Skin-Homing Th2/Th22 Cells in Papuloerythroderma of Ofuji

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Introduction

Papuloerythroderma of Ofuji (PEO) is a distinct clinical disease that was first described by Ofuji et al. [1] in 1984. PEO is characterized by pruritic, erythroderma-like lesions formed by the coalescence of solid, red to brown papules with sparing of the skin folds (the so-called deck-chair sign) [1–3]. This condition primarily occurs in elderly men and its course is chronic. PEO may be associated with underlying conditions such as malignancy [2, 3], infection [2] and drug ingestion [4]; however, its pathogenesis remains unclear. Peripheral blood eosinophilia and increased serum IgE levels are observed in most patients [2, 3]. The histopathology of PEO skin lesions exhibits epidermal acanthosis, occasional spongiosis and inflammatory dermal infiltrates primarily composed of lymphocytes and eosinophils. Moreover, effective PEO treatments include oral and topical corticosteroids [2], PUVA [5] and cyclosporine [3, 6]. These facts suggest that PEO is a T cell-mediated skin disease.

We report two cases of PEO, and to clarify the role of T cells in the pathogenesis of PEO, we examine the cytokine profile and expression of skin-homing receptors by circulating T cells in these patients.

Case Reports

Brief clinical profiles of cases 1 and 2 were previously reported [3].

Case 1
A 97-year-old man with a 3-year history of a pruritic eruption involving his trunk and extremities was referred to our dermatology clinic. Physical examination revealed peripheral blood eosinophilia (1,519/μl) and increased serum IgE levels (7,970 IU/ml). Serum levels of thymus and activation-regulated chemokine (TARC)/CCL17 (SRL Inc., Tokyo, Japan) were 67,120 pg/ml (normal <450 pg/ml). Skin biopsy revealed acanthosis of the epidermis, focal spongiosis and inflammatory dermal infiltrates primarily composed of lymphocytes and eosinophils. Moreover, effective PEO treatments include oral and topical corticosteroids [2], PUVA [5] and cyclosporine [3, 6]. These facts suggest that PEO is a T cell-mediated skin disease.

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Case 2
A 69-year-old man presented with a 10-year history of pruritic eruptions on his entire body. Physical examination revealed erythroderma formed by the coalescence of flat-topped, red to brown papules with a...
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Cobblestone-like appearance on the trunk and extremities with deck-chair sign (fig. 1). Skin biopsy revealed hyperkeratosis and acanthosis of the epidermis, focal spongiosis and perivascular infiltrates of lymphocytes and eosinophils in the superficial dermis. No underlying diseases, including internal malignancy, were present. Treatment with oral cyclosporine was initiated, which resulted in improvement of the skin lesions. However, the skin lesions recurred whenever cyclosporine was discontinued. When the skin lesions reappeared after the discontinuation of cyclosporine, laboratory examinations revealed peripheral blood eosinophilia (2,768/μl) and increased serum IgE levels (2,594 IU/ml). Serum TARC levels were 28,360 pg/ml. Treatment was switched to oral betamethasone (1.0 mg/day) and topical tacrolimus, which resulted in complete remission of the skin lesions within 4 months. Peripheral blood eosinophil count and serum TARC levels decreased to 84/μl and 357 pg/ml, respectively. Complete remission is currently maintained by 0.25 mg betamethasone daily.

Subjects and Methods

To clarify the phenotype of T cells implicated in the pathogenesis of PEO, the cytokine profile and expression of homing receptors were examined at a single-cell level in the peripheral blood T cells of patients with PEO. In case 2, the cytokine profile and homing receptors were additionally examined after treatment with systemic corticosteroids. Informed consent was obtained from both patients. Nine healthy subjects (average age 37 years, three males, six females) served as controls. This study was conducted according to the principles of the Declaration of Helsinki.

Monoclonal Antibodies

Monoclonal antibodies (MAbs) against the cutaneous lymphocyte antigen (CLA) (fluorescein isothiocyanate-conjugated), CC chemokine receptor 4 (CCR4) (phycoerythrin-Cy7-conjugated), CD4 (allophycocyanin-Cy7-conjugated), CD8 (peridinin chlorophyll protein-Cy5.5-conjugated), IFN-γ (fluorescein isothiocyanate-conjugated), interleukin 4 (IL-4) (phycoerythrin-conjugated), IL-13 (phycoerythrin-conjugated) and isotype controls were obtained from BD Biosciences (Franklin Lakes, N.J., USA). MAbs against IL-17 (phycoerythrin-conjugated) and IL-22 (Alexa Fluor® 647-conjugated) and isotype controls were obtained from eBioscience (San Diego, Calif., USA).

