

CONCISE COMMUNICATION

CCR4 is expressed on infiltrating cells in lesional skin of early mycosis fungoides and atopic dermatitis

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ABSTRACT

CCR4 is expressed on tumor cells of mycosis fungoides (MF) and Sézary syndrome (SS). In MF, most infiltrating cells in patches and plaques express CXCR3, while tumor cells express CCR4 in advanced stages. Poteligeo Test IHC (CCR4 staining kit) is a newly developed staining kit that can examine the presence of CCR4 expressed on tumor cells of adult T-cell leukemia/lymphoma, peripheral T-cell lymphoma and cutaneous T-cell lymphoma before treatment of anti-CCR4 antibody using paraffin-embedded samples. In this study, we analyzed CCR4 expression in lesional skin of MF, SS, atopic dermatitis (AD) and psoriasis with this new kit. CCR4 was expressed on infiltrating cells in lesional skin of patch, plaque, tumor MF and SS, and the number of positive cells increased as the disease progressed. Immunohistochemistry with frozen sections also showed some positive cells scattered in the dermis, although the quality was not high enough to quantify positive cells. There were significant positive correlations between CCR4⁺ cells and serum lactate dehydrogenase levels. Interestingly, CCR4⁺ cells were also detected in AD skin, whose number was larger than that in psoriatic skin. Previous studies showed only scattered CCR4⁺ cells in skin samples by standard immunohistochemical staining. The new, sensitive CCR4 staining kit has revealed that CCR4 is expressed on infiltrating cells in lesional skin of early MF and AD as well as advanced MF and SS. These cells can be therapeutic targets for patients who are resistant to standard treatments.

Key words: atopic dermatitis, CCR4, mycosis fungoides, psoriasis, Sézary syndrome.

INTRODUCTION

CCL17, also called thymus and activation-regulated chemokine, is a member of the CC chemokine family. CCL17 is designated a T-helper (Th)2 type chemokine and serum levels of this chemokine reflect disease activities of various skin diseases such as atopic dermatitis (AD), bullous pemphigoid and mycosis fungoides (MF).^{1,2} CCR4, a receptor of CCL17, is expressed on tumor cells of cutaneous T-cell lymphoma such as MF, Sézary syndrome (SS) and anaplastic large cell lymphoma.^{2–5} In MF, most infiltrating cells in patches and plaques express CXCR3, while tumor cells transform into much larger cells and express CCR4 in advanced stages.^{2–4} CCR4 is also expressed in more than 90% of adult T-cell leukemia/lymphoma (ATLL)⁶ and mogamulizumab, an anti-CCR4 antibody, is now approved for the treatment of recurrent/relapsed ATLL, peripheral T-cell lymphoma and cutaneous T-cell lymphoma in Japan when tumor cells are proved to express this chemokine receptor.⁷ Poteligeo Test IHC (CCR4 staining kit) is a newly developed staining kit that can examine the presence of CCR4 expressed by tumor cells before treatment of mogamulizumab using paraffin-embedded samples. In this study, we analyzed CCR4 expression in lesional skin of MF, SS, AD and psoriasis

with this new kit and the results were compared with those obtained with standard immunohistochemical staining using frozen sections plus ABC staining kit. We also investigated the relationship between the numbers of CCR4⁺ cells and clinical stages or serum levels of lactate dehydrogenase (LDH), soluble interleukin-2 receptor (sIL-2R) or CCL17, which are all good disease markers of MF/SS.

METHODS

Clinical samples

Biopsy was performed in patients with MF (25 cases; eight patch, eight plaque, nine tumor), SS (five cases), AD (four cases) and psoriasis (five cases). Skin samples of 23 MF/SS cases and four AD cases were cut into halves after biopsy and one half was formalin-fixed and paraffin-embedded, while the other half was snap-frozen. Clinical stages of MF/SS patients were as follows: three cases of IA, nine cases of IB, two cases of IIA, six cases of IIB, two cases of IIIA, eight cases of IVA, and no cases of IIIB and IVB. The medical ethics committee of the University of Tokyo approved all described studies and the study was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from participants.

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Immunohistochemistry

Formalin-fixed and paraffin-embedded samples were stained with a CCR4 staining kit according to the manufacturer's instruction (Kyowa Medex, Tokyo, Japan). Frozen sections were stained with anti-CCR4 antibody (Kyowa Medex) or anti-CXCR3 antibody (1C6; BD Biosciences Pharmingen, Heidelberg, Germany) plus ABC staining kit (Vector Lab, Burlingame, CA, USA). CCR4⁺ cells per high-power field ($\times 400$) were counted in lesional skin for paraffin-embedded samples. As the quality of staining with frozen samples was not high enough to quantify positive cells, samples were categorized into three groups: negative, scattered positive cells and positive.

