Increased Serum CCL28 Levels in Patients with Atopic Dermatitis, Psoriasis Vulgaris and Bullous Pemphigoid

To the Editor:

Atopic dermatitis is an inflammatory skin disease that is characterized by pruritic and eczematous lesions persisting chronically. Histopathologically, the skin lesions in atopic dermatitis show infiltration of T lymphocytes, especially cutaneous lymphocyte antigen-positive memory T cells, as well as eosinophils and macrophages (Novak et al., 2003). Previous studies revealed that increased amounts of thy-mus and activation-regulated chemokine (CCL17) and cuta-neous T cell-attracting chemokine (CTACK)/CCL27 were detected in the serum and lesional keratinocytes of patients with atopic dermatitis (Kakinuma et al., 2001, 2003). These results suggest involvement of these chemokines in the pathogenesis of this disease.

Psoriasis vulgaris is also a chronic and relapsing inflammatory skin disease characterized as a T cell-mediated autoimmune disorder (Chang, 1992). CTACK/CCL27 was strongly detected in the serum and lesional keratinocytes of patients with psoriasis vulgaris (Kakinuma et al., 2003). It has been proposed that interaction between T cells and epidermal keratinocytes plays a central role in the pathogenesis of psoriasis vulgaris (Chang, 1992).

Bullous pemphigoid is a blistering autoimmune skin disease. It often provokes lesional eosinophil infiltration (Pierard et al., 1961). Eotaxin/CCL11 was strongly detected in blister fluid of bullous pemphigoid and strongly expressed in keratinocytes around blisters of this disease (Wakugawa et al., 2000).

Mucosae-associated epithelial chemokine (MEC)/CCL28 is a novel chemokine ligand for CC chemokine receptor (CCR) 10 and CCR3 (Pan et al., 2000). MEC/CCL28 is most homologous to CTACK/CCL27, displaying about 40% identity, and is most abundant in the salivary gland, with strong expression in other tissues associated with mucosal epithelial surfaces, including colon, trachea, and mammary gland. MEC/CCL28 attracts subsets of memory lymphocytes and eosinophils (Pan et al., 2000). Wang et al. (2000) showed that MEC/CCL28 mRNA was expressed predominantly in psoriasis patient skin samples and to a lesser degree in healthy human skin. But MEC/CCL28 production at protein levels in skin is totally unknown. Furthermore, there has been no report of serum MEC/CCL28 levels in patients with skin diseases. This prompted us to investigate the relationship between MEC/CCL28 and skin diseases. Therefore, we examined serum MEC/CCL28 levels of patients with atopic dermatitis, psoriasis vulgaris, and bullous pemphigoid. Furthermore, in order to determine which cells produced MEC/CCL28 in skin, we performed

Abbreviations: CCR, CC chemokine receptor; CTACK, cutaneous T cell-attracting chemokine; MEC, Mucosae-associated epithelial chemokine
immunohistochemical staining of this chemokine in the lesional skin of patients with these diseases.

Forty-eight patients with atopic dermatitis (mean ± standard deviation age: 28.7 ± 6.7 y), 20 patients with psoriasis vulgaris (28.6 ± 6.3 y), 28 patients with bullous pemphigoid (68.2 ± 20.1 y), and 20 healthy controls (28.5 ± 8.4 y) were enrolled in this study. The medical ethical committee of the University of Tokyo approved all described studies and the study was conducted according to Declaration of Helsinki Principles. Informed consent was obtained to use the sera and skin tissues from the patients and healthy controls. The 20 healthy controls had no history of allergy, psoriasis or bullous disease. All sera were stored at −20 °C until use. ELISA was performed using MEC/CCL28 immunoassay kits obtained from R&D Systems (Minneapolis, Minnesota). Serum levels of MEC/CCL28 were measured according to the manufacturer's instructions. Optical densities were measured at 450 nm with a Bio-Rad Model 550 microplate reader (Bio-Rad Laboratories, Hercules, California). The concentrations were calculated from the standard curve generated by a curve-fitting program. Data obtained from the ELISA are presented as mean ± standard error. Data were analyzed using Mann–Whitney’s U test and paired t test. A p-value less than 0.05 was considered to be statistically significant.

Biopsy specimens obtained from patients and healthy controls were used for immunohistochemical staining. To detect MEC/CCL28, affinity-purified goat polyclonal antihuman MEC/CCL28 (Dako Cytomation, Glostrup, Denmark) was used. Normal goat IgG1 (Santa Cruz Biotechnology, Santa Cruz, California) was used as an isotype-matched control.

