Am J Transl Res 2009;1(2):203-210
www.ajtr.org/AJTR901010

Original Article

Lesional skin chemokine CTACK/CCL27 expression in mycosis fungoides and disease control by IFN-α and PUVA therapy

Gaia Goteri[1], Serena Rupoli[2], Anna Campanati[3], Antonello Costagliola[1], Simona Sabato[1], Daniela Stramazzotti[1], Paola Picardi[2], Lucia Canafoglia[2], Stefano Pulini[4], Giulia Ganzetti[3], Anna Maria Offidani[3], Pietro Leoni[2]

[1]Anatomia Patologica, [2]Clinica Ematologia and [3]Clinica Dermatologica, Azienda Ospedaliera Ospedali Riuniti di Ancona/Università Politecnica delle Marche, Ancona, Italy; [4]Dipartimento Ematologia, Ospedale Spirito Santo", Pescara, Italy

Received January 26, 2009; accepted January 29, 2009; available online January 31, 2009

Abstract: Recruitment of neoplastic T cells to skin is a critical step in the pathogenesis of mycosis fungoides (MF) lesions. Cutaneous T-cell attracting chemokine (CTACK)/CCL27 attracts memory T cells to skin, resulting in increased cutaneous expression. The interactions between neoplastic cells and skin immune system require further elucidation. CTACK/CCL27 expression and density of dendritic cells (DC), CD8+ and CD4+ lymphocytes were investigated in skin lesions of 52 early-stage MF patients treated by interferon (IFN)-α in combination with photochemotherapy (psoralen plus ultraviolet A, PUVA). Skin lesion biopsies obtained at diagnosis and after treatment were studied by immunohistochemistry. Initial CTACK/CCL27 expression was abnormal/suprabasal in 36 patients. Normal/basal CTACK/CCL27 expression tended to correlate with a high DC density and low CD4+ cell density in the neoplastic infiltrate. Treatment induced a significant increase in CTACK/CCL27 expression (χ^2 test: P=0.004). Overall, 33 patients relapsed [median event-free survival (EFS), 46 months] during follow-up (median, 92.5 months, range, 43-165). Normal/basal CTACK/CCL27 expression at the end of treatment correlated with lower rates of recurrence and a longer median EFS (111 months *vs.* 39 months with suprabasal expression; log rank test: P=0.031). CTACK/CCL27 overexpression in early-stage MF might thus be related to a balance between neoplastic cells and immunomodulant DC. Normal CTACK/CCL27 expression after treatment designates a subset of patients with favorable disease behavior. The mechanisms underpinning CTACK/CCL27 overexpression after therapy in the remaining patients, who are at greater risk of recurrence, warrant further investigation.

Key Words: Chemokine, CTACK/CCL27, mycosis fungoides, lymphocyte, dendritic cells

Introduction

Cutaneous lymphocyte recruitment is a highly complex process involving extravasation, migration through the dermis and, eventually, localization to the epidermis. A network of cytokines and chemokines provides the road signs for leukocyte migration, while various adhesion receptors orchestrate the dynamic events of cell-cell and cell-substrate interactions, resulting in cutaneous localization of T cells [1]. Cutaneous T-cell attracting chemokine (CTACK)/CCL27 is a skin-associated chemokine that attracts skin-homing memory T cells [2, 3]. CTACK/CCL27, the ligand for CCR10, is produced in various diseases, mainly by activated keratinocytes [4]. It is also expressed by dermal components and by the microvasculature, and has an important role in recruiting T cells to skin in several conditions [5, 6]. Neoplastic T-cell recruitment to skin is a critical step in the pathogenesis of mycosis fungoides (MF), the most common type of cutaneous T-cell lymphoma (CTCL), where gradual clonal expansion of CD3+/CD4+/CD8- atypical

