Analysis of Blood Follicular Helper T cells in Patients with Psoriasis

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Analysis of Blood Follicular Helper T cells in Patients with Psoriasis

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The Master's Thesis
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of Master of Medical Science

Dongyun Shin

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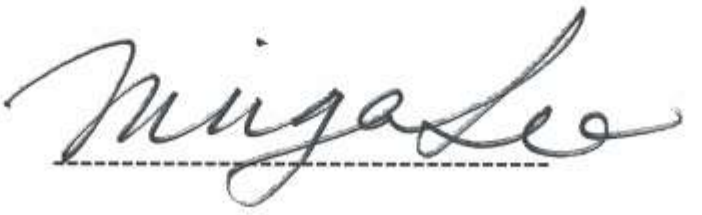
This certifies that the Master's Thesis of
Dongyun Shin is approved
By



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Thesis Committee Member : Professor You Chan Kim

The Graduate School
Yonsei University

December 2014

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I thank my colleagues in lab and clinic, and also the patients who consented to the utilization of their samples for this study.

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Psoriasis is a common, chronic inflammatory skin disease affecting about 2% of the worldwide population. Substantial clinical and basic research observations indicate that the cellular innate and adaptive immune responses, especially the activation of Th1 and Th17 cells, play a critical role in the pathogenesis of psoriasis. However, the role of B cells to pathogenesis of psoriasis is sparsely reported and controversial.

Follicular helper T (Tfh) cell is a recently characterized subset of helper T cells, found in the germinal centers of the B cell follicles. The major function of Tfh cells is to help B cell activation and antibody production during humoral immune responses. Recently, several studies indicate that blood Tfh cells are

frequently present in patients with autoimmune disease, such as systemic lupus erythematosus, rheumatoid arthritis and bullous pemphigoid. However, there is no report about Tfh cells in psoriasis.

This study sought to analyze the blood Tfh cells in patients with psoriasis. I found no increased frequencies of circulating $CXCR5^+$ Tfh cells, in disagreement with previous studies from several autoimmune diseases. However, the frequency of $PD\text{-}1^+$ subset of activated Tfh cells decreased significantly in patients with psoriasis. Furthermore, the proportion and the absolute numbers of $PD\text{-}1^+$ subset of activated Tfh cells negatively correlated with ESR levels. The proportion of $PD\text{-}1^+$ Tfh cells and the absolute numbers of $PD\text{-}1^+$ and $ICOS^+$ Tfh cells were lower in psoriatic patients with high ASO titers. Meanwhile, the proportion of $PD\text{-}1^+$ Tfh cells and the absolute numbers of $PD\text{-}1^+$ and $ICOS^+$ Tfh cells positively correlated with the disease duration of psoriasis.

These findings suggest that the activated Tfh cells decrease in severe status and early phase of psoriasis. Furthermore, it also implies that B cell immunity weakens in psoriasis as a result of predominance of Th1 and Th17 cytokine axises. I expect that this elucidation of the altered frequency of activated Tfh cells will help the further investigation of pathogenesis and potential therapeutic targets in psoriasis.

Key words : Follicular helper T (Tfh) cell, chemokine (C-X-C motif) receptor 5 (CXCR5), programmed cell death 1 (PD-1), inducible T-cell co-stimulator (ICOS), B cell immunity, psoriasis

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I. INTRODUCTION

Psoriasis is a common, chronic inflammatory skin disease affecting about 2% of the worldwide population.[1] Until the late 1970s, psoriasis had been considered a primary keratinocyte disorder.[2] However, substantial research observations indicate that the cellular innate and adaptive immune responses, especially the activation of T cells, play a critical role in the pathogenesis of psoriasis. Recently, the discovery of interleukin (IL)-23/Th17 axis in the pathophysiology of psoriasis shifted the cytokine paradigm from Th1 to Th17 cytokines.[3] Th17 or T helper 17 cells are distinct from Th1 and Th2 cells in their differentiation and maintenance conditions, as well as in their cytokine profiles. Integration of IL-23/Th17 axis into a revised concept of psoriasis pathogenesis has been translated into novel therapeutic strategies.

On the other hand, the role of B cells in pathogenesis of psoriasis is sparsely reported and controversial. Recently, it has been shown that IL-10^{+} B cells suppressed T-cell-mediated contact hypersensitivity to oxazolone in mice, suggesting that this subtype of B cells might play a role in the regulation of T cells.[4] Similarly, the number of these regulatory B cells in spleen decreased during imiquimod-induced skin inflammation, thus regulating IFN-γ and IL-17 production in mice.[5] In humans, B cell depletion using rituximab led to the development of psoriasis in patients with no previous history of psoriasis, supporting this model.[6] In contrast, Chang et al.[7] and Jimenez-Boj et al.[8] reported an improvement of psoriasis and psoriatic arthritis, respectively, after rituximab treatment. Additionally, it has also been reported that circulating B cells increased significantly in patients with psoriasis.[9]

Follicular helper T (Tfh) cell is a recently characterized subset of helper T cells, which is found in the germinal centers of the B cell follicles. The major function of Tfh cells is to help B cell activation and antibody production during humoral immune responses, specifically via interactions between molecules on the surface of Tfh cells and receptors or ligands located on the surface of B cells. The phenotypic and functional features of Tfh cells include surface expression of the chemokine (C-X-C motif) receptor 5 (CXCR5), IL-21, and B cell lymphoma-6 (Bcl-6).[10,11] Further, Tfh cells also express inducible T-cell co-stimulator (ICOS), programmed cell death 1 (PD-1), CD40 ligand (CD40L),

OX40, and SLAM-associated protein (SAP).[12] High levels of CXCR5 expression facilitate the homing of Tfh cells to B cell follicles, Bcl-6 is essential for the generation of Tfh cells and functions in a gene dose-dependent manner.[13] IL-21 produced by Tfh cells serves as an important regulator of humoral responses by directly regulating B cell proliferation and class switching.[14,15] Although the identification of Tfh cells in human blood remains controversial, it has been reported that human blood contains memory $CXCR5^+$ $CD4^+$ T cells that share phenotypic and functional properties with Tfh cells and therefore are called "blood Tfh cells".[16] Although the exact nature of human blood Tfh cells remains unclear, studies indicate that a high frequency of human blood Tfh cells and high level of serum IL-21 are observed in patients with systemic autoimmune disease, such as systemic lupus erythematosus, rheumatoid arthritis, juvenile dermatomyositis and autoimmune thyroid disease.[17]

The objective of this study is to analyze the blood Tfh cells in patients with psoriasis. There is no report on Tfh cells in patients with psoriasis, so this study might suggest a possible role of Tfh cells in pathogenesis of psoriasis. Furthermore, Tfh cells can give us a clue for the involvement of B cell immunity in pathogenesis of psoriasis.

II. MATERIALS AND METHODS

1. Patients

A total of 20 patients diagnosed of psoriasis were enrolled in the present study. Another 12 healthy controls (HC) were recruited. Peripheral blood samples were obtained from all patients and healthy controls. Additionally, skin samples were obtained from the 2 patients with psoriasis and 2 healthy controls. Individual patients with psoriasis were diagnosed based on the typical clinical and histological presentations. The major inclusion criteria are the following: no systemic treatment for at least 4 weeks prior to blood sampling; no significant infection or immune suppression; and no significant renal, hepatic or other medical disease. Clinical severity of the disease was evaluated with the Psoriasis Area and Severity Index (PASI) and Body Surface Area (BSA). Disease duration of psoriasis was defined as the interval from the time of symptom appearance to this study.

2. Laboratory examinations

Peripheral blood samples were obtained from individual subjects. Erythrocyte sedimentation rate (ESR), the concentrations of serum C-reactive protein (CRP) and anti-streptolysin O (ASO) of individual subjects were measured.

3. Cell isolation

Plasma was collected through centrifugation and stored at −80°C. Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation using Ficoll-Paque Plus (Amersham Biosciences, Piscataway, NJ, USA).

4. Flow cytometric analysis

For phenotypic analysis, APC-eFluor-780-anti-CD4 (eBioscience, San Diego, CA, USA), Alexa-Fluor-488-anti-CXCR5 (BD Pharmingen, San Diego, CA, USA), APC-Cy7-anti-ICOS (eBioscience) and PE-Cy7-anti-PD-1 (eBioscience) were purchased. All the staining was performed according to the manufacturer's protocol. Corresponding isotype control antibodies were also used for flow cytometry. The stained cells were analyzed by flow cytometer (FACSVerse, BD Bioscience, NJ, USA) and CELLQUEST software.

5. Immunohistochemical stain

Immunohistochemical staining was performed for skin samples, using antibodies against PD-1 (1:10; mouse monoclonal antibody, Abcam, Cambridge, UK), ICOS (1:100; rabbit monoclonal antibody, Abcam), Bcl-6 (1:100; mouse monoclonal antibody, eBioscience), CXCR5 (1:1000; rabbit polyclonal antibody, Abcam) and CXCL13 (1:10; goat polyclonal antibody, R&D Systems, Minneapolis, MN, USA).

6. Statistical analysis

Data were expressed as the median and range or individual values. Statistical differences were considered to be significant at a value $p < 0.05$ as determined by Mann-Whitney U test using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Correlation of nonparametric paired data was tested using Spearman's rank correlation, and the significance was evaluated using the t statistic. GraphPad Prism 5 was used to complete the figures.

III. RESULTS

1. Patients Characteristics

Overall, 20 patients with psoriasis and 12 healthy controls were involved in the study. There was no significant difference in the distribution of age and sex between patients with psoriasis and healthy controls (Age, p=0.207; Sex, p=0.581). Clinical parameters for psoriasis severity and laboratory data on blood including ESR, CRP and ASO were described in Table 1.

Table 1. Patients characteristics

Characteristics	Psoriasis patients	Healthy controls
No. subjects	20	12
Age, yrs (range)	34.6 (14-58)	31.6 (27-36)*
Male, n (%)	15 (75)	10 (83.3)*
Disease duration, yrs (range)	6.3 (1-26)	
ESR, mm/h (range)	16 (2-49)	
CRP, mg/dl (range)	1.5 (0.3-13.9)	
ASO titer, IU/mL (range)	135 (10-692)	
BSA, % (range)	9.9 (1.4-38)	
PASI, score (range)	10.9 (3.4-28.8)	

* There was no significant difference in the distribution of age and sex between patients with psoriasis and healthy controls (Age, p=0.207; Male, p=0.581).
Abbreviations : ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ASO, anti-streptolysin O; BSA, body surface area; PASI, psoriasis area and severity index

2. The proportion of $CXCR5^+PD\text{-}1^+$ cells among $CD4^+$ T cells and the absolute numbers of $CXCR5^+PD\text{-}1^+CD4^+$ T cells were significantly lower in patients with psoriasis.

The percentages of Tfh cells in the PBMCs of the patients with psoriasis and healthy controls were detected and analyzed by flow cytometry. Also, the percentages of activated Tfh cells subsets were analyzed (Figure 1). There was no significant difference in the percentages of $CXCR5^+$ cells among the $CD4^+$ T cells between the patients with psoriasis and healthy controls (p=0.363, Figure 2A). Although there was no statistically significant difference, the percentages of $CXCR5^+ICOS^+$ population, and activated subset of Tfh cells, among the $CD4^+$ T cells were lower in the patients with psoriasis (p=0.2297, Figure 2B). Furthermore, the percentages of $CXCR5^+PD\text{-}1^+$ population, another activated subset of Tfh cells, among the $CD4^+$ cells in patients with psoriasis were significantly lower than those in the healthy controls (p=0.0434, Figure 2C).

I also analyzed the absolute numbers of Tfh cells and their subsets among PBMCs. There was no significant difference in the absolute numbers of $CXCR5^+CD4^+$ T cells (p=0.4152, Figure 2D) and $CXCR5^+ICOS^+CD4^+$ T cells (p=0.2239, Figure 2E). However, the absolute numbers of $CXCR5^+PD\text{-}1^+CD4^+$ T cells were significantly lower in patients with psoriasis (p=0.0490, Figure 2F).

