Letter to the Editor

Increased population of central memory T cells in circulating peripheral blood of psoriasis patients

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Central memory T cells; Psoriasis; Th17; Th22; CCR7; CXCR5

Psoriasis is a common human skin disease mediated by T helper type-17 cells (Th17) expressing interleukin (IL)-17A and IL-22. Circulating Th17 are increased in the blood of psoriasis patients [1], and serum IL-22 levels correlate with the psoriasis activity severity index (PASI) [2].

There are two distinct populations of memory T cells. Effector memory T cells are similar to effector cells, express tissue-homing receptors, develop effector function, and rapidly produce cytokines upon restimulation. Central memory T cells (T_{CM}) are similar to naive T cells and express the lymph node homing receptor L-selectin (CD62L) and CC chemokine receptor (CCR) 7, but generally lack receptors for homing to peripheral tissues. T_{CM} have lower effector function levels but proliferate vigorously and develop into effector T cells when restimulated with antigens [3]. Here we demonstrated the effect of CCR7 on T cells *in vivo* in psoriasis patients. $CD4^+CCR7^+$ T cells are increased in psoriasis patients [4]. Approximately 20–25% of $CD4^+CCR7^+$ T_{CM} in human blood express C-X-C chemokine receptor 5 (CXCR5) [5]. CD4 and CCR7 are also expressed on naïve T cells, making CXCR5 a more suitable marker than CD45 for differentiating naïve T cells and T_{CM} [6].

Th17 or Th22 might be related to T_{CM}. T_{CM} generate Th17 in the presence of tobacco smoke extract, which has clinical implications for psoriasis patients exposed to tobacco smoke [7]. Human $CD4^+$ memory T cells produce Th22 [8].

We investigated $CD4^+CCR7^+$ T and $CD4^+CCR7^+CXCR5^+$ T cell levels in healthy controls and psoriasis patients, and the relationship between T_{CM} and Th17 or Th22. After obtaining written informed consent, 115 psoriasis patients and 18 healthy controls were enrolled. The Ethics Committee of Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan, approved the study. All patients in this open-label study were diagnosed with psoriasis clinically and histologically. Psoriasis patients were grouped according to PASI score: $0 \leqq \text{PASI} < 10$, $n = 21$; $10 \leqq \text{PASI} < 20$, $n = 44$; $20 \leqq \text{PASI} < 30$, $n = 29$; and $30 \leqq \text{PASI}$, $n = 21$.

Peripheral blood mononuclear cells were obtained from psoriasis patients and healthy controls; stained with antibodies against CD4 (DAKO, Carpinteria, CA), CCR7 (eBioscience, San Diego, CA), and CXCR5 (BD Bioscience, San Jose, CA); and subjected to fluorescence-activated cell sorting analysis (FACS Calibur™, Becton Dickinson, Franklin Lakes, NJ). $CD4^+CCR7^+$ and $CD4^+CCR7^+CXCR5^+$ cells were defined as T_{CM}.

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For Th17 and Th22 analysis, $CD4^+$T cells were isolated using magnetic beads (Miltenyi Biotec, Auburn, CA); incubated with phorbol 12-myristate 13-acetate and ionomycin (Sigma–Aldrich, St. Louis, MO) for 4 h in the presence of brefeldin A (BD Bioscience); and stained with antibodies against CD3 (BD Bioscience), IL-17A (eBioscience), and IL-22 (R&D Systems, Minneapolis, MN); and then subjected to FACS. The population of each plot was defined as (1) $IL\text{-}17A^+IL\text{-}22^+$ double-positive T cells, (2) $IL\text{-}17A^+IL\text{-}22^-$ T cells, or (3) $IL\text{-}17A^-IL\text{-}22^+$ T cells in the $CD3^+$ gate (Supplementary Figure 1). Correlations among all possible combinations were evaluated.

$CD4^+CCR7^+$ and $CD4^+CCR7^+CXCR5^+$ cell levels in psoriasis patients and healthy controls were compared using Student's *t* test. $CD4^+CCR7^+$ cell levels in healthy controls and psoriasis patients grouped by PASI score were compared by Tukey's test. Correlation analyses were performed to establish the relationship among some parameters using the Pearson product–moment correlation coefficient. Results are presented as mean ± SD. $p < 0.05$ was considered significant.

