Effect of Cyclosporine on Peripheral Blood and Lesional Skin in Psoriatic Patients

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Background: Cyclosporine effectively suppresses immune responses and inhibits skin homing T cell responses in psoriasis. E-selectin is known to be up-regulated on vascular endothelium of inflammatory skin lesions such as psoriasis.

Purpose: Based on our previous study that cyclosporine decreased lesional cutaneous lymphocyte antigen (CLA)+ T cells in psoriatic patients, we tried to find any change of CLA+ T cells in peripheral blood in psoriatic patients, since psoriasis is a disease of systemic T cell activation.

Subjects and Methods: Peripheral blood of 8 patients with chronic plaque type psoriasis at 0, 3, 6, 12, 18 weeks after cyclosporine was examined by flow cytometry using anti-CLA antibody. Five skin biopsy samples at 0, 3, 6, 12, 18 weeks were immunohistochemically stained with anti E-selectin antibody.

Results: Our results demonstrate that the number of CD3+ CLA+ and CD4+CLA+ T cells was significantly reduced in the peripheral blood at week 3, but gradually increased to the level of baseline at 18 weeks. In psoriatic skin lesions, with decrease of PASI score and CLA+ T cells number, the expression of E-selectin on the endothelial cells was gradually decreased throughout 18 weeks of therapy.

Conclusion: These results suggest that cyclosporine suppresses the migration of skin homing T cells to psoriatic skin lesions, in part, through the inhibition of E-selectin on the endothelial cells.

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INTRODUCTION

T cells are thought to play a critical role in the pathogenesis of psoriasis1. The tissue-selective homing of T cells to psoriatic skin lesions is regulated primarily by interaction of T cell homing receptors with adhesion molecules on the endothelial cells2. The molecules mediating the varied degrees of adhesion between leukocyte and endothelium include the small selectin family which mediates the very early transient adhesions as well as the rolling interactions. Among these, E-selectin is known to be up-regulated on the vascular endothelium of inflammatory skin lesions such as psoriasis. Cutaneous lymphocyte antigen (CLA) is expressed in a subset of circulating memory T cells and in the majority of skin-infiltrating T cells and is thought...
to mediate the homing of circulating skin-associated T cells to cutaneous inflammatory sites by interacting with endothelial cell ligand E-selectin\textsuperscript{16}. The immunosuppressive effect of cyclosporine is mediated by the inhibition of IL-2 production by activated T cells. Cyclosporine downregulates the expression of ICAM-1 and E-selectin on the endothelial cells and reduces the number of CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells in dermal infiltrates\textsuperscript{39}. The tissue-selective homing of memory T cells in psoriatic lesions is regulated primarily by interaction of T-cell homing receptors with vascular adhesion molecules. In psoriatic skin lesions, the number of CLA\textsuperscript{+} T cells and expression of E-selectin on the endothelial cells were increased\textsuperscript{0,11}, CLA/E-selectin is critical for trafficking skin-associated T cells to psoriatic skin lesions\textsuperscript{12,14}. We examined the effect of cyclosporine on expression of E-selectin on psoriatic lesional endothelial cells and also evaluated E-selectin immunohistochemical expression in recurrent lesions. We also examined the effect of cyclosporine on CLA\textsuperscript{+} T cells in peripheral blood using FACS analysis.

**MATERIALS AND METHODS**

**Patients**

Eight patients (5 females, 3 males, mean age 42.5 years) with severe chronic plaque psoriasis were included in this study.

**Antibodies and reagents for staining**

Primary unconjugated and fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, or biotin-conjugated monoclonal antibodies (mAbs)/reagents used in this study are as follows; anti-cutaneous lymphocyte antigen (CLA, Becton-Dickinson, Lincoln Park, NJ), anti-E-selectin (CD62E, R&D Systems, Minneapolis, MN), anti-CD3 (Dako, Kyoto, Japan), anti-CD4 (Dako) and anti-CD8 (Dako). Irrelevant monoclonal antibodies of the appropriate immunoglobulin isotype were used as negative controls. mAbs were titrated and diluted in staining buffer (5% FBS, IVGG, NAN3).

**Immunohistochemistry**

Five adult patients (2 females and 3 males, mean age 42.5) were biopsied. 9 paraffin-embedded, formalin-fixed skin samples were subject to each time-sequential skin biopsies (at baseline, 3 weeks, 6 weeks, 18 weeks following initiation of treatment, recurrence after treatment termination). Two normal skins were used for comparison. Paraffin-embedded, formalin-fixed skin samples were used in immunohistochemical staining by the standard streptavidin-biotin peroxidase method. Results were recorded as the mean number of each marker-positive cells in 10 high power fields (×400) by 2 dermatologists and 1 pathologist. Anti E-selectin antibody (CD62E, Novocastra, Vector Laboratories, Burlingame, U.S.A) was used as a primary antibody in immunohistochemical staining using standard streptavidin-biotin peroxidase method. Results were interpreted as follows; (3 +): strong intensity on more than 90% of upper dermal vessels, (2 +): moderate intensity on more than 30% and less than 90% of upper dermal vessels, (+): weak intensity on less than 30% of upper dermal vessels.

