

SHORT COMMUNICATION

Absence of Full-length Neurokinin-1 Receptor Protein Expression by Cutaneous T Cells: Implications for Substance P-mediated Signaling in Mycosis FungoidesMarina Tuzova^{1#}, Tara Conniff^{2#}, Clara Curiel-Lewandowski³, Keri Chaney⁴, William Cruikshank¹ and Deon Wolpowitz^{2*}¹The Pulmonary Center, ²Department of Dermatology and Section of Dermatopathology, Boston University School of Medicine, Boston, MA 02118, ³Arizona Cancer Center, Arizona State University, Tucson, and ⁴Dana Farber Cancer Institute, Harvard Medical School, Boston, USA. *E-mail: dewolpow@bu.edu[#]These authors contributed equally to this work and should be considered as first authors.

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Mycosis fungoides (MF) is a cutaneous T-cell lymphoma composed of epidermotropic atypical effector memory T lymphocytes (1). MF patients can suffer from debilitating pruritus (1). Clinical and experimental observations implicate signaling by the neurokinin-1 receptor (NK1R) and its highest affinity ligand, substance P (SP), in the epidermotropism and pruritus of MF. In early-stage skin lesions of MF, malignant epidermotropic T cells line up at the dermal–epidermal junction (DEJ), the anatomic site of termination of SP-expressing nerves (2). SP induced chemotaxis and proliferation of normal T lymphocytes *in vitro*, and this effect utilized the NK1R (3, 4). The pruritus of MF remits with treatments that reduce or eradicate malignant T cells (5). The pruritus in some MF patients, based on case reports, can respond rapidly to aprepitant, an oral NK1R antagonist (6). NK1R expression and signaling has been demonstrated in T cells in other settings (7), and SP binding was reported in the dermis in MF (8). These data suggest that *in vivo*, MF T-cell proliferation, migration, and/or pruritus signaling may be modulated by SP/NK1R signaling. We hypothesized that such signaling would occur directly through SP activation of NK1R on malignant T cells. To test this hypothesis, we examined the immunohistochemical expression of full-length NK1R (fl-NK1R) and of SP in the skin and blood of CTCL-MF subjects.

MATERIALS AND METHODS

Appendix S1¹ provides complete details of the reagents, immunohistochemistry protocols, image and data analysis, ELISA measurements, statistical analyses, and tissue and blood samples obtained with IRB approvals from Boston University, Arizona Cancer Center, and the Dana Farber Cancer Institute.

RESULTS

We performed immunohistochemistry with a C-terminus-directed antibody (see Appendix S1¹) to detect fl-NK1R expression in combination with CD3 for T cells and mast-cell tryptase for mast cells. In normal human skin (NHS), surgically-induced scars (SIS), and in skin from 17 MF subjects (see Table S1¹), fl-NK1R was detected in upper

spinous and granular cell layer keratinocytes, endothelial cells, and mast cells in accordance with previous studies ([9] and see Appendix S1¹, Fig. S1¹ and data not shown). In NHS, SIS, and in all MF subjects, epidermal or papillary dermal CD3⁺ T lymphocytes lacked fl-NK1R (Fig. 1 and Fig. S1¹). Fl-NK1R was detected on circulating CD3⁺ T cells from peripheral blood cytopins in normal donors and 4 of 4 MF subjects (both early and later stages of MF) (Fig. S2¹), randomly selected from the ELISA samples (Table SII¹). Circulating versus organ-specific T cells can differ in their synthesis and cell surface expression of proteins, and NK1R-expression has been reported in circulating T lymphocytes (10, 11).

Given the presence of NK1R-expressing circulating T cells, we measured systemic SP levels in MF subjects whose demographics are presented in Table SII¹. These samples were divided into early stage (stages Ia, Ib) and later stages (IIa–III). Compared to healthy controls (median, 25–75%: 194, 102.5–212.8 pg/ml), early (499, 267.9–574 pg/ml; $p=0.0106$) and later-stage MF groups (541.7, 395.1–582.7 pg/ml; $p=0.0032$) showed a significant increase in serum SP levels (Fig. 1). Expansion or proliferation of SP nerves in the papillary dermis did not account for the elevated systemic SP in MF subjects. SP(+) nerves are a minority of the total percent of PGP9.5+ intra-epidermal and papillary dermal nerves in MF subjects (median; 25–75%: 4.8%, 1.7–6.6%), similar to NHS (8.1%, 4.4–12.8%) (2). SP-expressing nerves were located predominantly in the papillary dermis and rarely entered the epidermis in MF subjects (0%, 0–2.2% of PGP9.5+ intra-epidermal nerves) (Fig. 1). SP expression was not detectable in CD3⁺ T cells or in mast cells in NHS or MFS ((2), Fig. 1 and Fig. S1¹).

