Original Research Article

Expression of CD127 suppresses T regulatory cells in psoriasis

ABSTRACT

Background: Psoriasis is a genetically regulated autoimmune skin disease associated with the impaired suppressive function of Treg cells. Foxp3 is the accepted marker of Treg cell, but CD127 (IL-7Rα), a surface marker of activated T cell, can distinguish the functional status of $CD4^+CD25^+FoxP3^+$Treg cells. The study aimed to demonstrate the functional status of Treg cells in peripheral blood of psoriasis patients, to analyze their association with disease severity and duration, serum IL-17 level, and IL-23R gene polymorphism to observe the relationship with susceptibility to psoriasis. **Methods:** Thirty-five psoriasis patients and 35 healthy controls were enrolled. Demographical details were recorded, severity was assessed by Psoriasis Area and Severity Index (PASI) scoring. Treg cells were quantified by flowcytometric immunophenotyping, serum IL-17 level by ELISA, and IL-23R gene polymorphism by Real-Time PCR. **Results:** Increased circulatory $CD4^+CD25^+FoxP3^+$Treg and $CD4^+CD25^+FoxP3^+CD127^+$/pTreg cells observed in psoriatics than in controls ($P<0.05$) but $CD4^+CD25^+FoxP3^+CD127^-$/tTreg cells significantly decreased ($P<0.001$). IL-17 levels were elevated in psoriatics ($P=0.002$). In severe patients, FoxP3 expression decreases in tTreg cells and are $CD127^+$. Out of 35 patients, 23 (65.71%) had an AA genotype of IL23Rrs10889677 gene polymorphism, associated with increased Treg, tTreg, pTreg cells, and IL-17 levels revealed susceptibility to psoriasis. **Conclusion:** This study shows the loss of tTreg function and pTreg cell activation in psoriasis, supporting the autoimmune disease model. CD127 expression can better distinguish Treg cells and augmenting the $CD127^{-/low}$ population of Treg cells to restore suppressive activity by anti-CD127 mAb therapy can be a potential treatment option for psoriasis patients.

Keywords: Psoriasis, CD127, FoxP3, Treg.

1. Introduction

The balance between immunity and tolerance is essential for immune system homeostasis. Cells like $CD4^+$ regulatory T cells (Tregs), $CD8^+CD28^-$ T cells, $CD4^-CD8^+$T cells, and NKT cells control the exaggerated immune response by suppressing autoimmune reactivation and regulating chronic inflammation [1]. Recent advancements in T-cell lineage development pointed out that functional and quantitative disruption of Treg cells can result in autoimmune and inflammatory diseases [2-7]. Specific markers for identifying Treg cells remain variable. For classic Treg population $CD4^+CD25^+FoxP3^+$ T-cells are widely considered for many years. $CD4^+$ T cell subset expressing the high levels of CD25/IL-2α (termed CD25 high) has in vitro suppressive activity [8,9]. However, Foxp3 (forehead box P3 transcription factor) is an intracellular marker that must be evaluated in a fixed and permeabilized cell. Fixed cells cannot be used [10]. Researchers have evaluated different functional markers to match a better fit for Treg cells. It has been demonstrated that surface expression of CD127 (IL-7Rα) in combination with CD25 and FoxP3 can better distinguish Treg cells. CD127 expression is not an intrinsic characteristic of Treg cells. Rather differential CD127 expression on Treg cells depends on their localization, functional, and activation status. IL-7 dependent peripherally activated Tregs cells express much more CD127 than IL-2 dependent thymic Tregs cells. Stable expression of FoxP3 is crucial for Treg cell function. Interestingly CD127 expression inversely correlates with Foxp3 and human CD4+ Treg cells [11-13]. Thus, conventional T reg cells (tT-reg, $CD4^+CD25^+FoxP3^+CD127^{-/low}$) has much more suppressive activity than peripherally active Treg cell population (pT-reg, $CD4^+CD25^+FoxP3^+CD127^{+/high}$). It has been recently noticed that $CD4^+CD25^{high}Foxp3^+$ pT-reg cells can be converted into inflammation-associated Th17 cells under certain conditions (e.g., IL-23), which triggers IL-23/Th17 axis [7,13]. Thus, rather than suppression of inflammatory events, dysfunctional Treg cells can trigger and potentiate chronic inflammatory events such as psoriasis in humans.

