Original Research

Turk J Immunol 2024;12(2):71-6
DOI: 10.4274/tji.galenos.2024.85856

An Immunological and Molecular Study of Regulatory T-cell Activity in Iraqi Patients with Psoriasis Treated by Topical and Biological Therapy

Shawq Raafat Alnaqqash[1], Zainab Thamer Showait Al-Asady[2], Mohammed Mahdi Jawad[3]

[1]Mustansiriyah University College of Science, Department of Biology, Baghdad, Iraq
[2]Al-Karkh University of Science, College of Science, Department of Microbiology, Baghdad, Iraq
[3]University of Baghdad, College of Education for Pure Science (Ibn-al Haitham), Department of Biology, Baghdad, Iraq

Cite as: Alnaqqash SR, Showait Al-Asady ZT, Jawad MM. The Immunological and Molecular Study of Regulatory T-cell Activity in Iraqi Patients with Psoriasis Treated by Topical and Biological Therapy. Turk J Immunol. 2024;12(2):71-6

Received: 19.12.2023 **Accepted:** 04.07.2024

Corresponding Author: Shawq Raafat Alnaqqash, Mustansiriyah University College of Science, Department of Biology, Baghdad, Iraq
Phone: +964 773 825 89 04 **E-mail:** shawq.r.m@uomustansiriyah.edu.iq **ORCID:** orcid.org/0009-0006-8310-1932

Abstract

Objective: Transforming growth factor-beta (TGF-β), an anti-inflammatory cytokine, plays a crucial role in restraining the proliferation and differentiation of T effector cells in autoimmune diseases. Glycoprotein A repetitions predominant (GARP), belonging to the leucine-rich repeat protein family, functions in facilitating the presentation of TGF-β on the surface of regulatory T-cells. The study aims to evaluate the activity of regulatory T-cell in Iraqi patients with psoriasis, treated with topical and biological therapy considering the level of TGF-β1 and *LRRC32* gene (GARP) expression.

Materials and Methods: In this study 22 blood samples of psoriasis patients and 24 healthy samples as a control group were analyzed to measure the level of TGF-β1 by enzyme-linked immunosorbent assay and the gene expression level of the LRRC32 (*GARP* gene) by quantitative reverse transcription polymerase chain reaction technique.

Results: The results showed a significant decrease (p=0.004) in the level of TGF-β1 in the serum of patients with psoriasis treated with topical therapy and others treated with biological therapy compared to the control group. A significant increase (p=0.05) in the folding of *LRRC32* gene expression was detected in psoriasis patients compared to the control percentage (100%). Moreover, there was a significant increase (p=0.000) in the percentage of the gene expression folding of *LRRC32* gene in patients treated with biological therapy and patients treated with topical therapy compared to control percentage (100%). **Conclusions:** The high expression of the *LRRC32* gene in patient samples is an indication of the effective role of regulatory T-cells under biological and topical treatment.

Keywords: Psoriasis, *LRRC32* gene, GARP, TGF-β1, biological therapy, topical therapy, Treg

Introduction

Factor forkhead box P3 (FOXP3$^+$) regulatory T-cells (Tregs) can be generated during the development of T-cell in the thymus and are consequently known as thymus-derived Tregs (tTregs) or naturally occurring Tregs (nTregs) (1). It is characterized by the presence of surface biomarkers such as CD4$^+$, CD25$^+$, and expression of transcription FOXP3. These cells secrete anti-inflammatory cytokines, including interleukin-10 and transforming growth factor-beta (TGF-β) (2), to balance autoimmune responses and express types of markers such as FOXP3, CD25, CD127, HLA-DR, CD103, CD45RO/CD45RA, LAG-3, glycoprotein A repetitions predominant (GARP), CD39 and Helios (3).

GARP, also known as leucine rich repeat containing 32 (LRRC32), is a transmembrane protein receptor associated with latency associated peptide TGF-β (LTGF-β) on the surface of FOXP3 regulatory T-cell and on the human cancer cell to promote TGF-β activation (4). The human *LRRC32* gene is situated in the chromosomal region 11q13.5-14 (5) and consists of two coding exons: The initial one encodes the signal peptide and nine additional residues, the second exon which is considered the majority of the coding region

