Expression of CD127 suppresses T regulatory cells in psoriasis

ABSTRACT

Background: Psoriasis is a genetically regulated autoimmune skin disease, considered to be associated with 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 functional status of $CD4^+CD25^+FoxP3^+$Treg cells. The study aimed to demonstrate functional status of Treg cells in peripheral blood of psoriasis patient, to analyze their association with disease severity and duration, serum IL-17 level and IL-23R gene polymorphism to observe 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 level 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 AA genotype of IL23Rrs10889677 gene polymorphism, associated with increased Treg, tTreg, pTreg cells and IL-17 level and revealed susceptibility to psoriasis. **Conclusion:** Loss of tTreg function and pTreg cell activation in psoriasis is evident in this study which also supports autoimmune disease model. CD127 expression can better distinguish Treg cells and by augmenting $CD127^{-/low}$ population of Treg cells to restore suppressive activity by anti-CD127 mAb therapy can be a potential treatment option for psoriasis patients.

1. Introduction

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 exaggerated immune response by suppressing autoimmune reactivation and by regulating chronic inflammation [1]. Recent advancement with regard to T-cell lineage development pointed that functional and quantitative disruption of Treg cells can results 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 has to be evaluated in a fixed and permeabilized cell. To study the functional status fixed cells cannot be used [10]. Considering the fact researchers has 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 that of Foxp3 and the suppressive function of 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 condition (eg: IL-23), which in turn triggers IL-23/Th17 axis [7,13]. Thus, rather 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 genitically susceptible person, along with other T cells, T reg cells also increased in psoriasis patients both in skin and peripheral circulation and positively correlated with severity and duration [16]. Genome wide association have confirmed many HLA and non-HLA genes are related with psoriasis. It has been reported that IL23R rs10889677 gene polymorphism can affect mRNA stability, localization and translational efficacy leading to increase IL-17 level [17]. In severe psoriasis patients, Treg cells particularly prone to differentiate into IL-17 producing cells. These FoxP3/ IL-17 double positive cells gradually lost expression of FoxP3 and remain the prime source of IL-17 that create a feed forward inflammatory response to sustain chronic events in psoriasis [18]. These findings pointed researchers to focus on the actual role of Treg cells in psoriasis.

Still the role of central and peripheral Treg cell in psoriasis is controversial due to a little work on it. On global report 2016, WHO recommends research on new treatments should focus on options which can be applicable globally, 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 a way in 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 institutional review board. Written informed consent was obtained from the study subjects for research and publication of this study. Study subjects enrolled according to the Declaration of Helsinki (revised in 2013). A total 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 without 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 heparinized vial for isolation of peripheral blood mononuclear cells (PBMCs), 3 ml in a tube without anticoagulant and separated serum was stored at $-20^{0}C$ 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 37^{0} C in the presence of 1µl Golgi plug protein transport inhibitor. Then 1µg BD Fc block added in cell media to prevent nonspecific binding of monoclonal antibodies. Cells were separated in two tube. 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^{TM} Plus solution for 20 minutes at 4º C. Then cells were washed for two times with wash buffer and pellet with final volume of 250 µl. Then 20 µl Foxp3 – PE added in both tube. Tube 1 represents CD4+CD25+FoxP3+/T-reg cells. Tube 2 represents

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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 tube were used for 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 were 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 manufacturer's instruction (Thermo Fisher Scientific GeneJET Genomic DNA Purification Kit #K0721, #K0722, USA). The genotyping for SNPs of IL-23R rs10889677 (A/C) performed using TaqMan 5´allele discrimination assay. Allele specific fluorogenic probes (Thermofisher Scientific) amplified the target genes in a system. Homozygous major and minor variants and heterozygous minor variant genotypes was 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 were done by Mann-Whitney-U-test (nonparametric test for skewed data). Pearson correlation was done to see the level of significance between variables. For all test a P value <0.05 was considered as 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 expression of the FoxP3 in CD4$^+$CD25$^+$ T cell proportions in psoriatic patients and then compare 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 were positively correlated with disease severity (Table 1) though it was not statistically significant. These data focused 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 subpopulation of 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, increased frequency of circulatory Treg cells in psoriasis patient represent 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 lead 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 were found, P> 0.05 (Table 1).