Statistical analysis

Statistical analysis was performed using the Mann-Whitney *U*-test for comparison of two groups. For testing equality of population means among three or more groups, Kruskal-Wallis test and Scheffé's *F*-test were used. Correlation coefficients were determined by using Spearman's rank correlation coefficient. $P < 0.05$ was considered statistically significant (StatView version 5; SAS Institute, Tokyo, Japan).

RESULTS

Immunological staining with the CCR4 staining kit revealed that CCR4 was expressed on infiltrating cells in lesional skin of patch, plaque, tumor MF and SS, and that the number of positive cells increased as the disease progressed (Figs 1,2a), which was consistent with previous reports.²⁻⁴ Immunohistochemistry with frozen sections also showed some positive cells scattered in the dermis, although the quality was not high enough to quantify positive cells (Fig. 1). CXCR3⁺ cells were detected in the dermis of any stage of MF and SS. The CCR4⁺/CXCR3⁺ ratio in MF increased as the disease progressed, which was consistent with previous papers.²⁻⁴ As expected, the number of CCR4⁺ cells identified by the CCR4 staining kit was strongly associated with the results using frozen sections (Fig. 2b). When we compared the numbers of CCR4⁺ cells in different clinical stages, skin lesions at stage IIB and IVA contained a large number of positive cells (Fig. 2c). There were significant positive correlations between CCR4⁺ cells and serum LDH levels, but not serum sIL-2R or CCL17 levels (Fig. 2d and data not shown). Interestingly, CCR4⁺ cells were also detected in lesional skin of AD and psoriasis, and their number in AD skin was larger than that in psoriatic skin (Fig. 2a).

DISCUSSION

CCR4 and CCR10 are expressed on CLA⁺ lymphocytes in the peripheral blood, which suggests that these two chemokine receptors play important roles for skin-homing.⁸ Previous studies, however, showed only scattered CCR4⁺ cells in the skin samples by standard immunohistochemical staining, which made it difficult to judge whether they were positive or negative.²⁻⁴ Our study clearly showed that CCR4 was expressed on infiltrating cells not only in advanced MF or SS but also in early MF and AD (Figs 1,2a). Many CCR4⁺ cells were detected by

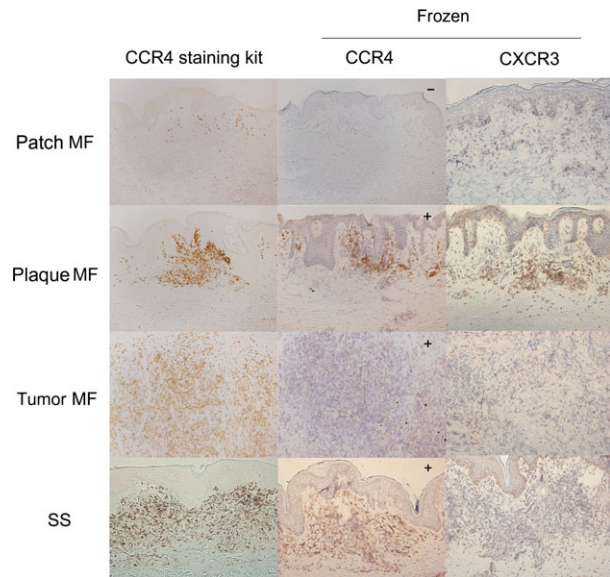


Figure 1. Immunohistochemical staining using paraffin-embedded samples with CCR4 staining kit (left column), frozen sections with anti-CCR4 antibody plus ABC staining kit (center column) and frozen sections with anti-CXCR3 antibody plus ABC staining kit (right column). Representative pictures of patch mycosis fungoides (MF), plaque MF, tumor MF and Sézary syndrome (SS) are shown ($\times 200$). Assessments for figures in the right column are shown in the upper right corner: –, negative; +, positive.

the CCR4 staining kit even in cases which were categorized as negative by standard staining with frozen samples (Fig. 2b). It is not easy to distinguish between the CCR4⁺ tumor cells and non-tumor CCR4⁺ infiltrating cells, especially in patch/plaque MF. We previously showed that from one-third to a half of CCR4⁺ cells in patch/plaque MF expressed thymocyte selection-associated high mobility group box factor (TOX), a molecular marker for histological diagnosis of early stage MF.⁹ In this study, we showed CCR4⁺ cells infiltrating into the epidermis (Fig. 1), suggesting that at least some tumor cells in patch/plaque MF expressed CCR4. As the disease progressed, the CCR4⁺/CXCR3⁺ ratio increased (Fig. 1), suggesting that the number and proportion of tumor cells in the skin increased in the advanced stages. Significant positive correlation between TOX expression and CCR4 expression in our previously study also support the idea.⁹ Although it is possible that the frequency of CCR4 expression on tumor cells increased in the advanced stages, our results have suggested that CCR4 is expressed on tumor cells even in early stages of MF/SS.

