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CCR6$^+$ MCAM$^+$ Th17 Cell Numbers Increase in Patients with Psoriasis and Correlate with Disease Severity

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Abstract: Psoriasis is a chronic immune-mediated disease in which the interleukin (IL)-23/IL-17 axis plays a key role in the inflammatory cascade. We recently reported that co-expression of CCR6 and melanoma cell adhesion molecule (MCAM) in effector memory CD4 T cells (T_{EM}) of peripheral blood might be a useful marker for T helper (Th) 17 cells. In this study, we monitored changes in T_{EM} expressing CCR6 and MCAM using the Psoriasis Area and Severity Index (PASI) score during anti-tumor necrosis factor (TNF) therapy. We also studied CCR6$^+$ MCAM$^+$ Th17 cells histologically in skin biopsy samples from psoriasis patients. In psoriasis patients treated with anti-TNF therapy, the PASI score and the percentage of CCR6$^+$ MCAM$^+$ T_{EM} cells in the blood changed almost in parallel. In immunohistochemical analyses, the proportions of IL-17$^+$ T cells and MCAM$^+$ T cells in the lesional skin of severely psoriatic patients were significantly higher than in mildly psoriatic patients ($P < 0.05$), and the number of IL17$^+$ T cells correlated with the PASI score ($r = 0.400$, $P < 0.05$). Taken together, these results indicate that CCR6$^+$ MCAM$^+$ Th17 cells in peripheral blood and lesional skin might play an important role in the pathology of psoriasis.

Key words: psoriasis, Th17 cells, CCR6, MCAM, IL-17

Introduction

Psoriasis is a chronic immune-mediated disease that affects 2–4% of the general population worldwide[1–3]. Over the past two decades, increased research into the physiopathology of psoriasis has resulted in novel therapeutic options[4–6]. T helper (Th)17 cells have been shown to play a key role in the inflammatory cascade central to the disease, via the so-called interleukin (IL)-23/IL-17 axis[7–8]. Accordingly, psoriasis is sensitive to biological agents targeting IL-17 and IL-23[9–11].

Effector memory CD4 T cells (T_{EM}) migrate into inflamed tissues, produce various cytokines, and aggravate psoriasis[12]. In addition, Th17 cells, a subset of CD4$^+$ T helper cells, characteristi-

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cally secrete cytokines such as IL-17 and play important roles in the pathogenesis of several inflammatory and autoimmune diseases[13]. Although several cell surface markers for human Th17 cells have been reported (including CCR6, MCAM, and CD161)[14–16], none has demonstrated practical utility in determining the severity of psoriasis. For example, although Th17 expresses CCR6[13], this is not a reliable clinical marker of psoriasis.

The CD146 / melanoma cell adhesion molecule (MCAM or MUC18) belongs to the immunoglobulin superfamily, and has been identified initially as a useful marker of melanoma progression and metastasis[17]. In normal adult tissues, MCAM is expressed primarily by vascular endothelium and smooth muscle, where it plays roles in cellular adhesion. MCAM was also reported to be a marker of activated T cell groups that are enriched in T_{EM}[18], and to aid the migration of Th17 cells into the central nervous system of patients with multiple sclerosis[14]. Previously, we reported that $CCR6^+$ $MCAM^+$ T_{EM} produced more IL-17 than other T_{EM} subsets in a study designed to clarify whether the $CCR6^+$ $MCAM^+$ phenotype could identify pathogenic Th17 cells in patients with psoriasis[19]. In the present study, $CCR6^+$ $MCAM^+$ T_{EM}, that enriches Th17 cells in both peripheral blood and $CCR6^+$ $MCAM^+$ $IL\text{-}17^+$ CD4 T cells in lesional skin, seemed to correlate with psoriatic disease severity. We also discuss the pathogenic roles played by these markers in the Th17 cell-mediated responses to disease.

Materials and methods

Patients

Blood samples from healthy controls and psoriatic patients

We studied two naïve patients with psoriasis vulgaris who were treated with anti-TNF therapy (infliximab) at Showa University Hospital. Before and after treatment, we collected blood samples and determined the Psoriasis Area and Severity Index (PASI) scores to evaluate the severity of psoriasis.