ORCID: S.R. Alnaqqash 0009-0006-8310-1932, Z.T. Showait Al-Asady 0000-0002-8442-9781, M.M. Jawad 0000-0003-4090-3055

encodes 20 repeats of the leucine-rich motif (2). Initially, 662 amino acid protein human product sequence analysis suggested that it was almost completely extracellular with 20LRR in the protein extracellular portion, followed by proteins hydrophobic stretch as a transmembrane domain, followed by a short (15 amino acids) cytoplasmic domain (6). It has been found that GARP expression has been detected in platelets and in activated ($FOXP3^+$) Treg to suppress effector cell activation (7), it has also been found that GARP is expressed in brain tumors (8). TGF-β is an essential regulator that plays a crucial role in cellular and physiologic processes, including proliferation, differentiation, angiogenesis, migration, cell survival, angiogenesis, and immunosurveillance (9). It inhibits effector T-cell proliferation and cytokines production and it also suppresses the differentiation of effector T-cell into T helper (Th)1 and Th17 cells (10). It is produced by lymphocytes, macrophages, and dendritic cells, and its expression serves to control immune cell differentiation, proliferation, and activation through autocrine and paracrine modes (11). Interestingly, numerous studies have shown that GARP plays a role in TGF-β activation by mediating both the surface expression and latent TGF-β integrin-mediated activation (12). GARP associates with latency-associated peptide to create an alternative cell surface for presenting LTGF-β and this demonstrates that GARP participates in the regulatory function of activated Treg as evidenced by reduced suppressive activity upon *GARP* gene silencing (12). In addition to GARP function, as a marker for Treg activation to regulate the bioavailability of TGF-β, the recent studies have shown that there is a strong connection between GARP protein and cancer due to the pro-tumorigenic function of this protein in several human malignancies such as breast cancer and oral squamous cell carcinoma (13). A study examined the mRNA expression of GARP in $CD4^+$ $CD25^+$ Tregs through a microarray screen, following T-cell receptor stimulation (14). This study observed the detection of GARP protein on the surface of activated polyclonal human $CD4^+CD25^+$ Tregs (14). In this study, we aimed to evaluate the activity of regulatory T-cell in Iraqi patients with psoriasis, who were treated with topical and biological therapy considering the level of (TGF-β1) and *LRRC32* gene (GARP) expression.

Materials and Methods

Blood Sample Collection

The current study included 46 samples taken from 22 Iraqi patients diagnosed with psoriasis and 24 healthy people as control samples, whose ages ranged from 18 to 72 years. The blood samples were collected from the patients for the period between August and November 2019 in Baghdad Teaching Hospital dermatology consultation clinic of the medical city Baghdad. The group of patients was divided into two subgroups, as those including 7 patients treated with salicylic acid, betnosam, dermovate and vaseline as a topical therapy and those including 15 patients treated with anti-tumour necrosis factor (TNF) (etanercept) 50 mg/weekly as a biological therapy. About 5 mL of venous blood was taken from the patients via a medical syringe, 4.5 mL of the blood was placed in a gel tube and separated by centrifuge 5000 rpm. The study approved by Ethics Committee of Baghdad University (approval number: 4624; date: 24.07.2019).

Measurement of TGF-β1 by ELISA

A sandwich enzyme-linked immunosorbent assay kit was used to measure TGF-β1 level according to the manufacturer's instruction (Elabscience, USA).

RNA Isolation and Real Time-qPCR

RNA Isolation

The concentration of the extracted RNA was detected by Quantus Florometer (Promega, USA) for revealing the quality of samples; 199 µL of diluted Quanty Flour Dye (Promega, USA) was mixed with 1 µL of RNA. After 5 min. incubation at room temperature and in a dark place, RNA concentration values were detected.

Detailed qPCR Method

RNA was isolated from samples according to the protocol of TRIzol™ (Thermo Scientific, USA) reagent by following those steps:

1. Sample Lysis

Blood Sample

For each tube, 0.4 mL of blood was added to 0.6 mL of TRIzol™ reagent, and the lysate was homogenized by pipetting up and down several times.

2. For Three Phase's Separation

• For each tube, 0.2 mL of chloroform was added to the lysate, then the tube cap was secured.

• All mixes were incubated for 2-3 minutes and then centrifuged for 10 minutes at 12.000 rpm, the mixture was separated into a lower organic phase, interphase, and a colorless upper aqueous phase.

• The aqueous phase containing the RNA was transferred to a new tube.

3. For RNA Precipitation

• 0.5 mL of isopropanol was added to the aqueous phase and incubated for 10 minutes and then centrifuged for 10 minutes at 12.000 rpm.