The etiology of psoriasis is complex [14]. For many years psoriasis was characterized as a Th1 driven chronic inflammatory disease. It was hypothesized that imbalance between Th1 and Th2 cells is due to dysfunctional Treg cells, whereas their frequency and anergic phenotype appeared unchanged 15]. But recent studies showed that in genetically susceptible persons, along with other T cells, T reg cells also increased in psoriasis patients both in the skin and peripheral

circulation and positively correlated with severity and duration [16]. The genome-wide association has confirmed many HLA and non-HLA genes are related to psoriasis. It has been reported that IL23R rs10889677 gene polymorphism can affect mRNA stability, localization, and translational efficacy leading to increase IL-17 levels [17]. Treg cells are particularly prone to differentiate into IL-17 producing cells in severe psoriasis patients. These FoxP3/ IL-17 double-positive cells gradually lost expression of FoxP3 and remained the prime source of IL-17 that created a feed-forward inflammatory response to sustain chronic events in psoriasis [18]. These findings pointed researchers to focus on the essential role of Treg cells in psoriasis.

Still, the role of central and peripheral Treg cells in psoriasis is controversial due to little work on it. In the global report 2016, WHO recommends research on new treatments should focus on global options on a large scale. Hence, the study aimed to demonstrate the Treg cell subsets, serum IL-17 level, and IL23R rs10889677 gene polymorphism to correlate with clinical parameters, thus providing an overview of their role in pathogenesis. This will also pave the way for the development of future treatment modalities.

2. Methods

2.1 Clinical samples

This cross-sectional study was conducted from March 2019 to February 2020 and was approved by the institutional review board. Written informed consent was obtained from the study subjects for research and publication. According to the Declaration of Helsinki (revised in 2013). 35 clinically confirmed psoriatic patients diagnosed by an expert dermatologist were enrolled. Patients with cutaneous or extra-cutaneous inflammation and infections, malignancies, diabetes, pre-existing thyroid disease, hypertension, smoking, and undergoing systemic therapy in the last three months and topical treatment for 1 month were excluded from the study. A total of 35 age and gender-matched healthy individuals, without any skin and infectious diseases and a family history of autoimmune diseases, were recruited as healthy controls [19]. The clinical characteristics, including disease severity as assessed by psoriasis area and severity index (PASI) scoring [20], duration of psoriasis, past therapies, associated comorbidities, and the detailed family history, were recorded. Nine ml of peripheral venous blood was collected from each study subject. 3ml taken in a heparinized vial for isolation of peripheral blood mononuclear cells

(PBMCs), 3 ml in a tube without anticoagulant, and separated serum were stored at -20ºC for cytokine analysis and 3 ml in EDTA tube for genetic analysis.

2.2 Antibodies

The following conjugated antibodies were used. FITC conjugated anti-CD3 (UCHT1), and PC7 conjugated anti CD25 (PC61.5) were obtained from Abcam, UK. PC5 conjugated anti-CD4 (13B8.2) and ECD conjugated anti-CD45 (J33) were obtained from BECKMAN COULTAR. FITC conjugated anti-CD127 (A019D5) was obtained from Bio Legend. PE-conjugated anti-FoxP3 (259D/C7) was obtained from BD Biosciences.

2.3 Cell isolation and flow cytometric analysis

From heparinized blood, peripheral blood mononuclear cells **(**PBMCs) were isolated using density-gradient centrifugation on Ficoll Hypaque (Invitrogen, Germany) and adjusted at a concentration of 10^6 /ml in 1ml of RPMI 1640 medium (Invitrogen, Germany). To maintain a stable P^H, 10 µl HEPES was added. Then the cells were stimulated by 50 ng Phorbal-12-myristate-13-acetate/ PMA for 5 hours at 37º C in the presence of 1µl Golgi plug protein transport inhibitor. Then 1µg BD Fc block was added in cell media to prevent nonspecific binding of monoclonal antibodies. Cells were separated into two tubes. 50 µl staining buffer and 20 µl of each cell surface monoclonal antibody CD3 - FITC, CD4-PC5, and CD45- ECD were added in 1st tube. CD127 - FITC, CD4- PC5 and CD45- ECD, CD25- CY5 were added in 2nd tube. Both tubes were then incubated for 30 minutes at 4º C then re-suspended with 250 µl Cytofix/Cytoperm™ Plus solution for 20 minutes at 4º C. Then cells were washed two times with wash buffer and pellet with a final volume of 250 µl. Then 20 µl Foxp3 – PE was added in both tubes. Tube 1 represents CD4+CD25+FoxP3+/T-reg cells. Tube 2 represents CD4+CD25+FoxP3+CD127-/ tTreg cell and CD4+CD25+FoxP3+CD127+/ pT-reg cell. Flow cytometry was done in BECKMAN COULTER CYTOMICS FC 500. Defining four-color flow cytometer tubes were used for the analysis of cells. Data analysis was done by CXP software. Minimum 10,000 events were taken for interpretation**.**

2.4 Detection of serum level of IL-17 by Enzyme-Linked Immunosorbent Assay (ELISA)

Frozen serum was thawed, and the level of IL-17 was measured by ELISA kit (Ray-Biotech, USA; Catalog #: ELH-IL17) as per standard protocol following manufacturer's instruction.