Skin samples from psoriatic patients

We obtained skin samples from 34 patients (21 males, 13 females, aged 21–87 years ; mean age, 59.2 years) newly diagnosed with psoriasis vulgaris in the Department of Dermatology at Showa University Hospital from 2010 to 2014. Medical records were also reviewed to extract clinical information and laboratory data. We divided the patients into two groups : those with mild ($PASI < 12$) and severe ($PASI \geq 12$) disease. The mean PASI score was 11.7 ; the mean scores of the mild and severe groups were 4.97 ± 0.60 (mean ± SEM) and 19.3 ± 1.07, respectively.

Written informed consent to participate in the study was obtained from all patients. The Ethics Committee of Showa University School of Medicine approved this study.

Flow cytometric analysis

Peripheral mononuclear blood cells (PMBCs) in phosphate-buffered saline (PBS) with 0.2% (w / v) bovine serum albumin (BSA) were incubated on ice for 30 min with the following murine monoclonal antibodies against human antigens (50-fold dilutions) : Pacific Blue anti-CD3

(clone OKT3), Brilliant Violet 510 anti-CD4 (clone OKT4), PerPC / Cy5.5TM anti-CD45RA (clone HI100), Alexa Fluor 488 anti-CCR7, PE / Cy7TM anti-CCR6 (clone G034E3), and PETM anti-MCAM (clone P1H12). All anti-human protein antibodies used in flow cytometry were purchased from BioLegend (San Diego, CA, USA). Cells were washed twice with ice-cold PBS containing 0.2% (w / v) BSA. After filtration, cells were analyzed using a FACSAria II running the FACS Diva software (ver. 6.1 ; BD Biosciences, San Jose, CA, USA).

Immunohistochemistry

Immunohistochemical analysis was performed on 3 μm sections from formalin-fixed, paraffin wax-embedded specimens, using antibodies against the following proteins : CD3 (clone PS1, Novocastra, Newcastle, UK), CD4 (clone 1F6, Novocastra), CD8 (clone C8 / 144B, DakoCytomation A / S, Glostrup, Denmark), CD146 / MCAM (clone EPR3028, Millipore Bedford, MA, USA), CCR6 (clone 53103, R&D), and IL-17 (polyclonal, goat ; LifeSpan Biosciences, Seattle, WA, USA).

Evaluation of immune cells detected microscopically

We determined the percentages of CCR6$^+$ cells in CD3$^+$ T cells, and the percentages of IL17$^+$ and MCAM$^+$ cells in 300 CD3$^+$ T cells in tissue sections (×400).

Statistical analysis

Paired or unpaired two-tailed Student's *t*-tests were used to compare differences between groups. A *P*-value of 0.05 was considered to indicate statistical significance. Spearman's ordered correlation coefficients were calculated.

Results

The percentage of CCR6$^+$ MCAM$^+$ T_{EM} cells paralleled the PASI score in psoriasis patients treated with biologics

The PASI scores and percentages of CCR6$^+$ MCAM$^+$ T_{EM} cells in seven psoriasis patients who were treated with biologics, such as infliximab, adalimumab, and ustekinumab (Table 1), were monitored. Six patients responded to the treatments and showed decreases in both PASI scores and percentages of CCR6$^+$ MCAM$^+$ T_{EM} cells. One patient (no. 5 in Table 1) was worse clinically after the treatments and the PASI scores and percentages of CCR6$^+$ MCAM$^+$ T_{EM} cells were still increased. Fig. 1 shows a typical case of parallel changes in PASI scores and percentages of CCR6$^+$ MCAM$^+$ T_{EM} cells during treatment for psoriasis. The patient was treated with infliximab and responded transiently, and then the treatment was changed to adalimumab. Subsequently, the symptoms improved. Whether the shift was up or down, all changes in PASI score and the percentage of CCR6$^+$ MCAM$^+$ T_{EM} were almost in parallel. These results suggest that percentages of CCR6$^+$ MCAM$^+$ T_{EM} in peripheral blood reflected the therapeutic response.

Localization of T cells and expression of cellular markers in psoriasis and the examination of CCR6$^+$ MCAM$^+$ IL-17$^+$ cells by IF microscopy

Table 1. Changes in PASI scores and percentages of $CCR6^+$ $MCAM^+$ T_{EM} in psoriatic patients treated with biologics

		PASI score		% $MCAM^+$ $CCR6^+$ T_{EM}	
Patient	Therapy	before	after	before	after
1	adalimumab (4 weeks)	20.9	4.8	7.7	0.6
2	adalimumab (14 weeks)	34.5	15.9	4.2	1.8
3	adalimumab (4 weeks)	26	5.4	8	1.5
4	ustekinumab (4 weeks)	14.3	8.1	4.7	2.1
5	ustekinumab (48 weeks)	5.8	15	3.3	7.5
6	infliximab (24 weeks)	13.5	0.8	6	2.8
7	infliximab (28 weeks) and adalimumab (30 weeks)	32	6	6.5	1.2

Seven patients were treated with biologics (adalimumab, ustekinumab, and/or infliximab) at Showa University Hospital. Before, during, and after these treatments, PASI scores and % $CCR6^+$ $MCAM^+$ T_{EM} were determined at least twice.