lymphoid cells with skin-homing properties results in the appearance of cutaneous patches and plaques. Lesions present as accumulations of band-like infiltrates in the papillary dermis; variable numbers of cells also infiltrate the epidermis, sometimes forming Pautrier's microabscesses [7]. The neoplastic infiltrate in such lesions contains varying numbers of non-neoplastic T cells, predominantly of the CD8+ phenotype, which are regarded as ''reactive'' [8]. Recently, Fujita et al. have documented increased CTACK/CCL27 serum levels and epidermal expression in MF patients compared to normal controls, hypothesizing that CCR10-CTACK/CCL27 interactions play an early role in the evolution from patch to tumor stage [9]. However, the interactions between neoplastic cells and the cutaneous immune system need to be further elucidated. We investigated whether CTACK/CCL27 expression is related to the density of dendritic cells (DC), CD8+ lymphocytes and CD4+ neoplastic lymphocytes, whether it influences the clinical response to therapy with IFN-α or photochemotherapy (psoralen plus ultraviolet A, PUVA), and whether it is affected by this therapy; we also investigated whether changes in CTACK/CCL27 expression may predict the rate of recurrence and event-free survival (EFS), since although early stage (IA, IB, IIA) MF is highly responsive to common skin-directed therapies, treatment suspension is often followed by relapse [10,11]. In this study skin biopsies from 52 patients in the patch or plaque stage were examined for cells expressing CTACK/CCL27, CD1a, CD4, and CD8.

Materials and methods

Skin biopsies and patients' characteristics

Subjects were from a prospective cohort of early-stage MF patients referred to the Clinic of Hematology, Polytechnic University of Marche Medical School, Ancona, Italy, for staging, treatment and follow-up by participants in the Marche Regional Multicenter Study Group of Cutaneous Lymphomas. Patients were treatment naive or had undergone 4-month washout after systemic therapy or 4-week washout after topical therapy. Subjects with clinical stages I–IIA, age ≤ 75, and a Performance Status (PS) >60% were included; those aged ≥ 75 were included only if their PS was 100%. Exclusion criteria were unilesional MF, severe cyclothymic syndromes, active autoimmune disorder, hepatic, cardiac, respiratory or renal disease contraindicating IFN-α or PUVA, and pregnancy. Patients with MF showing a cytotoxic phenotype were also excluded, to evaluate the prognostic impact of reactive CD8+ lymphocytes. The protocol was approved by the ethics committee of our institution. A biopsy of a skin lesion was collected at diagnosis and then, from an adjacent site, at the end of treatment, which consisted of an induction and a maintenance phase lasting 14 months, as described previously [12, 13]. Participants received physical examination and assessment of skin lesion evolution at 8-week intervals during the protocol, at the end of treatment, 2 months post treatment and then every 3 months thereafter. Overall 52 patients were included.
Response to treatment was defined as complete remission (CR), i.e. complete clearance of all skin lesions, or as partial remission (PR), i.e. a >50% reduction of the skin lesions as determined by the sum of the largest perpendicular diameters of all measurable lesions and no evidence of new lesions. The response—which was to be of at least 4 weeks' duration—was assessed by two independent observers blind to each other's evaluation. Relapse was defined as clinically detectable disease after a period of complete clearance.

Immunohistochemistry

Immunohistochemical analyses were performed on paraffin blocks. Conventional 6-μm-thick histological sections were obtained with a microtome and mounted on slides pretreated with poly-l-lysine (Sigma Chemicals, St Louis, MO). Slides were then deparaffinized and rehydrated in a gradient of ethanol and xylene. Two antigen retrieval methods were applied, by incubating sections 1) with a 0.01M EDTA solution, pH 8.0, at 95-98° C for 30 min before staining with anti-CD1a, -CD3, -CD4 and -CD8, and 2) with TUF (Target Unmasking Fluid; Alexis Biochemical, Lausen, Switzerland) solution at 90° C for 10 min, before staining with anti-CCR10 and CCL27. After washing in H2O and then in Tris buffer solution sections were incubated with the following monoclonal antibodies: mouse anti-dendritic cell marker CD1a (clone O10, Immunotech, Marseille, France), mouse anti-CD4 (1F6; dil 1:40, Novocastra Laboratories Ltd, Newcastle, UK), mouse anti-CD8 (4B11,