• Total RNA precipitate formed a white gel-like pellet at the bottom of the tube.

• Supernatant then discarded.

4. For RNA Washing

• For each tube, 0.5 mL of 70% ethanol was added and vortexed briefly, and then centrifuged for 5 minutes at 10.000 rpm.

• Ethanol was then aspirated and the pellet was air-dried.

5. For RNA Solubility

• Pellet was rehydrated in 100 µL of nuclease-free water and then incubated in a water bath or heat block set at 55-60°C for 10-15 minutes or overnight at 4°C.

The Gene Expression Level of the *LRRC32* Gene by RT-qPCR

The *LRRC32* gene was amplified by GoTaq® 1-Step RT-qPCR System (Promega, USA) in an RT-qPCR program including the reverse transcription step (37°C for 15 min.), initial denaturation step (95°C for 10 min.), denaturation step with (95°C for 20 sec.) annealing step (60°C for 20 sec.) and extension step with (72°C for 20 sec). The gene expression level of LRRC32 in psoriatic patients under different therapeutic modes was calculated according to the following equation and the gene expression in control represented as 100%:

ΔCT=CT gene-CT housekeeping gene

ΔΔCT (threshold cycle)=ΔCT Treated patients-ΔCT Control

Folding=$2^{-\Delta\Delta CT}$

Folding=Gene expression

CT=Threshold cycle

This is the calculations I used in the research

$$\text{Gene expression level (\%)} = \frac{\text{Folding of gene expression in patients group}}{\text{Folding of gene expression in control}} \times 100$$

(Relative expression, $2^{(-\Delta\Delta CT)}$)

Statistical Analysis

The statistical analysis of the results was performed by using a Statistical Package for the Social Sciences. A comparison was made between the different pathological groups with the control group and with each other in a One-Way ANOVA and the findings were considered statistically significant at a p-value of 0.05 or less. In the figures, the results were represented as mean ± standard error (post-hoc test). The t-test was used to detect the statistical differences between the arithmetic averages of the samples and the Pearson correlation coefficient was employed to determine the extent of the relationship between the studied parameters (15).

Results

The current study revealed a significant decrease (p=0.004) in the TGF-β1 levels in the serum of psoriasis patients treated with topical therapy and other patients treated with biological therapy compared to the control group, where the levels of TGF-β1 were 0.3 ± 0.05 and 0.4 ± 0.1 pg/mL, respectively, and its level in the control group was 0.6 ± 0.05 pg/mL and there was non-significant decreasing (p=0.410) in the patients treated with topical therapy compared to those treated with biological therapy. The results are shown in Figure 1.

With respect to age in psoriasis patients, the results showed no significant differences (p=0.763) in serum levels between patients under 40 and those over 40, with TGF-β1 levels of 0.4 ± 0.04 pg/mL and 0.3 ± 0.1 pg/mL, respectively.

Regarding gender, the frequency of male psoriasis patients was higher than that of females (63.3% male, 36.3% female). The results indicated no significant differences (p=0.441) in TGF-β1 levels between females and males, with levels of 0.3 ± 0.05 pg/mL and 0.4 ± 0.1 pg/mL, respectively. The results are shown in Table 1.

The results demonstrated significantly higher percentage (p=0.05) of *LRRC32* gene expression (1030.7%) in psoriasis patients compared to the control group (100%) (Figure 2).

Based on the type of therapy, there was a significant increase (p>0.001) in the percentage of *LRRC32* gene expression in psoriasis patients treated with biological therapy (1192.3%) and topical therapy (682.6%) compared to the control group (100%). However, there were no significant differences (p=0.103) between patients treated with biological therapy and those treated with topical therapy.

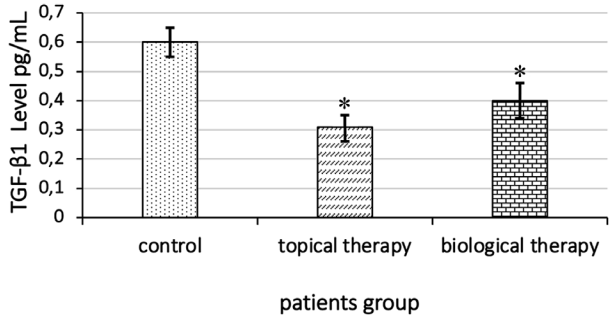

Figure 1. The levels of TGF-β1 pg/mL in psoriasis patients under topical therapy, biological therapy, and control group.
TGF-β: Transforming growth factor-beta
*The sign is referred to the significancy p≤0.05
p=0.004 between topical therapy, biological therapy compared to control group; p=0.410 between topical therapy compared to biological therapy