2.5 Genotyping of IL23

Genomic DNA extraction from stored blood was performed according to the manufacturer's instruction (Thermo Fisher Scientific GeneJET Genomic DNA Purification Kit #K0721, #K0722, USA). The genotyping for SNPs of IL-23R rs10889677 (A/C) was performed using TaqMan 5'allele discrimination assay. Allele-specific fluorogenic probes (Thermofisher Scientific) amplified the target genes. Homozygous major and minor variants and heterozygous minor variant genotypes were distinguished based on the fluorescence emission from the corresponding fluorescent dyes. Amplification was performed on ABI 7500 Fast Real-time PCR platform (Applied Bio-system Tm 7500 Fast Real-time PCR system).

2.6 Statistical analysis

Collected data were analyzed with SPSS software package version-22 (Strata Corporation, College Station, Texas). Continuous parameters were expressed as mean ± SD and categorical parameters as frequency and percentage. The independent sample t-test was done to compare baseline characteristics in normally distributed data. Comparison between groups was made by Mann-Whitney-U-test (nonparametric test for skewed data). Pearson correlation was done to see the level of significance between variables. For all tests, a P-value <0.05 was considered statistically significant.

3. Results

3.1 Increased frequency of $CD4^+CD25^+FoxP3^+$ Treg cells in the peripheral blood of psoriasis patients

To examine whether Treg cells have a role in disease pathogenesis in psoriasis, we first analyzed the expression of the FoxP3 in $CD4^+CD25^+$ T cell proportions in psoriatic patients and then compared it with healthy controls. Interestingly there was a higher frequency of circulating $CD4^+CD25^+FoxP3^+$Treg cells in psoriasis patients compared to healthy controls (10.87 ± 9.18 Vs. 5.41 ± 7.71, P= 0.02) (Figure 1a, Supplementary Table 1). Furthermore, we analyzed the association between $CD4^+CD25^+FoxP3^+$Treg cells with disease severity and disease duration. The frequency of $CD4^+CD25^+FoxP3^+$Treg cells was positively correlated with disease severity (Table 1) though it was not statistically significant. These data focused on the possibility that, instead of limiting the inflammatory response, $CD4^+CD25^+FoxP3^+$Treg cells could play a vital role in disease pathogenesis in psoriasis.

3.2 CD127 expression increased in $CD4^+CD25^+FoxP3^+$ Treg population in psoriasis patients

Given the discovery of greater frequency of $CD4^+CD25^+FoxP3^+$ Treg cells in psoriasis patients, we approach to determine whether expression of CD127 on these cells can identify a subpopulation Treg cells. Interestingly peripherally active $CD4^+CD25^+FoxP3^+CD127^+$ dysfunctional Treg cells were increased in psoriasis patients when compared to healthy controls (51.76 ± 19.58 Vs. 30.09 ± 23.13, P<0.001) (Figure 1b, Supplementary Table 1). Whereas conventional $CD4^+$ $CD25^+FoxP3^+CD127^-$ cells were more prevalent Treg population in healthy controls (Figure 1c, Supplementary Table 1). These findings suggest that the increased frequency of circulatory Treg cells in psoriasis patients represents the peripherally active $CD4^+CD25^+FoxP3^+$T cells. Thus, it is likely that inappropriate immune response due to both qualitative and quantitative defects of conventional Treg cells leads to chronic inflammatory events in psoriasis.

3.3 Correlation of $CD4^+CD25^+FoxP3^+CD127^+$/pTreg $CD4^+CD25^+FoxP3^+CD127^-$/tTreg cells of psoriatic patients with PASI and disease duration

We further analyzed the correlation of $CD4^+CD25^+FoxP3^+CD127^+$ and $CD4^+CD25^+FoxP3^+CD127^-$ Treg cells with disease severity and disease duration. In our study,

we observed conventional $CD4^+CD25^+FoxP3^+CD127^-$/tTreg cells tended to correlate with disease severity and duration. However, $CD4^+CD25^+FoxP3^+CD127^+$/pTreg cells were inversely correlated with disease severity and disease duration. But no statistical significance was found, P> 0.05 (Table 1).

