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CXCL13 and CCL21 Are Expressed in Ectopic Lymphoid Follicles in Cutaneous Lymphoproliferative Disorders

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TO THE EDITOR

B-cell-attracting chemokine 1/CXCL13 and secondary lymphoid tissue chemokine/CCL21 are constitutively expressed within secondary lymphoid organs, regulating homing of lymphocyte and dendritic cells to these organs (Nagira *et al.*, 1997, Gunn *et al.*, 1998a; Legler *et al.*, 1998; Yoshida *et al.*, 1998). CXCL13 is a ligand for CXCR5 (Dobner *et al.*, 1992), which is mainly expressed on B cells (Forster *et al.*, 1994). CXCL13 is expressed by follicular dendritic cells and other stromal cells located in the B-cell areas of secondary lymphoid organs (Gunn *et al.*, 1998a; Ansel *et al.*, 2000). CCL21 is a ligand for CCR7 (Burgstahler *et al.*, 1995), which is highly expressed on naïve T cells and mature dendritic cells (Sozzani *et al.*, 1998; Yanagihara *et al.*, 1998; Saeki *et al.*, 1999). CCL21 is constitutively expressed by stromal cells within the T-cell zones of secondary lymphoid organs, endothelial cells of high endothelial venules (HEVs), and lymphatic vessels (Gunn *et al.*, 1998b; Nagira *et al.*, 1998). Recently, several researchers have recognized that CXCL13 and CCL21 are also involved in some human diseases. CXCL13 and CCL21 expression was shown in lymphoid follicle-like structures in salivary glands of Sjögren syndrome and inflamed synovial specimens of rheumatoid arthritis (Shi *et al.*, 2001; Manzo *et al.*, 2005; Barone *et al.*, 2005). Expression of CXCL13 was also shown in lymphoproliferative disorders such as

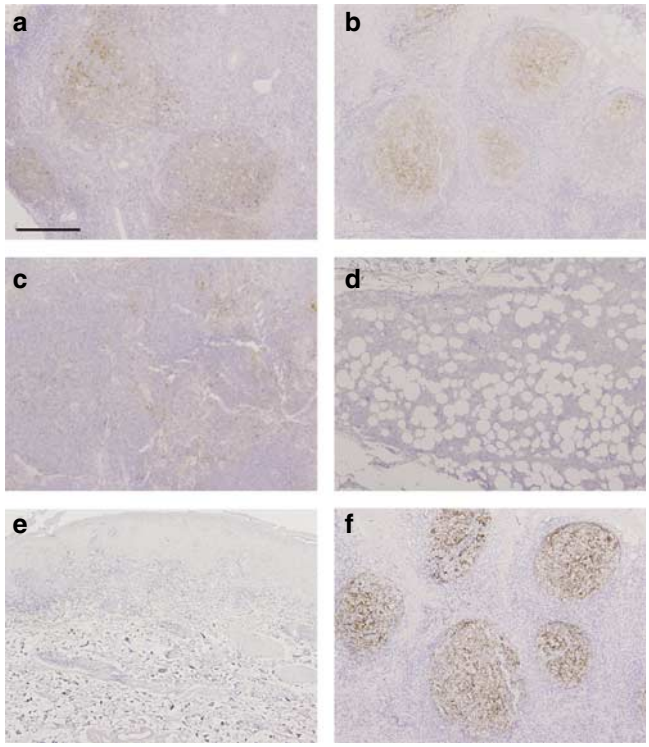

Figure 1. Ectopic follicular structures of the skin are positive for CXCL13 and CD21. Immunohistochemistry for formalin-fixed paraffin-embedded sections was performed as described previously (Barone *et al.*, 2005). Immunohistochemical staining for CXCL13 (AF801, R&D Systems, Abingdon, UK) of (**a**) reactive lymph nodes, (**b**) Kimura's disease, (**c**) primary cutaneous follicle center lymphoma, (**d**) primary cutaneous diffuse large B-cell lymphoma, and (**e**) cutaneous T-cell lymphoma. Positive staining for CD21 (1F8 Dako, Cambridge, UK) in follicular structures of (**f**) Kimura's disease. Bar = 500 μm.