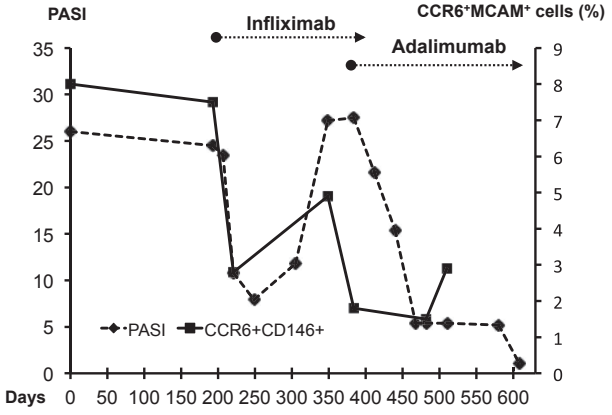
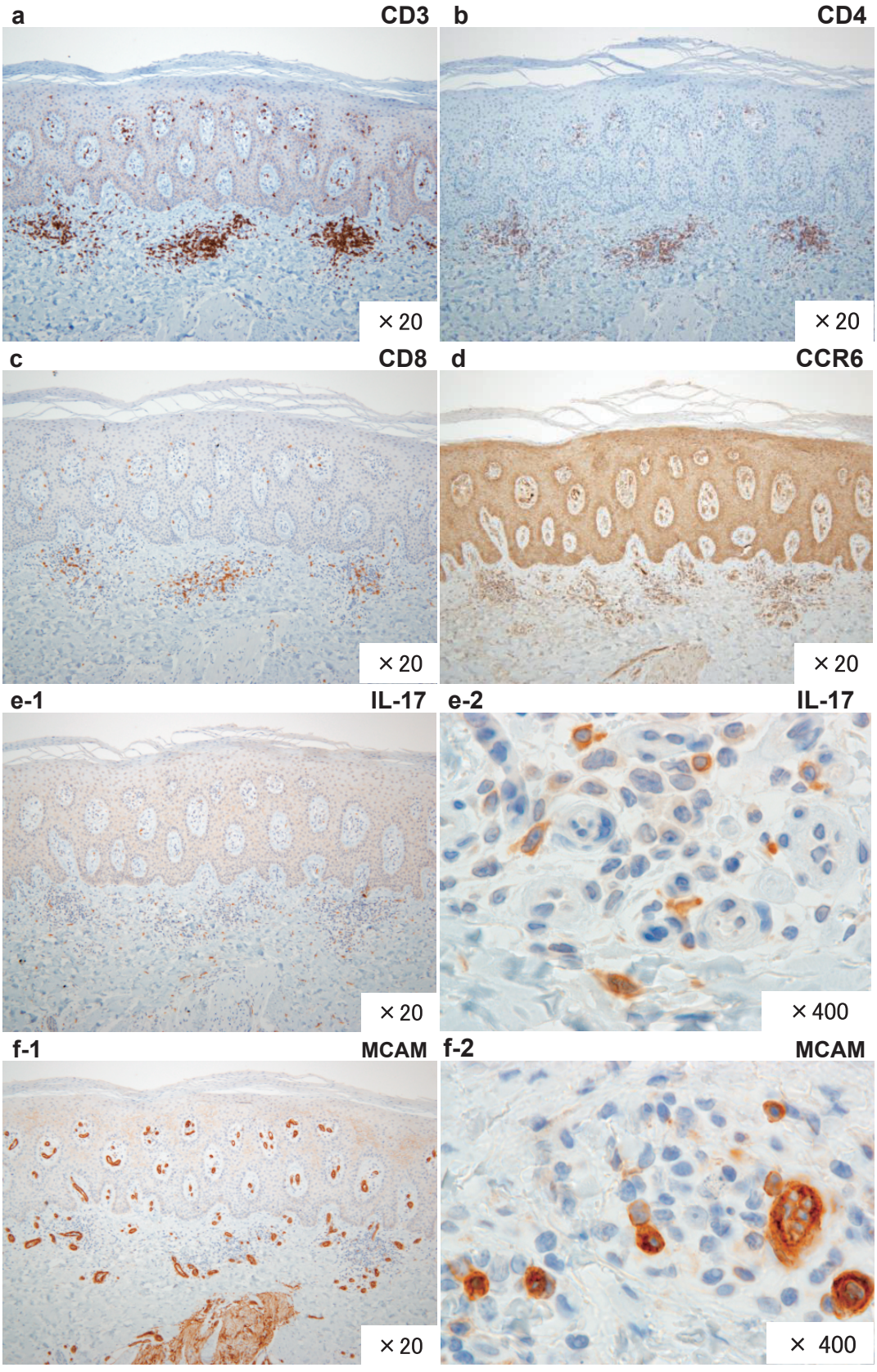