3.4 Expression of CD127 on $CD4^+CD25^+FoxP3^+$ T-reg cells from peripheral blood of a psoriatic patient with PASI score

While analyzing PBMCs for expression of CD127 on $CD4^+CD25^+FoxP3^+$ Treg cells from peripheral blood of psoriatic patients, we observed its variation along with PASI. We found the expression of FoxP3 was 34.9% in a patient with a PASI score of 7.2, and CD127 expression was 77.2% (Figure 2c and 2d). These $CD4^+CD25^+FoxP3^+CD127^+$ cells represent pTregs (peripherally active dysfunctional Treg cells). In another patient with a PASI score, 27.2 dot plot diagram showed FoxP3 expression 8.1%, and of it, 93.1% was expressing CD127 (Figure 3c and 3d). So, in psoriasis patients, tTreg cells increase in milder cases but decline with severity, and most Treg cells are dysfunctional pTreg cells. It indicates decreasing trends of self-tolerance, thus supporting the autoimmune disease model.

3.5 Correlation of serum IL-17 level and $CD4^+CD25^+FoxP3^+CD127^-$/tTreg cells in psoriasis patients

As IL-17 is thought to be the prime driver of psoriatic pathogenesis, we further estimated the serum IL17 level of study subjects. As expected, the IL-17 level was elevated in psoriatic patients. Interestingly we found both $CD4^+CD25^+FoxP3^+$Treg and $CD4^+CD25^+FoxP3^+CD127^-$/tTreg cells were inversely correlated with serum IL-17 levels in psoriasis patients (Figure 4a and 4b). In contrast, $CD4^+CD25^+FoxP3^+CD127^+$/pTreg cells were correlated with serum IL-17 levels in psoriasis patients (Figure 4c). Though none of it was statistically significant (P> 0.05).

3.6 Treg cells and serum IL-17 level in psoriasis patient with AA genotype of IL23R rs10889677 gene

We further do genetic analysis to see if Treg cells and cytokine show any variation. We found 65.7% of psoriatic patients had AA genotype of IL23R rs10889677 gene. Analyzing the Flowcytometric and cytokine study, we see increased FoxP3 and CD127 expression in moderate to severe psoriasis patients with AA genotype of IL23R rs10889677 gene compared to a milder one. FoxP3 expression also increases with disease duration in these patients. We also observed a notifiable change in serum IL-17 level in chronic cases in psoriasis patients with AA genotype of IL23R rs10889677 gene. (Table 2 and 3).

4. Discussion

Regulatory T cells (Treg) are central to maintaining self-tolerance and tissue homeostasis. Quantitative and functional impairments and conversion into effector cells in response to inflammation of Treg cells in psoriasis have been reported. But discrepancies are seen in literature as the choice of study participants, technical challenges associated with investigating this cell population, and the methodology used to define and isolate putative regulatory cells to assess their suppressive function. That's why the role of central and peripheral Treg cells in psoriasis is still controversial. Recent studies showed that the expression of CD127 along with FoxP3 better distinguishes the functional status of Treg cells.

To examine whether activation and functional status of Treg cells have a role in chronic inflammatory events in psoriasis, we first analyze Treg cells in peripheral blood of psoriasis. In past years, it has been reported that the frequency of Treg cells remains unchanged in psoriasis [15]. We observed circulatory $CD4^{+}CD25^{+}FoxP3^{+}$ Treg cells elevated and correlated with PASI in psoriasis patients. A study in China concluded a significant increase in Treg cell frequency among psoriasis patients [16]. This is by the results of this study. A study regarding this observed proportion of Treg cells in the control group was significantly higher than psoriasis patients [6.4% ± (5.4-7.6) vs. 4.1% (3.1 -5.8), P <0.001] [21]. Similar findings were also observed in a

study carried out in India [10]. That contradicts the observation of this study. But no significant correlation between the levels of T-reg cells and PASI was seen in psoriatics in the aforementioned studies, which strongly matches this study's findings. It has been hypothesized that increased T cells in psoriasis patients are accompanied by T-reg cell accumulation that correlates with disease severity. It was also reported that keratinocytes in the skin produce pro-inflammatory cytokine "thymic stromal lymphopoietin (TSLP)" to activate T-reg cells to contain skin inflammation locally and prevent its systemic spread [16,22]. This can be the cause of the increased population of T-reg cells in psoriasis patients in this study.

It has been demonstrated that FoxP3 act as a repressor on the CD127 promoter and likely contributes to the reduced expression of CD127 in Treg cells. $CD4^{+}CD127^{low/-}$ T cell subset is anergic and suppresses alloantigen responses in vitro. tTreg (thymic Treg) cells are $CD4^{+}CD25^{+}FoxP3^{+}CD127^{low/-}$ that express the highest level of Foxp3 that effectively suppress the proliferation of T effector cells ($CD4^{+}CD25^{-}$). Whereas pTreg cells express a high level of CD127 only upon activation with a low Fox P3 level. Thus, a state of low-to-absent levels of CD127 expression combined with surface staining for CD25 may better distinguish Treg cells from the non-suppressive activated cells [23-25]. It has been reported that CD127 is associated with various diseases like psoriasis, rheumatoid arthritis, idiopathic juvenile arthritis, spondyloarthritis, primary Sjogren's syndrome, colitis, type 1 diabetes, and multiple sclerosis. Ellis et al. reported upon full target engagement, anti-CD127 monoclonal antibodies effectively modulate the auto inflammatory activity of pathogenic T cells in diseased tissue [26].

