

Abnormal expression of interleukin-23 in mycosis fungoides/Sézary syndrome lesions

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Abstract Progression of mycosis fungoides (MF) to Sézary syndrome (SS) is accompanied by a shift from a T_H1 to a T_H2 cytokine profile. Interleukin (IL)-23 is a novel cytokine that shares a common p40 subunit with the T_H1 inducer, IL-12. IL-23 induces a third profile, T_H17 , that is dominant in inflammation and autoimmunity. Although IL-23 induces an eczematous-like skin reaction in mice, and is expressed in T_H1 -mediated skin disorders such as psoriasis, it has not been evaluated in MF/SS. To study the role of IL-23 in MF/SS development, 40 MF/SS lesions of all stages were immunohistochemically analyzed with a novel anti-human IL-23 antibody raised against full-length human IL-23. IL-23 was detected with the catalyzed signal amplification system. The intensity and frequency of IL-23 staining were semi-quantitatively graded in both the dermal infiltrate and the epidermis. Increased expression of IL-23 was observed throughout the epidermal keratinocytes and in dermal lymphocytes compared to normal skin. IL-23 intensity did not differ

significantly among the stages of MF/SS; however, in stage IVB patients, we observed lower frequency of IL-23 expression in dermal lymphocytes than in other stage patients [$P = 0.13$, analysis of variance (ANOVA)]. Interestingly, clusters of atypical lymphocytes, especially the epidermotropic tumor cells, demonstrated weak or absent IL-23 staining in 18 of 40 (45%) lesions. This finding was present in 4 of 5 (80%) of the stage IVB lesions and 7 of 11 (64%) of the lesions from Sézary patients. These findings indicate that abnormal IL-23 expression may play a role in the pathogenesis and progression of MF/SS.

Keywords Interleukin-23 · Mycosis fungoides · Cutaneous T-cell lymphoma

Introduction

Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of peripheral, extranodal, non-Hodgkin's lymphomas. Mycosis fungoides (MF), the most common form of CTCL, arises in skin-homing $CD4^+$, $CD45RO^+$ helper/memory T-lymphocytes [9]. MF has a complex cytokine profile that evolves from a T_H1 (cell-mediated) profile in early stages to a T_H2 (humoral) profile as the disease progresses to the leukemic Sézary syndrome (SS) [6, 15]. Interleukin (IL)-12 is the predominant cytokine for transforming naïve $CD4^+$ T-cells to T_H1 effector cells [5] and has been shown to be active for treating MF/SS, inducing $CD8^+$ cytotoxic T-cell infiltrates [14].

Interleukin-23 is a novel cytokine, related to IL-12. They are both heterodimeric molecules that share the common p40 subunit, which is covalently bound to a

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unique p35 subunit or p19 subunit to form biologically active IL-12 and IL-23, respectively [10]. Although the effects of IL-23 have been shown to mimic those of IL-12, IL-23 has novel actions, inducing a subset of T-cells (T_H IL-17 cells) that produce predominantly IL-17 [1]. IL-17 has been shown to increase keratinocyte production of pro-inflammatory cytokines and may be in part responsible for the pro-inflammatory functions attributed to IL-23 [16]. The study by Piskin et al. found that the keratinocytes-derived IL-23 enhances the interferon (IFN)- γ production by memory T cells [13]. Kopp et al. demonstrated that IL-23 injection in mice resulted in an eczematous skin reaction. They concluded that IL-23 regulates the induction of cutaneous immune responses toward increased immunogenicity and may be abnormally expressed in T-cell-mediated skin disorders [7].

Elevated levels of IL-23 have been reported in many autoimmune and inflammatory disorders [5]. The research involving IL-23 and the skin diseases has been done in psoriasis, which is a T_H 1-mediated disease [8, 12, 13]. Increased expression of IL-23 has been found the psoriatic lesions with antibody to the common p40 subunit shared with IL-12 or antibody to the p19 subunit. However, IL-23 has not previously been examined in MF/SS, although MF has similarities to psoriasis including epidermotropic T-cells causing epidermal hyperproliferation. In order to understanding of the role of IL-23 in the MF/SS development, we examined 40 MF/SS lesions with all stages using a novel anti-human IL-23 antibody raised against full-length human IL-23.