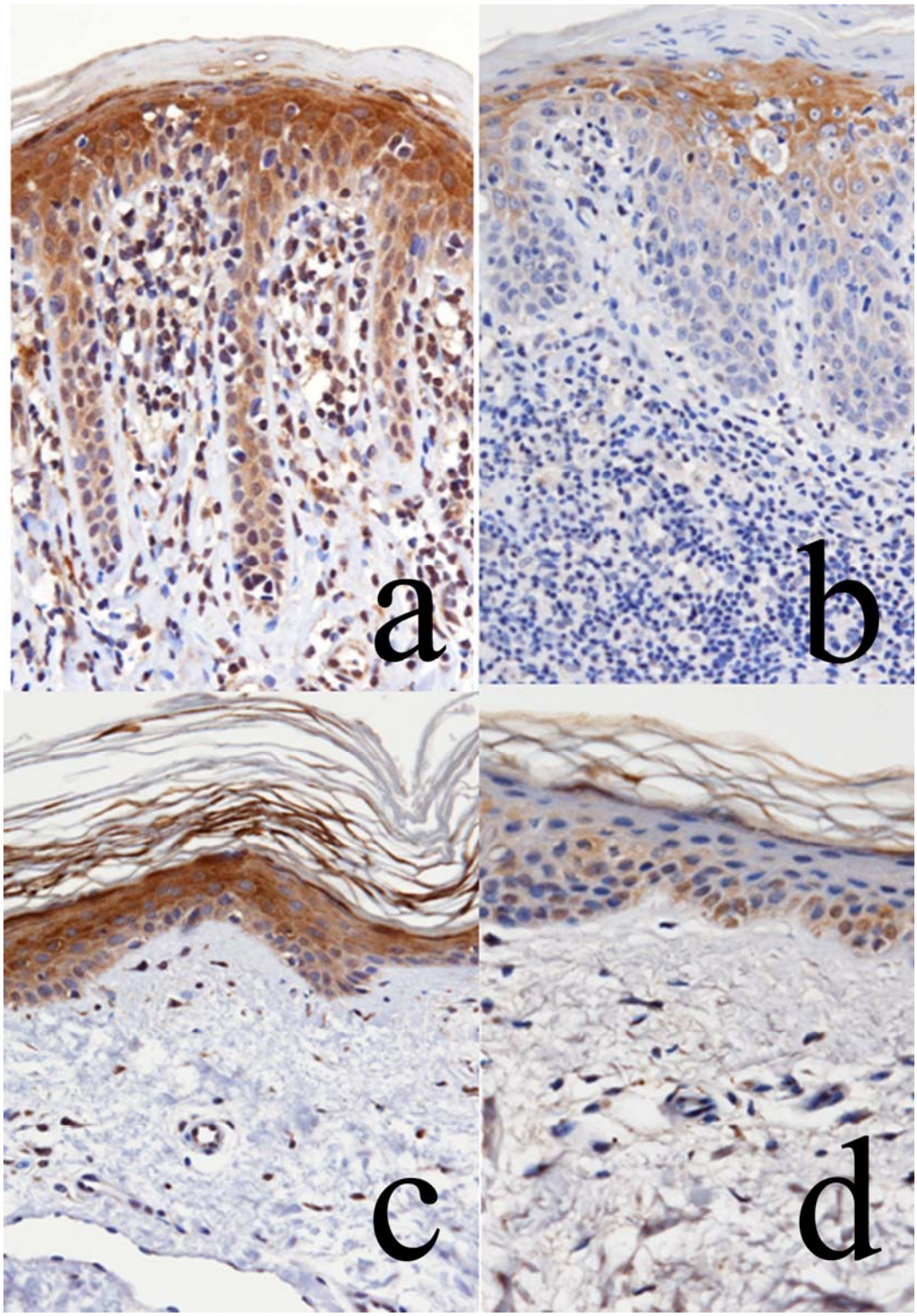

Figure 1. CTACK/CCL27 immunostaining in MF lesions before (a,b) and after (c,d) IFN-α and PUVA therapy. a) strong immunostaining in epidermis infiltrated by atypical lymphocytes (score +++) and in several vessels in the dermis; b) fainter immunostaining confined to few epidermal suprabasal layers (score ++). c) regression of the lymphoid infiltrate after therapy and strong immunostaining throughout the epidermal layers (score +++); d) weak basal staining (score +).

