INTRODUCTION

Over the past decade, much has been learned and much more to discover about regulatory T-cells (Treg cells) that develop from uncommitted (naive) CD4 T helper cells.[1] Disturbed effector functions of Treg cells can lead to (sometimes fatal) autoimmune and allergic inflammation.[2] It is now recognized that there are two major subsets of Treg cells, “natural” Treg (nTreg) cells that develop in the thymus (1%–10%) and “induced” Treg (iTreg) cells that arise in the periphery from CD4+ T-cells.[3] Both nTreg cells and iTreg cells have their own, nonredundant roles in the immune system, with nTreg cells mainly maintaining tolerance toward self-structures and iTreg developing in response to externally delivered antigens or commensal microbes.[4] The forkhead box protein lead to (sometimes fatal) autoimmune and allergic inflammation.[5] It is now recognized that there are two major subsets of Treg cells, “natural” Treg (nTreg) cells that develop in the thymus (1%–10%) and “induced” Treg (iTreg) cells that arise in the periphery from CD4+ T-cells.[3] Both nTreg cells and iTreg cells have their own, nonredundant roles in the immune system, with nTreg cells mainly maintaining tolerance toward self-structures and iTreg developing in response to externally delivered antigens or commensal microbes.[4] The forkhead box protein

Paucity of forkhead box protein 3+ regulatory T-cells in psoriatic skin compared to other inflammatory dermatoses

Marwa Zohdy, Laila Ahmed Sharaf, Samia E. Abdelnaby, Khaled Refaat Zalata, Hanan Fathy Mohamed

Department of Dermatology, Andrology and STDs, Mansoura University Hospitals, Mansoura, Egypt

ABSTRACT

Introduction: Forkhead box protein 3 (Foxp3+) regulatory T-cells (Treg cells) are essential to maintain balance between pro- and anti-inflammatory responses. They play a role in maintaining homeostasis by locally suppressing other skin-resident T-cells thus protecting against autoimmune reactions. Thus, they have been the focus of authors’ attention in skin T-cell-mediated diseases in the last decade. The Foxp3 gene encodes a transcription factor thought to be important for the function of Treg cells and represents a reliable marker. Contradictory results have been reported in literature about Treg cell densities in the skin of different inflammatory dermatoses including psoriasis, eczema, pityriasis lichenoides chronica (PLC), and cutaneous lupus erythematosus.

Patients and Methods: This was a cross-sectional study on lesional skin biopsies from 10 cases of psoriasis, 10 of spongiotic dermatitis, and 16 cases of lichenoid dermatoses (10 of PLC and 6 of discoid lupus erythematosus). We compared the densities of Foxp3+ Treg cells in relation to CD4+ cells in the epidermis and dermis between these groups using Foxp3 and CD4 monoclonal antibodies.

Results: Epidermal Foxp3+ CD4+ Treg cells were lower in psoriasis and lichenoid groups than spongiotic group and dermal Foxp3+ CD4+ Treg cells were lower in psoriasis than lichenoid and spongiotic groups.

Conclusions: Treg cells have been proved to suppress other skin-resident T-cells and prevent autoimmunity. Being an autoimmune inflammatory dermatosis, psoriasis showed an overall lower density of Foxp3+ CD4+ Treg cells than spongiotic or lichenoid dermatitis. This supports the theory of Treg cell consumption in psoriatic skin due to conversion to interleukin 17 producers.

Key words: Discoid lupus erythematosus, forkhead box protein 3, inflammatory dermatoses, pityriasis lichenoides, psoriasis, regulatory T-cell, spongiotic dermatitis