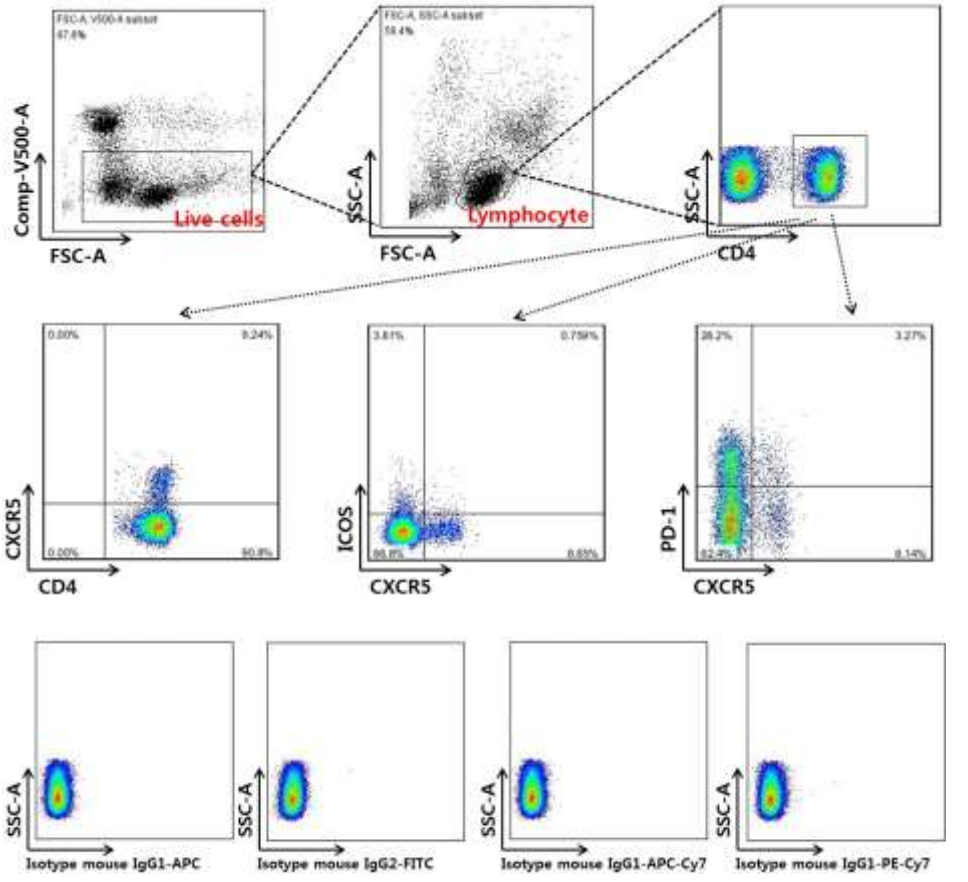
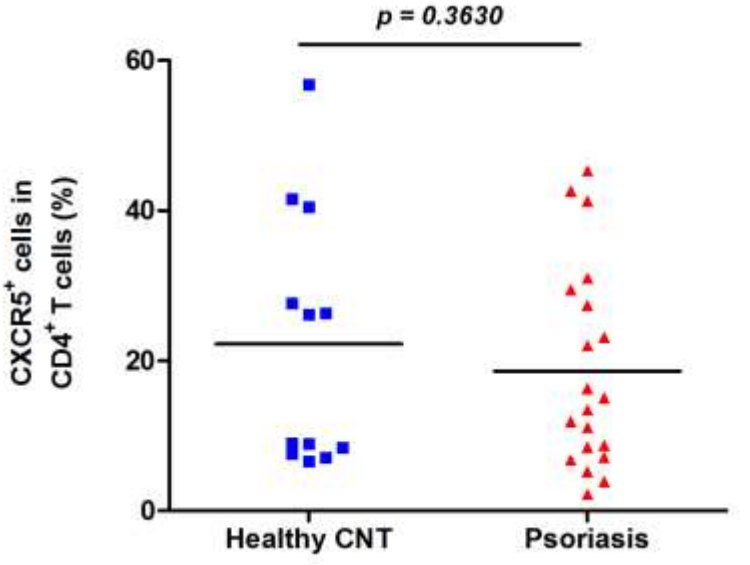
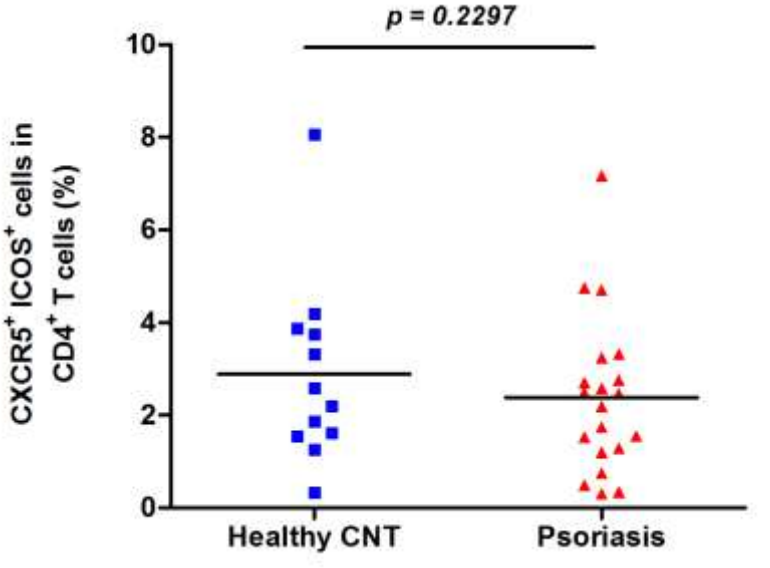


Figure 1. Flow cytometric analysis and gating methods. Viable lymphocytes were chosen using a two-dimensional scatter plot chart composed of forward scatter (FSC) and side scatter (SSC). The percentages of $CXCR5^+$ cells, $CXCR5^+ICOS^+$ cells and $CXCR5^+PD\text{-}1^+$ cells were determined among $CD4^+$ T cells. Flow cytometric analysis of corresponding isotype control antibodies were also shown (Bottom Panels). The cells were then analyzed using the supporting flow cytometry software.

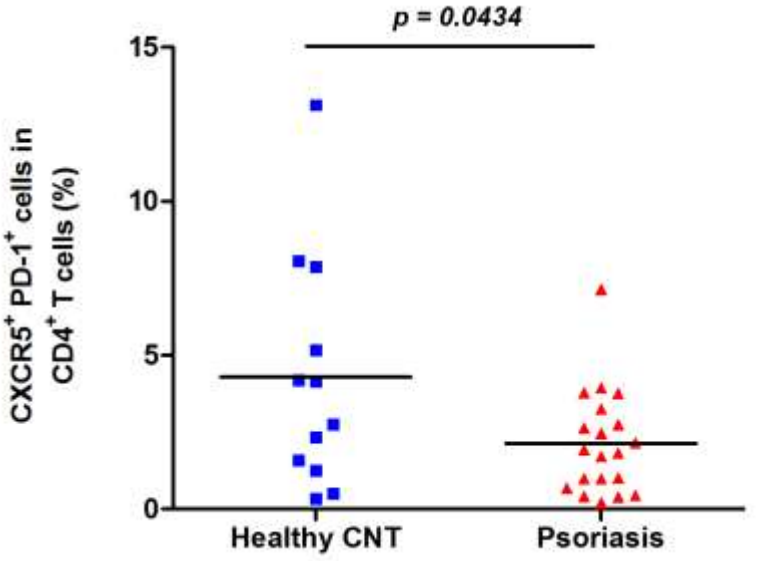
A)


p = 0.3630
60
40
20
0
CXCR5+ cells in CD4+ T cells (%)
Healthy CNT
Psoriasis

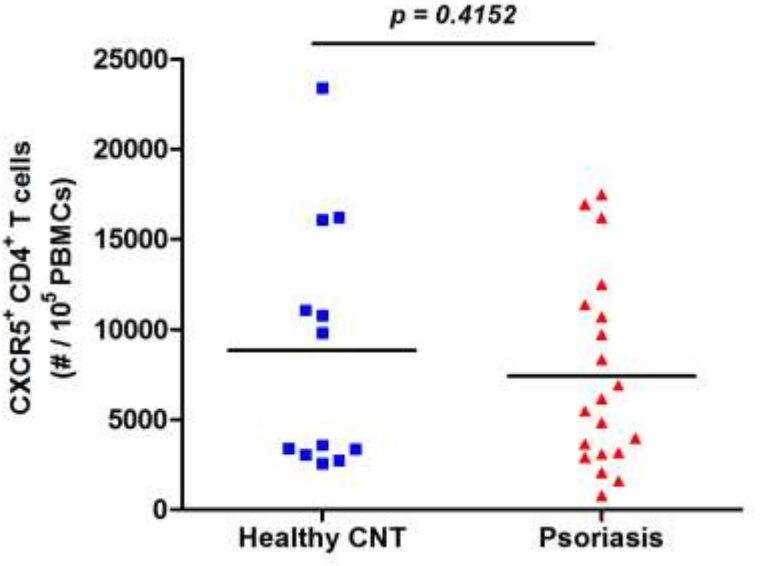
B)


p = 0.2297
10
8
6
4
2
0
CXCR5+ ICOS+ cells in CD4+ T cells (%)
Healthy CNT
Psoriasis

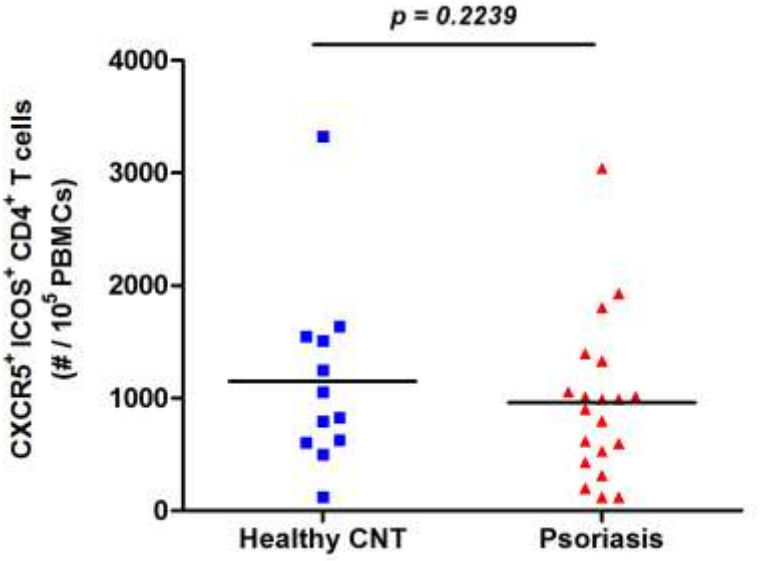
C)


p = 0.0434
15
10
5
0
CXCR5+ PD-1+ cells in CD4+ T cells (%)
Healthy CNT
Psoriasis

D)


p = 0.4152
25000
20000
15000
10000
5000
0
CXCR5+ CD4+ T cells (# / 10^5 PBMCs)
Healthy CNT
Psoriasis

E)


p = 0.2239
4000
3000
2000
1000
0
CXCR5+ ICOS+ CD4+ T cells
(# / 10^5 PBMCs)
Healthy CNT
Psoriasis

F)