**FACS**

The lymphocytes expressing CD3, CD4, CD8, or CLA were detached briefly with 0.02% trypsin and incubated for 20 min in ice with each monoclonal antibody (CD3, CD4, CD8, or CLA) or normal human IgG, followed by FITC-conjugated goat anti-mouse IgG or anti-human secondary Abs. After fixation in 3.7% formaldehyde, cells were analyzed by flow cytometry using a Becton Dickinson FACS machine (San Jose, CA) and the data was analyzed using CellQuest software (Becton Dickinson).

**Statistical analysis**

The results were expressed as mean ± standard deviation. Statistical analysis of the difference between each group was made by Wilcoxon rank sum and signed rank test. \( p<0.05 \) was accepted as significant.

**RESULTS**

The number of CLA\textsuperscript{+} T cells is significantly higher in peripheral blood of psoriatic patients than in normal control subjects

We first compared the number of CD3\textsuperscript{+}CLA\textsuperscript{+}, CD4\textsuperscript{+}CLA\textsuperscript{+}, and CD8\textsuperscript{+}CLA\textsuperscript{+} T cells in peripheral blood of psoriatic patients with those of normal control subjects using FACS analysis. The percentages of CD3\textsuperscript{+}CLA\textsuperscript{+}, CD4\textsuperscript{+}CLA\textsuperscript{+}, and CD8\textsuperscript{+}
CLA⁺ T cells were significantly higher in psoriatic patients than in normal control subjects \((p<0.01, p<0.01, p<0.05, \text{ respectively}; \text{ Fig. } 1)\).

**Effect of cyclosporine on the number of CLA⁺ T cells in peripheral blood of psoriatic patients**

To investigate the effect of cyclosporine on CLA⁺ T cells, we performed FACS analysis on peripheral blood of psoriatic patients taken at 0 (baseline, before treatment), 3, 6, 12, and 18 weeks after the initiation of cyclosporine treatment. In the early phase of cyclosporine therapy at 3 weeks after the initiation of treatment, the percentages of CD3⁺ CLA⁺ and CD4⁺ CLA⁺ T cells were significantly reduced in comparison with those of the baseline \((p<0.01, \text{ Fig. } 2 \text{A} \text{ and } \text{B}). The suppressive effect of cyclosporine on CLA⁺ T cells in peripheral blood of psoriatic patients was gradually diminished after 3 weeks and the percentages of CD3⁺ CLA⁺ and CD4⁺ CLA⁺ T cells were restored to the level of baseline (before treatment) at 18 weeks after the initiation of cyclosporine treatment in all patients. The percentage of CD8⁺ CLA⁺ T cells showed change similar to those of CD3⁺ CLA⁺ and CD4⁺ CLA⁺ T cells but after 6 weeks of cyclosporine treatment. There was no significant difference between the percentage measured and the baseline (Fig. 2C). Fig. 3 is a representative two-dimensional flow cytometric profile of CD4⁺ CLA⁺ T cells from peripheral blood of one psoriatic patient of this study.

![Fig. 1](image1.png)

*Fig. 1. Comparison of CD3⁺ CLA⁺, CD4⁺ CLA⁺, and CD8⁺ CLA⁺ T cells in peripheral blood between psoriatic patients and normal control subjects using FACS analysis. The percentages of CD3⁺ CLA⁺, CD4⁺ CLA⁺, and CD8⁺ CLA⁺ T cells were significantly higher in psoriatic patients than normal control subjects \((p<0.01, p<0.01, p<0.05, \text{ respectively})\). Each dot represents one individual and central bars are the median values of eight subjects in each group.*

![Fig. 2](image2.png)

*Fig. 2. Changes of CD3⁺ CLA⁺ (Fig. 2A), CD4⁺ CLA⁺ (Fig. 2B) and CD8⁺ CLA⁺ (Fig. 2C) T cells in peripheral blood of psoriatic patients by cyclosporine treatment. Dosage of cyclosporine was 3 mg/kg per day. At 3 weeks after the initiation of cyclosporine therapy, the percentages of CD3⁺ CLA⁺, CD4⁺ CLA⁺ and CD8⁺ CLA⁺ T cells examined by FACS were significantly reduced in comparison with those of the baseline \((p<0.01)\). But at the end of the study (at 18 weeks), the percentages of CD3⁺ CLA⁺, CD4⁺ CLA⁺ and CD8⁺ CLA⁺ T cells were restored to the level of the baseline and there was no significant difference between baseline and at 18 weeks. Data are the mean ± SD of eight psoriatic patients.*
Cyclosporine reduces expression of E-selectin on endothelial cells in psoriatic skin lesions