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

While analyzeing 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, expression of FoxP3 was 34.9% in a patient with PASI score 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 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 increases in milder cases but tends to decline with severity and most of 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 prime driver of psoriatic pathogenesis we further estimated serum IL17 level of study subjects. As expected, 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 level in psoriasis patients (Figure 4a and 4b). Whereas $CD4^+CD25^+FoxP3^+CD127^+$/pTreg cells were correlated with serum IL-17 level in psoriasis patients (Figure 4c). Though none of it were 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 with it. We found 65.7% psoriatic patients had AA genotype of 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 of IL23R rs10889677 gene comparing to 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 of IL23R rs10889677 gene. (Table 2 and 3).

4. Discussion

Regulatory T cells (Treg) are central to the maintenance of self-tolerance and tissue homeostasis. Quantitative and functional impairments along with conversion into effector cells in response to inflammation of Treg cells in psoriasis has been reported. But discrepancies is seen in literatures as the choice of study participants, technical challenges associated with investigating this cell population and also in terms of 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 cell in psoriasis is still controversial. Recent studies showed that expression of CD127 along with FoxP3 better distinguish the functional status of Treg cells.

To examine whether activation and functional status of Treg cells have role in chronic inflammatory events in psoriasis, we first analyze Treg cell in peripheral blood of psoriasis. In past years, it has been reported that the frequency of Treg cells remain unchanged in psoriasis [15]. We observed circulatory $CD4^+CD25^+FoxP3^+$ Treg cells elevated and correlated with PASI

in psoriasis patients. A study carried out in China concluded that a significant increase in Treg cell frequency among psoriasis patients [16]. This is in accordance with 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 aforementioned studies, which strongly match with findings of this study. It has been hypothesized that increase T cells in psoriasis patients are accompanied by T-reg cell accumulation that correlate with disease severity. It was also reported that, keratinocytes in 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]. Which can be the cause of increased population of T-reg cells in psoriasis patients in this study.

It has been demonstrated that FoxP3 act as a repressor on 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 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 disease 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 antibody 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 majority of the Treg cells of psoriatic patients were $CD127^+$. From the observation, we can conclude that though the quantity of Treg cell is increased but the most effective suppressor sub-population is deficit in psoriasis patients. Recent works on different human and animal model provide further information that anti-CD127 mAb promotes antitumor activity against T-cell acute lymphoblastic leukemia, improves allergic airway

inflammation and also effective in autoimmune encephalomyelitis and low CD127 expression is not an intrinsic characteristic of Treg cells [27-29]. Therefore, using therapies aimed at augmenting the $CD4^+CD25^+FoxP3^+CD127^{-/low}$ population of Treg cells can be a potential treatment option for the psoriasis patients.

Upon stimulation of IL-1, IL-6 and TGFβ both $FOXP3^+$Treg and $RORϒt^+$Th17 lineage cell population can be differentiated. But FOXP3 antagonizes RORϒt by directly binding with it, thus maintaining immune balance. In recent times it has been found that in genetically susceptible person Treg cells are particularly prone to differentiate into IL-17 producing cells. While presence of IL-23 in skin leading chronic inflammatory events and these $Foxp3^+IL-17^+$ Treg cells gradually lost expression of Foxp3 and remain the true source of IL-17. Thus, in spite of 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 Bangladeshi patient population we further assess IL23R rs10889677 gene polymorphism of study subjects. Genotypic distribution for IL23R rs10889677 gene showed AA genotype do 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 considered to be associated with impaired suppressive function of Treg cells. This study further provides evidence to confirm the association of dysfunctional T-reg in pathogenesis of psoriasis underscoring the importance of CD127 as activation marker. In genitically 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 Bangladeshi patient population. Further research on large scale should be done to provide more precise data of it.

Ethical approval

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.

References

1. Rouse BT. Regulatory T cells in health and disease. J Intern Med 2007;262:78-95.

2. Zhou X, Bailey-Bucktrout SL, Jeker LT, Penaranda C, Martinez-Llordella M, Ashby M, *et al*. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. Nat Immunol 2009;10:1000-7.

3. Voo KS, Wang YH, Santori FR, Boggiano C, Wang YH, Arima K, *et al.* Identification of IL-17-producing FOXP3+ regulatory T cells in humans. PNAS USA 2009;106:4793-8

4. Beriou G, Costantino CM, Ashley CW, Yang L, Kuchroo VK, Baecher-Allan C, *et al*. IL-17–producing human peripheral regulatory T cells retain suppressive function. Blood 2009; 113:4240-49.