Abbreviation: HEV, high endothelial venule

gastric mucosa-associated lymphoid tissue lymphoma and central nervous system lymphoma (Mazzucchelli *et al.*, 1999; Smith *et al.*, 2003). Here, we analyzed the relationship between CXCL13 and CCL21 expression and ectopic lymphoid structures in cutaneous lymphoproliferative disorders.

Skin and lymph node specimens were obtained from the patients with Kimura's disease (five cases), pseudolymphoma (six cases), primary cutaneous diffuse large B-cell lymphoma (five cases), primary cutaneous marginal zone B-cell lymphoma (two cases), primary cutaneous follicle center lymphoma (four cases), cutaneous T-cell lymphoma (seven cases), lichen planus (five cases), and reactive lymphadenopathy (two cases) over a period from 1985–2006 in our department. All cases of cutaneous lymphoma were diagnosed by the criteria of World Health Organization-European Organization for Research and Treatment of Cancer (WHO-EORTC) classification (Willemze *et al.*, 2005). The medical ethical committee of the University of Tokyo approved all described studies and the study was conducted according to the Declaration of Helsinki Principles. Informed consent was obtained to use tissue specimens from patients and controls.

We first investigated the relationship between CXCL13 expression and follicular formation in the skin. There was strong expression of CXCL13 within follicular structures of Kimura's disease (Figure 1b) and pseudolymphoma with prominent follicular formation similar to reactive lymph nodes (Figure 1a). In primary cutaneous marginal zone B-cell lymphoma and primary cutaneous follicle center lymphoma, CXCL13+ cells were observed, but their localization was not so distinct (Figure 1c). CXCL13+ cells were very sparse or almost negative in primary cutaneous diffuse large B-cell lymphoma (Figure 1d). No CXCL13+ cells were detected in cutaneous T-cell lymphoma (Figure 1e), lichen planus, and some cases of pseudolymphoma in which follicular formation was inconspicuous. As a source of CXCL13, CD21+ follicular dendritic cells were detected in lymphoid follicles of reactive lymph nodes and skin specimens with prominent follicular formation such as Kimura's disease (Figure 1f). CD20+ B cells were located in these lymphoid follicles as expected (data not shown). These results suggested that CXCL13 expressed by follicular dendritic cells induces chemotaxis of B cells, playing important roles in lymphoid follicular formation in the skin.

Expression of CXCL13 in cutaneous lymphoid follicles is consistent with recent reports suggesting that this chemokine may be involved in the pathogenesis of several lymphocyte-mediated diseases such as mucosa-associated lymphoid tissue lymphoma (Mazzucchelli *et al.*, 1999). It was indicated that the degree of expression of CXCL13 was related to size and the degree of follicular aggregates (Shi *et al.*, 2001). In our study, cases with clear follicular structures showed follicular localization of large numbers of CXCL13+ cells. By contrast, cases with moderate follicular structures showed smaller numbers of CXCL13+ cells. These results indicate that expression of CXCL13 is associated with follicular formation in the skin.

We next investigated CCL21 expression because CCL21 is also critical for lymphoid tissue organization. We observed two types of CCL21+ dermal vessels. One type had wide cavities and flat one layer of endothelial cells, which were also positively stained with D2-40 (Figure 2a and b). These vessels were observed in all specimens and were regarded as lymphatic vessels. The other type had smaller cavities with cuboidal endothelial cells whose nuclei were large and prominent, which were also positively stained with peripheral lymph node addressin (PNAd) (Figure 2c and d), a good marker for HEVs (Michie *et al.*, 1993). These vessels were localized to perifollicular area in reactive lymph nodes, 4/5 of Kimura's disease, and pseudolymphoma with prominent follicular formation. Closer examination revealed that CCL21 staining was observed on the outside of PNAd+