Fig. 1.

In psoriatic lesional skin, most $CD3^+$ T cells (including $CD4^+$ cells and $CD8^+$ cells) were localized to certain regions of the upper dermis (Fig. 2a, b, c); such accumulations of skin-infiltrating T cells are often observed in the perivascular areas of psoriatic lesional skin[20]. Large numbers of $CCR6^+$ cells as well as $CD4^+$ cells were present in the same area (Fig. 2d), as well as some IL-17^+ cells and $CCR6^+$ cells (Fig. 2e, f).

We performed double staining to evaluate the consistency in numbers of $CD4^+$, $CCR6^+$ $MCAM^+$, IL-17^+ $MCAM^+$, and $CCR6^+$ IL-17^+ cells among patients and controls. As shown in Fig. 2g, h, antibodies to MCAM (red) and IL-17 (green), or antibodies to MCAM (green) and CCR6 (red), stained $CD4^+$ cells in the upper dermis. We concluded that the large $MCAM^+$ $CD4^+$ cells were not of the macrophage lineage, because they were negative for CD68 (data not shown), and cells of the myeloid lineage do not express MCAM[15]. These data suggest that $CCR6^+$ $MCAM^+$ Th17 cells were present in the lesional skin of patients with psoriasis.


a
CD3
× 20
b
CD4
× 20
c
CD8
× 20
d
CCR6
× 20
e-1
IL-17
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e-2
IL-17
× 400
f-1
MCAM
× 20
f-2
MCAM
× 400

Fig. 2.

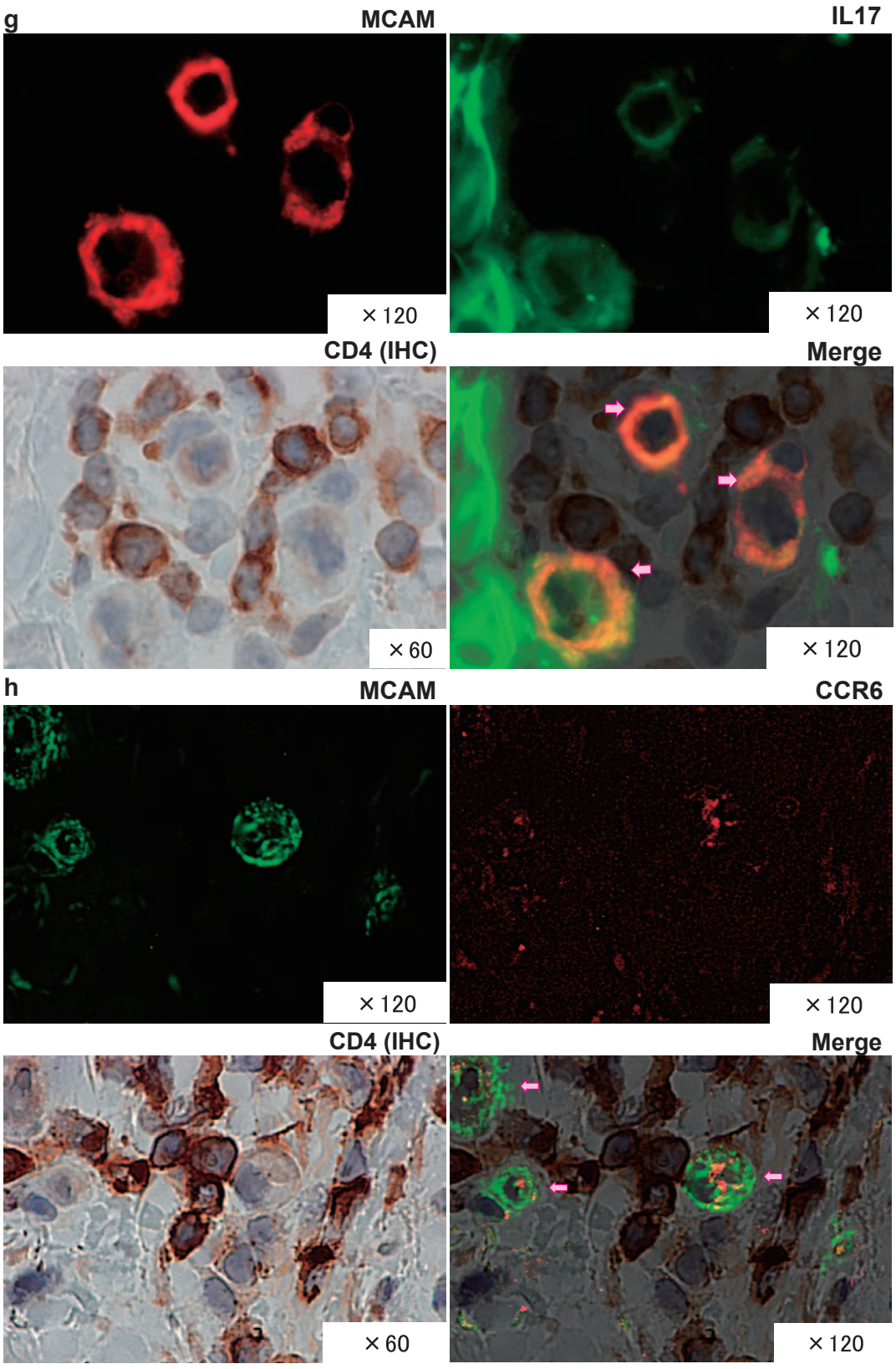
g
MCAM
IL17
× 120
× 120
CD4 (IHC)
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Fig. 2.