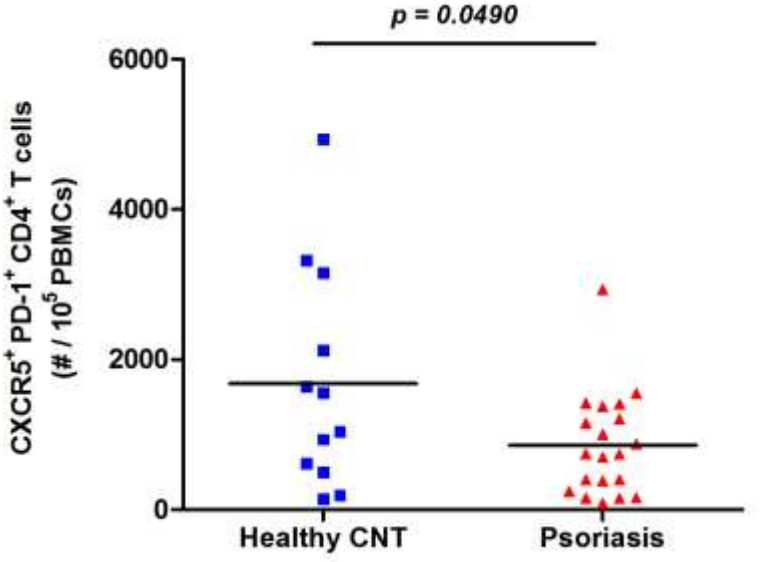

p = 0.0490
6000
4000
2000
0
CXCR5+ PD-1+ CD4+ T cells
(# / 10^5 PBMCs)
Healthy CNT
Psoriasis

Figure 2. The proportion of (A) $CXCR5^+$ cells, (B) $CXCR5^+ICOS^+$ cells, and (C) $CXCR5^+PD\text{-}1^+$ cells among $CD4^+$ T cells and the absolute numbers of (D) $CXCR5^+CD4^+$ T cells, (E) $CXCR5^+ICOS^+CD4^+$ T cells, and (F) $CXCR5^+PD\text{-}1^+CD4^+$ T cells in the peripheral blood of the patients with psoriasis and healthy controls. Percentages of indicated Tfh cell subsets in $CD4^+$ T cells and absolute numbers of indicated Tfh cell subsets in 10^5 PBMCs from individuals were illustrated as individual dots. The average value of each distribution was marked as a horizontal bar. The statistical significance for the difference between patients with psoriasis and healthy controls was calculated by Mann-Whitney U test.

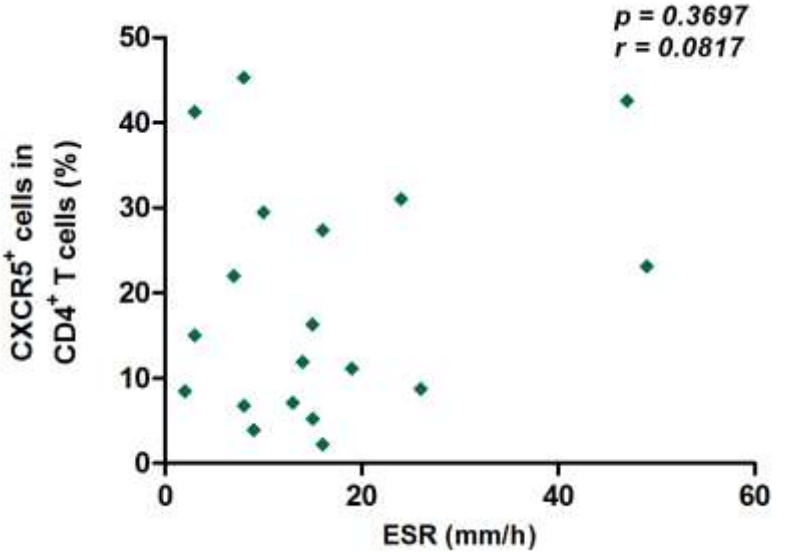
3. The level of ESR is negatively correlated with the proportion of $CXCR5^+PD1^+$ cells among $CD4^+$ T cells and the absolute numbers of $CXCR5^+PD1^+CD4^+$ T cells in patients with psoriasis.

I then analyzed the correlation between the laboratory parameters of psoriasis and the percentages of Tfh cells. There was no significant correlation between the percentages of $CXCR5^+$ cells and $CXCR5^+ICOS^+$ cells among $CD4^+$ T cells and the levels of ESR in patients with psoriasis (Figure 3A & 3B). However, the percentages of $CXCR5^+PD\text{-}1^+$ cells among $CD4^+$ T cells showed a negative correlation with the level of ESR (r=-0.5464, p=0.0078, Figure 3C).

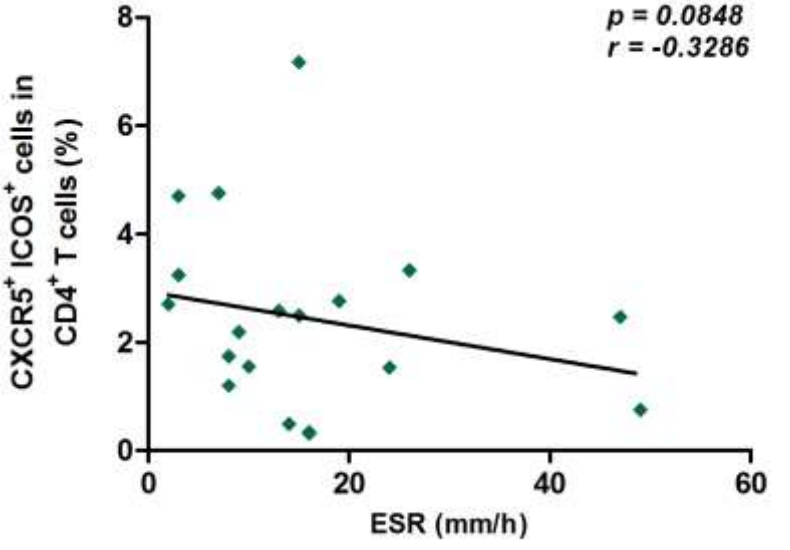
There was also no significant correlation between the absolute numbers of $CXCR5^+CD4^+$ T cells and $CXCR5^+ICOS^+CD4^+$ T cells and the levels of ESR in patients with psoriasis (Figure 3D & 3E). However, the absolute numbers of $CXCR5^+PD\text{-}1^+CD4^+$ T cells showed a negative correlation with the level of ESR (r=-0.5554, p=0.0068, Figure 3F).

The correlation with CRP level was inappropriate for comparison since the most patients showed lower levels of CRP (<2.0mg/dl).

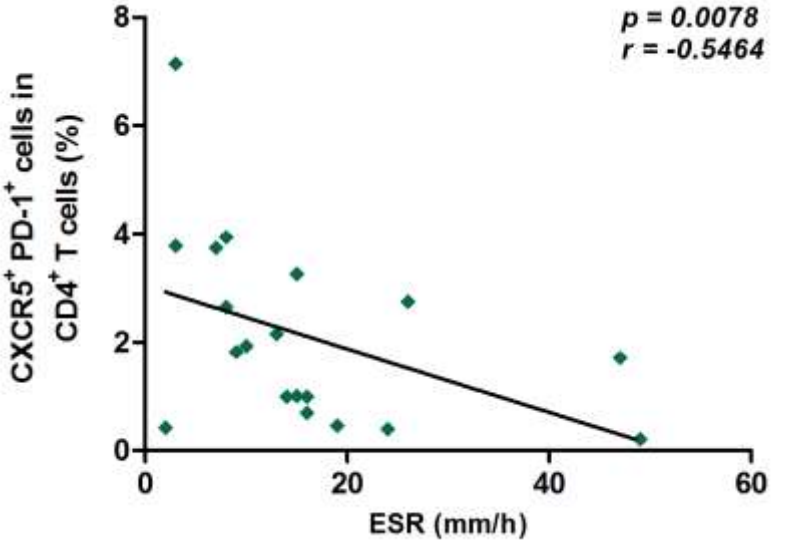
A)


p = 0.3697
r = 0.0817
CXCR5+ cells in CD4+ T cells (%)
50
40
30
20
10
0
0
20
40
60
ESR (mm/h)

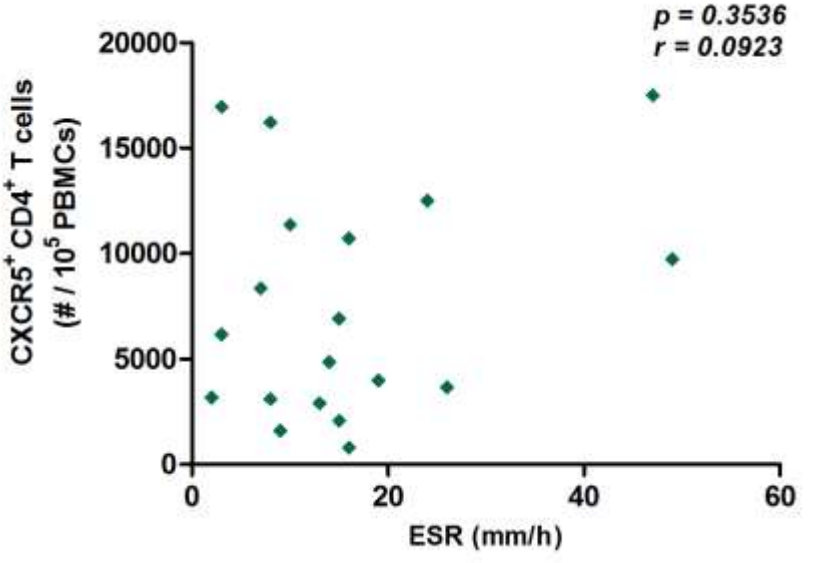
B)


p = 0.0848
r = -0.3286
CXCR5+ ICOS+ cells in CD4+ T cells (%)
8
6
4
2
0
0
20
40
60
ESR (mm/h)

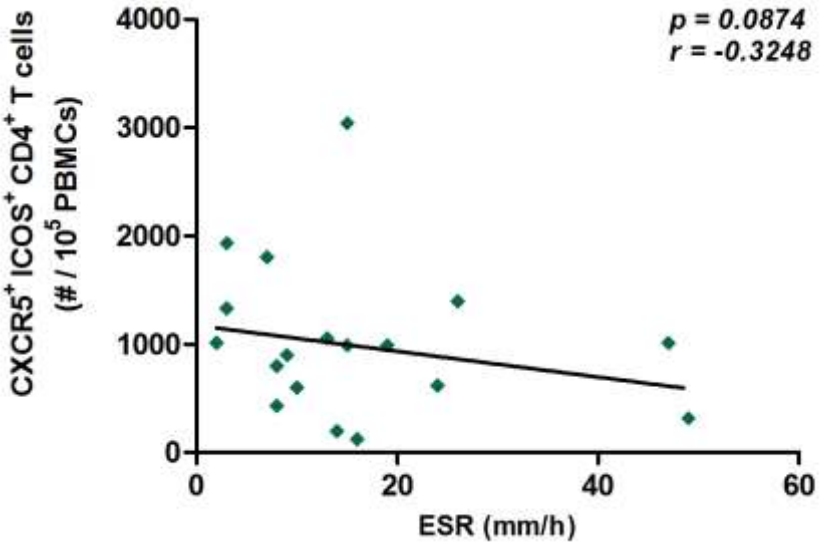
C)


p = 0.0078
r = -0.5464
CXCR5+ PD-1+ cells in CD4+ T cells (%)
0
2
4
6
8
0
20
40
60
ESR (mm/h)

D)


p = 0.3536
r = 0.0923
CXCR5+ CD4+ T cells (# / 10^5 PBMCs)
0
5000
10000
15000
20000
0
20
40
60
ESR (mm/h)

E)


p = 0.0874
r = -0.3248
CXCR5+ ICOS+ CD4+ T cells
(# / 10^5 PBMCs)
4000
3000
2000
1000
0
0
20
40
60
ESR (mm/h)

F)

