Cutaneous T-cell Lymphoma and Pruritus: The Expression of IL-31 and its Receptors in the Skin

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 Approximately 88% of cutaneous T-cell lymphoma (CTCL) patients are affected by pruritus that responds poorly to current antipruritic therapies. Interleukin (IL)-31, a Th2 cytokine, has been found to be increased in the serum of CTCL patients and to correlate with itch severity. This study investigated the role of IL-31 and its receptors (IL-31 receptor-alpha [IL-31RA] and OSMRβ) in the skin of CTCL patients with mild versus moderate/severe pruritus. Expression levels of IL-31, IL-31RA, and OSMRβ in the skin were measured using immunohistochemistry and correlated to pruritus severity and disease stage. In CTCL patients with moderate/severe pruritus, IL-31 was significantly elevated in the epidermis and dermal infiltrate, while IL-31RA and OSMRβ were significantly elevated only in the epidermis. Furthermore, epidermal IL-31 levels correlated to itch severity. These results show that IL-31 may play a role in CTCL pruritus by exerting indirect effects on sensory nerves through epidermal neoplastic T cells and keratinocytes to transmit itch. Key words: CTCL; skin; pruritus; IL-31; IL-31RA; OSMRβ.

METHODS

Subjects

Skin biopsies were obtained from CTCL patients and healthy subjects at the dermatology clinics of Temple University, Northwestern University, and the University of Pennsylvania in accordance with the Declaration of Helsinki and with approval by each institution’s Institutional Review Board.

Patients were diagnosed based on clinical, histopathological, and immunohistological criteria and staged using the Tumor-Node-Metastasis-Blood (TNMB) 2007 International Society for Cutaneous Lymphomas (ISCL) and European Organization of Research and Treatment of Cancer (EORTC) revised classifica-
Histology and immunohistochemistry

Researchers were blinded to the identity of the biopsies and the results were only decoded after the analysis was fully performed. For histology to confirm disease stage, 5-μm thick sections of paraffin-embedded skin tissue were processed for standard hematoxylin and eosin (H&E) staining. For immunohistochemistry, a total of 12 20-μm thick sections of paraffin-embedded skin tissue were deparaffinized and then underwent antigen retrieval using Target Retrieval Solutions (DAKO, Glostrup, Denmark) heated in a humidified oven overnight at 60°C, then washed in PBS. Sections were blocked with 5% normal donkey serum and 0.2% Triton X-100 in PBS for 2 h and then incubated with primary antibodies overnight at 4°C. Primary antibody combinations were: anti-PCP9.5 (1:50; Abcam, Cambridge, MA) and anti-IL-31 (1:100; Abcam); anti-IL-31RA (1:200, Abcam) and anti-OSMRβ (1:100; Santa Cruz, Dallas, Texas); Alexa Fluor (488 & 594, 1:300; Molecular Probes, Eugene, OR) secondary antibodies were used for detection. The slides were mounted with Vectashield with DAPI (Vector Laboratories, Burlingame, CA) and imaged under a fluorescence microscope. Sections treated without any primary antibodies were used as negative controls. Furthermore, specificity of the IL-31 and IL-31RA antibody was confirmed by pre-absorbing the full length IL-31 and IL-31RA peptides (20 μg/ml; Abcam) in blocking solutions with their respective antibodies overnight at 4°C with gentle agitation. Solutions were centrifuged, and the supernatant was used for IHC as described above, which resulted in blocking the IL-31 and IL-31RA immunoreactivity.

Quantification

Three fields (20X objective magnification) were measured for every section. The total field and selected field (epidermis) fluorescence area (in μm²) were measured and normalized to background staining using ImageJ Software. Data is presented as the mean epidermal fluorescence and mean dermal infiltrate fluorescence, which was calculated as the total field – selected field fluorescence. Inter-epidermal nerve fibers were counted and normalized to tissue length as previously described (17). The immunofluorescence staining was further compared to the H&E staining to examine localization of positive staining to lymphocytes.