dil. 1:40, Novocastra Laboratories), rabbit anti-CD3 (dil. 1:100, Dakocytomation, Milano, Italy), and mouse anti-CCL27 (dil. 1:200, R&D Systems, Minneapolis, MN). Incubations were carried out in humidified atmosphere at room temperature for 1 hour (anti-CD1a, -CD3, -CD4, -CD8) or overnight at 4° C (anti-CCL27). Sections were incubated with anti-mouse Ig/HRP (ENVISIONTM/HRP, Dakocytomation) for 30 min at room temperature. Slides were then washed with TBS and treated with 3,3'diaminobendizidine until brown staining was visible, counterstained with Mayer hematoxylin, dehydrated and mounted in permount. Each series included appropriate negative control sections without the primary antibody reaction.

CD8+ and CD4+ T lymphocytes, DC positive for CD1a+, and CTACK/CCL27+ epidermal cells were evaluated with a light microscope by scanning the entire lesion. The intensity of CTACK/CCL27 immunoreactivity in epidermis was expressed semiquantitatively as follows: + (faint/moderate staining confined to the basal layer, as in normal skin); ++ (faint/moderate suprabasal staining; strong staining up to the lower two-thirds of epidermis); or +++ (strong, full-thickness staining). CD1a+ DC and CD8+ lymphocyte density was rated as + (few/isolated cells), ++ (small, ≤ 5 cell clusters), or +++ (larger, > 5 cell clusters). CD4+ lymphocyte density was graded as + (perivascular infiltrate), ++ (discontinuous subepidermal band), or +++ (continuous band).

Slides were analyzed separately by two pathologists (GG and SS); discordant scores were discussed until an agreement was reached.

Statistical methods

Patients' characteristics and descriptive data were expressed as median and range for continuous variables and by frequency tabulations for categorical variables. Factors affecting the achievement of CR and relapse were assessed using the χ^2 test (or Fisher's exact test) for contingency tables. EFS was defined as the time from the beginning of treatment to any significant event or to the last follow-up. EFS curves were plotted using the Kaplan–Meier method. Differences between curves were assessed using the log-rank test. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using the SPSS statistical package (SPPS Inc., Chicago, IL).

Results

A total of 52 patients, 34 M and 18 F, with a median age of 63.5 yrs (range, 23-84) were included in the study. The skin disease was limited in 10 (T1) and diffuse in 42 (T2). Nine patients were stage IA, 40 were stage IB and 3 were stage IIA. During follow-up (median, 92.5 months; range, 43-165), 33 patients relapsed (median EFS, 46 months). There were 50 CR and 2 PR. At the last follow-up, 2 patients had died from unrelated causes and 40/50 was still in CR.

At diagnosis a superficial lymphoid infiltrate with solely perivascular distribution (score +) was disclosed in 21 patients; in 13 the infiltrate formed a discontinuous subepidermal band (score ++), and in 18 participants it formed a continuous band (score +++).

Few/isolated CD8+ lymphocytes were detected in 27 patients (score +), small clusters (score ++) were seen in 20, and larger clusters (score ++) in 5. Epidermal DC were few and sparse (score +) in 25 patients and were grouped into small (score ++) or larger clusters (score +++) in 27.

CTACK/CCL27 expression was confined to the basal epidermal layer, as in normal skin, in 16 subjects; in the other 36 it was also detected in the suprabasal epidermal layers (**Figure 1a-b**). In 12 patients staining was strong throughout the epidermis. A normal/basal CTACK/CCL27 expression tended to correlate with high epidermal DC density and with low CD4+ density in the neoplastic infiltrate (albeit P=NS). Since all patients responded to treatment (50 CR, 2 PR), the correlation, if any, between CTACK/CCL27 expression and clinical response could not be assessed.