The serum MEC/CCL28 levels of patients with atopic dermatitis (148.9 ± 13.6 pg per mL), psoriasis vulgaris (167.8 ± 34.2 pg per mL), and bullous pemphigoid (89.2 ± 45.3 pg per mL) were significantly higher than those in healthy controls (44.7 ± 5.9 pg per mL) (Fig 1). Because patients with bullous pemphigoid were much older than the other cohorts, it will be necessary to compare them with much older healthy controls in a future study in order to confirm the conclusion. The serum MEC/CCL28 levels did not correlate with disease activity of these three conditions (data not shown). The elevated serum MEC/CCL28 levels of patients with atopic dermatitis tended to decrease after treatments (n = 6, before: 169.8 ± 75.9 pg per mL, after: 65.8 ± 22.7 pg per mL), although the change was not significant (data not shown).

MEC/CCL28 was strongly expressed in epidermal keratinocytes of patients with atopic dermatitis and psoriasis vulgaris, and weakly expressed in epidermal keratinocytes of patients with bullous pemphigoid, insect bite, healthy controls, and non-lesional skin of patients with psoriasis vulgaris (Fig 2). Thus, MEC/CCL28 was strongly expressed only in the lesional skin of systemic skin diseases, such as atopic dermatitis and psoriasis vulgaris.

This is the first report describing MEC/CCL28 expression by epidermal keratinocytes in skin and elevated levels of this chemokine in the serum of patients with some inflammatory skin diseases.

Both CTACK/CCL27 and MEC/CCL28 attract CCR10+ lymphocytes. The CCR10+ lymphocytes invading the skin in atopic dermatitis and psoriasis have different Th1/Th2 profiles (Vestergaard et al, 2000). Therefore, CTACK/CCL27 and MEC/CCL28 may contribute to the pathogenesis of both a Th2-dominant disease like atopic dermatitis and a Th1-dominant disease like psoriasis vulgaris. MEC/CCL28, but not CTACK/CCL27, stimulates migration of CCR3 transfectants as well as eosinophils (Pan et al, 2000). In this context, MEC/CCL28 may play a role, in conjunction with other regulatory elements, in the recruitment of eosinophils and potentially, of rare CCR3+ T cells into epidermis. MEC/CCL28 seems to be one of the important chemokines in the lesional formation of eosinophil infiltrating diseases like atopic dermatitis and bullous pemphigoid.

These results suggest that MEC/CCL28 is involved in the pathogenesis of these chronic inflammatory skin diseases in cooperation with other chemokinases.

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To The Editor:

Mounting evidence suggests that adult stem cells have greater plasticity than previously thought. Among stem cells derived from mesenchymal tissues (e.g., Toma et al., 2001; Zuk et al., 2001; Jiang et al., 2002), one population from the skin dermis has received considerable prominence, owing to its accessibility and wide spectrum of differentiation activities in culture (Toma et al., 2001).

Within the adult hair follicle dermis, two developmentally active cell populations, the dermal papilla (DP) and dermal sheath (DS), have well-established instructive powers in relation to the control of hair follicle cycling and induction. Although the stem cell activity of the hair follicle epithelium has been studied intensively (Taylor et al., 2000; Morris et al., 2004; Tumbar et al., 2004), until recently the follicle dermis has been largely overlooked in this context.

We have previously suggested that follicle dermal cells have extensive stem or progenitor cell activities within the skin, including an important role in wound healing (Jahoda and Reynolds, 2001). Using rodent models we have demonstrated that the follicle dermis harbors hematopoietic stem cell activity (Lako et al., 2002). We recently directed clonally derived papilla and sheath cell lines towards adipocyte and osteocyte phenotypes (Jahoda et al., 2003). For follicle dermal stem cells to be useful in any therapeutic context, we need, however, to show that human hair follicle dermal stem cells have similar capabilities. Here, we report adipogenic and osteogenic differentiation in human hair follicle-derived DP and DS cultures. Human scalp or beard follicles were isolated from skin biopsies from four individuals (aged 23, 48, 89, and 91 y). DP and DS tissues were dissected from amputated end bulbs as previously described (Reynolds et al., 1999). Briefly, amputated follicle end bulbs were inverted, and the exposed epithelial matrix was detached. Adherent epithelial cells and debris were removed, the DP and DS were separated and explant cultures initiated in MEM plus 20% FBS from a minimum of 10 isolated DP or DS samples from each biopsy.

Cells between passage 3 and 7 (50,000 cells per 35 mm plate) were allowed to adhere overnight in MEM plus 10% FBS from a minimum of 10 isolated DP or DS samples from each biopsy. For each of the osteogenic and adipogenic differentiation experiments, the DP and DS cell lines were established from two different human samples. All established cell lines could be directed towards osteogenesis (five of five) and, in contrast to a previous report (Iguchi et al., 2001), adipogenesis (five of five). These differentiation events occurred irrespective of the origin of the cell line or patient age. Interestingly, as repeat experiments were

Abbreviations: DP, dermal papilla; DS, dermal sheath