Levels of the two T_{CM} subsets were compared between psoriasis patients and healthy controls. $CCR7^+$ T cell levels and $CCR7^+CXCR5^+$ T cell levels were significantly higher in psoriasis patients than in healthy controls (Fig. 1A).

T_{CM} levels were compared between healthy controls and psoriasis patients stratified by PASI score. $CCR7^+$ T cell levels and $CCR7^+CXCR5^+$ T cell levels were significantly higher in all groups of psoriasis patients than in healthy controls (Fig. 1B).

We analyzed the correlation between T_{CM} and IL-17A or IL-22 producing T cells. $CCR7^+$ T cell levels were not significantly correlated with $IL\text{-}17A^+$ T cell [1]+[2] levels. In contrast, $CCR7^+$ T cell levels in patients with PASI scores >20 were significantly correlated with $IL\text{-}22^+$ T cell [1]+[3] levels ($r = 0.310$, $p = 0.036$, Fig. 2A). There was no correlation with other combinations and T_{CM}.

$CCR7^+$ T cell levels of patients with $\text{PASI} > 20$ ($r = 0.354$, $p = 0.018$) and $\text{PASI} > 30$ ($r = 0.463$, $p = 0.049$) were significantly correlated with $IL\text{-}17A^+IL\text{-}22^+$ double-positive T cell levels. The $CCR7^+CXCR5^+$ T cell levels with $\text{PASI} > 20$ ($r = 0.480$, $p = 0.001$) and $\text{PASI} > 30$ ($r = 0.659$, $p = 0.002$) were significantly correlated with $IL\text{-}17A^+IL\text{-}22^+$ double-positive T cell levels (Fig. 2B). $CCR7^+CXCR5^+$ T cell levels seems to be more highly correlated with $IL\text{-}17A^+IL\text{-}22^+$ double-positive cell levels than $CCR7^+$ T cell levels in severe psoriasis patients.

We focused on circulating $CD4^+CCR7^+$ cells and $CD4^+CCR7^+CXCR5^+$ cells in psoriasis patients. CCR7 is a defining factor for T_{CM} and is necessary for migration from the skin to lymph nodes. CXCR5 is a chemokine receptor binding CXCL13 that defines follicular T cells. $CXCR5^+$ T cells co-express the lymph node homing receptor CCR7 and L-selectin (CD62L), enabling their recruitment to secondary lymphoid tissue [5]. CCR7 expression on $CD4^+$ cells is higher in psoriasis patients than in healthy controls [4], consistent with the present findings. In the present study, $CCR7^+$ and $CCR7^+CXCR5^+$ T cell levels were significantly higher in almost all psoriasis patients than in controls, suggesting that $CCR7^+$ and