Statistical analysis was carried out using one-way ANOVAs with Bonferroni post hoc tests and non-parametric two-tailed Spearman correlations with linear regression; significance was set at \( p < 0.05 \) (GraphPad Prism; La Jolla, CA).

RESULTS

Cutaneous T-cell lymphoma characteristics and pruritus

The mean ages of the 3 groups (Table I) did not significantly differ from one another. There was a statistically significant difference \( (p < 0.0001) \) in pruritus VAS ratings among healthy (VAS 0), mildly pruritic CTCL (VAS 2.5 ± 1.5), and moderately/severely pruritic CTCL (VAS 7.5 ± 1.2) subjects (Fig. 1A). No correlation was found between age and VAS ratings (Fig. 1B). The stage of CTCL correlated to VAS ratings \( (r = 0.85, p = 0.04) \) in mildly pruritic CTCL subjects only (Fig. 1C).

### Table I. Sample demographic data

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Age, years, mean ± SD</th>
<th>Sex (M:F), n</th>
<th>VAS rating, mean ± SD</th>
<th>Diagnosis, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>32 ± 1.5</td>
<td>8</td>
<td>Healthy</td>
<td></td>
</tr>
<tr>
<td>Moderate pruritic</td>
<td>28 ± 1.8</td>
<td>15</td>
<td>7.5 ± 1.2</td>
<td>CTCL</td>
</tr>
<tr>
<td>CTCL</td>
<td>65 ± 13.9</td>
<td>8</td>
<td>2.5 ± 1.5</td>
<td>8</td>
</tr>
</tbody>
</table>

\*Stage (Tumor-Node-Metastasis-Blood).

PCGDTCL: primary cutaneous gamma delta T-cell lymphoma.

Fig. 1. Pruritic VAS rating and its correlation to cutaneous T-cell lymphoma (CTCL) stage. (A) The pruritic VAS rating was significantly higher in CTCL patients when compared to healthy controls (***\( p < 0.001 \), ****\( p < 0.0001 \)). (B) The pruritic rating did not correlate to subject age. (C) The pruritic ratings correlated to CTCL disease stage in the mildly pruritic CTCL group only.

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**IL-31 and epidermal innervation (PGP 9.5)**

IL-31 was significantly ($p<0.0001$) increased in the epidermis and lymphocytic infiltrate of CTCL subjects, with the moderately/severely pruritic CTCL subjects having the highest levels of IL-31 in the skin (Fig. 2A). The mean ± SD IL-31 fluorescence levels in the epidermis of moderately/severely pruritic CTCL subjects were $1,859 ± 271$, while the infiltrate levels were $1,049 ± 164$. In mildly pruritic CTCL subjects, the IL-31 levels were $1,489 ± 123$ in the epidermis and $625 ± 129$ in the infiltrate. Healthy controls did present with some constitutive IL-31 in the epidermis, with levels of $239 ± 72$, but little to none in the dermis ($5 ± 15$). Interestingly, in all subjects, IL-31 expression was limited to the lymphocytic infiltrate in the dermis and in the epidermis (representative image: Fig. 2B). No significant difference among groups was found in the number of epidermal nerve fibers, and IL-31 did not appear to co-localize to any afferent fibers (data not shown). Epidermal IL-31 levels significantly correlated ($r = 0.94; p< 0.0001$) with VAS ratings (Fig. 2C), but not with CTCL stage ($r = 0.04; p= 0.86$; Fig. 2D).