Materials and methods

Tissue specimens

Forty patients with either newly diagnosed or established MF/SS signed informed consent for the use of tissue for research, and all research was approved by the M.D. Anderson Cancer Center Institutional Review Board. Biopsy specimens were taken from patients of the following clinical stages: 7 stage IA, 4 stage IB, 10 stage IIB, 2 stage III, 12 stage IVA, and 5 stage IVB. Eleven patients fit blood B2 criteria for SS among stage III–IVB patients. Normal control skin was obtained from adult breast surgery. Psoriatic lesions were available from a previous study [2].

Immunohistochemistry

Mycosis fungoides/Sézary syndrome lesional specimens, psoriatic skin lesions (positive controls), and

normal skin (negative controls) were reacted with monoclonal mouse anti-human IL-23 antibody raised against full-length human IL-23 (clone HTL 2376, 10 μ g/ml, Biolegend, San Diego, CA, USA) and detected by immunohistochemistry with a tyramine-catalyzed signal amplification system (DAKO, Carpinteria, CA, USA). Hematoxylin counterstain was performed. The negative control was performed with purified mouse IgG1, κ (clone MOPC-21, 10 μ g/ml, Biolegend, San Diego, CA, USA).

Semi-quantitative analysis of staining intensity

The staining frequency and intensity in epidermal keratinocytes and dermal lymphocytic infiltrates were graded by two separate blinded graders. The staining intensity was graded as 0 (no staining), 1 (faint staining), 2 (moderate staining), or 3 (intensely staining). The frequency of staining of the keratinocytes and dermal infiltrates were separately graded as 0 (<5%), 1 (5–33%), 2 (33–66%), or 3 (>66%).

Statistical analysis

Means and standard deviations are given in the summary of data. Variations in grading between stages were analyzed with an ANOVA with $\alpha = 0.05$.

Results

Interleukin-23 was strongly stained throughout epidermal keratinocytes and dermal infiltrates in MF/SS lesions compared with normal skin (Fig. 1). The IL-23 staining was predominantly located in the cytoplasm of both the keratinocytes and the dermal infiltrates. Dendritic-like cells, sweat glands, and hair follicles within the MF/SS lesions were also noted to stain strongly.

The mean grades of the frequency and intensity of IL-23 staining in all MF/SS lesions are summarized in Table 1. IL-23 intensity did not differ significantly among the stages of MF/SS. The frequency of positive dermal infiltrates in stage IVB lesions (1.30 ± 0.45) was lower than that for other stages (IA: 2.14 ± 0.90 , IB: 2.05 ± 0.10 , IIB: 2.44 ± 0.73 , III: 2.50 ± 0.71 , IVA: 2.12 ± 0.80), although not statistically different ($P = 0.13$, ANOVA). Among five lesions from stage IVB patients, three lesions showed < 33% of the positive dermal infiltrate.

Interestingly, clusters of atypical lymphocytes, especially the epidermotropic tumor cells, demonstrated weak or absent IL-23 staining as shown in Fig. 2. This finding was present in 18 of all 40 MF/SS

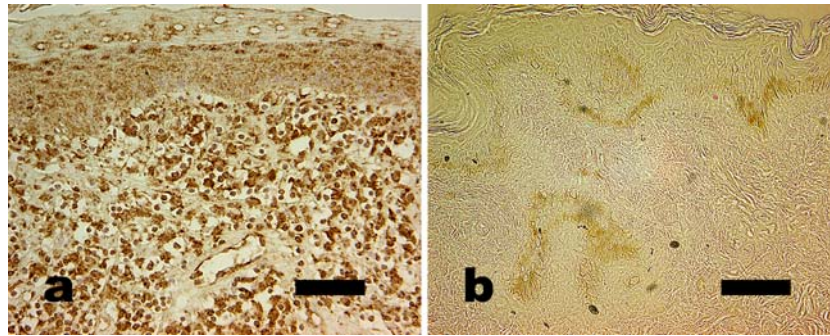


Fig. 1 IL-23 expression in mycosis fungoides lesions and control normal skin. Paraffin-embedded sections were labeled with purified anti-human IL-23 (clone HTL 2736, 10 µg/ml, Biolegend, San