The treatment induced a significant increase in CTACK/CCL27 expression, which was suprabasal in 42 patients (**Figure 1c**) and strong, throughout the epidermal layers, in 24 (χ^2 test: P=0.004). CTACK/CCL27 expression at the end of treatment, but not at diagnosis, correlated with recurrence and influenced EFS. Normal/basal CTACK/CCL27 expression at the end-of treatment (**Figure** 1d) correlated with

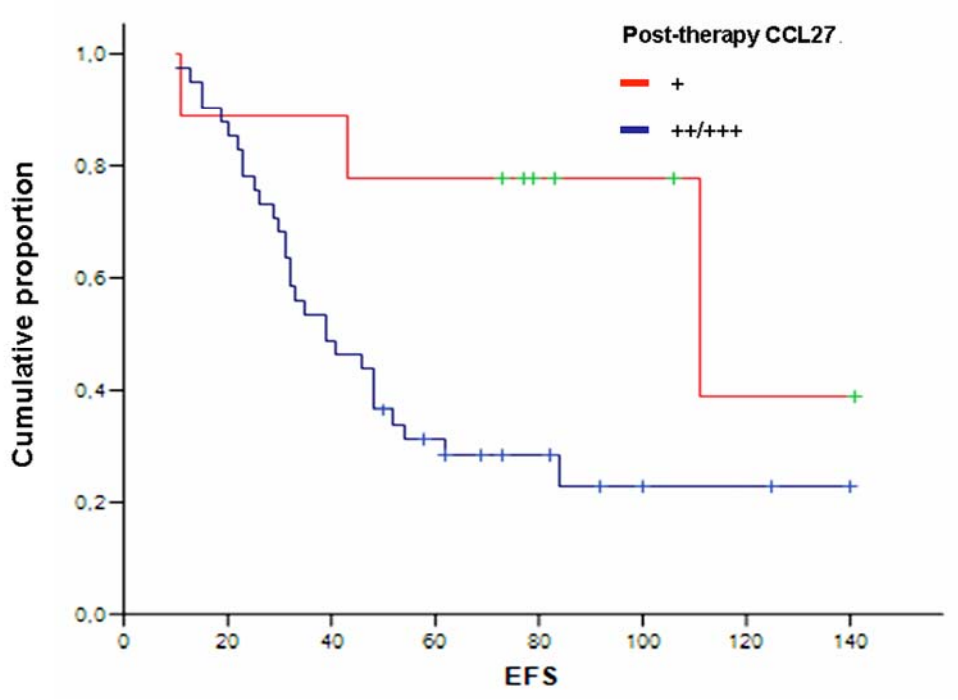

Figure 2. Plot of EFS curves in relation to suprabasal CTACK/CCL27 expression at the end of treatment. Low scores (+) correlate with a longer median EFS (111 months) compared with higher scores (++/+++ :39 months; log rank test: P=0.031).

lower rates of MF recurrence (3/9 compared to 30/41 in patients with suprabasal expression; χ^2 test: P=0.022) and a longer median EFS (111 months vs 39 months with suprabasal expression; log rank test: P=0.031; **Figure 2**).

Discussion

In our experience 14-month combination therapy with low-dose IFN-α and PUVA has proved effective in achieving high rates of long-term CR, even though it does not cure early-stage MF [13]. Its action in early MF is exerted via biological mechanisms including induction of tumor cell apoptosis, direct anti-proliferation activity, a shift toward Th1-mediated immune response, suppression of cytokine production by keratinocytes, and release of oxygen-free radicals [14]. Although response to combination therapy is adversely affected by the neoplastic cell burden, CR induction and clinical course after treatment seem to be favorably influenced by the host immune surveillance. In previous studies [8, 13] we focused on accessory cells in the neoplastic lymphoid infiltrate, and hypothesized that a high density of epidermal DC and CD8+ lymphocytes could sustain therapeutic strategies directed at suppressing MF cell proliferation and at preventing disease progression. Here we addressed the possible links between neoplastic and immunemodulant cells and the chemokine CTACK/CCL27. Epidermal CTACK/CCL27 overexpression was seen in 70% of early MF lesions at diagnosis. This finding is in line with a previous study also showing an increase in chemokine serum levels [9] and with the fact that CTACK/CCL27 participates in the recruitment of neoplastic T cells to skin. Other Th1 and Th2 chemokines are also overexpressed in MF sera and are thus considered critical for MF pathogenesis and for progression from early to advanced stages. They include thymus and activation-regulated chemokine (TARC)/CCL17 and macrophage derived chemokine (MDC)/CCL22, which bind to the CC chemokine receptor 4 (CCR4) on Th2 cells [15,16], the interferon-inducible protein-10 (IP10)/CXCL10, and the monokine induced by interferon-γ (MIG)/CXCL9, which bind to CXC chemokine receptor 3 on Th1 cells (CXCR3) [15-18]. Similarly to CTACK/CCL27, increased TARC/