5. Ayyoub M, Deknuydt F, Raimbaud I, Dousset C, Leveque L, Bioley G, *et al*. Human memory FOXP3+ Tregs secrete IL-17 ex vivo and constitutively express the TH17 lineage-specific transcription factor RORγt. PNAS USA 2009;106:8635-40.

6. Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP GJ, *et al*. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. Immunity 2008; 29:44-56.

7. Koenen HJ, Smeets RL, Vink PM, Van Rijssen E, Boots AM, Joosten I. Human CD25highFoxp3pos regulatory T cells differentiate into IL-17–producing cells. Blood, The Journal of the American Society of Hematology 2008;112:2340-52.

8. Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. CD4+ CD25high regulatory cells in human peripheral blood. J Immunol 2001;167:1245-53.

9. Takahashi T, Kuniyasu Y, Toda M, Sakaguchi N, Itoh M, Iwata M, *et al*. Immunologic self-tolerance maintained by CD25+ CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. Int Immunol 1998;10:1969-80.

10. Zheng Y, Rudensky AY. Foxp3 in control of the regulatory T cell lineage. Nat Immunol 2007; 8:457-62.

11. Liu W, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, Zhu S, *et al*. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. J Exp Med 2006;203:1701-11.

12. Seddiki N, Santner-Nanan B, Martinson J, Zaunders J, Sasson S, Landay A J, *et al*. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J Exp Med 2006;203:1693-00.

13. Yu N, Li X, Song W, Li D, Yu D, Zeng X *et al*. CD4+ CD25+ CD127 low/− T cells: a more specific Treg population in human peripheral blood. Inflamm 2012;35:1773-80.

14. Takeshita J, Grewal S, Langan SM, Mehta NN, Ogdie A, Van VoorheesAS, *et al*. Psoriasis and comorbid diseases: epidemiology. J Am Acad Dermatol 2017;76:377-90.

15. Sugiyama H, Gyulai R, Toichi E, Garaczi E, Shimada S, Stevens SR E *et al*. Dysfunctional blood and target tissue CD4+ CD25high regulatory T cells in psoriasis: mechanism underlying unrestrained pathogenic effector T cell proliferation. J Immunol 2005;174:164-73.

16. Zhang L, Yang XQ, Cheng J, Hui RS, Gao TW. Increased Th17 cells are accompanied by FoxP3+ Treg cell accumulation and correlated with psoriasis disease severity. J Clin Immunol 2010;135:108-17.

17. Safrany E, Szabo M, Szell M, Kemeny L, Sumegi K, Melegh BI, *et al*. Difference of interleukin-23 receptor gene haplotype variants in ulcerative colitis compared to Crohn's disease and psoriasis. Inflamm Res 2013; 62:195-200.

18. Bovenschen HJ, Van DeKerkhof PC, Van Erp PE, Woestenenk R, Joosten I, Koenen HJ, *et al*. Foxp3+ regulatory T cells of psoriasis patients easily differentiate into IL-17A-producing cells and are found in lesional skin. J Invest Dermatol 2011;131:1853-60.

19. Priyadarssini M, Divya Priya D, Indhumathi S, Rajappa M, Chandrashekar L, Thappa DM, *et al*. Immunophenotyping of T cells in the peripheral circulation in psoriasis. Br J Biomed Sci 2016;73:174-9.

20. Langley RG, Ellis CN. Evaluating psoriasis with psoriasis area and severity index, psoriasis global assessment, and lattice system physician's global assessment. J Am Acad Dermatol 2004;51:563-9.

21. Karamehic J, Zecevic L, Resic H, Jukic M, Jukic T, Ridjic O, *et al*. Immunophenotype lymphocyte of peripheral blood in patients with psoriasis. Medical Archives 2014; 68:236.

22. Kashiwagi M, Hosoi J, Lai JF, Brissette J, Ziegler SF, Morgan BA, *et al*. Direct control of regulatory T cells by keratinocytes. Nat Immunol 2017;18:334-43.

23. Jacob SE, Nassiri M, Kerdel FA, Vincek V. Simultaneous measurement of multiple Th1 and Th2 serum cytokines in psoriasis and correlation with disease severity. Med Inflamm 2003;12:309-13.