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Zohdy M, Sharaf LA, Abdelnaby SE, Zalata KR, Mohamed HF. Paucity of forkhead box protein 3+ regulatory T-cells in psoriatic skin compared to other inflammatory dermatoses. Indian J Dermatopathol Diagn Dermatol 2016;3:52-6.
3 (Foxp3) gene encodes a transcription factor thought to be important for the development and function of Treg cells and represents a reliable marker. Patients carrying rare loss-of-function mutations in the Foxp3 gene develop a range of autoimmune and inflammatory disorders referred to as immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, also known as X-linked autoimmunity and allergic dysregulation.[3] Transforming growth factor (TGF)-beta is needed for the Foxp3 expression in Treg cells.[1] Numerous mechanisms are employed by Treg cells to mediate their function including contact-mediated suppression that dampens the immunostimulatory properties of dendritic cells (DCs), metabolic disruption of effector T-cells, and secretion of inhibitory cytokines such as interleukin (IL)-10, IL-35, and TGF-β1, which inhibit both T-cells and DCs.[6] Resident Treg cells represent between 5% and 10% of the total resident skin T-cells. Under normal conditions, the function and homeostasis of human skin-resident memory Treg cells remain uncharacterized. Programed trafficking pathways that promote T-cell-mediated allergic inflammation are also used by Treg cells.[7] In psoriasis, Treg cells can differentiate toward IL17-expressing Treg cells. The combination of psoriatic Treg cells dysfunction and a propensity for differentiating into IL17-producing regulatory cells contributes to the perpetuation of chronic autoimmunity.[8] nTreg cells are responsible for the downregulation of eczema in allergic patients.[9] The role of Treg cells in atopic dermatitis is still unclear. Several pathways allow Treg cells to control and modify the development of allergic reactions in atopic patients. They block the migration of effector T-cells into inflamed tissue, suppress the production of IgE, induce IgG4 in B-cells, and limit Th17-mediated inflammation.[10] In some lichenoid tissue reactions such as pityriasis lichenoides chronica (PLC), Treg cells may play an important role in controlling clonally restricted CD4+ T-cell proliferations.[11] Among several factors involved in the pathogenesis of cutaneous lupus erythematosus (CLE), decreased number of Treg cells at the site of inflammation and increased expression of pro-inflammatory cytokines such as TNF-α were recently hypothesized.[12] This study was done to compare the densities of Treg cells between psoriatic, spongiotic, and some lichenoid dermatoses (PLC and discoid lupus erythematosus [DLE]) aiming to clarify Treg cell role in their pathogenesis.

**PATIENTS AND METHODS**

This study was carried out as a comparative cross-sectional trial on lesional biopsies from 36 patients with inflammatory dermatoses divided into 10 patients with psoriasis, 10 patients with eczema, and 16 patients with lichenoid dermatoses (10 with pityriasis lichenoides and 6 with discoid lupus erythematosus). Patients were selected from Dermatology outpatient clinic and biopsied in Dermatopathology laboratory at Dermatology, Andrology and STDs Department, Mansoura University Hospital, Mansoura, Egypt, from February 2012 to November 2014. Oral consent was taken from all patients. Patients with psoriasis were either new cases or those who stopped topical treatment for at least 4 weeks or stopped systemic therapy (PUVA, methotrexate, cyclosporine, and retinoids) for at least 12 months. Patients with eczema, pityriasis lichenoides, and DLE were selected as newly diagnosed cases. Patients with concurrent autoimmune disease or any other systemic disease with immune dysregulation that may alter Treg cells were excluded from the study. Skin punch biopsies were fixed with 10% formaldehyde and paraffin-embedded sections and stained with hematoxylin and eosin (H and E) and examined. Prior to immunohistochemistry, antigen retrieval process was done by placing the 3 µm cut skin specimens on positively charged slides. Specimens were then deparaffinized, and rehydrated skin sections were rated through a series of xylenes and graded alcohols. Specimens were then quenched for endogenous peroxidase in 3% H₂O₂ for 5 min. After that, the slides were placed in a vegetable steamer in Dako’s target retrieval solution. All skin specimens were stained with anti-CD4 antibody (mouse monoclonal anti-CD4, clone 4B12, dilution 1:30; Genemed, CA, USA) and anti-Foxp3 mAb (FoxP3 mAb, clone NB100-39002, dilution 1:400; Novus, Missouri, USA) to define Treg cells. Quantification of the frequency of immune-stained cells in the epidermis and upper dermis was performed in single-stained sections. In each run, negative and positive controls were applied. The negative control was a section from the tested case but lacking the Ab. Positive control was taken from tonsil and lymph node tissues. CD4 expressed a membranous reaction, while in case of Foxp3, the applied clone expressed nuclear and/or cytoplasmic reactions. The slides stained with CD4 and Foxp3 were examined for each case in one session in association with the H and E stained slides. The specifically stained lymphocytes were calculated in three different high-power fields (×40), and then the number was recorded both in the epidermis and upper dermis separately. The mean number of each specifically immune-stained lymphocytes (CD4+ and Foxp3+) was calculated by dividing the sum in the three fields by 3. CD4 was considered as the reference
standard for which a ratio for Foxp3+ was calculated. Divided numbers were approximated for the highest value. Data were entered and statistically analyzed using the SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc. Qualitative data were described as numbers and percentages. Chi-square test was used for comparison between groups. Quantitative data were described as medians after testing normality by Kolmogorov–Smirnov test. Kruskal–Wallis test was used for comparison between several groups.