Table 1. The level of TGF-β1 and *LRRC32* gene expression according to age and gender

Disease	Clinical characteristics		Mean ± SE (TGF-β1)	p-value	Mean ± SE (LRRC32)	p-value
Psoriasis	Age	<40	0.4 ± 0.04	0.763	11.6 ± 2.5	0.453
		>40	0.3 ± 0.1		7.6 ± 4.7	
	Gender	Female	0.3 ± 0.05	0.441	13.9 ± 3.6	0.277
		Male	0.4 ± 0.1		8.9 ± 2.7	

SE: Standard error, TGF-β: Transforming growth factor-beta

With respect to age, the results indicated no significant differences (p=0.453) in *LRRC32* gene expression between patients younger than 40 and those older than 40 (11.6 ± 2.5 vs. 7.6 ± 4.7, respectively). Similarly, there were no significant differences (p=0.277) in LRRC32 gene expression between females and males (13.9 ± 3.6 vs. 8.9 ± 2.7, respectively), as shown in Table 1.

We have found a negative correlation (p=0.022) between the *LRRC32* gene and TGF-β1 expression in the topical therapy-treated patients (r=-0.446)* while there was a non-significant (p=0.193) negative correlation (r=-0.229) between the expression of these two genes as shown in Table 2.

Discussion

The results of this study showed a significant reduction in the level of TGF-β1 in the serum of psoriasis patients under treatment with topical therapy and those treated with biological therapy compared to the control group. There were non-significant differences in the level of TGF-β1 in female patients with psoriasis compared to males and also, there were non-significant differences in the patients younger than 40 years and older than 40 years in all treatment groups. According to the molecular detection, the results indicated a significant increase in the gene expression of *LRRC32* gene (GARP) in psoriasis patients treated with biological therapy than those who were treated with topical therapy. We have found a negative correlation between TGF-β1 level and its receptor on the Treg cell surface (all results should be given in the first paragraph).

Previous studies indicated that TNF-α amplified Treg populations, and had CD25 and FOXP3 up-regulated expression, expanding the suppressive activity of Tregs (16). The reduced level of TGF-β1 in the serum of patients in relation to etanercept binds to the pro-inflammatory cytokines such as TNF-α, inhibits its interaction with cell surface receptors and declines its action (please correct the sentence). The mechanism of the effect of these drugs on TGF-β1 cannot be determined only by measuring the TGF-β1 levels in the serum, but it is probably based on the association of the activity of T-cells and TNF-α. It appears that this cytokine may be helpful as a biomarker for monitoring biological-drug treatment of psoriasis (17),

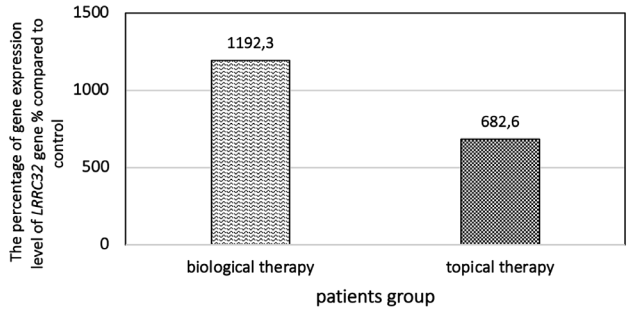

Figure 2. The percentage % of gene expression levels of *LRRC32* gene (GARP) in psoriasis patients according to the type of therapy.
GARP: Glycoprotein A repetitions predominant
p=0.000 between topical therapy and biological therapy compared to control group; p=0.103 between topical therapy compared to biological therapy. No significant differences

Table 2: Correlation coefficient between TGF-β1 and LRRC32 according to the type of therapy

TGF-β1 and *LRRC32* gene	Biological therapy	p-value	Topical therapy	p-value
	-0.229	0.193	-0.446*	0.022

TGF-β: Transforming growth factor-beta

which were in line with agent the results of our study in which anti-TNF-α agent (etanercept) blocks TNF-α and inhibits its binding to its receptors on other cells and those cells may be the source of TGF-β1 production and will lead to decrease its level in the serum. It was also shown (18) that active topical treatment (salicylic acid and/or sulfur followed by dithranol ointment) caused a significant reduction in plasma TGF-β1 levels only in patients with more severe cases.