CCL17 expression has also been detected in MF lesional keratinocytes [15]. Because chemokine production and receptor expression on lymphoid cells change during MF progression, the pathogenic role of these chemokines is influenced by their links to the relevant receptors in lymphocyte populations. While CCR4 is highly expressed on neoplastic Th2 cells both in the patch/plaque and in the tumor stage, CXCR3 tends to decrease in transformed dermal MF neoplastic cells, as does the amount of reactive CD8+ lymphocytes positive for CCR4 [19-23]. Our data indicate that an increase in neoplastic cells and a decline in immunomodulant DC in early MF cutaneous lesions is related to the increased CTACK/CCL27 expression seen in epidermal keratinocytes, confirming its role in the recruitment of neoplastic T cells to skin and making it a potential marker of local skin MF activity.

As regards the clinical significance of CTACK/CCL27 in the course of MF, treatment may modify chemokine expression in sera and tissues. Our study documents the effects of combined IFN-α and PUVA treatment on tissue CTACK/CCL27. In this study of early MF patients CR did not correlate with a reduction in CTACK/CCL27 expression in skin: in fact keratinocyte expression continued to be high, or even increased, in most patients even though the lymphocyte infiltrate disappeared. Because in experimental models single or multiple UV irradiation downregulates CTACK/CCL27 in mouse skin [24], it may be hypothesized that either the combination with IFN is responsible for local Th2 chemokine production in such cases, despite the disappearance of the neoplastic infiltrate, or that other biological mechanisms sustain its overexpression. The local effects seem to parallel systemic effects, since persistence of high serum levels of other Th2 chemokines, like TARC/CCL17 and MDC/CCL22, has been reported in MF patients responding to IFN and narrow-band UVB therapy (18). However, in another clinical report narrow-band UVB reduced TARC/CCL17 and CTACK/CCL27 serum levels in Sezary syndrome, indicating that IFN therapy might be responsible for Th2 chemokine overexpression [25]. Hence the interest in exploring the clinical significance of local changes in Th2 chemokine expression in patients with a long follow-up. Our data seem to indicate that low CTACK/CCL27 expression after CR might be related to a different clinical behavior, designating a subset of patients (ca. 20%) with a lower risk of relapse and a longer EFS. The mechanisms involved in local CTACK/CCL27 overexpression after therapy in the other 80% of patients, who achieve CR but are at greater risk of a recurrence, warrant further investigation, since restoration of cutaneous CTACK/CCL27 levels in early MF promises to be a key end-point in the treatment of the disease.

Acknowledgements

The authors are indebted to the following colleagues of the Marche Regional Multicenter Study Group of Cutaneous Lymphomas: S. Lanari, L. Morresi, I. Cataldi, A. Cellini, G. Ricotti, S. Serresi, MG. Tucci, A. Giacchetti, G. Mozzicafreddo, M. Giangiacomi, D. Brancorsini, R. Ranaldi (Ancona); M. Ottaviani (Fabriano); G. Ciattaglia, L. Bugatti, G. Filosa (Jesi); A. Bettacchi, M. Simonacci (Macerata); S. Barulli, B. Guiducci (Pesaro); V. Agostini (Rimini); A.Tassetti (Civitanova Marche); E. Grilli Cicilioni (S. Severino); N. Novelli (San Benedetto), and V. Mambelli (Ascoli Piceno). The study was partly supported by AIL Onlus (Italian Leukemia, Lymphoma and Myeloma Association), Ancona section. The authors have no commercial or other relationships that could constitute a conflict of interest.

Please address correspondences to: Gaia Goteri, MD, PhD, Anatomia Patologica, Università Politecnica delle Marche, Via Conca 71 I-60020 Torrette di Ancona (Ancona) – Italy, Tel. +39 071 596 4811; Fax +39 071 889 985; e-mail: g.goteri@univpm.it

References

[1] Schön MP, Zollner TM, Boehncke WH. The molecular basis of lymphocyte recruitment to the skin: clues for pathogenesis and selective therapies of inflammatory disorders. J Invest Dermatol 2003; 121:951-962.