So to assess the suppressive capacity of Treg cells, we further differentiated them by the expression of CD127. We observed the majority of the Treg cells of psoriatic patients were $CD127^{+}$. From the observation, we can conclude that though the quantity of Treg cells is increased, the most effective suppressor sub-population is a deficit in psoriasis patients. Recent works on different human and animal models provide further information that anti-CD127 mAb promotes antitumor activity against T-cell acute lymphoblastic leukemia, improves allergic airway inflammation, and is also effective in autoimmune encephalomyelitis and low CD127 expression is not an intrinsic characteristic of Treg cells [27-29]. Therefore, using therapies to augment the $CD4^{+}CD25^{+}FoxP3^{+}CD127^{-/low}$ population of Treg cells can be a potential treatment option for psoriasis patients.

Upon stimulation of IL-1, IL-6, and TGFβ, both FOXP3^{+}Treg and RORϒt^{+}Th17 lineage cell populations can be differentiated. But FOXP3 antagonizes RORϒt by directly binding with it, thus maintaining immune balance. In recent times, it has been found that Treg cells are particularly prone to differentiate into IL-17 producing cells in genetically susceptible persons. While the presence of IL-23 in the skin leading chronic inflammatory events and these Foxp3^{+}IL-17^{+} Treg cells gradually lost the expression of Foxp3 and remained the true source of IL-17. Thus, despite immune suppression, they rather potentiate the chronic inflammatory cascades [17,30,31]. We also found IL-17 level in psoriasis patients increased with less FoxP3 expression in severe patients. Conversion of CD4^{+}FoxP3^{+} cells to IL-17^{+} cells might be the cause behind it.

To see the genetic basis with these cellular events in psoriasis in the Bangladeshi patient population, we further assess the IL23R rs10889677 gene polymorphism of study subjects. Genotypic distribution for the IL23R rs10889677 gene showed AA genotype does not appear to be protective in Bangladeshi patients with psoriasis. As expected, serum IL-17 level and CD127 expression in Treg cells were increased in these psoriasis patients. Elevated serum IL-17 level in psoriasis patients with AA genotype of this study may be due to IL23R rs10889677 gene polymorphism.

5. Conclusion

Psoriasis has been associated with the impaired suppressive function of Treg cells. This study further provides evidence to confirm the association of dysfunctional T-reg in the pathogenesis of psoriasis, underscoring the importance of CD127 as an activation marker. In genetically susceptible person, CD4^{+}CD25^{+}FoxP3^{+}CD127$^{low/-}$/tT-reg cell along with CD4^{+}CD25^{+}FoxP3^{+}CD127^{+}/pT-reg cell activation is also evident for its autoimmune disease model. This is the first evidence of IL23R gene polymorphism in psoriasis in the Bangladeshi patient population. Further research on a large scale should be done to provide more precise data.

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Conflict of interest

The authors have no conflicts of interest to declare.

Ethical approval and consent:

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by institutional review board (IRB) of Bangabandhu Sheikh Mujib Medical University (BSMMU). Written informed consent was obtained from the patients for research and publication of this study.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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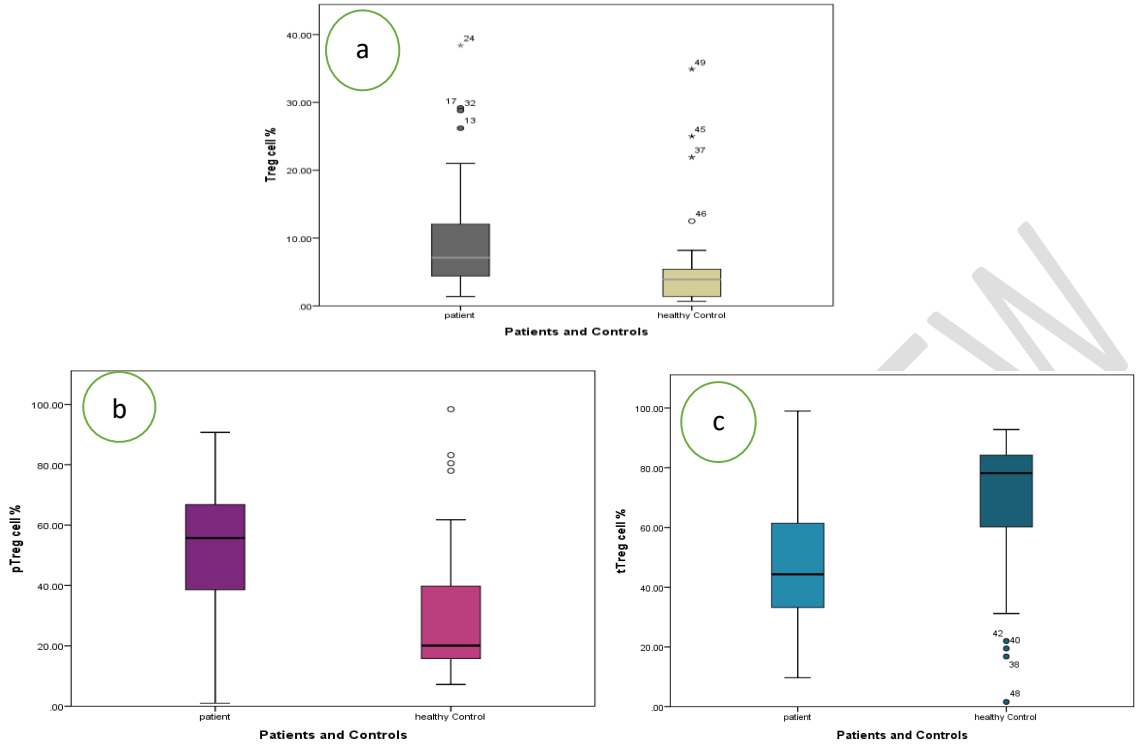