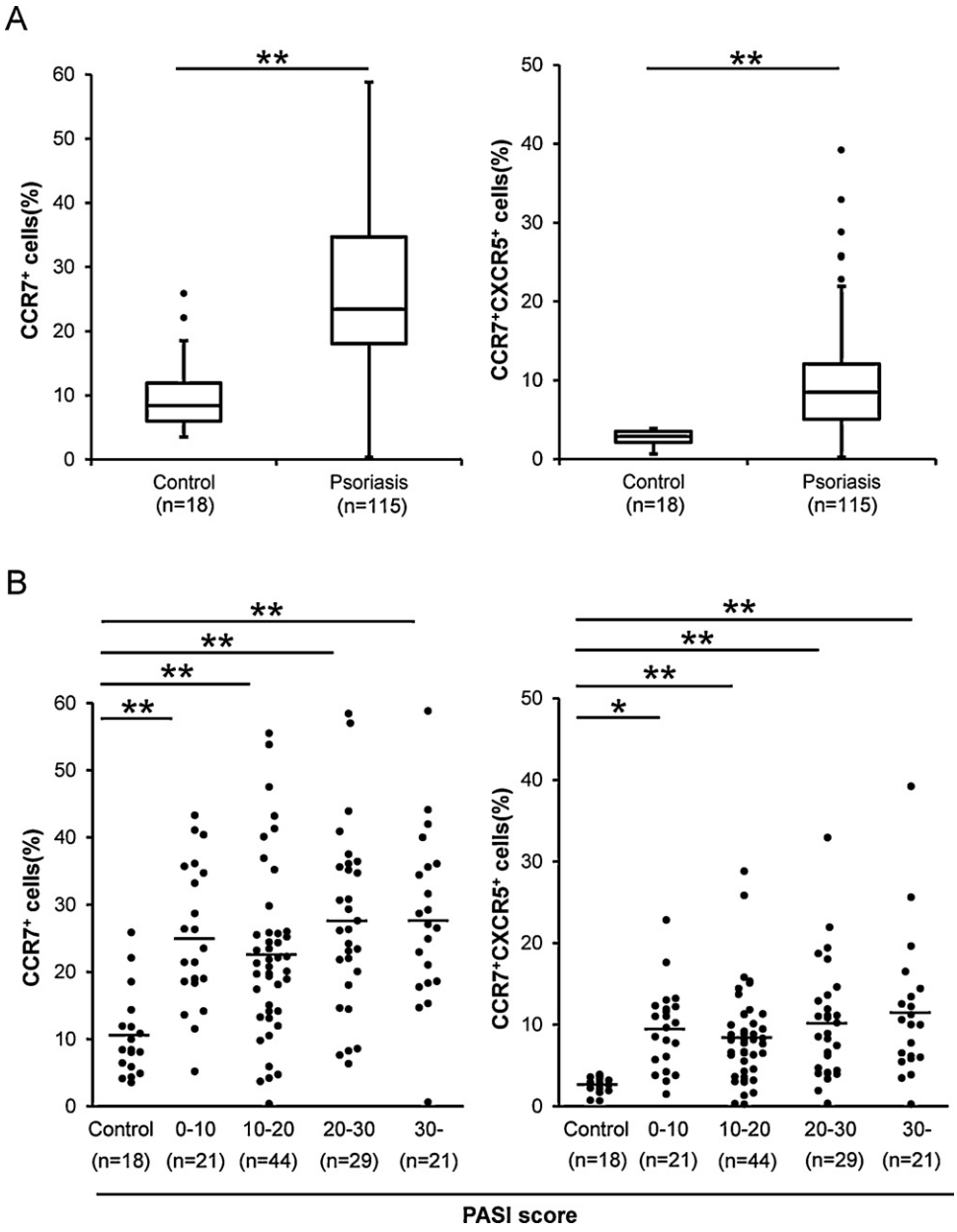

Fig. 1. (A) Central memory T cell levels are higher in psoriasis patients. Peripheral blood mononuclear cells from psoriasis patients and healthy controls were stained for CD4, CCR7, and CXCR5, and examined by fluorescence-activated cell sorting. Data were gated on $CD4^+$ T cells. The $CCR7^+$ T and $CCR7^+CXCR5^+$ T cell levels were significantly higher in psoriasis patients than in healthy controls.$^{**}p < 0.01$. (B) $CCR7^+$ and $CCR7^+CXCR5^+$ T cell populations are increased in almost all psoriasis patients. $CCR7^+$ T cell levels were significantly higher in psoriasis patients with PASI scores of 0–10, 10–20, 20–30, and >30 than in healthy controls. $CCR7^+CXCR5^+$ T cell levels of patients with PASI scores of 0 to 10, 10–20, 20–30, and >30 were significantly higher than in healthy controls.$^{*}p < 0.05$. Horizontal bar indicates mean value.

$CCR7^+CXCR5^+$ T cells are related to the psoriatic pathology. CCR7 also has an important role in regulatory T cell (Treg) function and migration to lymph nodes [9]. In this study, the $CCR7^+$ T cell population that we analyzed might be part of the $CCR7^+$ Treg population.

$CCR7^+$ T cell levels correlated significantly with IL-22 producing cells in severe psoriasis. Th17 and Th22 secrete IL-22. A Th22 subset of T cells was recently found to secrete IL-22, but not IL-17 or interferon-γ [10]. Circulating Th22 cells are increased in psoriasis patients [1]. Our results suggest that T_{CM} are related to the pathogenesis of psoriasis and are precursors of Th22 and Th17.

In conclusion, levels of CCR7 and CXCR5 cells expressing T_{CM} were significantly increased in patients with psoriasis and correlated with IL-22 producing T cells in patients with severe psoriasis. These findings suggest that T_{CM} is strongly associated with psoriasis pathogenesis and severity. T_{CM} might be precursors of pathogenic T cells in psoriasis.