RESULTS

This comparative study included 16 male and 20 female patients with age range at presentation from 2 to 75 years (median = 28). They were clinically diagnosed as follows: Ten patients with psoriasis (four cases with psoriasis vulgaris, four with guttate, and two cases with erythrodermic psoriasis), ten patients with eczema (five atopic, four contact, and one seborrheic dermatitis), ten patients with PLC, and six with discoid lupus erythematosus. In the terms of histologic findings, within the 36 biopsies, there were 10 cases with typical pathologic features of psoriasis (hyperkeratosis, acanthosis, regular elongation of rete ridges, and diminished granular cell layer), 10 cases with variable features of spongiotic dermatitis (intraepidermal vesiculation, serum crusts, lymphocytic exocytosis, and collections of Langerhans cell microvesicles within the epidermis), and 16 cases of lichenoid interface changes with occasional extravasated erythrocytes in 10 cases of PLC and variable epidermal atrophy with follicular plugging in 6 cases of DLE. Comparing the densities of Treg cells between the three groups, a marked statistically significant difference was observed in Foxp3+/CD4+ ratios between psoriatic, spongiotic, and lichenoid dermatitis subgroups in the epidermal and dermal Foxp3+/CD4+ ratios (P = 0.004 and <0.001, respectively) [Table 1]. The ratios were lower in psoriasis and lichenoid groups than spongiotic dermatitis group in the epidermis and lower in psoriasis group than both lichenoid and spongiotic groups in the dermis [Figures 1-4].

Table 1: Comparison of forkhead box protein 3+/CD4+ Treg cell ratios between the studied groups

| Group       | Psoriasis (n=10) | Spongiotic (n=10) | Lichenoid (n=16) | Kruskal–Wallis test P value |
|-------------|------------------|-------------------|------------------|---------------------------|
| Median ratio (%) Epidermal  | 50<sup>a</sup> | 100<sup>a,b</sup> | 50<sup>b</sup> | 0.004                     |
| Foxp3/CD4 Epidermal  | 53<sup>a</sup> | 100<sup>a,c</sup> | 82<sup>b,c</sup> | <0.001                   |
| Dermal Foxp3/CD4    | 53<sup>a</sup> | 100<sup>a</sup> | 82<sup>b</sup> | <0.001                   |

<sup>a,b,c</sup> Chi square= 17.34 for the P=0.001; <sup>a</sup> Similar letters indicate significant differences. Foxp3: Forkhead box protein 3
DISCUSSION

Since the importance of Treg cells in the maintenance of peripheral immunologic tolerance has been well established,[2] we attempted to compare the densities of Treg cells in the skin of different dermatoses. Our results showed significantly lower epidermal Foxp3/CD4 ratios in psoriasis and lichenoid groups than spongiotic groups. This was consistent with the finding that Treg cells are defective in psoriasis[13] possibly due to their conversion to IL17-producing Treg cells,[8] and with the finding that the number of Foxp3+ Treg cells in CLE was significantly reduced.[14] Our results were discordant with a study that evaluated the percentage of epidermal Foxp3+ cells among CD3+, CD4+, CD8+, and CD25+ cell populations in skin biopsies of psoriasis (n = 16), eczematous dermatitis (n = 18) in addition to normal skin (n = 10). They denoted lower percentage of epidermal Foxp3+ cells in eczematous dermatitis than in psoriasis vulgaris and significantly lower dermal Foxp3+ cells in psoriasis vulgaris than in eczematous dermatitis (P < 0.05).[15] The previous study showed concordance with our results regarding dermal findings. The discrepancy in epidermal findings between the previous study results and ours could be attributed to the method of calculation of Foxp3+ Treg cells among CD3+, CD4+, CD8+, and CD25+ cells in addition to CD4+ cells. Moreover, it is known that activated CD8+CD25+ nTreg lymphocytes are thymus-derived cells that share phenotypic and functional characteristics of the CD4+ Treg cells[16] which could substantially increase the calculated number of Treg cells. Many other factors could explain the discrepancy, such as the difference in the pathological spectrum of the studied groups, as the previous study did not include lichenoid dermatoses among the studied groups; instead, it included normal skin as a control and the use of different antibodies to FoxP3 (rabbit versus mouse monoclonal antibodies).