A previous study clarified the potential role of TGF-β1in the pathogenesis of psoriasis pretreatment, which is associated with the inhibition of T-cell's adhesiveness to dermal microvascular endothelial cells, so the decrease in the expression of TGF-β1and its function may contribute to lymphocyte infiltration to psoriatic plaques (19).

The results of our study align with a previous study that found no significant differences in the application of topical therapy between men and women. The study noted, "Men are more likely than women to receive intensive treatments for severe psoriasis, at least in part due to the teratogenic potential of these treatments" (20).

In the psoriasis group, the results showed no significant differences in TGF-β1 levels between patients younger than 40 and those older than 40. These findings are consistent with a study that examined the effectiveness of biological therapy (etanercept) in elderly patients with moderate to severe psoriasis, revealing that it had a similar impact on quality of life in both elderly and younger patients (21).

Studies showed that increasing expression of the *LRRC32* gene in naive human T-cells appeared to provide a limited Treg phenotype, but low expression of LRRC32 in extended Treg resulted in only uncertain absence in the suppressive function of Treg, and downregulation of FOXP3 (22). In addition, fresh human Tregs showed a selective GARP mRNA and rapidly upregulated after activation (13). The results of this study pointed out that increased folding of *LRRC32* gene expression indicated the effectiveness of Treg cells in the blood sample of patients with psoriasis despite being treated with different therapeutic mechanisms.

The high gene expression of GARP is an indication of the inhibition activity of Tregs in the peripheral blood (22).

According to recent studies, in certain cell types, the latent associated peptide in the TGF-β complex becomes disulfide-linked to the transmembrane protein GARP and fastens it to the TGF-β producing cell surface (23). Previous research revealed an indirect correlation between LTGF-β1 on activated Treg and the kinetics of *LRRC32* gene (GARP) expression (24). Freshly isolated Tregs transfected with TGF-β1 siRNA were able to express GARP after 48 h of activation, while Tregs transfected with GARP siRNA failed to express LTGF-β; this result indicates that GARP is required for the TGF-β1 expression (25), which agrees with the result of this study, indicating that there was a significant decrease in the level of TGF-β1 in the serum of psoriasis patients under different therapeutic mode compared to the high gene expression of its receptor (GARP) on the Treg surface, since there was a negative correlation between them under topical therapy.

Conclusion

According to the findings of the present study, it is concluded that Tregs are highly effective in patients with psoriasis treated with biological therapy than those who had topical therapy, by increasing the expression of GARP, accompanied by a significant reduction of TGF-β1. The high production of GARP in turn activates FOXP3 which enhances the inhibitory action of Treg.

Ethics

Ethics Committee Approval: The study approved by Ethics Committee of Baghdad University (approval number: 4624; date: 24.07.2019).

Informed Consent: Informed consent was obtained from individual participants or legal guardians for subjects unable to consent.

Authorship Contributions

Surgical and Medical Practices: S.R.A., Z.T.S.A., M.M.J., Concept: S.R.A., Z.T.S.A., M.M.J., Design: S.R.A., Z.T.S.A., M.M.J., Data Collection or Processing: S.R.A., Z.T.S.A., M.M.J., Analysis or Interpretation: S.R.A., Z.T.S.A., M.M.J., Literature Search: S.R.A., Z.T.S.A., M.M.J., Writing: S.R.A., Z.T.S.A., M.M.J.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare that they have no relevant financial.