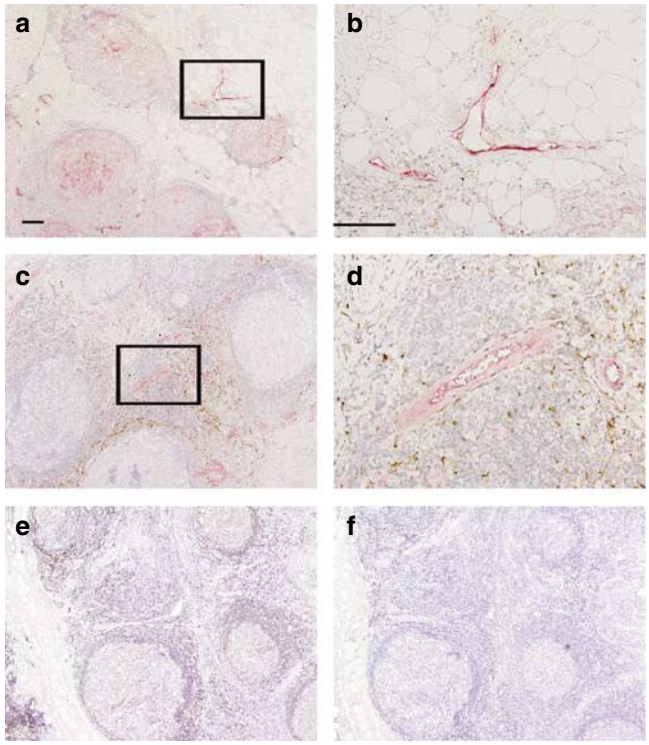

Figure 2. CCL21 is positive in D2-40+ lymphatics and on the outer side of PNAd+ HEV-like vessels. Double staining for CCL21 (brown; AF366 R&D Systems, Abingdon, UK) and D2-40 (red; Nichirei, Tokyo, Japan) of (**a** and **b**) Kimura's disease. (**b**) Higher magnification of double-positive vessels with large cavities and flat endothelial cells. Double staining for CCL21 (brown) and PNAd (red; MECA-79, PharMingen, Oxford, UK) of (**c** and **d**) Kimura's disease. (**d**) Higher magnification of double-positive vessels with cubic endothelial cells. Immunohistochemical staining for (**e**) CD45RA (4KB5 Dako, Cambridge, UK) and (**f**) CD45RO (UCHL1 Dako, Cambridge, UK) of Kimura's disease. Bar = 100 μm.

vessels. In 4/7 of cutaneous T-cell lymphoma, PNAd+ HEV-like vessels were distributed sparsely in the dermis, but they were negative for CCL21. PNAd+ HEV-like vessels were not detected in lichen planus, and some cases of pseudolymphoma in which follicular formation was inconspicuous. With regard to T-cell sub-populations (CD45RA+ naïve T cells and CD45RO+ memory T cells), CD45RA+ cells were mainly located around lymphoid follicles in reactive lymph nodes, Kimura's disease (Figure 2e), and pseudolymphoma with prominent follicular formation. The number of CD45RO+ cells was extremely small (Figure 2f). By contrast, most T cells infiltrating in cutaneous T-cell lymphoma and lichen planus were CD45RA− and CD45RO+ (data not shown). These results suggested that CCL21 expressed on PNAd+ HEV-like vessels induces chemotaxis of CD45RA+ naive T cells, playing important roles in lymphoid follicular formation in the skin.

In all cases, some dermal vessels were positively stained with CCL21, which is natural considering that CCL21 is known to be expressed by endothelial cells of afferent lymphatic vessels (Gunn *et al.*, 1998b). Endothelial cells of some vessels positively stained with CCL21, however, were cuboidal and had large and prominent nuclei, which is characteristic of HEV (Figure 2c and 2d). These double-positive vessels were detected only in cases showing follicular structures. It suggests that dermal HEV-like vessels expressing CCL21 is responsible for formation of ectopic follicular structures in the skin.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Hanako Ohmatsu[1], Makoto Sugaya[1], Takafumi Kadono[1] and Kunihiko Tamaki[1]

[1]Department of Dermatology, Faculty of Medicine, University of Tokyo, Tokyo, Japan
E-mail: sugayam-der@h.u-tokyo.ac.jp

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