**IL-31RA and OSMRβ**

IL-31RA was significantly ($p<0.0001$) elevated in only the epidermis of CTCL subjects (Fig. 3A; representative image: Fig. 3C). The mean ± SD IL-31RA fluorescence levels were highest in moderately/severely pruritic subjects, with levels of $1,394 ± 183$ in the epidermis and $50 ± 12$ in the lymphocytic infiltrate. Mildly pruritic CTCL subjects had expression levels of $620 ± 42$ in the epidermis and $21 ± 29$ in the infiltrate, while healthy controls had levels of only $377 ± 56$ in the epidermis and $12 ± 3$ in the dermis. Epidermal IL-31RA levels significantly correlated ($r=0.84; p<0.0001$) with VAS ratings (Fig. 3B), but not with CTCL stage ($r=−0.01; p=0.96$).

Furthermore, OSMRβ was also significantly ($p<0.0001$) higher in the epidermis of CTCL subjects (Fig. 3D; representative image: Fig. 3F). In moderately/severely pruritic CTCL subjects, the mean ± SD OSMRβ fluorescence levels were $693 ± 184$ in the epidermis and $142 ± 93$ in the lymphatic infiltrate. Mildly pruritic CTCL subjects had levels of $421 ± 83$ in epidermis and $174 ± 123$ in the lymphocytic infiltrate. The lowest levels of OSMRβ were observed in healthy controls (epidermis $183 ± 26$; dermis $78 ± 15$). Epidermal OSMRβ levels significantly correlated ($r=0.85; p<0.0001$) with VAS ratings (Fig. 3E), but not with CTCL stage ($r=−0.16; p=0.47$).

**DISCUSSION**

Although several recent studies have found increased levels of IL-31 in CTCL, these studies largely focus on serum analysis and do not address the crucial question of IL-31’s role in the skin (13, 14, 18). This is the first study to show the expression levels and patterns of IL-31 and both of its receptors within the skin of CTCL subjects with pruritus. Similar to most of the previous serum studies, this study shows that elevated IL-31 is positively correlated to itch intensity (13, 18). We also report that IL-31 was not correlated to disease stage, suggesting that IL-31 does not play an essential role in disease progression.
role in the pathogenesis of CTCL. However, our study was limited by the low number of early stage CTCL patients and by the lack of CTCL patients without itch.

This study also found increased expression of the IL-31 receptors, IL-31RA and OSMRβ. The previous findings for these receptors in CTCL is limited to one serum and cancer cell line study, which did not find evidence of significant elevation of these receptors, possibly due to their focus on the blood compartment of CTCL cells (18). The IL-31RA and OSMRβ receptors are known to be located on keratinocytes, rather than serum, making this study better suited to assess the role of IL-31 signaling in CTCL. Interestingly, a recent study in mice showed that repeated IL-31 exposure caused elevated expression of IL-31RA and OSMRβ in the dorsal root ganglia (19). This mechanism could explain the increase in receptor density we found in the epidermis. In another study, a single intradermal exposure of IL-31 did not cause pruritus in healthy subjects (20). Therefore, the increase in IL-31RA and OSMRβ expression after chronic exposure to IL-31 may be necessary in order for a subsequent exposure to IL-31 to induce pruritus.

Our study showed elevated expression of IL-31 in the epidermis. This result is similar to studies performed in atopic dermatitis subjects, which localized IL-31 to lymphocytes infiltrating the skin (11), and in lichen planus, which found overexpression of IL-31 in the epidermis. However, IL-31 expression in lichen planus did not correlate to pruritus ratings (21). It is thought that IL-31 can induce keratinocytes and infiltrating cells to release additional mediators involved in pruritus. Furthermore, IL-31 has also been shown to cause the release of pro-inflammatory cytokines from eosinophils, monocytes, and macrophages (22, 23). It would be of great interest to investigate the role of other pruritic mediators and cell types involved in CTCL pruritus.

The finding of elevated IL-31 and its receptors in the skin of pruritic CTCL subjects adds the missing link to previous studies investigating the role of IL-31 in CTCL pruritus. As pruritus treatments begin to focus on reducing IL-31 in CTCL patients, it will be interesting to correlate the therapeutic response with epidermal IL-31, as well as possible changes in the expression of IL-31 receptors (13, 24, 25).

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REFERENCES