Figure 1: Comparison of percentage of circulating T regulatory cells in peripheral blood of study population (a) higher frequency of circulating $CD4^{+}CD25^{+}FoxP3^{+}$Treg cells in psoriasis patients compared to healthy controls (b) peripherally active dysfunctional $CD4^{+}CD25^{+}FoxP3^{+}CD127^{+}$/pTreg cells increased in psoriasis patients when compared to healthy controls (c) Conventional $CD4^{+}CD25^{+}FoxP3^{+}CD127^{-}$/tTreg cell cells were more prevalent Treg population in healthy controls. Dark line within each box indicates the median value. Fifty percent of the data lied inside the box. Line/whisker extended from box indicates distribution of data. Indicators along this line with number indicates outliers (abnormally distributed data.

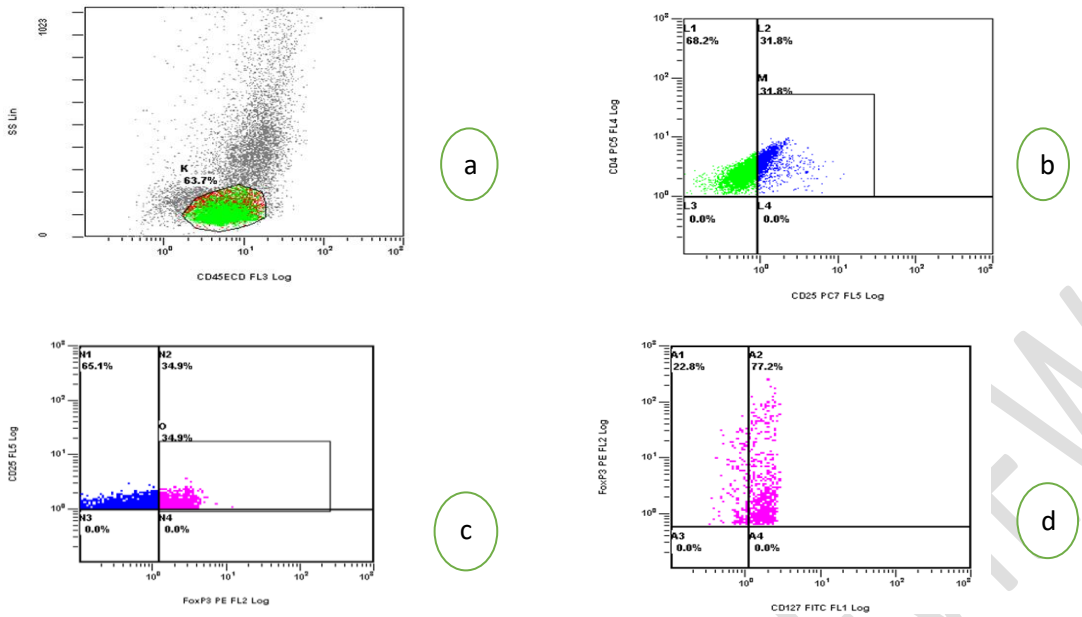

Figure 2: Expression of CD127 on $CD4^{+}CD25^{+}FoxP3^{+}$ T-reg cell from peripheral blood of psoriatic patient with a PASI score 7.2. PBMCs were firstly stimulated by PMA in the presence of Golgi plug protein transport inhibitor and analyzed for CD4, CD25, FoxP3 and CD127 expression. Dot plots shows expression of marker as percentage of cells. (a) For analysis, the PBMCs were first gated on lymphocytes (based on forward and side light scatter). (b) Within this gate $CD4^{+}CD25^{+}$ cells were ascertained. (c) These double positive cells were further sub gated to see regulatory activity by intracellular expression of FoxP3. The gated cells are regulatory population of T cell. (d) Here all $FoxP3^{+}$ T regulatory cells representing pTreg cells (peripherally active dysfunctional Treg cells)