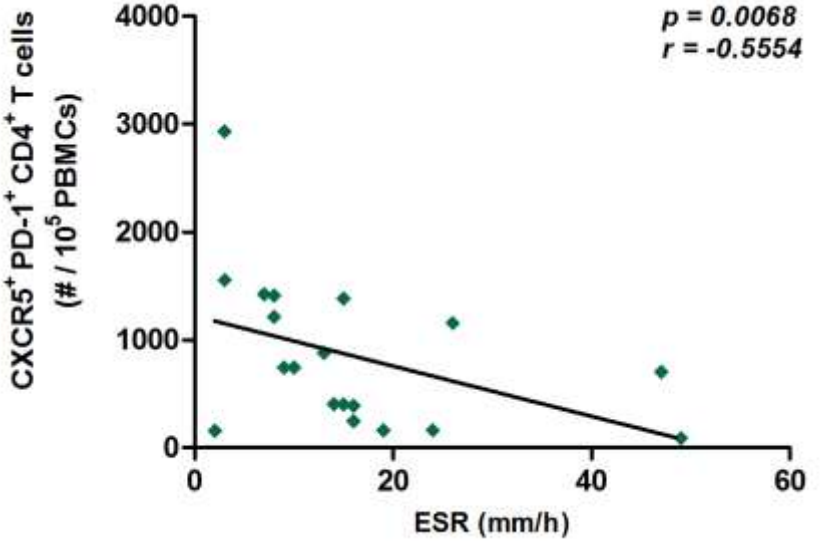

p = 0.0068
r = -0.5554
CXCR5+ PD-1+ CD4+ T cells
(# / 10^5 PBMCs)
4000
3000
2000
1000
0
0
20
40
60
ESR (mm/h)

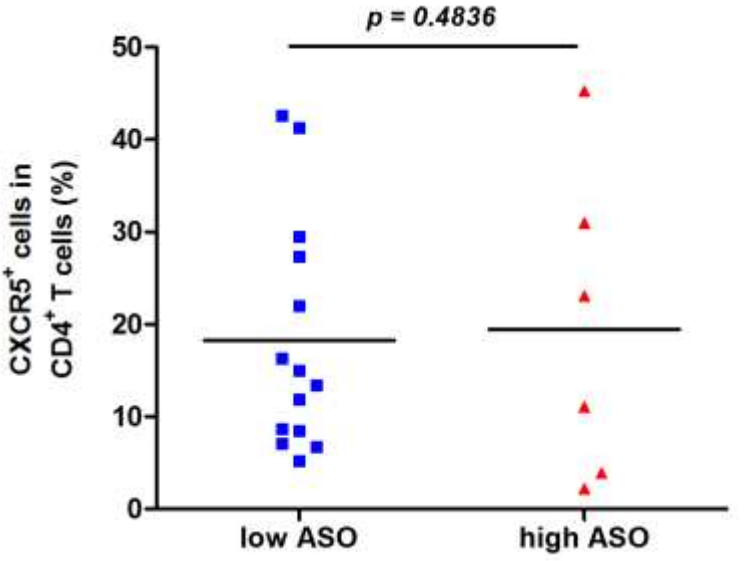
Figure 3. The correlation of ESR level and proportions of (A) $CXCR5^+$ cells, (B) $CXCR5^+ICOS^+$ cells, (C) $CXCR5^+PD\text{-}1^+$ cells among $CD4^+$ T cells, absolute numbers of (D) $CXCR5^+CD4^+$ T cells, (E) $CXCR5^+ICOS^+CD4^+$ T cells, and (F) $CXCR5^+PD\text{-}1^+CD4^+$ T cells in the peripheral blood of the patients with psoriasis. Correlation was tested using Spearman's rank correlation, and the significance was evaluated using the t statistic.

4. The proportion of $CXCR5^+PD1^+$ cells in $CD4^+$ T cells and the absolute numbers of $CXCR5^+ICOS^+CD4^+$ T cells and $CXCR5^+PD\text{-}1^+CD4^+$ T cells were significantly lower in psoriasis patients with high ASO titers.

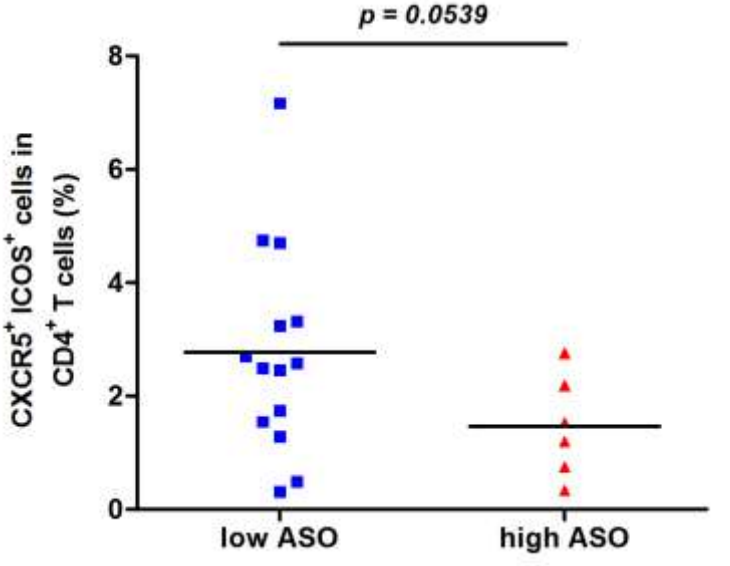
I analyzed the correlation between the ASO titers (high ASO>150 IU/mL versus low ASO≤150 IU/mL) and the levels of Tfh cells among these psoriatic patients. There was no significant correlation between the ASO titers and the percentages of $CXCR5^+$ cells or $CXCR5^+ICOS^+$ cells among $CD4^+$ T cells (Figure 4A & 4B). However, the level of $CXCR5^+PD\text{-}1^+$ cells among $CD4^+$ T cells was significantly lower in patients with high ASO titers (p=0.038, Figure 4C).

There was also no significant correlation between the absolute numbers of $CXCR5^+CD4^+$ T cells and the ASO titers in patients with psoriasis (Figure 4D). However, the absolute numbers of $CXCR5^+ICOS^+CD4^+$ T cells and $CXCR5^+PD\text{-}1^+CD4^+$ T cells were significantly lower in patients with high ASO titers (p=0.0347, Figure 4E & p=0.0381, Figure 4F).

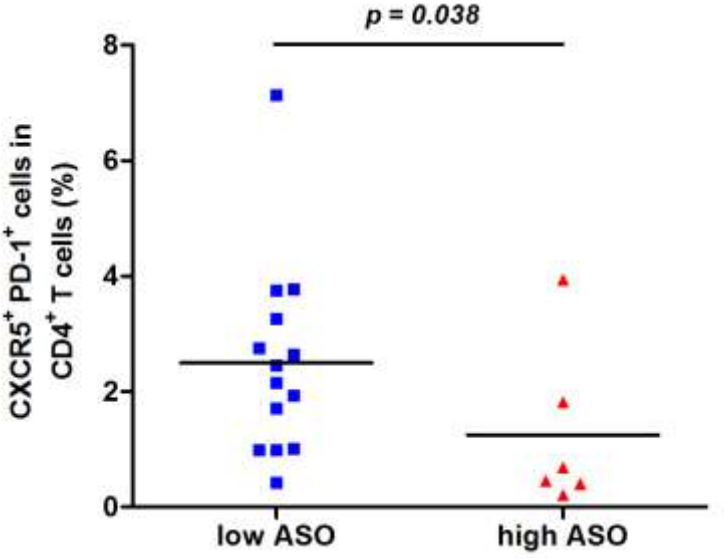
A)


p = 0.4836
CXCR5+ cells in CD4+ T cells (%)
50
40
30
20
10
0
low ASO
high ASO

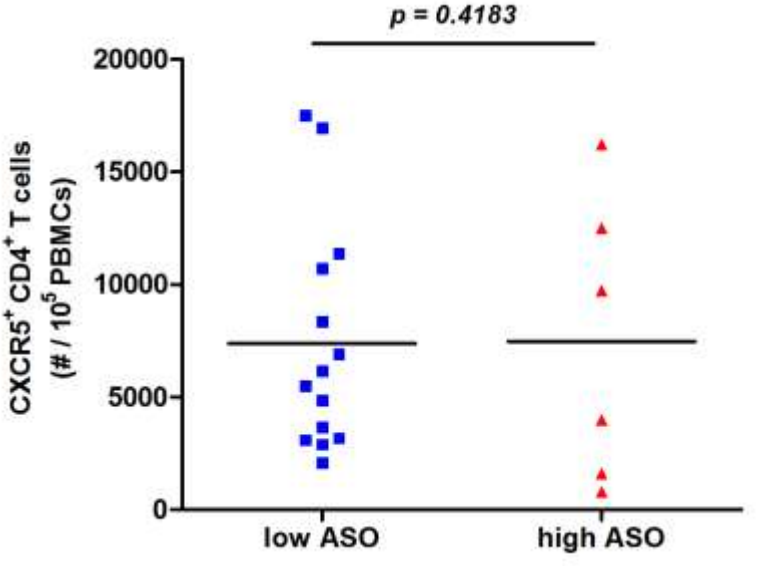
B)


p = 0.0539
CXCR5+ ICOS+ cells in CD4+ T cells (%)
8
6
4
2
0
low ASO
high ASO

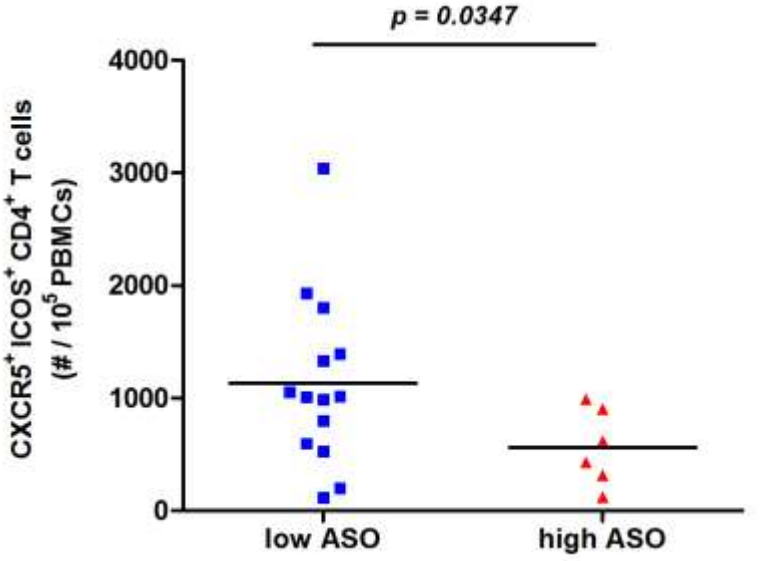
C)


p = 0.038
CXCR5+ PD-1+ cells in CD4+ T cells (%)
8
6
4
2
0
low ASO
high ASO

D)


p = 0.4183
CXCR5+ CD4+ T cells (# / 10^5 PBMCs)
20000
15000
10000
5000
0
low ASO
high ASO

E)


p = 0.0347
CXCR5+ ICOS+ CD4+ T cells
(# / 10^5 PBMCs)
4000
3000
2000
1000
0
low ASO
high ASO

F)