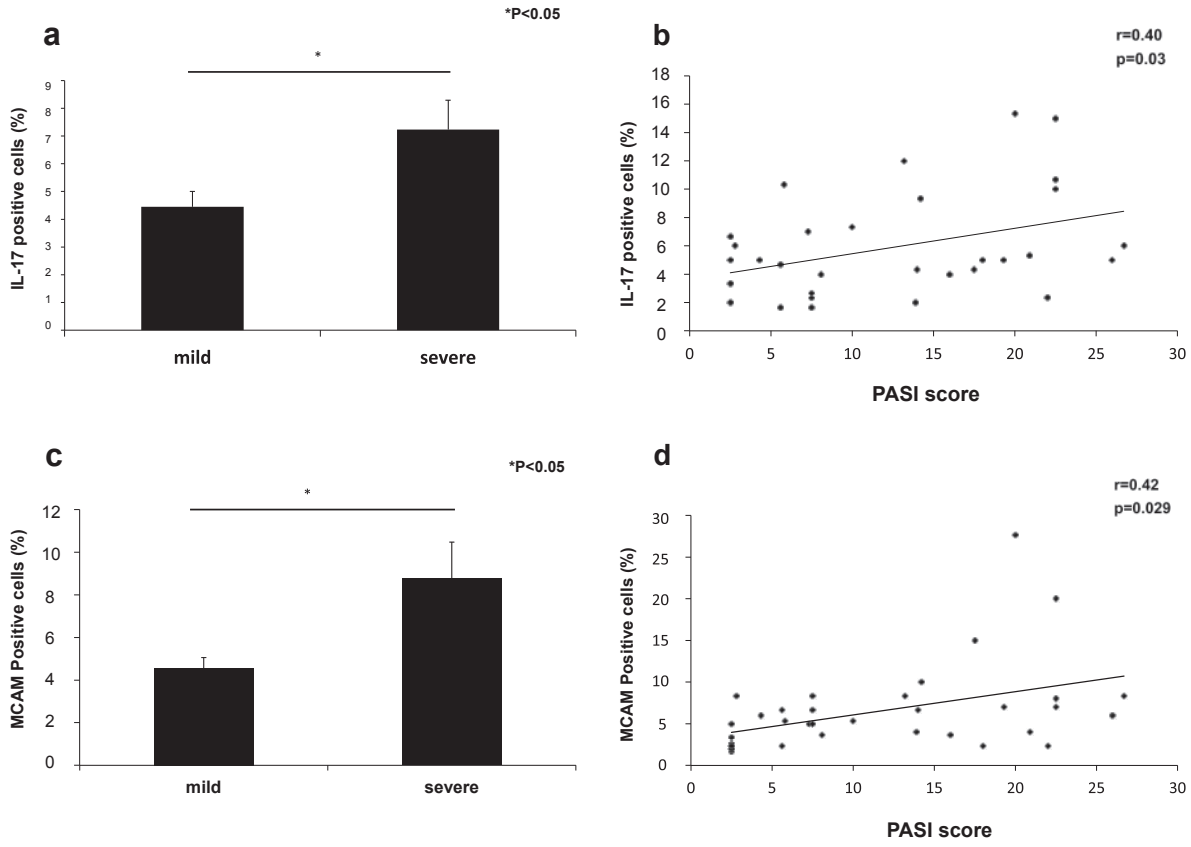

Fig. 3.

Because CCR6 and MCAM are both involved in the migration of inflammatory T cells[14, 18], the elevated numbers of CCR6$^+$ MCAM$^+$ T_{EM} cells in peripheral blood may migrate to lesional skin.

Correlations between IL-17$^+$ and MCAM$^+$ cell numbers in lesional skin and the severity of psoriasis

We calculated the percentages of IL17$^+$ cells among 300 CD3$^+$ T cells evident in tissue sections, and compared these numbers to the disease severity in 34 psoriasis patients. A significant difference was evident between patients with mild (PASI $<$ 12) and severe (PASI $\geq$ 12) disease (Fig. 3a). Moreover, we found a significant correlation between the PASI score and IL17$^+$ cell numbers ($r = 0.400$; Fig. 3b). Next, we determined the proportions of MCAM$^+$ cells among CD3$^+$ cells in psoriatic lesional skin, and found significantly higher proportions of MCAM$^+$ cells in the severe disease group than in patients with mild disease (Fig. 3c). There was also a moderate and significant correlation between the PASI score and MCAM$^+$ cell numbers ($r = 0.42$; Fig. 3d).

Discussion

A recent study found that the number of circulating MCAM$^+$ CD4 T cells was increased in patients with inflammatory autoimmune diseases, including Behçet's disease, sarcoidosis, and

Crohn's disease, and that such numbers are correlated with disease severity[21]. Moreover, anti-MCAM antibody therapy seemed to be potentially effective for the treatment of various Th17-mediated diseases[22], suggesting that MCAM could play pathogenic roles in disease. Indeed, the MCAM level in endothelial cells is increased by TNF, and it acts to enhance both angiogenesis and migration of activated monocytes into inflamed tissues, suggesting that MCAM may augment the pathogenesis of psoriasis[18]. As shown in Table 1 and Fig. 1, the percentages of $CCR6^+$ $MCAM^+$ T_{EM} cells in the blood reflected the therapeutic response in parallel with PASI scores, supporting the notion of MCAM with a specific pathological role. Thus, Th17 subtypes expressing MCAM might augment disease pathogenesis. However, the number of patients tested in this study was low, and studies using more patient are needed.