24. Liu W, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, Zhu S, *et al*. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. J Exp Med. 2006;203:1701-11.

25. Seddiki N, Santner-Nanan B, Martinson J, Zaunders J, Sasson S, Landay A, *et al*. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J Exp Med 2006;203:1693-00.

26. Ellis J, van Maurik A, Fortunato L, Gisbert S, Chen K, Schwartz A, *et al*. Anti-IL-7 receptor α monoclonal antibody (GSK2618960) in healthy subjects–a randomized, double-blind, placebo-controlled study. Br J Clin Pharmacol 2019;85:304-15.

27. Dunham J, Lee LF, van Driel N, Laman JD, Ni I, Zhai W, *et al*. Blockade of CD127 exerts a dichotomous clinical effect in marmoset experimental autoimmune encephalomyelitis. J Neuroimmune Pharmacol 2016;11:73-83.

28. Akkapeddi P, Fragoso R, Hixon JA, Ramalho AS, Oliveira ML, Carvalho T, *et al*. A fully human anti-IL-7Rα antibody promotes antitumor activity against T-cell acute lymphoblastic leukemia. Leukemia 2019; 33:2155-68.

29. Mai HL, Nguyen TV, Bouchaud G, Henrio K, Cheminant MA, Magnan AG, *et al*. Targeting the interleukin-7 receptor alpha by an anti-CD127 monoclonal antibody improves allergic airway inflammation in mice. Clin Exp Allergy 2020; 50:824-34.