Diego, CA, USA). Mycosis fungoides lesions (**a**) stained more strongly throughout both the epidermis and the dermal infiltrate than the control normal skin (**b**). Scale bar 100 µm

Table 1 Mean frequency and intensity of staining of IL-23 in mycosis fungoides lesions of various stages

Stage	Frequency in dermal infiltrate	Intensity in dermal infiltrate	Frequency in epidermis	Intensity in epidermis
Stage IA	2.14 ± 0.90 ^a	1.71 ± 0.95	2.14 ± 1.07	1.86 ± 0.90
Stage IB	2.05 ± 0.10	1.25 ± 0.50	2.25 ± 0.96	1.62 ± 0.48
Stage IIB	2.44 ± 0.73	2.00 ± 0.76	2.80 ± 0.42	2.15 ± 0.82
Stage III	2.50 ± 0.71	1.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
Stage IVA	2.12 ± 0.80	1.62 ± 0.71	2.25 ± 0.87	2.00 ± 0.83
Stage IVB	1.30 ± 0.45	1.20 ± 0.45	2.60 ± 0.55	2.10 ± 0.89
<i>P</i> -value ^b	0.13	0.31	0.40	0.84

^a All data reported as mean ± standard deviation

^b Analysis of variance with $\alpha = 0.05$

lesion (45%), more in lesions from stage IVB patients (4/5 lesions, 80%), and 7 of 11 lesions from Sézary patients (64%).

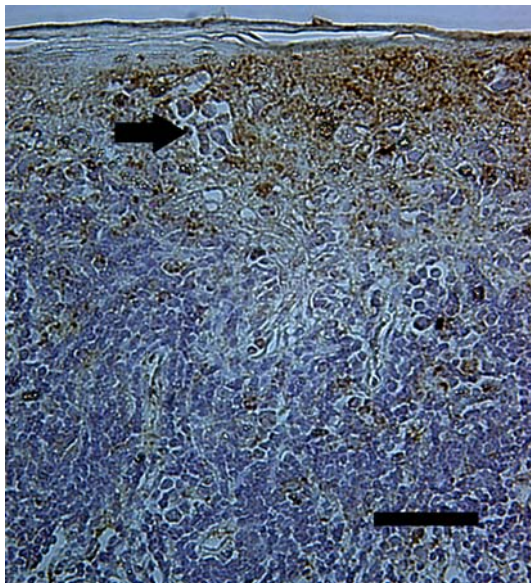


Fig. 2 Decreased expression of IL-23 in epidermotropic lymphocytes in the dermis of SS lesions. Paraffin-embedded sections were labeled with purified anti-human IL-23 (clone HTL 2736, 10 µg/ml, Biolegend, San Diego, CA, USA) and counterstained with hematoxylin. Note the Pautrier microabscess (arrow). Scale bar 100 µm

Discussion

By using a novel antibody raised against full-length human IL-23, we demonstrate that increased expression of IL-23 is present in the epidermal keratinocytes and in dermal lymphocytes in MF/SS lesions of all stages compared to normal skin. We also found that the weak or absent expression of IL-23 is present in atypical lymphocytes in most advanced lesions. These findings confirm the involvement of IL-23 in the development and progression of MF/SS.

In our study, the stronger, diffuse epidermal and dermal staining of IL-23 was seen in MF/SS lesions. Interestingly, a similar observation has reported in psoriatic lesions [13]. The study done by Piskin et al. found that diffuse strong epidermal and dermal perivascular staining of IL-23 were seen in psoriatic lesions using an anti-p19 subunit polyclonal antibody. Keratinocytes contribute to cutaneous immune responses through the expression of cytokines. Piskin et al. reports that IL-23 is expressed by keratinocytes in healthy skin and psoriatic lesions, and significant higher in psoriatic lesional skin [13]. They also found that the keratinocytes-derived IL-23 enhances the IFN- γ production by memory T cells. Psoriasis is a chronic inflammatory skin disease mediated by IFN- γ -expressing type 1 memory T cells. MF/SS results from a clonal malignant proliferation of epidermotropic memory T cells causing persistent,

eczematous skin lesions. We have previously proposed that the epidermotropism in MF/SS may be mediated by IFN-inducible protein 10 and we have shown that a potent inducer of IFN-inducible protein 10 is IFN- γ [3]. Therefore, this similarity of IL-23 expression in the early stage of MF/SS and psoriasis might imply that a similar immunopathogenesis exist during the chronic inflammatory course.