Cell Preparation

Peripheral blood mononuclear cells were isolated from heparinized venous blood of both patients using density gradient sedimentation and stored at −80°C until use.

Flow Cytometry

For intracellular cytokine staining, peripheral blood mononuclear cells were stimulated for 4 h in RPMI medium containing 25 ng/ml phorbol 12-myristate 13-acetate (Sigma-Aldrich, Japan), 1 μM ionomycin (Sigma-Aldrich) and 10 μg/ml Brefeldin A (Sigma-Aldrich). After harvesting, cells were directly labeled with MAbs against the cell surface markers CLA, CCR4, CD4 and CD8 for 25 min at room temperature. Cells were washed and then incubated in 0.5 ml lysing solution and 0.5 ml permeabilizing solution (BD Biosciences) at room temperature. Finally, cells were incubated for 30 min at 4°C with MAbs specific to IL-4, IL-13, IL-17, IL-22 and IFN-γ. Samples were analyzed using a FACSCanto II 6-color flow cytometer (BD Biosciences).

Results

The percentages of CD4+ and CD8+ T cells in the circulation of case 1 were 56.8 and 16.6%, respectively, and in that of case 2 were 65.9 and 19.2%, respectively. The percentages of CLA-expressing CD4+ and CD8+ T cells in the circulation of case 1 (67.1 and 34.8%, respectively) and case 2 (68.7 and 61.7%, respectively) were markedly higher than those in the circulation of healthy subjects (13.8 ± 1.5 and 8.0 ± 1.4%, respectively). The percentages of CCR4-expressing CD4+ and CD8+ T cells in the circulation of case 1 (64.4 and 35.7%, re-
spectively) and case 2 (69.9 and 44.4%, respectively) were also markedly higher than those of healthy subjects (23.6 ± 2.2% and 5.9 ± 1.7%, respectively). The majority of CLA-expressing CD4+ and CD8+ T cells co-expressed CCR4 (fig. 2).

The percentages of IL-4-, IL-13- and IL-22-producing CD4+ and CD8+ T cells in the circulation of case 1 (IL-4, 3.3 and 13.1%; IL-13, 27.5 and 23.3%; IL-22, 26.3 and 3.0%, respectively) and case 2 (IL-4, 4.8 and 6.5%; IL-13, 23.5 and 15.3%; IL-22, 16.3 and 23.2%, respectively) were markedly higher than those in the circulation of healthy subjects (IL-4, 1.5 ± 0.2% and 1.1 ± 0.2%; IL-13, 1.1 ± 0.2% and 0.6 ± 0.2%; IL-22, 0.7 ± 0.1% and 0.1 ± 0.0%, respectively). The percentages of IFN-γ-producing CD4+, but not CD8+ T cells, in the circulation of case 1 (13.9 and 48.5%, respectively) and case 2 (7.5 and 45.5%, respectively) were lower than those in the circulation of healthy subjects (20.6 ± 3.8% and 45.2 ± 7.5%, respectively). There were no significant differences in the proportions of IL-17-producing CD4+ and CD8+ T cells between case 1 (2.1 and 1.3%, respectively) and case 2 (1.7 and 0.6%, respectively) and healthy subjects (1.5 ± 0.2% and 0.2 ± 0.0%, respectively) (fig. 3).

In case 2, the expression of CLA and CCR4 as well as cytokine production were examined after complete remission of PEO. The percentages of CLA- and CCR4-expressing CD4+ and CD8+ T cells in the circulation decreased to 22.2 and 28.1% and to 24.9 and 10.3%, respectively. The percentages of IL-4-, IL-13- and IL-22-producing CD4+ and CD8+ T cells decreased significantly to 1.2 and 3.3%, 0.6 and 1.1%, and 4.7 and 1.7%, respectively, however; no significant changes in the percentages of IFN-γ- and IL-17-producing CD4+ and CD8+ T cells were observed (IFN-γ, 9.1 and 46.1%; IL-17, 1.4 and 0.6%, respectively) (fig. 4).