30. Safrany antagonism and plasticity of regulatory and inflammatory T cell programs. Immunity 2008; 29:44-56.

31. Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, et al. TGF-β-induced Foxp3 inhibits TH 17 cell differentiation by antagonizing RORγt function. Nature 2008; 453:236-40.
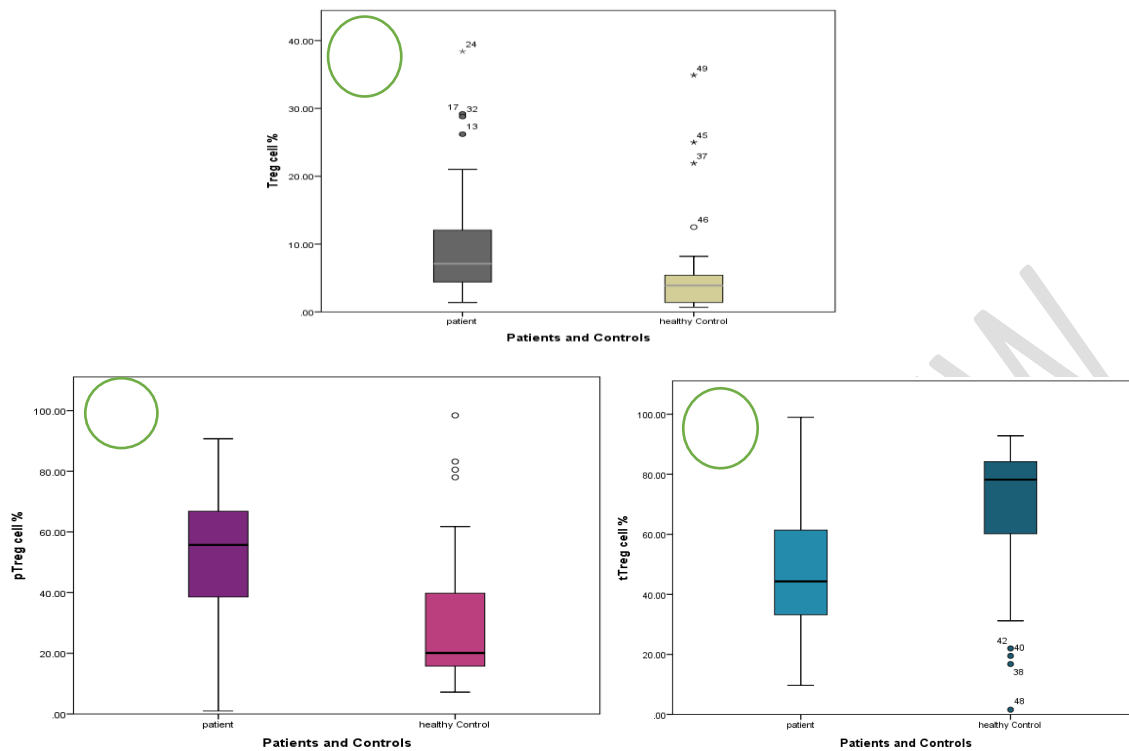
Figure 1: Comparison of percentage of circulating T regulatory cells in peripheral blood of study population (a) higher frequency of circulating CD4$^+$CD25$^+$FoxP3$^+$Treg cells in psoriasis patients compared to healthy controls (b) peripherally active dysfunctional CD4$^+$CD25$^+$FoxP3$^+$CD127$^+$/pTreg 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.
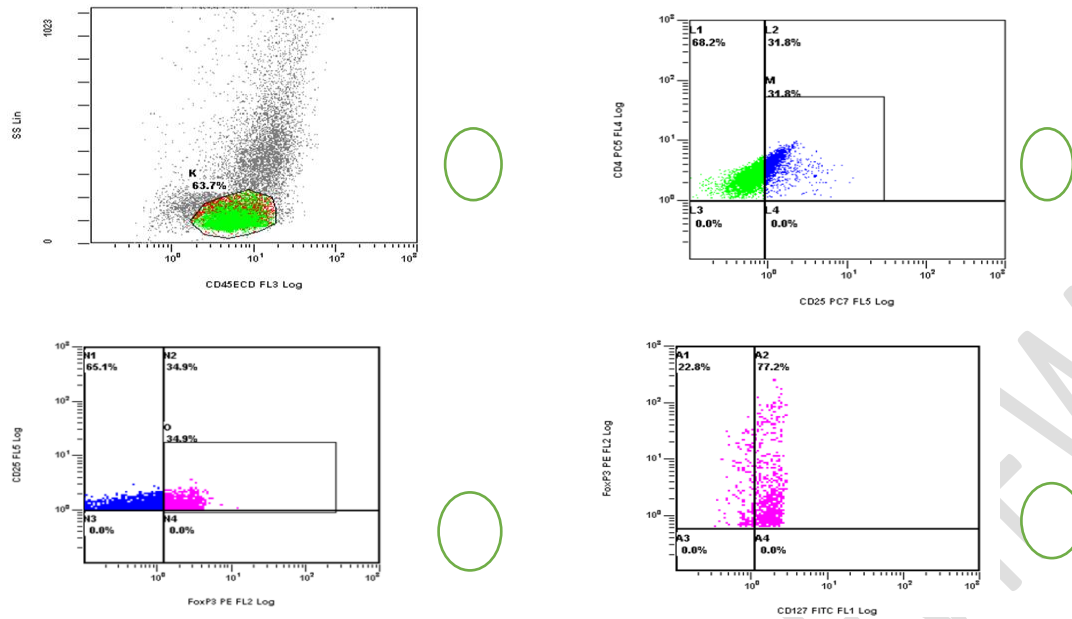
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Figure 2: Expression of CD127 on CD4$^+$CD25$^+$FoxP3$^+$ T-reg cell from peripheral blood of psoriatic patient with a PASI score 7.2. PBMCs were firstly stimulated by PMA in the presence of Golgi plug protein transport inhibitor and analyzed for CD4, 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)