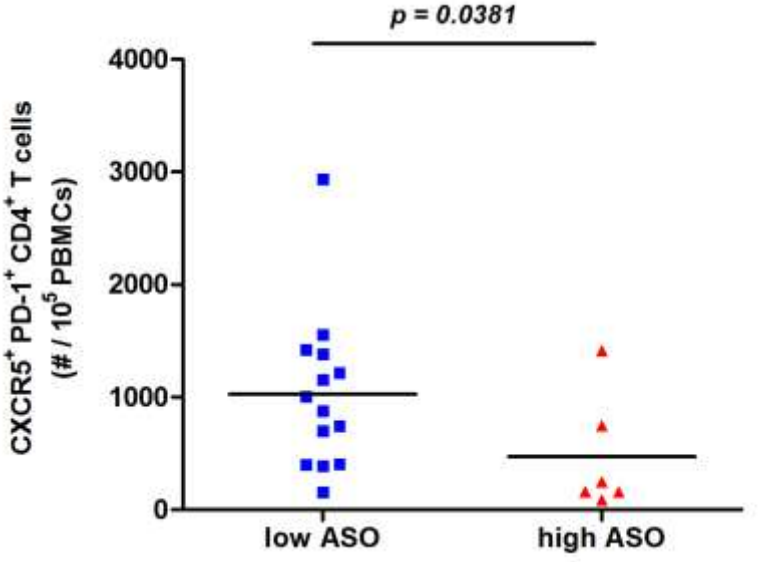
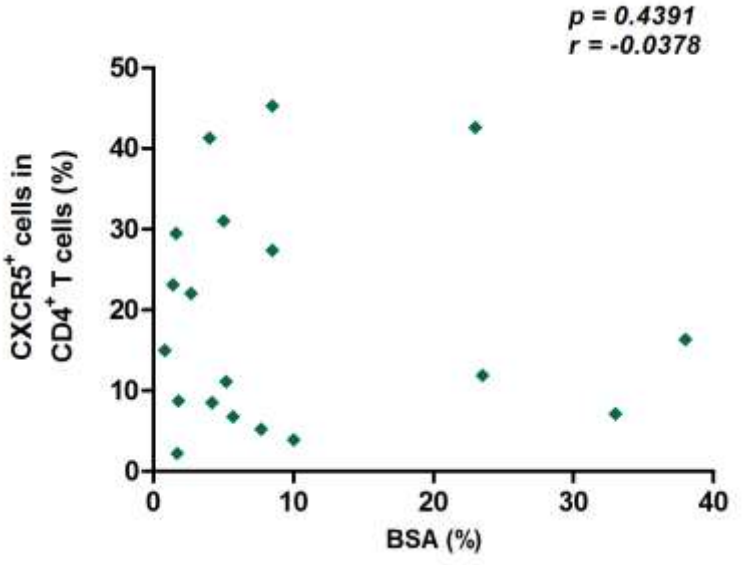

p = 0.0381
CXCR5+ PD-1+ CD4+ T cells
(# / 10^5 PBMCs)
4000
3000
2000
1000
0
low ASO
high ASO

Figure 4. The proportion of (A) $CXCR5^+$ cells, (B) $CXCR5^+ICOS^+$ cells, and (C) $CXCR5^+PD\text{-}1^+$ cells among $CD4^+$ T cells and the absolute numbers of (D) $CXCR5^+CD4^+$ T cells, (E) $CXCR5^+ICOS^+CD4^+$ T cells, and (F) $CXCR5^+PD\text{-}1^+CD4^+$ T cells were compared between the psoriatic patients with high versus low ASO titers. The difference in the level of each blood Tfh subsets between high ASO (>150 IU/mL) and low ASO (≤150 IU/mL) was statistically analyzed as in Figure 2.

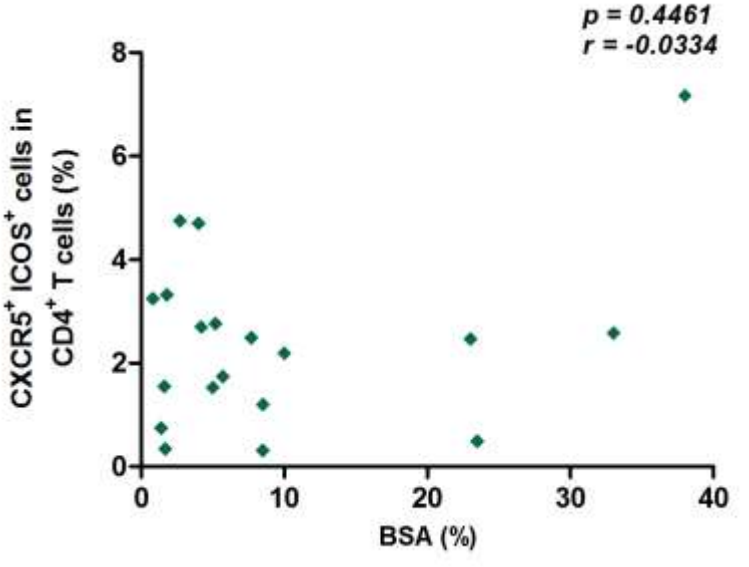
5. The disease severity of psoriasis showed no correlation with the levels of blood Tfh cell subsets.

Two commonly used clinical severity markers for psoriasis are Body Surface Area (BSA) and Psoriasis Area and Severity Index (PASI). Therefore, I analyzed the correlation between these clinical parameters of psoriasis and the levels of blood Tfh cell subsets. However, I found no significant correlation between the clinical severity markers of psoriasis and the proportions of blood Tfh cells (Figure 5A~5L).

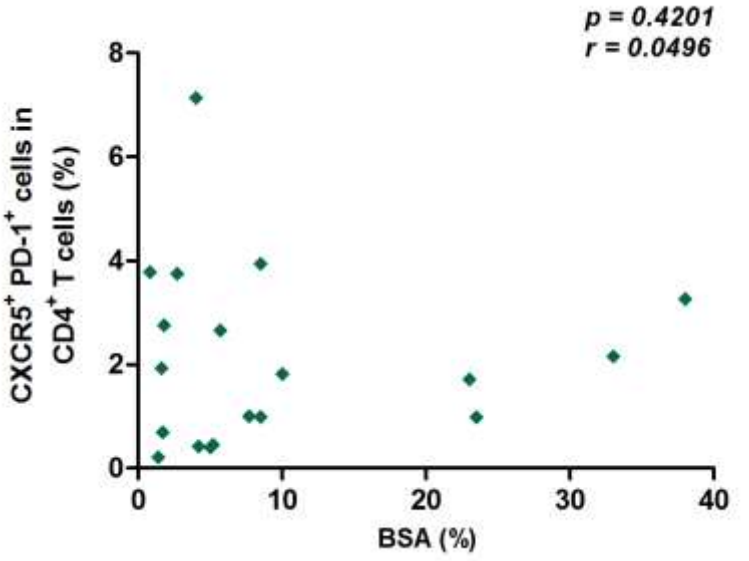
A)


p = 0.4391
r = -0.0378
CXCR5+ cells in CD4+ T cells (%)
BSA (%)
0
10
20
30
40
50

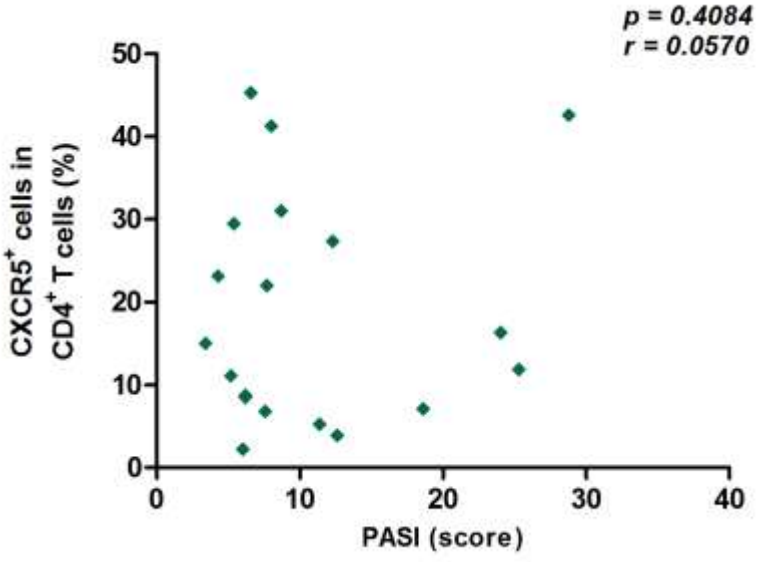
B)


p = 0.4461
r = -0.0334
CXCR5+ ICOS+ cells in CD4+ T cells (%)
BSA (%)
0
2
4
6
8
10
20
30
40

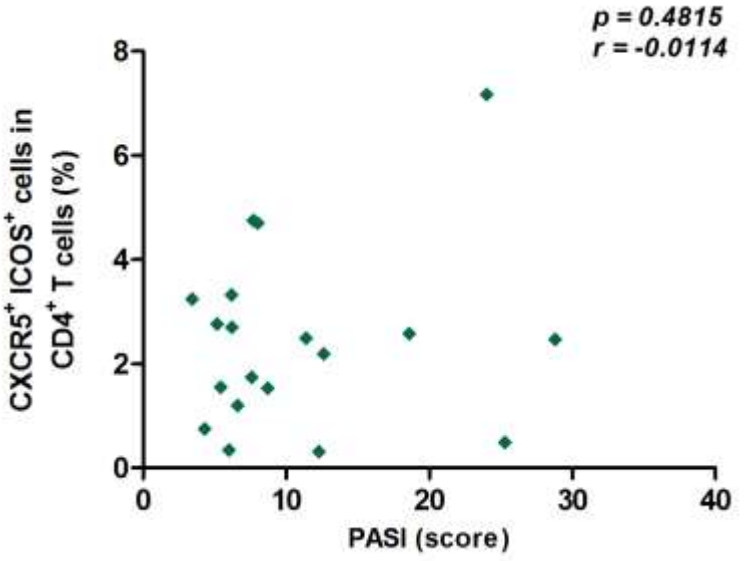
C)


p = 0.4201
r = 0.0496
CXCR5+ PD-1+ cells in CD4+ T cells (%)
BSA (%)
0
2
4
6
8
0
10
20
30
40

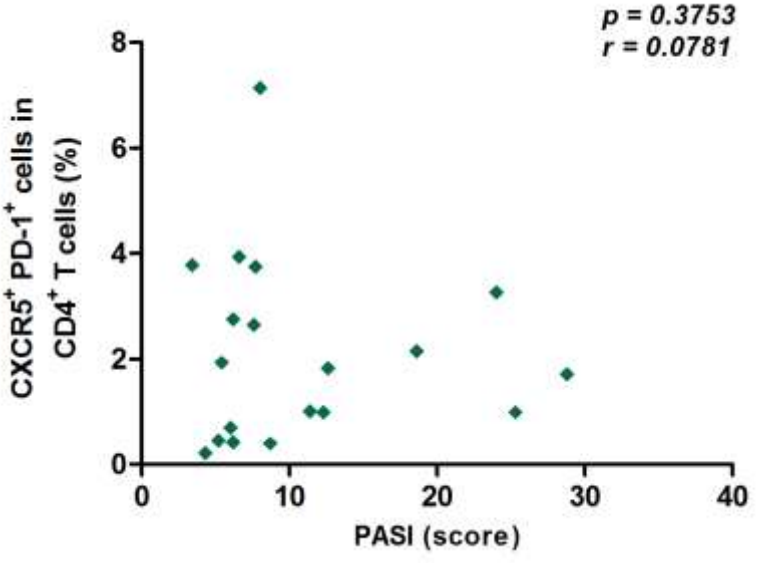
D)


p = 0.4084
r = 0.0570
CXCR5+ cells in CD4+ T cells (%)
PASI (score)
0
10
20
30
40
50
0
10
20
30
40