References

1. Fiyouzi T, Pelaez-Prestel HF, Reyes-Manzanas R, Lafuente EM, Reche PA. Enhancing regulatory T cells to treat inflammatory and autoimmune diseases. Int J Mol Sci. 2023;24:7797.
2. Singh R, Alape D, de Lima A, Ascanio J, Majid A, Gangadharan SP. Regulatory T cells in respiratory health and diseases. Pulm Med. 2019;2019:1907807.
3. Chan DV, Somani AK, Young AB, Massari JV, Ohtola J, Sugiyama H, et al. Signal peptide cleavage is essential for surface expression of a regulatory T cell surface protein, leucine rich repeat containing 32 (LRRC32). BMC Biochem. 2011;12:27.
4. Gao S, Liu K, Jiao Y, Chen P, Gu B, Liu Y, et al. Selective activation of TGFβ signaling by P. gingivalis-mediated upregulation of GARP aggravates esophageal squamous cell carcinoma. Am J Cancer Res. 2023;13:2013-29.
5. Ollendorff V, Noguchi T, deLapeyriere O, Birnbaum D. The GARP gene encodes a new member of the family of leucine-rich repeat-containing proteins. Cell Growth Differ. 1994;5:213-9.
6. Roubin R, Pizette S, Ollendorff V, Planche J, Birnbaum D, Delapeyriere O. Structure and developmental expression of mouse GARP, a gene encoding a new leucine-rich repeat-containing protein. Int J Dev Biol. 1996;40:545-55.
7. Wang R, Zhu J, Dong X, Shi M, Lu C, Springer TA. GARP regulates the bioavailability and activation of TGFβ. Mol Biol Cell. 2012;23:1129-39.
8. Zimmer N, Kim E, Sprang B, Leukel P, Khafaji F, Ringel F, et al. GARP as an immune regulatory molecule in the tumor microenvironment of glioblastoma multiforme. Int J Mol Sci. 2019;20:3676.
9. Elliott RL, Blobe GC. Role of transforming growth factor beta in human cancer. J Clin Oncol. 2005;23:2078-93.
10. Sun L, Jin H, Li H. GARP: a surface molecule of regulatory T cells that is involved in the regulatory function and TGF-β releasing. Oncotarget. 2016;7:42826-36.
11. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. Annu Rev Immunol. 1998;16:137-61.
12. Wu BX, Li A, Lei L, Kaneko S, Wallace C, Li X, et al. Glycoprotein A repetitions predominant (GARP) positively regulates transforming growth factor (TGF) β3 and is essential for mouse palatogenesis. J Biol Chem. 2017;292:18091-7.

13. Metelli A, Salem M, Wallace CH, Wu BX, Li A, Li X, et al. Immunoregulatory functions and the therapeutic implications of GARP-TGF-β in inflammation and cancer. J Hematol Oncol. 2018;11:24.

14. Stockis J, Colau D, Coulie PG, Lucas S. Membrane protein GARP is a receptor for latent TGF-beta on the surface of activated human Treg. Eur J Immunol. 2009;39:3315-22.

15. Griffith A. SPSS for Dummies (2nd ed). Wiley publishing-Inc. Indianapolis, Indiana;2007:363 pp.

16. Zaragoza B, Chen X, Oppenheim JJ, Baeyens A, Gregoire S, Chader D, et al. Suppressive activity of human regulatory T cells is maintained in the presence of TNF. Nat Med. 2016;22:16-7.

17. Kallimanis PG, Xenos K, Markantonis SL, Stavropoulos P, Margaroni G, Katsambas A, et al. Serum levels of transforming growth factor-β1 in patients with mild psoriasis vulgaris and effect of treatment with biological drugs. Clin Exp Dermatol. 2009;34:582-6.

18. Flisiak I, Chodynicka B, Porebski P, Flisiak R. Association between psoriasis severity and transforming growth factor beta(1) and beta (2) in plasma and scales from psoriatic lesions. Cytokine. 2002;19:121-5.

19. Cai JP, Falanga V, Taylor JR, Chin YH. Transforming growth factor-beta receptor binding and function are decreased in psoriatic dermal endothelium. J Invest Dermatol. 1996;106:225-31.

20. Hotard RS, Feldman SR, Fleischer AB Jr. Sex-specific differences in the treatment of severe psoriasis. J Am Acad Dermatol. 2000;42:620-3.

21. Militello G, Xia A, Stevens SR, Van Voorhees AS. Etanercept for the treatment of psoriasis in the elderly. J Am Acad Dermatol. 2006;55:517-9.

22. Probst-Kepper M, Geffers R, Kröger A, Viegas N, Erck C, Hecht HJ, et al. GARP: a key receptor controlling FOXP3 in human regulatory T cells. J Cell Mol Med. 2009;13:3343-57.

23. Massagué J, Sheppard D. TGF-β signaling in health and disease. Cell. 2023;186:4007-37.

24. Tran DQ, Andersson J, Hardwick D, Bebris L, Illei GG, Shevach EM. Selective expression of latency-associated peptide (LAP) and IL-1 receptor type I/II (CD121a/CD121b) on activated human FOXP3+ regulatory T cells allows for their purification from expansion cultures. Blood. 2009;113:5125-33.

25. Shevach EM. Garp as a therapeutic target for modulation of T regulatory cell function. Expert Opin Ther Targets. 2017;21:191-200.