DISCUSSION

We postulated that putative SP/NK1R signaling in migration, proliferation, and/or pruritus generation in MF would involve SP directly activating NK1R on malignant T cells (3, 5, 6). Fl-NK1R was not detected in T cells in normal skin or MF skin lesions, including in 5 MF skin samples with known and active pruritus of the specific lesion biopsied at the time of biopsy. Our findings are potentially limited by the inability of the C-terminus antibody to detect the shorter NK1R isoform

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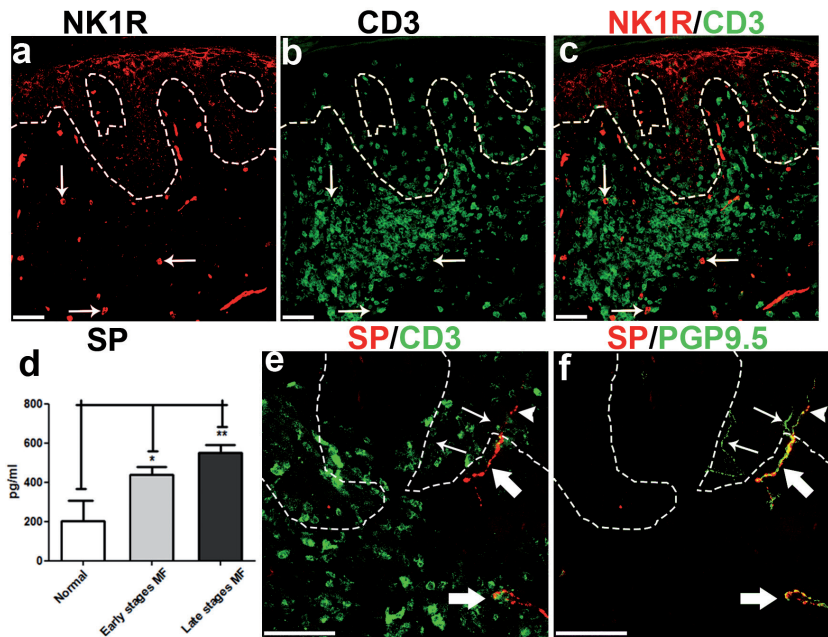


Fig. 1. Immunohistochemical expression of full-length NK1 receptor expression and substance P (SP) in skin and plasma SP levels in mycosis fungoides (MF) subjects. Double immunohistochemical staining of 4 μ m thick sections of (a-c) cutaneous MF with NK1R and CD3. (a, c) thin arrows indicate NK1R-expressing mast cells (a) that do not co-label with CD3-positive T cells (b, c). (d) Plasma levels of SP in normal donors ($n=4$), early stages (stages Ia, Ib; $n=19$), and later stages (stage IIa-III, $n=15$) of MF. Serum SP levels are significantly increased in both groups versus normal controls (one way ANOVA, $p=0.002$). (e, f) Triple-label immunohistochemical staining of 50 μ m thick skin sections of human MF with CD3, SP, and PGP9.5 (e) Composite image of CD3 (green) and SP (red). (f) Composite of PGP9.5 (green) and SP (red). (e, f) thick arrows highlight SP(+)/PGP9.5(+) papillary dermal nerves (yellow in (f)); arrowhead indicates SP(+)/PGP9.5(+) intraepidermal nerve fiber; thin arrows indicate SP(-)/PGP9.5(+) intraepidermal nerve fibers. (a-c, e, f) dotted lines indicate the dermoepidermal junction. (a-c, e, f) 200x; scale bars, 50 μ m.

missing the last 96 amino acids of the C-terminus (7). Given the absence of a functioning N-terminal antibody (see Appendix S1¹), we cannot exclude expression of truncated NK1R in MF subjects. Nevertheless, we found concordance between the expression pattern we obtained and that reported using labeled SP (9). SP/NK1R signaling in MF skin lesions may play a biological role independent of fl-NK1R in skin T cells. The skin remains an important site for generation of pruritogens in MF, and SP may contribute through fl-NK1R -expressing keratinocytes or mast cells, or through malignant T cells using an NK1R-independent mechanism (12).

The biological significance of the elevated systemic SP levels in MF is still unknown. In MF subjects, elevated SP levels occurred in the absence of a proliferation of SP-expressing papillary dermal nerves. Elevated SP levels correlated with itch intensity in atopic dermatitis (13). In MF, both the degree of pruritus (1) and systemic SP levels increased with disease stage. Our study is limited by the absence of prospective quantitation of pruritus severity to correlate with serum SP measurements. Nevertheless, our data provide biologic rationale for a future such investigation to assess the contribution of systemic SP to MF itch.

In conclusion, elevated systemic SP levels may contribute to the pathophysiology of MF using fl-NK1R -expressing skin cells, central nervous system cells (7), and now circulating lymphocytes. This complex distribution of NK1R expression highlights the need for both *in vitro* and prospective *in vivo* studies to ascertain further the contribution of SP and NK1R signaling to MF pathogenesis and pruritus.

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