Normal skin samples showed no detectable E-selectin expression in our study. Blind scoring of the samples by 3 dermatologists showed strong (3+) intensity of E-selectin in upper dermal endothelial cells before cyclosporine treatment (Fig. 3A). Moderate (2+) to strong (3+) expression of E-selectin in upper dermal endothelial cells was observed after 3 weeks of cyclosporine treatment (Fig. 3B). After 6 and 12 weeks of cyclosporine treatment, E-selectin positivity was not observed on endothelial cells (Fig. 3C and 3D). Among the 5 patients, 2 showed rapid recurrences within 1 month and skin samples were taken again. Anti-E-selectin staining showed strong (3+) positivity in upper dermal endothelial cells (Fig. 3E) although perivascular lymphocytic infiltrates were much less than in the skin samples taken before cyclosporine treatment.

DISCUSSION

In this study, we demonstrated that the percentage of CLA+ T cells was significantly higher in peripheral blood of psoriatic patients than in normal control subjects. Previous studies have reported that CLA was expressed by 10% to 20% of peripheral blood T cells in normal healthy adults and the differences of the percentage of CLA+ T cells between psoriatic patients and normal control subjects were not significant. But we found that only 3.6% and 2.1% of peripheral blood T cells expressed CD3+ CLA and CD4+ CLA, respectively, in normal subjects but psoriatic patients had significantly greater percentages of CD3+ CLA and CD4+ CLA+ T cells (median 8.7% and 5.7%, respectively) than those of normal control subjects. Such differences between this study and previous studies may be due to the characteristics of the recruited patients such as age, sex, severity of psoriasis, duration of the disease, previous treatment for psoriasis, number of patients and racial difference. Our data suggests that a certain portion of skin-homing CLA+ T cells in peripheral blood may be psoriasis-specific T cells especially in patients with very severe psoriasis. In one report, acute stage of psoriasis less than 6 weeks, CLA+ T cells were correlated with disease severity.

We found that cyclosporine significantly reduced the percentage of CLA+ T cells in peripheral blood of psoriatic patients during the early phase of cyclosporine therapy (at 3 weeks after the initiation of the study). But after 3 weeks, the number of CD3+ CLA+ and CD4+ CLA+ T cells increased gradually and there was no difference in the percentage of CLA+ T cells between baseline (before treatment) and at 18 weeks (at the end of the study). This finding has not been reported previously. The exact cause of gradual recovery of the initially reduced CLA+ T cell number by cyclosporine treatment was unknown. One possible hypothesis is that there may be two subsets of CLA+ T cells. One subset is

![Fig. 3. (A) Strong expression of E-selectin in upper dermal endothelial cells before cyclosporine treatment. (B) Moderate expression of E-selectin in upper dermal endothelial cells was observed after 3 weeks of cyclosporine treatment. (C, D) No expression of E-selectin after 6 weeks (C) and 18 weeks (D) of cyclosporine treatment. (E) Strong expression of E-selectin in the skin lesion at 4 weeks after recurrence (Immunoperoxidase with haematoxylin counterstain × 100).](image-url)
cyclosporine-sensitive and the other is cyclosporine-resistant. T cell proliferation involving CD28:B7 costimulatory signal pathway was associated with cyclosporine-resistant IL-2 gene expression. T cells infiltrated in upper levels of the dermis and epidermis may be of the cyclosporine-resistant subset and this would explain the ineffectiveness of topical cyclosporine for psoriasis treatment. In the early phase of cyclosporine therapy, reduction of cyclosporine-sensitive CLA+ T cells results in a decrease of total CLA+ T cell percentage. The gradual increase of CLA+ T cell percentage after the early phase may be due to proliferation of cyclosporine-resistant CLA+ T cell subset. As cyclosporine treatment was continued, the expression of E-selectin on the endothelial cells were gradually and persistently diminished. E-selectin may be involved in one of the action mechanisms of cyclosporine on psoriasis and may be used as a marker for disease activity. In contrast to the gradual recovery of the CLA+ T cell numbers in peripheral blood to the level of baseline, reduction of diminished expression of E-selectin on the endothelial cells were maintained at the end of this study (18 weeks after the initiation of cyclosporine therapy). These findings suggest that recruitment of skin-homing T cells to psoriatic skin lesions was inhibited by diminished expression of E-selectin on the endothelial cells. The average remission time for cyclosporine was only 6 weeks. E-selectin expression may be an earlier event in recurrence of psoriasis since strong re-expression of E-selectin on the endothelial cells in the early-recurred psoriatic skin lesions was observed. In summary, gradual increase of CLA+ T cell numbers to the level of baseline in peripheral blood at 18 weeks and re-expression of E-selectin on the endothelial cells in the recurred psoriatic skin lesion, may explain partly the short average relapse time of cyclosporine treatment for psoriasis.

REFERENCES