**Discussion**

In the present two PEO cases, we demonstrated that the percentages of IL-4- and IL-13-producing CD4+ and CD8+ T cells were markedly higher in the circulation of patients with PEO than in those of healthy subjects. The percentages of IL-4- and IL-13-producing CD4+ and CD8+ T cells decreased significantly after remission of PEO. The percentages of IFN-γ-producing CD4+ T cells, but not CD8+ T cells, decreased in the circulation of patients with PEO; however, no difference was observed in the proportions of IFN-γ-producing CD4+ and CD8+ T cells between the active stage of PEO and after remission. These results suggest that Th2 cells may be involved in the pathogenesis of PEO. Our results are consistent with the clinical findings that most patients with PEO exhibit peripheral blood eosinophilia and increased serum IgE levels [2, 3], because Th2 cytokines are critical mediators that influence IgE production and eosinophil survival [7]. Furthermore, the proportions of IL-22-producing CD4+ and CD8+ T cells were markedly higher in the circulation of patients with PEO than in those of healthy subjects, and they significantly decreased after remission of PEO. Although IL-22 was originally described as a Th17 cytokine [8, 9], recent studies showed that IL-22 can be produced independent of IL-17 by a specific T cell.
Fig. 3. a Flow cytometry plots of cytokine production by circulating CD4+ and CD8+ T cells (case 2). b The percentages of IL-4-, IL-13- and IL-22-producing CD4+ and CD8+ T cells were markedly higher in the patients with PEO than in the healthy subjects.
subset referred to as Th22 [10, 11]. In addition, Th22 cells do not release IL-4 or IFN-γ. In the present cases, the percentages of IL-17-producing CD4+ and CD8+ T cells were not increased in the circulation of patients with PEO compared with those in the circulation of healthy subjects. The majority of the IL-22-producing CD4+ and CD8+ T cells did not co-produce IL-17, IL-4 or IFN-γ, suggesting that the IL-22-producing cells found in the circulation of patients with PEO may belong to the Th22 cell subset. IL-22 has proinflammatory effects in the inflamed skin and promotes keratinocyte proliferation and epidermal hyperplasia [8, 9]. PEO is clinically characterized by erythroderma formed by the coalescence of flat-topped, solid, erythematous papules, whose histopathology reveals epidermal acanthosis and lymphocytic infiltration in the dermis. Therefore, Th22 cells may contribute to the inflammation and epidermal acanthosis in PEO.

The cytokine-producing T cells in the circulation migrate to the skin during cutaneous inflammation. Such skin-homing memory T cells are characterized by expression of CLA and chemokine receptors such as CCR4 and CCR10 [12]. In the present PEO cases, the percentages of CLA-expressing CD4+ and CD8+ T cells in the circulation were markedly higher than in those of healthy subjects. The majority of CLA-expressing CD4+ and CD8+ T cells co-expressed CCR4. Moreover, the proportions of CLA- and CCR4-expressing CD4+ and CD8+ T cells significantly decreased after remission of PEO, suggesting that these populations are involved in PEO inflammation. This is supported by the previous results showing that serum TARC levels are paralleled by the percentage of circulating CLA- and CCR4-expressing T cells, because TARC is a key chemokine in tissue migration of CCR4-expressing T cells [13]. Our results thus suggest that TARC and CCR4 may play an important role in the pathogenesis of PEO.

Recent studies have shown that atopic dermatitis (AD) is mediated by Th2 and Th22 cells [14, 15]. Moreover, TARC and CCR4 are believed to play a role in AD pathogenesis [13]. PEO is now recognized as a condition with multiple etiologies, which may occur in association with malignancy, infection and drug ingestion [2–4]. Atopic diathesis has been reported in a few cases of PEO [2]; however, neither of our patients had a history of AD. Although PEO primarily occurs in elderly men and the clinical picture of PEO is quite different from that of AD, it is interesting to note that AD and PEO have striking similarities with regard to cytokines, homing and chemokine receptors.

In conclusion, the number of Th2/Th22 cells, expression of CLA and CCR4 by T cells, and serum TARC levels were all increased in the circulation of patients with PEO and were strongly correlated with PEO disease activity. Although the analysis is based on only two patients, these findings suggest that skin-homing Th2/Th22 cells are likely to be important in the pathogenesis of PEO.

**Disclosure Statement**

The authors declare no conflict of interest.
References