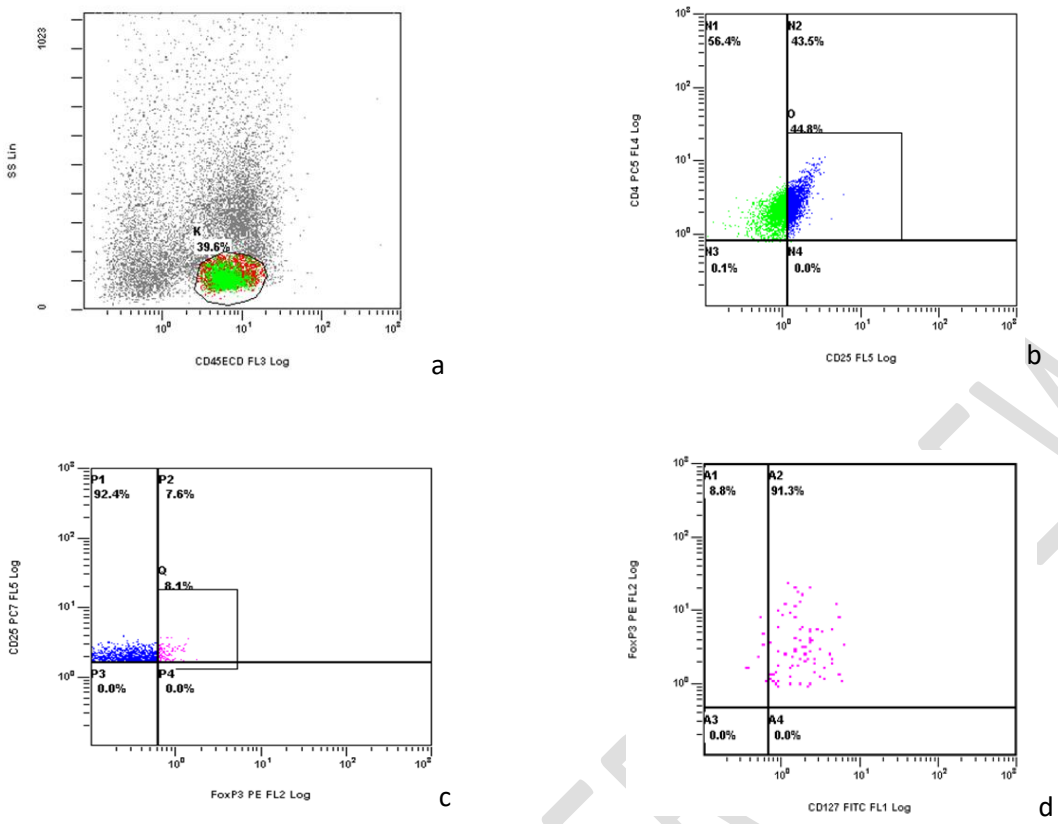

Figure 3: Expression of CD127 on $CD4^+CD25^+FoxP3^+$ T-reg cell from peripheral blood of psoriatic patient with a PASI score 27.2. (a) For analysis, the PBMCs were first gated on lymphocytes (based on forward and side light scatter). (b) Within this gate $CD4^+CD25^+$ cells were ascertained. (c) About 8.1% cells expressing FoxP3, that is a low FoxP3 expression. (d) 91.3% of $CD4^+CD25^+FoxP3^+$ regulatory cells are expressing CD127.