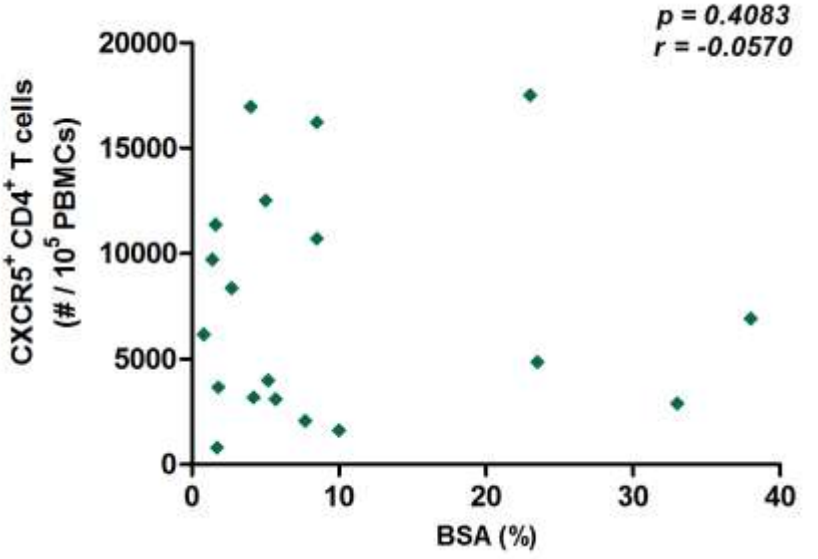
E)


p = 0.4815
r = -0.0114
CXCR5+ ICOS+ cells in CD4+ T cells (%)
0
2
4
6
8
0
10
20
30
40
PASI (score)

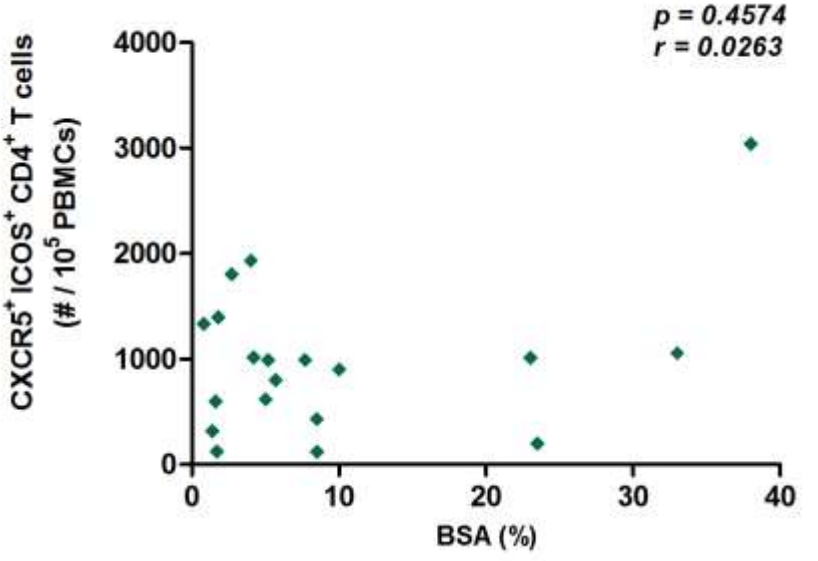
F)


p = 0.3753
r = 0.0781
CXCR5+ PD-1+ cells in CD4+ T cells (%)
0
2
4
6
8
0
10
20
30
40
PASI (score)

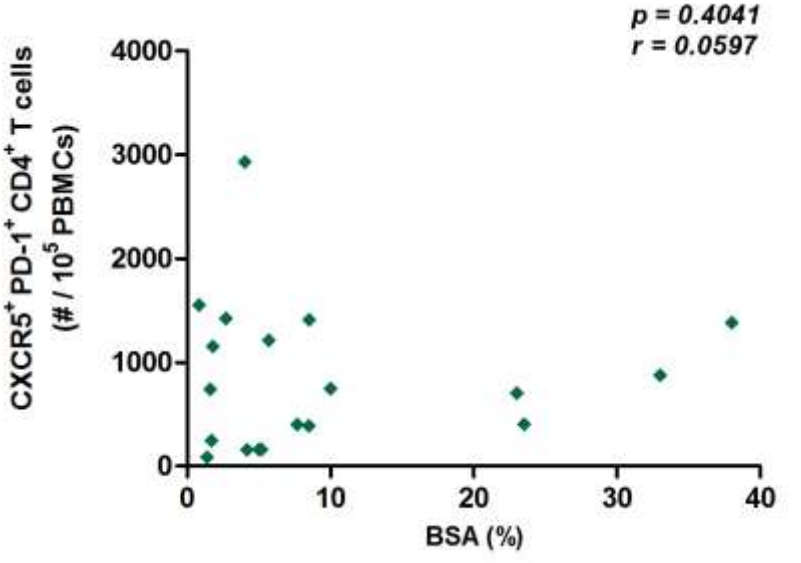
G)


p = 0.4083
r = -0.0570
CXCR5+ CD4+ T cells
(# / 10^5 PBMCs)
20000
15000
10000
5000
0
0
10
20
30
40
BSA (%)

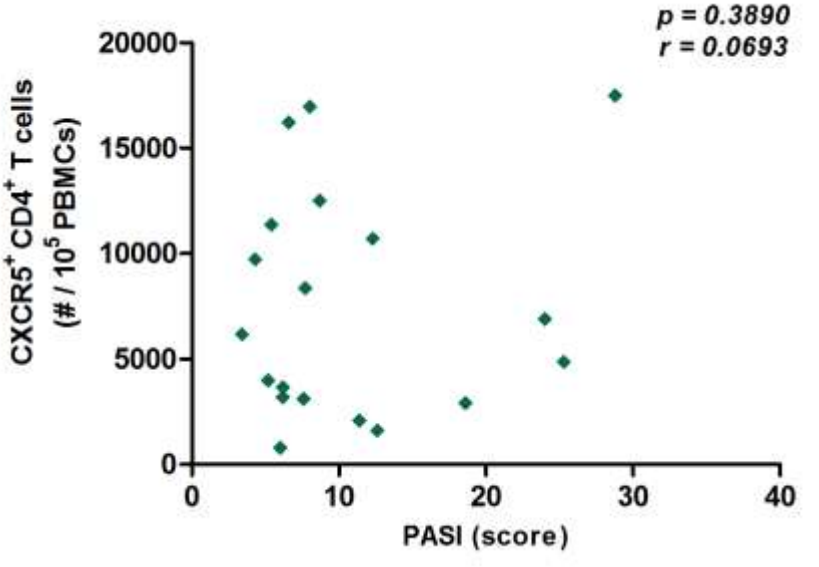
H)


p = 0.4574
r = 0.0263
CXCR5+ ICOS+ CD4+ T cells
(# / 10^5 PBMCs)
4000
3000
2000
1000
0
0
10
20
30
40
BSA (%)

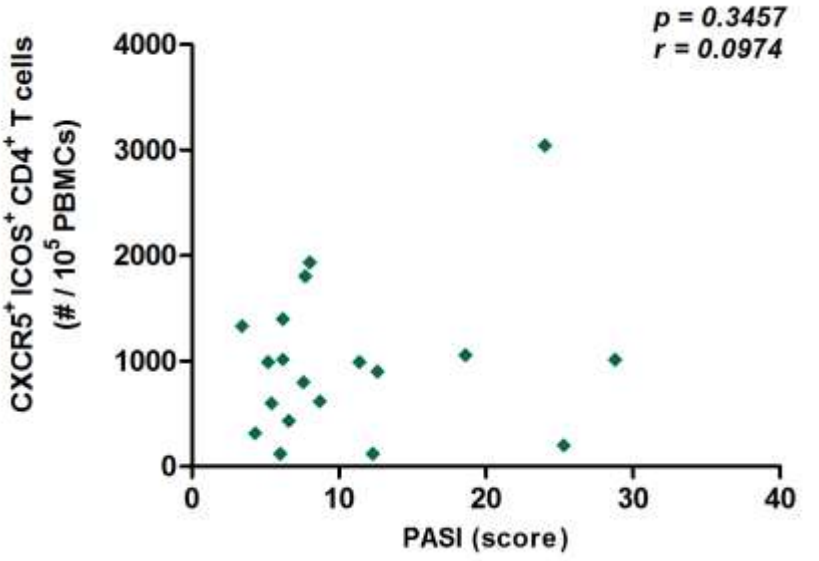
I)


p = 0.4041
r = 0.0597
CXCR5+ PD-1+ CD4+ T cells
(# / 10^5 PBMCs)
4000
3000
2000
1000
0
0
10
20
30
40
BSA (%)

J)


p = 0.3890
r = 0.0693
CXCR5+ CD4+ T cells
(# / 10^5 PBMCs)
20000
15000
10000
5000
0
0
10
20
30
40
PASI (score)

K)


p = 0.3457
r = 0.0974
CXCR5+ ICOS+ CD4+ T cells
(# / 10^5 PBMCs)
4000
3000
2000
1000
0
0
10
20
30
40
PASI (score)

L)

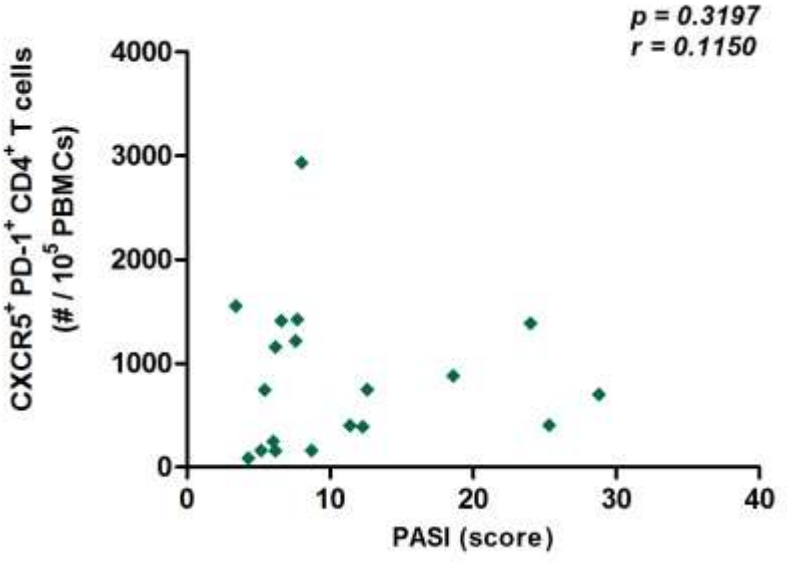

p = 0.3197
r = 0.1150
CXCR5+ PD-1+ CD4+ T cells
(# / 10^5 PBMCs)
4000
3000
2000
1000
0
0
10
20
30
40
PASI (score)

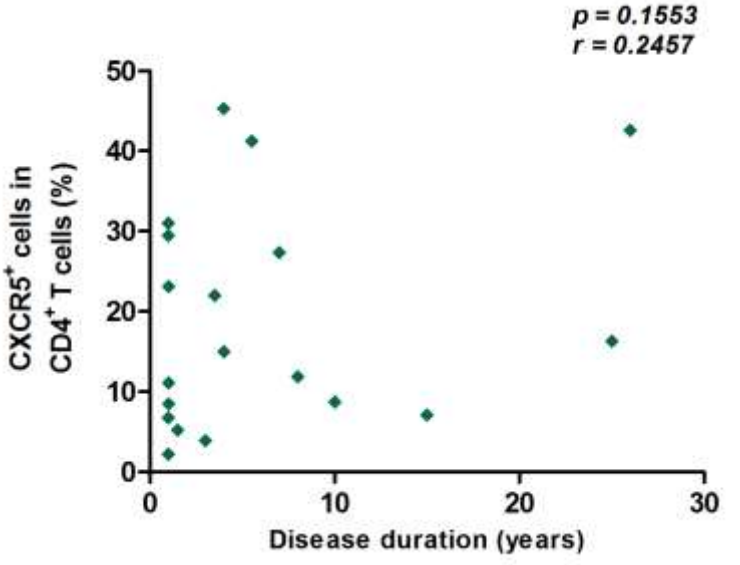
Figure 5. The correlation of disease severity markers and proportions of (A & D) $CXCR5^+$ cells, (B & E) $CXCR5^+ICOS^+$ cells, (C & F) $CXCR5^+PD\text{-}1^+$ cells among $CD4^+$ T cells, absolute numbers of (G & J) $CXCR5^+CD4^+$ T cells, (H & K) $CXCR5^+ICOS^+CD4^+$ T cells, and (I & L) $CXCR5^+PD\text{-}1^+CD4^+$ T cells in the peripheral blood of the patients with psoriasis. Correlation was tested using Spearman's rank correlation, and the significance was evaluated using the t statistic.