[2] Morales J, Homey B, Vicari AP, Hudak S, Oldham E, Hedrick J, Orozco R, Copeland NG, Jenkins NA, McEvoy LM, Zlotnik A. CTACK, a skin-associated chemokine that preferentially attracts skin-homing memory T cells. Proc Natl Acad Sci U S A 1999; 96(25):14470-14475.

[3] Vestergaard C, Johansen C, Otkjaer K, Deleuran M, Iversen L. Tumor necrosis factor-alpha-induced CTACK/CCL27 (cutaneous T-cell-attracting chemokine) production in keratinocytes is controlled by nuclear factor kappaB. Cytokine 2005; 29:49-55.

[4] Homey B, Alenius H, Müller A, Soto H, Bowman

EP, Yuan W, McEvoy L, Lauerma AI, Assmann T, Bünemann E, Lehto M, Wolff H, Yen D, Marxhausen H, To W, Sedgwick J, Ruzicka T, Lehmann P, Zlotnik A. CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. Nat Med 2002; 8:157-165.

[5] Kakinuma T, Saeki H, Tsunemi Y, Fujita H, Asano N, Mitsui H, Tada Y, Wakugawa M, Watanabe T, Torii H, Komine M, Asahina A, Nakamura K, Tamaki K. Increased serum cutaneous Increased serum cutaneous T cell-attracting chemokine (CCL27) levels in patients with atopic dermatitis and psoriasis vulgaris. J Allergy Clin Immunol. 2003; 111:592-597.

[6] Campanati A, Goteri G, Simonetti O, Ganzetti G, Giuliodori K, Stramazzotti D, Morichetti D, Bernardini ML, Mannello B, Fabris G, Offidani A. CTACK /CCL27 expression in psoriatic skin and its modification after administration of etanercept. Br J Dermatol 2007; 157:1155-1160.

[7] Willemze R, Jaffe ES, Burg G, Cerroni L, Berti E, Swerdlow SH, Ralfkiaer E, Chimenti S, Diaz-Perez JL, Duncan LM, Grange F, Harris NL, Kempf W, Kerl H, Kurrer M, Knobler R, Pimpinelli N, Sander C, Santucci M, Sterry W, Vermeer MH, Wechsler J, Whittaker S, Meijer CJ. WHO-EORTC classification for cutaneous lymphomas. Blood 2005; 105:3768-3785.

[8] Goteri G, Filosa A, Mannello B, Stramazzotti D, Rupoli S, Leoni P, Fabris G. Density of neoplastic lymphoid infiltrate, CD8+ T cells, and CD1a+ dendritic cells in mycosis fungoides. J Clin Pathol 2003; 56:453-458.

[9] Fujita Y, Abe R, Sasaki M, Honda A, Furuichi M, Asano Y, Norisugi O, Shimizu T, Shimizu H. Presence of circulating CCR10+ T cells and elevated serum CTACK/CCL27 in the early stage of mycosis fungoides. Clin Cancer Res 2006; 12:2670-2675.

[10] van Doorn R, Van Haselen CW, van Voorst Vader PC, Geerts ML, Heule F, de Rie M, Steijlen PM, Dekker SK, van Vloten WA, Willemze R. Mycosis fungoides: disease evolution and prognosis of 309 Dutch patients. Arch Dermatol 2000; 136:504-510.

[11] Kim YH, Chow S, Varghese A, Hoppe RT. Clinical characteristics and long-term outcome of patients with generalized patch and/or plaque (T2) mycosis fungoides. Arch Dermatol 1999; 135:26-32.

[12] Rupoli S, Barulli S, Guiducci B, Offidani M, Mozzicafreddo G, Simonacci M, Filosa G, Giacchetti A, Ricotti G, Brandozzi G, Cataldi I, Serresi S, Ceschini R, Bugatti L, Offidani A, Giangiacomi M, Brancorsini D, Leoni P. Low dose interferon-alpha2b combined with PUVA is an effective treatment of early stage mycosis fungoides: results of a multicenter study. Cutaneous-T Cell Lymphoma Multicenter Study Group. Haematologica 1999; 84:809-813.