Staining with paraffin-embedded samples allowed us to observe morphology much better than that with frozen sections, which made it possible to quantify positive cells. It may be helpful to set up a threshold number of CCR4⁺ cells for selecting patients suitable for treatment with anti-CCR4 antibody. Moreover, high detection sensitivity of the staining showed that the number of CCR4⁺ cells in AD was larger than that in psoriasis, which was consistent with the idea that the

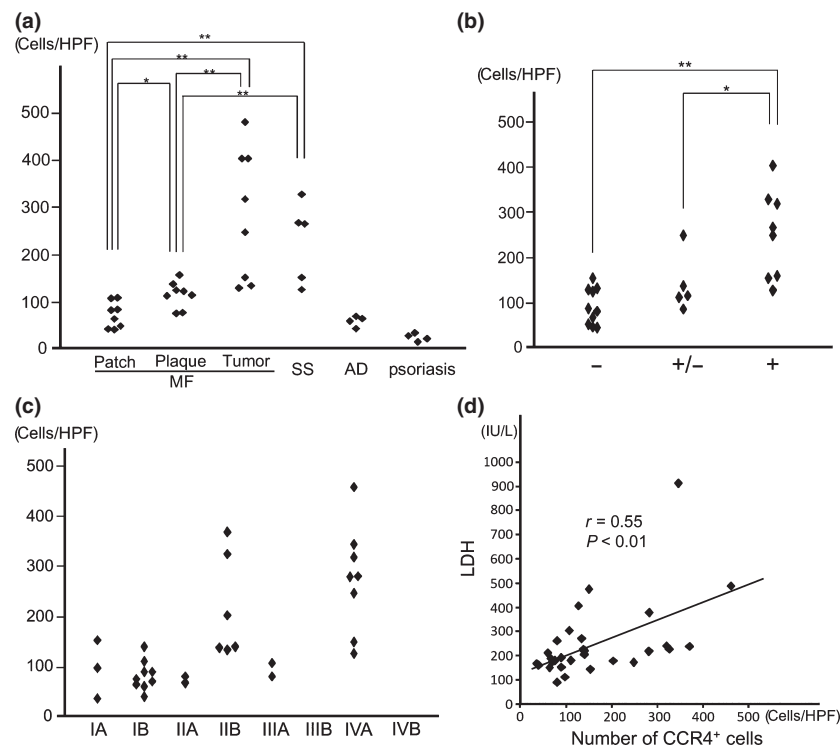


Figure 2. (a) The numbers of CCR4⁺ cells by CCR4 staining kit in patch mycosis fungoides (MF; $n = 8$), plaque MF ($n = 8$), tumor MF ($n = 9$), Sézary syndrome (SS; $n = 5$), atopic dermatitis (AD; $n = 4$) and psoriasis vulgaris ($n = 5$). * $P < 0.05$. ** $P < 0.01$. Statistical analysis performed only among MF/SS. (b) The numbers of CCR4⁺ cells by CCR4 staining kit categorized by the results using frozen sections for 23 cases of MF/SS. –, negative ($n = 10$); +/-, scattered positive cells ($n = 5$); +, positive ($n = 8$). * $P < 0.05$. ** $P < 0.01$. (c) The numbers of CCR4⁺ cells by CCR4 staining kit in different clinical stages. (d) Significant positive correlation between the numbers of CCR4⁺ cells by CCR4 staining kit and serum lactate dehydrogenase (LDH) levels. HPF, high-power field.

former is a Th2-dominant skin disease, while the latter is mediated by Th1/Th17.¹⁰

In summary, the new, sensitive CCR4 staining kit has revealed that CCR4 is expressed on infiltrating cells in lesional skin of early MF and AD as well as advanced MF and SS. These cells can be therapeutic targets for patients who are resistant to standard treatments.

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CONFLICT OF INTEREST: The authors have no conflict of interest to declare.

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