CONCLUSIONS

Foxp3+ Treg cells reside in normal human skin and play an important role in preventing autoimmunity by locally suppressing other skin-resident inflammatory cells. Human memory phenotype FoxP3+ Treg cells have been very recently isolated from psoriatic skin and shown to be defective in psoriasis. Contradictory results were reported in literature about Treg cell numbers in lesional skin from atopic dermatitis though nTreg cells are thought to be responsible for the downregulation of eczema in allergic patients. In PLC, Treg cells may play an important role in controlling clonally restricted CD4+ T-cell proliferations. The number of Foxp3+ Treg cells in CLE was shown to be significantly reduced. Our study compared the densities of Foxp3+ Treg cells in relation to CD4+ cells in lesional skin biopsies from 10 cases of psoriasis, 10 cases of spongiotic dermatitis, and 16 cases of lichenoid dermatoses (10 with PLC and 6 with DLE). We found that Foxp3+ CD4+ Treg cells were lower in psoriasis than spongiotic group and dermal Foxp3+ CD4+ Treg cells were lower than lichenoid and spongiotic groups. We concluded that a lower density of Foxp3+ CD4+ cells in psoriasis could attribute to autoimmunity, perhaps due to their consumption as a result of conversion to IL17-producing cells.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Coomes SM, Pelly VS, Wilson MS. Plasticity within the αβ+CD4+ T-cell lineage: When, how and what for? Open Biol 2013;3:120157.
2. O’Connor RA, Taams LS, Anderton SM. Translational mini-review series on Th17 cells: CD4+ T helper cells: Functional plasticity and differential sensitivity to regulatory T cell-mediated regulation. Clin Exp Immunol 2010;159:137-47.
3. Dharmne C, Chung Y, Alousi AM, Cooper LJ, Tran DQ. Peripheral and thymic foxp3+ regulatory T-cells in search of origin, distinction, and function. Front Immunol 2013;4:253.
4. Lehtimäki S, Lahesmaa R. Regulatory T cells control immune responses through their non-redundant tissue specific features. Front Immunol 2013;4:294.
5. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy,
enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 2001;27:20-1.
6. Schmitt EG, Williams CB. Generation and function of induced regulatory T cells. Front Immunol 2013;4:152.
7. Clark RA. Skin-resident T cells: The ups and downs of on-site immunity. J Invest Dermatol 2010;130:362-70.
8. Bovenschen HJ, van de Kerkhof PC, van Erp PE, Woestenenk R, Joosten I, Koenen HJ. 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.
9. Vocanson M, Hennino A, Rozières A, Poyet G, Nicolas JF. Effector and regulatory mechanisms in allergic contact dermatitis. Allergy 2009;64:1699-714.
10. Palomares O, Yaman G, Azkur AK, Akkoc T, Akdis M, Akdis CA. Role of Treg in immune regulation of allergic diseases. Eur J Immunol 2010;40:1232-40.
11. Magro CM, Crowson AN, Morrison C, Li J. Pityriasis lichenoides chronica: Stratification by molecular and phenotypic profile. Hum Pathol 2007;38:479-90.
12. Kuhn A, Sontheimer RD. Cutaneous lupus erythematosus: Molecular and cellular basis of clinical findings. Curr Dir Autoimmun 2008;10:119-40.
13. Sanchez Rodriguez R, Pauli ML, Neuhaus IM, Yu SS, Arron ST, Harris HW, et al. Memory regulatory T cells reside in human skin. J Clin Invest 2014;124:1027-36.
14. Franz B, Fritzsching B, Riehl A, Oberle N, Klemke CD, Sykora J, et al. Low number of regulatory T cells in skin lesions of patients with cutaneous lupus erythematosus. Arthritis Rheum 2007;56:1910-20.
15. Fujimura T, Okuyama R, Ito Y, Aiba S. Profiles of Foxp3+ regulatory T cells in eczematous dermatitis, psoriasis vulgaris and mycosis fungoides. Br J Dermatol 2008;158:1256-63.
16. Dinesh RK, Skaggs BJ, La Cava A, Hahn BH, Singh RP. CD8+ Tregs in lupus, autoimmunity, and beyond. Autoimmun Rev 2010;9:560-8.