A most striking finding of this study was that clusters of atypical lymphocytes in the dermis and especially the epidermotropic T-cells had lower or absent IL-23 staining. This was most pronounced in Sézary cells, which are known to express T_H2 cytokines [6]. How this change affect the cytokine shift from T_H1 to T_H2 in MF/SS and what is/are the phenotype(s) of these cells will need to further study with a larger sample size. Recent studies elucidating the complex interactions of IL-23 in inducing the differentiation of naïve T-cells into T_HIL-17-cells have demonstrated that IFN- γ also inhibits the development of T_HIL-17-cells and that IFN- γ potently inhibits IL-23 receptor expression [4, 11].

Further studies of T_HIL-17-cells and the IL-23 receptor may be helpful to gain a more complete understanding of the relative importance of IL-23 in MF/SS, psoriasis, and eczema.

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References

- Bettelli E, Kuchroo VK (2005) IL-12- and IL-23-induced T helper cell subsets: birds of the same feather flock together. *J Exp Med* 201:169–171
- Breuer-McHam JN, Ledbetter LS, Sarris AH, Duvic M (2000) Cytokine expression patterns distinguish HIV associated skin diseases. *Exp Dermatol* 9:341–350
- Daliani D, Ulmer R, Jackow C, Pugh W, Gansbacher B, Cabinillas F, et al (1998) Tumor necrosis factor- α and interferon- γ but not HTLV-I Tax, are likely factors in the epidermotropism of cutaneous T-cell lymphoma via induction of interferon-inducible protein-10. *Leuk Lymphoma* 29:315–328
- Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al (2005) Interleukin 17-producing CD4+ effector T-cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 6:1123–1132
- Hunter CA (2005) New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. *Nat Rev Immunol* 5:521–531
- Kim EJ, Hess S, Richardson SK, Newton S, Showe LC, Benoit BM, et al (2005) Immunopathogenesis and therapy of cutaneous T cell lymphoma. *J Clin Invest* 115:798–712
- Kopp T, Lenz P, Bello-Fernandez C, Kastelein RA, Kupper TS, Stingl G (2003) IL-23 production by cosecretion of endogenous p19 and transgenic p40 in keratin 14/p40 transgenic mice: evidence for enhanced cutaneous immunity. *J Immunol* 170:5438–5444
- Lee E, Trepicchio WL, Oestreicher JL, Pittman D, Wang F, Chamian F, et al (2004) Increased expression of interleukin 23p19 and p40 in lesional skin of patients with psoriasis vulgaris. *J Exp Med* 199:125–130
- Ni X, Hazarika P, Zhang C, Talpur R, Duvic M (2001) Fas ligand expression by neoplastic T lymphocytes mediates elimination of CD8+ cytotoxic T lymphocytes in mycosis fungoides: a potential mechanism of tumor immune escape? *Clin Cancer Res* 7:2682–2692
- Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, et al (2000) Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* 13:715–725
- Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al (2005) A distinct lineage of CD4 T-cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 6:1133–1141
- Piskin G, Tursen U, Sylva-Steenland RMR, Bos JD, Teunissen MBM (2004) *Exp Dermatol* 13(12):764–772
- Piskin G, Sylva-Steenland RMR, Bos JD, Teunissen MBM (2006) *J Immunol* 176(3):1908–1915
- Rook AH, Kuzel TM, Olsen EA (2003) Cytokine therapy of cutaneous T-cell lymphoma: interferons, interleukin-12, and interleukin2. *Hematol Oncol Clin North Am* 17:1435–1448
- Saad G, Fivenson DP, Naidu Y, Nickoloff BJ (1994) Mycosis fungoides exhibits a Th1-type cell-mediated cytokine profile whereas Sezary syndrome expresses a Th2-type profile. *J Invest Dermatol* 103:29–33
- Teunissen MB, Koomen CW, de Waal Malefyt R, Wierenga EA, Bos JD (1998) Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. *J Invest Dermatol* 111:645–649