 online, top). Patient 3 presented with diffuse intractable pruritus, a dominant detectable Vβ clone (>83% of CD4 T-cells were Vβ+), and 2.83% of CD4+ Vβ+ cells positive for IL-31. After similar multimodality treatment, the patient had resolution of pruritus, decreased Vβ+ cells (28.6% of CD4 cells Vβ+), and undetectable CD4+ Vβ+ IL-31+ cells (<0.10%) (Supplementary Figure S2 online, bottom).

In this report, we confirm that PBMC-derived IL-31 mRNA is increased in CTCL and that it significantly correlates with patients experiencing marked pruritus at the time of sample collection. Furthermore, 14 of 26 pruritic patients (53%) had detectable serum IL-31, whereas serum IL-31 protein was not detectable in 13 of 14 non-pruritic CTCL patients (92%). It should be noted that our ELISA was less sensitive than that used by Ohmatsu *et al.*, and we may have not identified some patients who produce less than 7.8 pg ml^{-1} of IL-31, the lower limit of our assay. In addition, it should be noted that we classified patients based on their stage at the time of diagnosis, and that some advanced-stage patients were probably non-pruritic when tested, given that they had already initiated multimodality therapy. Importantly, our flow cytometry results showing that in some patients only a small percentage of lymphocytes produce IL-31 are consistent with the report by Szegedi *et al.* (2012) in which, using the same IL-31 polyclonal antibody, only 1% of CD4 T cells were found to produce IL-31.

It is not yet fully clear as to what drives the production of IL-31. Although Sézary syndrome patients are frequently colonized by *Staphylococcus aureus*, which has been shown to increase IL-31 expression, this is probably not the primary mechanism of IL-31 production given that there were *S. aureus*–colonized Sézary syndrome patients included in this study who did not produce IL-31 (Sonkoly *et al.*, 2006; Talpur *et al.*, 2008).

In summary, in this study, we report that CD4+CD26- malignant cells specifically produce IL-31 and that clinical resolution of pruritus correlates with decreased IL-31 levels in the circulation. Given this strong association, IL-31 should be considered as a potential therapeutic target for anti-itch treatment in CTCL.

Studies were conducted in accordance with the Declaration of Helsinki and approved by the University of Pennsylvania's Institutional Review Board (IRB). All subjects provided written informed consent.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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