In this study, we showed that $CCR6^+$ $MCAM^+$ Th17 cells were present in psoriatic lesional skin and were more numerous in the peripheral blood of affected patients than in healthy controls. Although circulating Th17 cells can be recruited into lesional skin by CCR6 and MCAM, the source of the circulating Th17 cells remains unknown, and there are at least two possibilities. One is that inflammatory dendritic cells in lesional skin migrate into lymph nodes and drive Th17 cells produced in the lymph nodes into the circulation. Another is that Th17 cells produced by inflammatory dendritic cells in lesional skin return to the circulation. Because human adult naïve CD4 T cells have not been shown to develop into Th17 cells (unlike Th1 or Th2 cells)[23, 24], the latter possibility seems attractive. However, the former one is supported by the observation that inflammatory dendritic cells positive for IL-1β, IL-23, and CCR7 are present in psoriatic lesional skin (unpublished data).

In conclusion, we showed that $CCR6^+$ $MCAM^+$ T_{EM} cells are enriched in the Th17 cells of peripheral blood of psoriasis patients, and that the numbers of $CCR6^+$ $MCAM^+$ Th17 cells in lesional skin may correlate with disease severity. Moreover, the percentage of $CCR6^+$ $MCAM^+$ T_{EM} cells in the blood of psoriasis patients reflects the therapeutic response, and it could be a useful marker of the disease process. Our results contribute to a better understanding of the relationships between Th17 responses and the pathogenesis of psoriasis.

Conflict of interest disclosure

There are no conflicts of interest to disclose concerning this study.

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