6. The disease duration of psoriasis is positively correlated with the proportion of $CXCR5^+PD1^+$ cells among $CD4^+$ T cells and the absolute numbers of $CXCR5^+ICOS^+CD4^+$ T cells and $CXCR5^+PD1^+CD4^+$ T cells in patients with psoriasis.

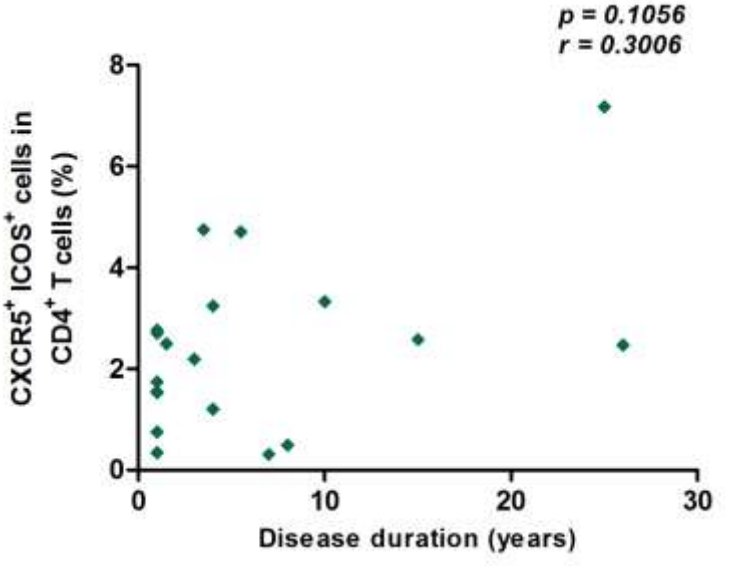
I analyzed the correlation between the disease duration of psoriasis and the percentages of Tfh cells. There was no significant correlation between the percentages of $CXCR5^+$ cells and $CXCR5^+ICOS^+$ cells among $CD4^+$ T cells and the disease duration in patients with psoriasis (Figure 6A & 6B). However, the percentages of $CXCR5^+PD\text{-}1^+$ cells among $CD4^+$ T cells showed a positive correlation with the disease duration of psoriasis (r=0.4988, p=0.0149, Figure 6C).

There was also no significant correlation between the absolute numbers of $CXCR5^+CD4^+$ T cells and the disease duration in patients with psoriasis (Figure 6D). However, the absolute numbers of $CXCR5^+ICOS^+CD4^+$ T cells and $CXCR5^+PD\text{-}1^+CD4^+$ T cells showed a positive correlation with the disease duration of psoriasis (r=0.4034, p=0.0434, Figure 6E & r=0.4968, p=0.0152, Figure 6F).

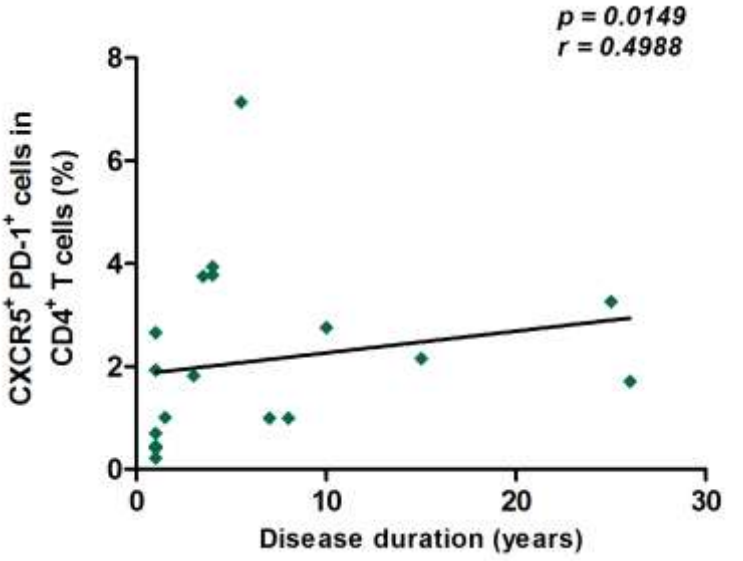
A)


p = 0.1553
r = 0.2457
CXCR5+ cells in CD4+ T cells (%)
50
40
30
20
10
0
0
10
20
30
Disease duration (years)

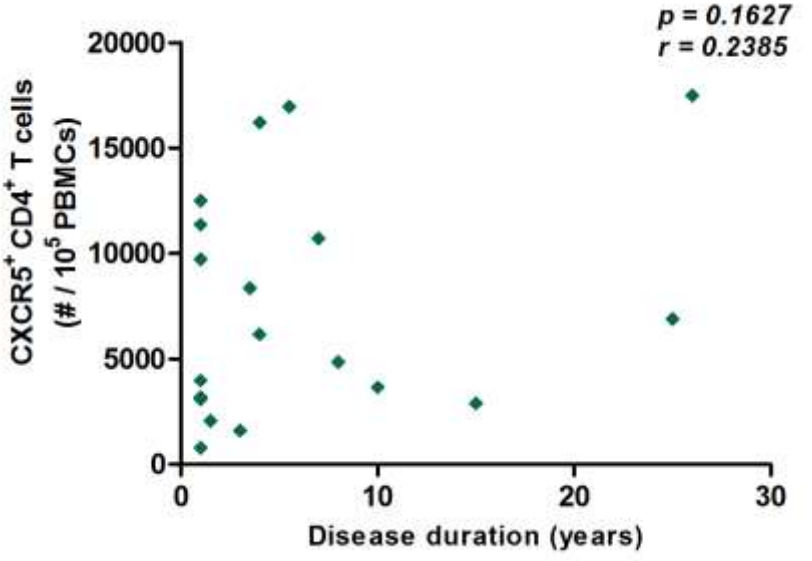
B)


p = 0.1056
r = 0.3006
CXCR5+ ICOS+ cells in CD4+ T cells (%)
8
6
4
2
0
0
10
20
30
Disease duration (years)

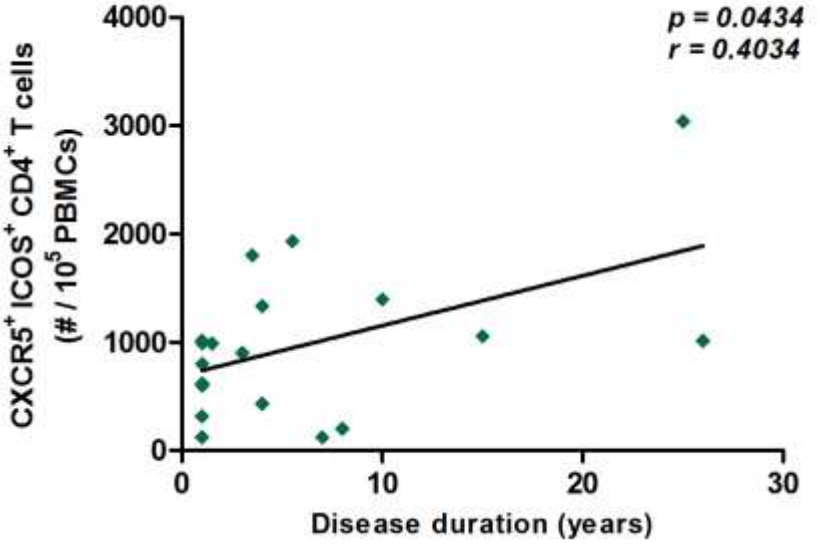
C)


p = 0.0149
r = 0.4988
CXCR5+ PD-1+ cells in CD4+ T cells (%)
0
2
4
6
8
0
10
20
30
Disease duration (years)

D)


p = 0.1627
r = 0.2385
CXCR5+ CD4+ T cells (# / 10^5 PBMCs)
0
5000
10000
15000
20000
0
10
20
30
Disease duration (years)

E)


p = 0.0434
r = 0.4034
CXCR5+ ICOS+ CD4+ T cells
(# / 10^5 PBMCs)
4000
3000
2000
1000
0
0
10
20
30
Disease duration (years)

F)

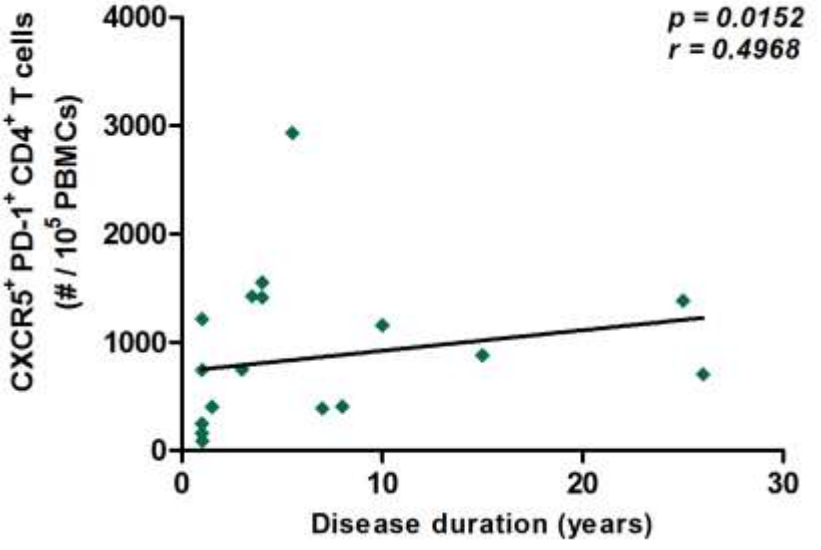

p = 0.0152
r = 0.4968
CXCR5+ PD-1+ CD4+ T cells
(# / 10^5 PBMCs)
4000
3000
2000
1000
0
0
10
20
30
Disease duration (years)

Figure 6. The correlation of the disease duration of psoriasis and proportions of (A) $CXCR5^+$ cells, (B) $CXCR5^+ICOS^+$ cells, (C) $CXCR5^+PD\text{-}1^+$ cells among $CD4^+$ T cells, absolute numbers of (D) $CXCR5^+CD4^+$ T cells, (E) $CXCR5^+ICOS^+CD4^+$ T cells, and (F) $CXCR5^+PD\text{-}1^+CD4^+$ T cells in the peripheral blood of the patients with psoriasis. Correlation was tested using Spearman's rank correlation, and the significance was evaluated using the t statistic.

7. Immunohistochemical analysis

I also performed immunohistochemical stain of CXCR5, CXCL13, Bcl-6, PD-1 and ICOS for the skin lesions obtained from psoriasis patients and the normal skin tissue of healthy controls. However, the results were inappropriate for comparison due to lack of infiltrating T cells in dermis (Figure 7).