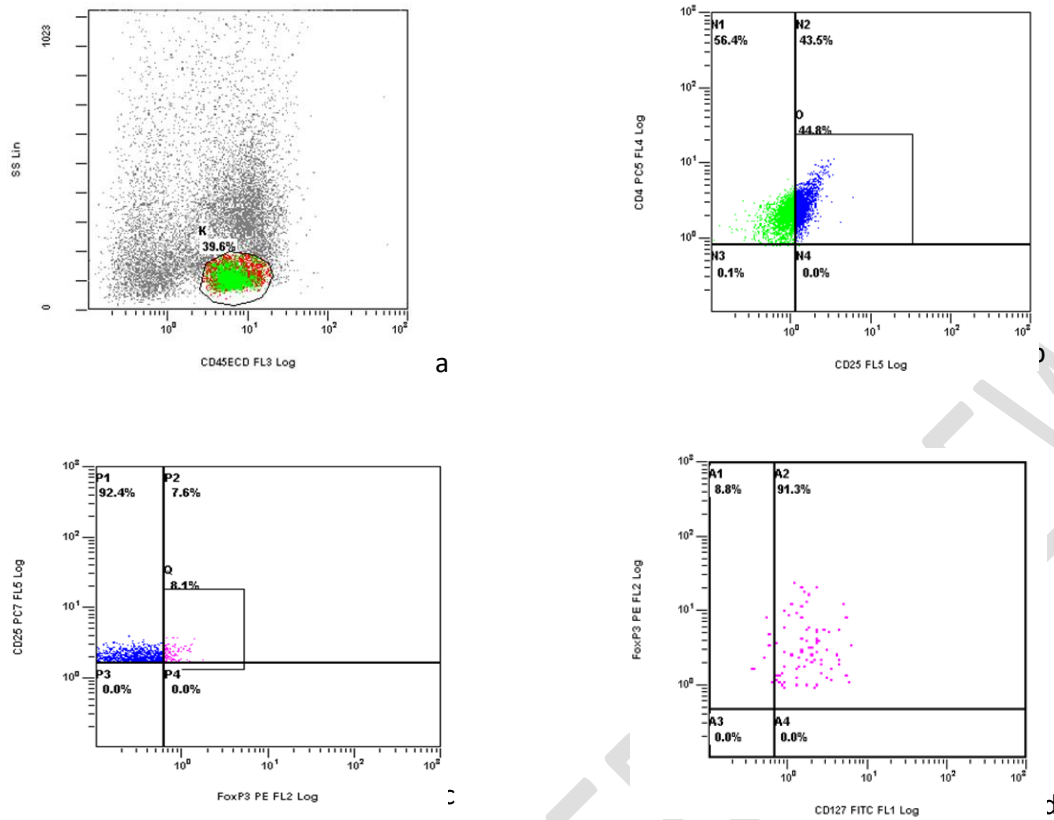
Figure 3: Expression of CD127 on CD4$^+$CD25$^+$FoxP3$^+$ T-reg cell from peripheral blood of psoriatic patient with a PASI score 27.2. (a) For analysis, the PBMCs were first gated on lymphocytes (based on forward and side light scatter). (b) Within this gate CD4$^+$CD25$^+$ cells were ascertained. (c) About 8.1% cells expressing FoxP3, that is a low FoxP3 expression. (d) 91.3% of CD4$^+$CD25$^+$FoxP3$^+$ regulatory cells are expressing CD127.
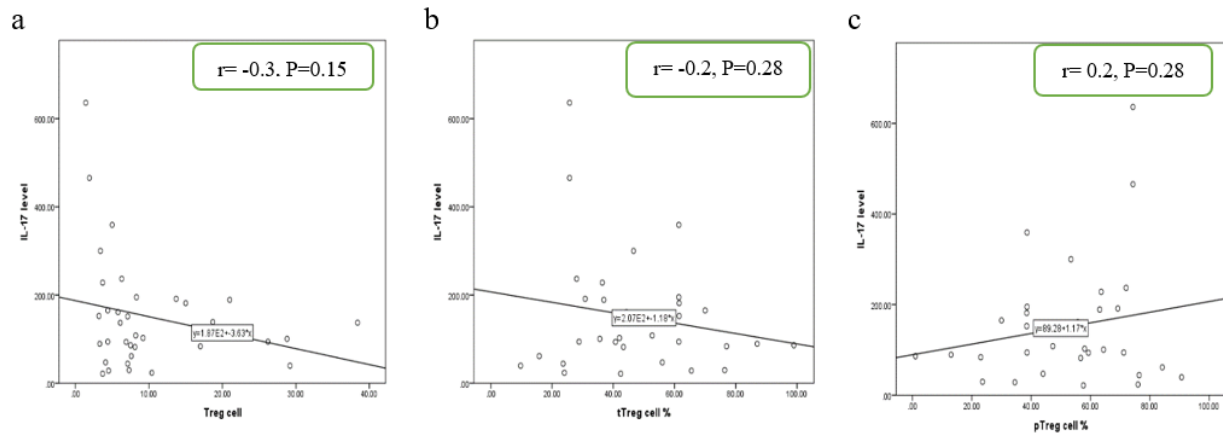
Figure 4: Correlation of serum IL-17 level with circulating Treg cells in psoriasis patients.
Treg cells and tTreg cells were inversely correlated with serum IL-17 level. pTreg cells were positively correlated with serum IL-17 level. Correlation was assessed by Pearson correlation test. 'r' value without 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.