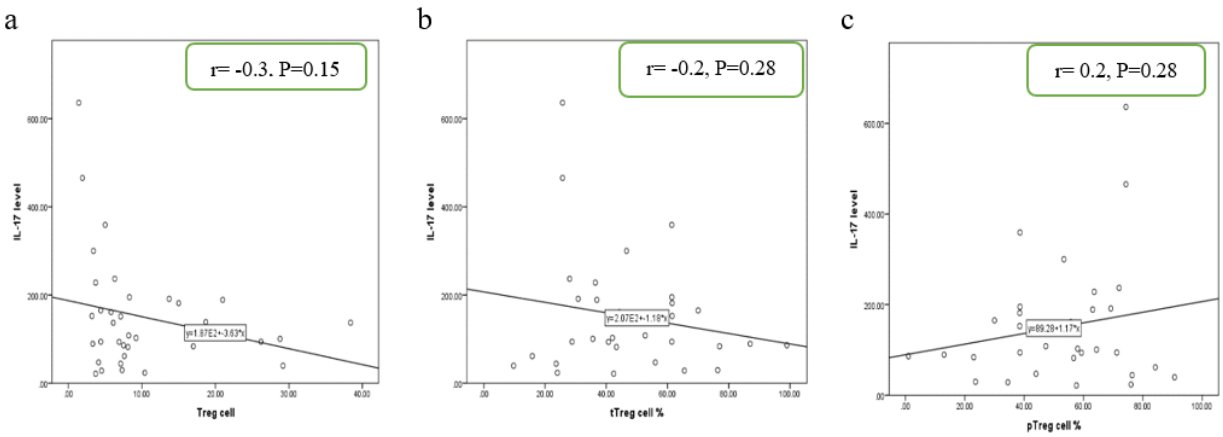

Figure 4: Correlation of serum IL-17 level with circulating Treg cells in psoriasis patients. Treg cells and tTreg cells were inversely correlated with serum IL-17 level. pTreg cells were positively correlated with serum IL-17 level. Correlation was assessed by Pearson correlation test. 'r' value without any sign indicates positive correlation and" -" indicates inverse correlation. Value > 0.7-1 indicates strong correlation.

Table 1: Correlation of PASI and disease duration of psoriasis patients with circulating Treg cells (n = 35), Related to figure 2.

	PASI		Duration of diseases	
	r value	**P value**	**r value**	**P value**
Treg cell	**0.15**	0.47	-0.09	0.61
tT-reg cell	**0.07**	0.69	**0.06**	0.72
pT-reg cell	-0.07	0.69	-0.06	0.71

Pearson correlation was done to see the level of significance (r value without any sign indicates positive correlation and "-" indicates inverse correlation. Value > 0.7-1 indicates strong correlation).

Table 2: Distribution of regulatory T cells and serum IL-17 level in psoriasis patients with AA genotype of IL23R rs10889677 gene according to PASI (n=23).

	PASI <10 (n= 5)	PASI >10 (n= 18)	P value
	Mean ± SD	Mean ± SD	
Treg cell (%)	4.52 ± 2.28	11.97 ± 9.07	0.80[a]
tTreg cell (%)	51.70 ± 18.91	46.92 ±18.44	**0.005[a]**
pTreg cell (%)	48.30 ± 18.91	53.10 ±18.49	0.63[a]
IL-17 (pg/ml)	174.32 ± 262.27	145.72 ± 80.39	0.68[b]

P value was calculated by [a] independent sample t test and [b] Mann-Whitney U-test. P value <0.05 was considered statistically significant.

Note:

P ASI range = 3.70- 27.2.
PASI ≤10 = Mild disease, PASI>10- 72 = Moderate to severe disease.

Table 3: Distribution of regulatory T cells and serum IL-17 level in in psoriasis patients with AA genotype of IL23R rs10889677 gene according to disease duration (n=23).

	<10 Years (n= 5)	>10 Years (n= 18)	P value
	Mean ± SD	Mean ± SD	
Treg cell (%)	10.05 ± 8.72	11.46 ± 9.17	0.76[a]
tTreg cell (%)	47.83 ± 19.49	48.42 ±14.56	0.94[a]
pTreg cell (%)	52.19 ± 19.53	51.58 ±14.56	0.95[a]
IL-17	169.81 ± 142.02	222.33± 29.86	0.23[b]

P value was calculated by [a] independent sample t test and [b] Mann-Whitney U-test. P value <0.05 was considered statistically significant.

Note:
Disease duration = 6 month- 28 years.

Supplemental Tables:

Supplementary Table 1: Circulating Treg cell subsets in peripheral blood in study population, Related to figure 1.

T cell subtypes	Patients (n = 31) Mean of % ± SD	Healthy Controls (n = 27) Mean of % ± SD	P value
T-regulatory ($CD4^+CD25^+FoxP3^+$)	10.87 ± 9.18	5.41 ± 7.71	**0.02**
t T-regulatory ($CD4^+$ $CD25^+FoxP3^+CD127^-$)	48.25 ± 19.56	69.25 ± 22.96	< **0.0001**
p T-regulatory ($CD4^+$ $CD25^+FoxP3^+CD127^+$)	51.76 ± 19.58	30.09 ± 23.13	< **0.0001**

Note:
P value was calculated by an independent sample t test.
Cell Percentage (%) are from CD4+T lymphocytes.
Samples of 4 patients and 8 healthy controls were discarded due to lack of their quality.