[13] Rupoli S, Goteri G, Pulini S, Filosa A, Tassetti A, Offidani M, Filosa G, Mozzicafreddo G, Giacchetti A, Brandozzi G, Cataldi I, Barulli S, Ranaldi R, Scortechini AR, Capretti R, Fabris G, Leoni P; Marche Regional Multicentric Study Group of Cutaneous Lymphomas. Long-term experience with low-dose interferon-alpha and PUVA in the management of early mycosis fungoides. Eur J Haematol 2005; 75:136-145.

[14] Dummer R. Immunomodulators in the treatment of cutaneous lymphomas. Expert Opin Biol Ther 2002; 2:279-286.

[15] Hino R, Shimauchi T, Tokura Y. Treatment with IFN-gamma increases serum levels of Th1 chemokines and decreases those of Th2 chemokines in patients with mycosis fungoides. J Dermatol Sci 2005; 38:189-195.

[16] Kakinuma T, Sugaya M, Nakamura K, Kaneko F, Wakugawa M, Matsushima K, Tamaki K. Thymus and activation-regulated chemokine (TARC/CCL17) in mycosis fungoides: serum TARC levels reflect the disease activity of mycosis fungoides. J Am Acad Dermatol. 2003; 48:23-30.

[17] Ferenczi K, Fuhlbrigge RC, Pinkus J, Pinkus GS, Kupper TS. Increased CCR4 expression in cutaneous T cell lymphoma. J Invest Dermatol 2002; 119:1405-1410.

[18] Shimauchi T, Sugita K, Nishio D, Isoda H, Abe S, Yamada Y, Hino R, Ogata M, Kabashima K, Tokura Y. Alterations of serum Th1 and Th2 chemokines by combination therapy of interferon-gamma and narrowband UVB in patients with mycosis fungoides. J Dermatol Sci 2008; 50:217-225.

[19] Kallinich T, Muche JM, Qin S, Sterry W, Audring H, Kroczek RA. Chemokine receptor expression on neoplastic and reactive T cells in the skin at different stages of mycosis fungoides. J Invest Dermatol 2003; 121:1045-1052.

[20] Yamaguchi T, Ohshima K, Tsuchiya T, Suehuji H, Karube K, Nakayama J, Suzumiya J, Yoshino T, Kikuchi M. The comparison of expression of cutaneous lymphocyte-associated antigen (CLA), and Th1- and Th2-associated antigens in mycosis fungoides and cutaneous lesions of adult T-cell leukemia/lymphoma. Eur J Dermatol 2003; 13:553-559.

[21] Lu D, Duvic M, Medeiros LJ, Luthra R, Dorfman DM, Jones D. The T-cell chemokine receptor CXCR3 is expressed highly in low-grade mycosis fungoides. Am J Clin Pathol 2001; 115:413-421.

[22] Shimauchi T, Kabashima K, Tokura Y. CXCR3 and CCR4 double positive tumor cells in granulomatous mycosis fungoides. J Am Acad Dermatol. 2006; 54:1109-1111.

[23] Yagi H, Seo N, Ohshima A, Itoh T, Itoh N, Horibe T, Yoshinari Y, Takigawa M, Hashizume H. Chemokine receptor expression in cutaneous T cell and NK/T-cell lymphomas: immunohistochemical staining and in vitro chemotactic assay. Am J Surg Pathol 2006; 30:1111-1119.

[24] Rundhaug JE, Hawkins KA, Pavone A, Gaddis S,

Kil H, Klein RD, Berton TR, McCauley E, Johnson DG, Lubet RA, Fischer SM, Aldaz CM. SAGE profiling of UV-induced mouse skin squamous cell carcinomas, comparison with acute UV irradiation effects. Mol Carcinog. 2005; 42:40-52.

[25] Masui Y, Sugaya M, Kagami S, Fujita H, Yano S, Nagao M, Komine M, Saeki H, Ihn H, Kikuchi K, Tamaki K. Sézary syndrome treated with narrowband ultraviolet B: time-course measurement of serum levels of CCL17/CCL27. Clin Exp Dermatol 2007; 32:57-59.