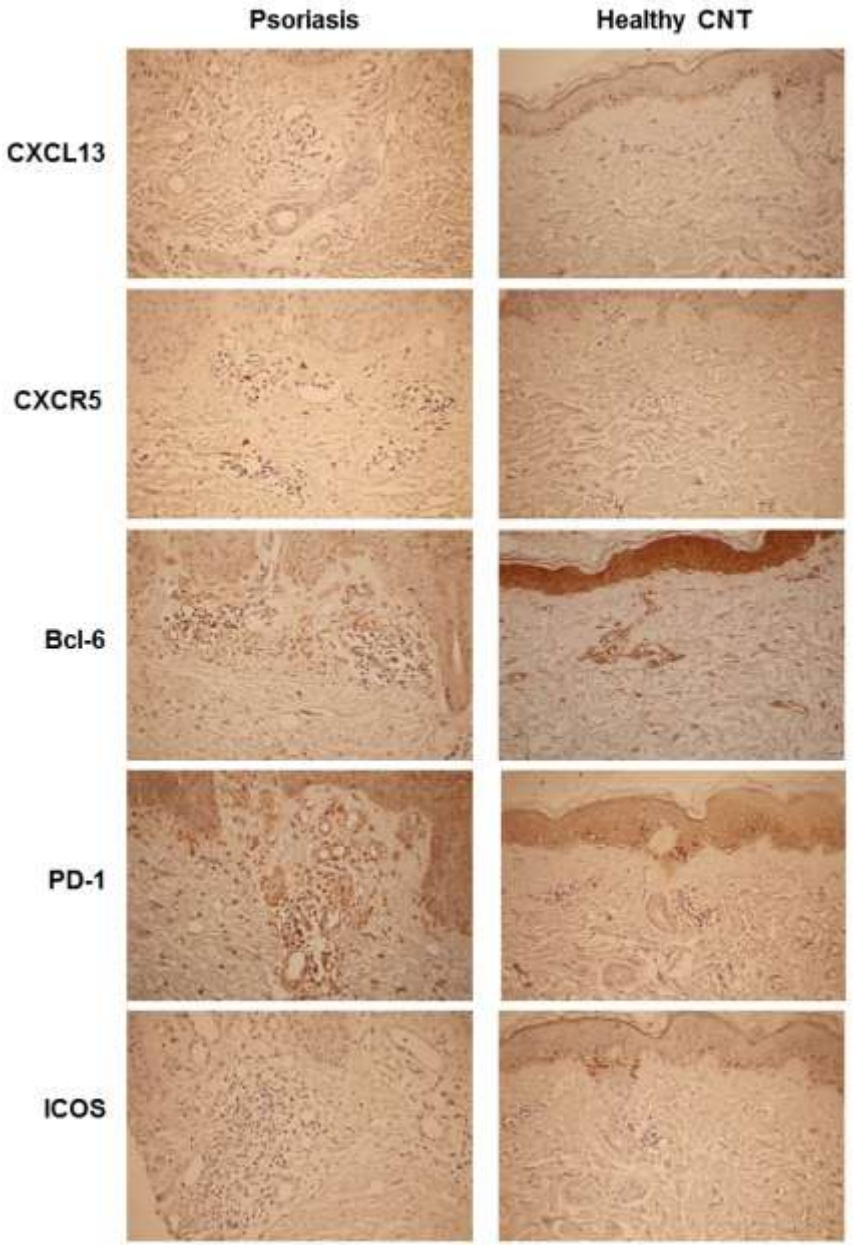


Figure 7. Results of immunohistochemical stain (original magnification x 100).

IV. DISCUSSION

Tfh cells have received more attention in recent years because they are the specialized $CD4^+$ T cell subset that provides help to B cells both to form germinal centers and to differentiate into memory B cells as well as plasma cells in the process of humoral responses. Meanwhile, Tfh cells have also been proved to increase in peripheral blood of several autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis, juvenile dermatomyositis and autoimmune thyroid disease.[16-20] For skin diseases, there was one recent report for the patients with bullous pemphigoid where an increased frequency of circulating Tfh cells was found.[21] However, there has been no report on blood Tfh cells in the literature for psoriasis yet. I have herein described that patients with psoriasis demonstrated no increase in frequencies of blood Tfh cells, but rather a decrease in the frequency of $PD\text{-}1^+$ activated subset of Tfh cells in psoriatic patients.

These findings were unexpected since the previous studies in patients with systemic autoimmune diseases, mentioned above, showed that the increased frequency of Tfh cells and their subsets in correlation with disease severity. Different nature of psoriasis from other systemic autoimmune diseases might be responsible for this result. Although some investigators consider psoriasis as an

autoimmune disease in a broad sense,[22] psoriasis is routinely regarded as a different disease entity from typical autoimmune diseases.

First of all, there is no disease specific autoantibody found in psoriasis. Typical autoimmune diseases have specific autoantibodies such as anti-cyclic citrullinated peptide (CCP) antibody in rheumatoid arthritis; anti-dsDNA, anti-SSA/Ro and anti-SSB/La antibodies in systemic lupus erythematosus; and anti-BP180 and anti-BP230 antibodies in bullous pemphigoid. The recent studies in those diseases suggested that increased follicular helper T cells might involve in pathogenesis via B cell activation and thus inducing disease specific autoantibodies.[23,24] In those diseases, although the Th1 and/or Th17 cytokine axises are known to play a pathogenic role similarly as in the pathogenesis of psoriasis, B cell immune response is still considered to be fundamental pathomechanism. In psoriasis, however, B cell immunity has not been clearly delineated. Our findings are meaningful to confirm the difference in disease nature between psoriasis and other typical autoimmune diseases in the respect of Tfh cells.

Interestingly, I found the proportion and the absolute numbers of PD-1^+ subset of activated Tfh cells negatively correlated with ESR levels. Besides, the proportion of PD-1^+ Tfh cells and the absolute numbers of PD-1^+ and $ICOS^+$ Tfh cells were lower in psoriatic patients with high ASO titers. These negative

correlations of Tfh subsets and several disease severity markers of psoriasis suggest that the function of Tfh cells may decline in the pathogenesis of psoriasis. Meanwhile, the proportion of PD-1^+ Tfh cells and the absolute numbers of PD-1^+ and $ICOS^+$ Tfh cells positively correlated with the disease duration of psoriasis (Table 2). These findings suggest that the activated Tfh cells were decreased in severe status and early phase of psoriasis. Furthermore, it also implies that B cell immunity weakens in psoriasis as a result of predominance of Th1 and Th17 cytokine axises.

However, there is a conflicting possibility of sequestration of circulating Tfh cells in psoriatic lesion of skin. Bautista-Caro et al. recently reported a similar result to our data in ankylosing spondylitis, where a decreased frequency of activated Tfh cells are found to circulate in those patients.[25] This result is in contrast with previous reports on ankylosing spondylitis, so they speculated that local sequestration of Tfh cells at the inflammatory sites would lead to decreased circulation of activated Tfh cells. Further investigations will be needed to figure out whether the altered frequency of activated subset of Tfh cells represents the reduced function or the local sequestration of these Tfh cells, or else.

Table 2. Summary of results

	$CXCR5^+$ / $CD4^+$ T cells (%)	$CXCR5^+$ $CD4^+$ T cells (#)	$CXCR5^+ICOS^+$ / $CD4^+$ T cells (%)	$CXCR5^+ICOS^+$ $CD4^+$ T cells (#)	$CXCR5^+PD\text{-}1^+$ / $CD4^+$ T cells (%)	$CXCR5^+PD\text{-}1^+$ $CD4^+$ T cells (#)
Psoriasis vs. healthy control	NS	NS	NS	NS	Lower in psoriasis patients	Lower in psoriasis patients
Correlation with ESR	NS	NS	NS	NS	Negative correlation	Negative correlation
Correlation with CRP	Inappropriate for comparison since most patients showed lower levels of CRP					
Correlation with ASO titer	NS	NS	NS	Lower in high ASO titers	Lower in high ASO titers	Lower in high ASO titers
Correlation with clinical severity (BSA & PASI)	NS	NS	NS	NS	NS	NS
Correlation with disease duration	NS	NS	NS	Positive correlation	Positive correlation	Positive correlation
Immunohistochemistry	Inappropriate for comparison due to lack of infiltrating T cells in dermis					

Abbreviations : NS, Not significant; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ASO, anti-streptolysin O; BSA, body surface area; PASI, psoriasis area and severity index

V. CONCLUSION

In this study, I have elucidated the altered frequency of circulating activated Tfh cells in psoriasis. PD-1^+ activated subset of Tfh cells was decreased in peripheral blood of psoriasis patients. Furthermore, the proportion and the absolute numbers of PD-1^+ subset of activated Tfh cells negatively correlated with ESR levels. The proportion of PD-1^+ Tfh cells and the absolute numbers of PD-1^+ and ICOS$^+$ Tfh cells were lower in psoriatic patients with high ASO titers. Meanwhile, the proportion of PD-1^+ Tfh cells and the absolute numbers of PD-1^+ and ICOS$^+$ Tfh cells positively correlated with the disease duration of psoriasis. These findings suggest that the activated Tfh cells decrease in severe status and early phase of psoriasis. I expect that this elucidation of the altered frequency of activated Tfh cells will help the further investigation of pathogenesis and potential therapeutic targets in psoriasis.

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ABSTRACT (IN KOREAN)

건선 환자의 혈액 내
Follicular Helper T cell 에 대한 분석 연구

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건선은 전세계 약 2%에 해당하는 인구에서 발병하는 만성 염증성 피부 질환이다. 건선의 병인은 아직까지 정확히 알려지지 않은 자극에 의해 선천면역이 염증을 일으키고 이는 결국 적응면역을 유발시켜 다양한 cytokine, chemokine, 그리고 growth factor들이 생성되어 표피의 증식을 일으키는 것으로 알려져 있다. 최근의 연구들은 특히 Th1과 Th17 cytokine 축이 병인에 핵심적인 역할을 하는 것으로 보고하였다. 반면, 아직까지 건선의 병인에 있어서 B 세포 면역의 역할에 대해서는 연구가 부족하다.

Follicular helper T (Tfh) 세포는 최근에 발견된 새로운 helper T 세포로, B cell follicle 의 germinal center 에 위치하는 것으로 알려져 있다. 주된

기능은 B 세포의 활성화를 도와 체액성 세포 반응 동안 항체 생성을 촉진시키는 것으로 밝혀져 있다. 최근에는 전신성 홍반성 루푸스, 류마티스성 관절염, 유천포창 등 다양한 자가면역 질환에서 혈액 내 Tfh 세포의 수가 증가되어 있음이 밝혀진 바 있다. 하지만 현재까지 건선 환자의 Tfh 세포에 대해서는 보고된 바 없다.

이번 실험을 통해 저자는 건선 환자의 혈액 내에서 Tfh 세포, 그리고 활성화된 Tfh 분획 세포의 증감에 대해 알아보고자 하였고, 또한 이들 세포가 건선의 다양한 임상적, 실험적 중증도 지표들과 관련성을 보이는지 고찰하고자 하였다. Flow cytometry를 통한 말초 혈액 분석을 통해 건선 환자의 혈액에서 Tfh 세포는 정상인과 비교하여 유의한 차이를 보이지 않음을 확인할 수 있었으며, 오히려 PD-1 양성을 띠는 활성화된 Tfh 분획 세포가 건선 환자의 혈액에서 감소되어 있음이 확인되었다. 또한 PD-1 양성을 띠는 활성화된 Tfh 분획 세포의 분율과 숫자가 건선 환자의 혈액 내 ESR 값과 음의 상관관계를 보였다. 또한, PD-1 양성을 띠는 활성화된 Tfh 분획 세포의 분율과 PD-1 양성, 그리고 ICOS 양성을 띠는 활성화된 Tfh 분획 세포의 분율과 숫자가 높은 ASO titer 를 보이는 환자군에서 낮게 관찰되었다. 반면, 이러한 활성화된 Tfh 분획세포는 건선의 유병 기간이 짧을수록 낮게 관찰되었다.

이 실험 결과는 건선의 중증도가 심한 상태, 그리고 건선의 유병기간이 짧은 경우 활성화된 Tfh 분획 세포가 감소되어 있음을 보여주는 연구 결과로써, 건선의 병리기전에 있어 Th1, Th17 축이 지배적으로 작용함에 따라 B 세포 면역은 약화되어 있음을 시사하는 소견이라 할 수 있다. 따라서 저자는 이 실험 결과가 기존에 보고되지 않은 건선에서의 Tfh 세포의 역할, 그리고 B 세포 면역의 역할에 대한 단서를 제공할 수 있으리라 생각하며, 추후 건선의 치료에 있어서 잠재적인 표적의 고려 대상이 될 수 있음을 조심스럽게 제안하는 바이다.

핵심되는 말 : Follicular helper T (Tfh) 세포, chemokine (C-X-C motif) receptor 5 (CXCR5), programmed cell death 1 (PD-1), inducible T-cell co-stimulator (ICOS), B 세포 면역, 건선